

ANTIBODIES AGAINST CD112R AND USES THEREOF

FIELD OF THE INVENTION

The present invention is in the field of immunotherapy and relates to antibodies and
5 fragments thereof which bind to the human protein CD112R (PVRIG), to polynucleotide
sequences encoding these antibodies and to cells producing them. Pharmaceutical
compositions comprising these antibodies and their uses are also included.

BACKGROUND OF THE INVENTION

10 Cancer immunotherapy is utilized for generating and augmenting anti-tumor immune
responses, e.g., by treatment with antibodies specific to antigens on tumor cells, by chimeric
antigen receptors T cells (CAR-T), or by specific activation of anti-tumor cells. The ability of
recruiting immune cells (e.g., T cells or NK cells) against tumor cells in a patient provides a
therapeutic modality of fighting tumors and their metastasis that otherwise were considered
15 incurable.

T cell mediated immune responses include multiple sequential steps regulated by co-
stimulatory and co-inhibitory signals leading to a net effect that controls the magnitude of the
immune response and its outcome. The inhibitory signals, referred to as immune checkpoints,
are crucial for the maintenance of self-tolerance and for the limitation of immune-mediated
20 collateral tissue damage.

The expression of immune checkpoint proteins is altered by tumors. For example,
upregulation of programmed death-ligand 1 (PD-L1) on the surface of cancer cells allows them
to bind to the checkpoint molecule PD-1, expressed on T cells. This leads to inhibition of T
cells, which might otherwise attack the tumor cells, and thus allows the cancer cells to evade
25 the host immune system. Hence, immune checkpoints represent significant barriers for the
activation of functional cellular immunity against cancer. Accordingly, antagonistic antibodies
specific for inhibitory ligands on T cells (e.g., PD-1) are examples of targeted agents against
immune checkpoint inhibitors (ICIs) that are being used in cancer therapy (e.g., Nivolumab,
Pembrolizumab). Another example for an immune checkpoint molecule is T cell
30 immunoreceptor with Ig and ITIM domains (TIGIT). TIGIT is a co-inhibitory molecule
expressed on various immune cells, including T cells and natural killer cells (NK cells). TIGIT

binds with high affinity to polio virus receptor (PVR, CD155) and with low affinity to Nectin-2 (CD112), both expressed by various cancer cells.

CD112R, also called PVRIG, binds with high affinity to Nectin-2. Upon interaction with CD112R, and/or TIGIT, Nectin-2 inhibits T-cell proliferation. Importantly, Nectin-2 can also serve as a costimulator of T-cell functions, upon binding to CD226 (DNAM-1). This interaction stimulates T-cell proliferation and cytokine production, such as IL-2, and IFN γ . These contradictory interactions are competitive, and their net effect impacts the resulting anti-cancer immune response.

WO2019232484 discloses anti-PVRIG, anti-TIGIT, and anti-PVRIG/anti-TIGIT bispecific antibodies, as well as compositions, and methods of using the antibodies for the treatment of cancer.

WO2018017864 discloses agents and bi-specific agents, including antibodies that specifically bind PVRIG. WO2018017864 also discloses methods of using the agents for enhancing the immune response and/or treatment of diseases such as cancer.

WO2016134333 discloses anti-PVRIG antibodies and methods of using same.

There is an unmet need for additional and more effective, specific, safe, and stable agents that alone, or in combination with other agents, may potentiate cells of the immune system to attack tumors or virus infected cells. Monoclonal antibodies (mAbs) inhibiting CD112R binding to Nectin-2, may be such agents.

SUMMARY OF THE INVENTION

The present invention provides antibodies and fragments thereof that recognize CD112R (PVRIG), bind it, prevent its binding to Nectin-2 (CD112) and inhibit the resulting suppressive activity on lymphocytes such as natural killer (NK) cells and T-cells. These antibodies and fragment thereof are characterized by having unique sets of CDR sequences, high affinity, and high specificity to human CD112R, and are useful in cancer immunotherapy for combating tumor immune evasion, as either a stand-alone therapy or in combination with other anti-cancer agents. The antibodies disclosed herein are also useful in treating viral infections and may be used for detection assays and cancer diagnosis.

It is now disclosed that the high affinity anti-CD112R monoclonal antibodies (mAbs) described herein block CD112R-Nectin-2 interactions and subsequently restore T and NK cell activities. These properties make the mAbs of the present invention valuable candidates for use in anti-cancer immune-therapy, enabling administration of lower doses with fewer side effects.

5 The anti-CD112R mAbs described herein were found to induce T cell activation similarly to that induced by anti-PD-1, anti-TIGIT, and anti-PVR mAbs. Moreover, the combination of some of the anti-CD112R mAbs, described herein, with other immuno-modulating agents, including anti-PD-1, anti-TIGIT or anti-PVR mAbs resulted in a significant increase of anti-cancer activity that exceeded the activity level induced by each of the individual mAbs.
10 CD112R mAbs disclosed herein were able to induce NK cell activation in the presence of target cancer cells and to synergize with anti-TIGIT and anti-PVR mAbs for the activation of NK cells. It is further disclosed that the antibodies described herein were found to be highly specific to human and cynomolgus CD112R, but not to rodent CD112R. It is further disclosed, according to some embodiments, that the anti-CD112R mAbs block CD112R interactions with
15 its ligand Nectin-2 and increase activation of immune cells.

Unexpectedly, the humanized forms of the anti-CD112R mAbs of the present invention were found to possess improved binding and productivity properties in comparison to the parental murine mAbs. Furthermore, the humanized mAb variants have a cross-reactivity with cynomolgus CD112R, which enables further studies in monkeys. The antibodies described
20 herein possess unique properties and improved potency over known anti-CD112R antibodies.

Surprisingly and advantageously, specific mutations in the complementarity determining region 1 (CDR1) of the light chain and in CDR1 of the heavy chain, introduced to remove deamidation and N-glycosylation motifs, respectively, preserved the affinity to human CD112R.

25 According to one aspect, the present invention provides an antibody, or an antibody fragment thereof comprising at least the antigen binding portion, which specifically binds to human CD112R, said antibody or fragment thereof have an affinity to human CD112R of at least $0.5 \times 10^{-9} \text{M}$.

According to some embodiments, the antibody specifically binds to human CD112R and
30 inhibits its binding to its ligand, human Nectin-2 (CD112).

According to some embodiments, the antibody or antibody fragment comprises a set of six CDR sequences selected from the group consisting of:

- i. three CDRs of a heavy-chain (HC) variable region comprising SEQ ID NO: 17 and three CDRs of a light-chain (LC) variable comprising SEQ ID NO: 19, or an analog or derivative thereof having at least 90% sequence identity with said antibody or fragment sequence; and
- ii. three CDRs of a heavy-chain variable region comprising SEQ ID NO: 21 and three CDRs of a light-chain variable region comprising SEQ ID NO: 23, or an analog or derivative thereof having at least 90% sequence identity with said antibody or fragment sequence.

There are several methods known in the art for determining the CDR sequences of a given antibody molecule, but there is no standard unequivocal method. Determination of CDR sequences from antibody heavy and light chain variable regions can be made according to any method known in the art, including, but not limited to, the methods known as KABAT, Chothia, and IMGT. A selected set of CDRs may include sequences identified by more than one method, namely, some CDR sequences may be determined using KABAT and some using IMGT, for example. According to some embodiments, the CDR sequences of the mAb variable regions are determined using the KABAT and/or Chothia methods.

According to some embodiments, the antibody or its fragment comprises the CDR sequences of a monoclonal antibody denoted clone 13, namely, the three CDR sequences contained in heavy chain variable region set forth in SEQ ID NO: 17 and the three CDR sequences contained in light chain variable region set forth in SEQ ID NO: 19, or the CDR sequences of a monoclonal antibody denoted clone 15, namely, the three CDR sequences contained in heavy chain variable region set forth in SEQ ID NO: 21 and the three CDR sequences contained in light chain variable region set forth in SEQ ID NO: 23.

The present invention comprises variants and analogs of the antibody denoted clone 13. According to some embodiments, the antibody or antibody fragment comprises a set of three CDR sequences of a heavy-chain variable region comprising SEQ ID NO: 28 and three CDRs of a light-chain variable comprising SEQ ID NO: 26, or an analog or derivative thereof having at least 90% sequence identity with said antibody or fragment sequence.

According to some embodiments, the antibody or the antibody fragment comprises heavy-chain CDR1 comprising the sequence GYX₁FX₂SY (SEQ ID NO: 1), wherein X₁ denotes N, D, A, Q, S, or T; and X₂ denotes T or A, or the sequence SYWIN (SEQ ID NO: 7). According to some embodiments, the antibody or the antibody fragment comprises heavy-chain CDR2 comprising the sequence YPGSYIP (SEQ ID NO: 2). According to some
5 embodiments, the antibody or the antibody fragment comprises heavy-chain CDR3 comprising the sequence GYFDV (SEQ ID NO: 3).

According to certain embodiments, the antibody or the antibody fragment comprises: (i) heavy-chain CDR1 comprising the sequence GYX₁FX₂SY (SEQ ID NO: 1) wherein X₁
10 denotes N, D, A, Q, S, or T; and X₂ denotes T or A, or the sequence SYWIN (SEQ ID NO: 7); (ii) heavy-chain CDR2 comprising the sequence YPGSYIP (SEQ ID NO: 2); and (iii) heavy-chain CDR3 comprising the sequence GYFDV (SEQ ID NO: 3).

According to some embodiments, the antibody or the antibody fragment comprises light-chain CDR1 comprising the sequence KSSQSLLXSGNQKNYLA (SEQ ID NO: 4), wherein
15 X denotes N or S. According to some embodiments, the antibody or the antibody fragment comprises light-chain CDR2 comprising the sequence GASTRES (SEQ ID NO: 5). According to some embodiments, the antibody or the antibody fragment comprises light-chain CDR3 comprising the sequence QNDHSYPYT (SEQ ID NO: 6).

According to certain embodiments, the antibody or the antibody fragment comprises: (i)
20 light-chain CDR1 comprising the sequence KSSQSLLXSGNQKNYLA (SEQ ID NO: 4) wherein X denotes N or S; (ii) light-chain CDR2 comprising the sequence GASTRES (SEQ ID NO: 5); and (iii) heavy-chain CDR3 comprising the sequence QNDHSYPYT (SEQ ID NO: 6).

According to some specific embodiments the antibody or fragment comprises heavy
25 chain CDR1 sequence comprising the sequence GYX₁FX₂SY (SEQ ID NO: 1), wherein X₁ denotes N, D, A, Q, S, or T; and X₂ denotes T or A or the sequence SYWIN (SEQ ID NO: 7), heavy chain CDR2 comprising the sequence YPGSYIP (SEQ ID NO: 2), heavy chain CDR3 comprising the sequence GYFDV (SEQ ID NO: 3), light chain CDR1 comprising the sequence KSSQSLLXSGNQKNYLA (SEQ ID NO: 4) wherein X denotes N or S, light chain CDR2
30 comprising the sequence GASTRES (SEQ ID NO: 5), and light chain CDR3 comprising the sequence QNDHSYPYT (SEQ ID NO: 6), or analogs thereof comprising no more than 5%

amino acid substitution, deletion and/or insertion in the hypervariable region (HVR) sequence that comprises the six CDRs.

According to some specific embodiments the antibody or fragment comprises a set of six CDR sequences consisting of:

- 5 i. heavy chain CDR1 having a sequence set forth in SEQ ID NO: 1 or 7;
- ii. heavy chain CDR2 having a sequence set forth in SEQ ID NO: 2;
- iii. heavy chain CDR3 having a sequence set forth in SEQ ID NO: 3;
- iv. light chain CDR1 having a sequence set forth in SEQ ID NO: 4;
- v. light chain CDR2 having a sequence set forth in SEQ ID NO: 5; and
- 10 vi. light chain CDR3 having a sequence set forth in SEQ ID NO: 6.

According to some embodiments, the heavy chain CDR1 sequence comprises the sequence GYX₁FX₂SY (SEQ ID NO: 1), wherein X₁ denotes N, D, A, Q, S, or T; and X₂ is T or A. According to some embodiments, the heavy chain CDR1 sequence comprising the sequence GYX₁FX₂SY (SEQ ID NO: 1), wherein X₁ denotes N, D, A, Q, S, or T and X₂ is T.

15 According to some embodiments, the heavy chain CDR1 sequence comprising the sequence GYTFTSY (SEQ ID NO: 13). According to additional embodiments, the heavy chain CDR1 sequence comprising the sequence GYNFASY (SEQ ID NO: 14). According to additional embodiments, the heavy chain CDR1 sequence comprising the sequence SYWIN (SEQ ID NO: 7).

20 According to some embodiments, the heavy chain CDR2 sequence comprising the sequence YPGSYIP (SEQ ID NO: 2) or DIYPGSYIPNYNEKFKN (SEQ ID NO: 8). According to certain embodiments, the heavy chain CDR2 sequence comprising the sequence DIYPGSYIPNYNEKFKN (SEQ ID NO: 8).

According to some embodiments, the light chain CDR1 sequence comprising the sequence KSSQSLLXSGNQKNYLA (SEQ ID NO: 4), wherein X is N. According to specific

25 embodiments, the light chain CDR1 sequence comprising the sequence KSSQSLLSSGNQKNYLA (SEQ ID NO: 15).

According to some embodiments, the antibody or fragment thereof comprises heavy chain variable region set forth in SEQ ID NO: 28, or an analog or derivative thereof having at

30 least 90% sequence identity with the heavy chain variable region sequence.

According to some embodiments, the antibody or fragment thereof comprises light chain variable region set forth in SEQ ID NO: 26, or an analog thereof having at least 90% sequence identity with the light chain variable region sequence.

5 According to a specific embodiment, the antibody or fragment thereof comprises a heavy chain variable region having a sequence set forth in SEQ ID NO: 28, and a light chain variable region having a sequence set forth in SEQ ID NO: 26, or an analog thereof having at least 90% sequence identity with the light and/or heavy chain sequence.

10 According to a specific embodiment, the antibody or fragment thereof comprises a heavy chain variable region having a sequence set forth in SEQ ID NO: 17, and a light chain variable region having a sequence set forth in SEQ ID NO: 19, or an analog thereof having at least 90% sequence identity with the light and/or heavy chain sequence.

15 According to some embodiments, the antibody or fragment comprises the CDR sequences of a monoclonal antibody denoted clone 13, namely, the three CDR sequences contained in heavy chain variable region set forth in SEQ ID NO: 17 and the three CDR sequences contained in light chain variable region set forth in SEQ ID NO: 19.

20 According to additional embodiments, the antibody or the antibody fragment comprises heavy-chain CDR1 comprising the sequence GYNFTSY (SEQ ID NO: 9) or SYWIN (SEQ ID NO: 7). According to some embodiments, the antibody or the antibody fragment comprises heavy-chain CDR2 comprising the sequence FPGSYS (SEQ ID NO: 10). According to some embodiments, the antibody or the antibody fragment comprises heavy-chain CDR3 comprising the sequence GYFDV (SEQ ID NO: 3).

25 According to certain embodiments, the antibody or the antibody fragment comprises: (i) heavy-chain CDR1 comprising the sequence GYNFTSY (SEQ ID NO: 9) or SYWIN (SEQ ID NO: 7); (ii) heavy-chain CDR2 comprising the sequence FPGSYS (SEQ ID NO: 10); and (iii) heavy-chain CDR3 comprising the sequence GYFDV (SEQ ID NO: 3).

According to some embodiments, the antibody or the antibody fragment comprises light-chain CDR1 comprising the sequence KSSQSLLNSGSQKNYLA (SEQ ID NO: 11). According to some embodiments, the antibody or the antibody fragment comprises light-chain CDR2 comprising the sequence GASTRES (SEQ ID NO: 5). According to some embodiments,

the antibody or the antibody fragment comprises light-chain CDR3 comprising the sequence QNDHSYPYT (SEQ ID NO: 6).

According to certain embodiments, the antibody or the antibody fragment comprises: (i) light-chain CDR1 comprising the sequence KSSQSLNLSGSQKNYLA (SEQ ID NO: 11); (ii) 5 light-chain CDR2 comprising the sequence GASTRES (SEQ ID NO: 5); and (iii) heavy-chain CDR3 comprising the sequence QNDHSYPYT (SEQ ID NO: 6).

According to some specific embodiments, the antibody or fragment comprises heavy chain CDR1 sequence comprising the sequence GYNFTSY (SEQ ID NO: 9) or SYWIN (SEQ ID NO: 7), heavy chain CDR2 comprising the sequence FPGSYS (SEQ ID NO: 10), heavy 10 chain CDR3 comprising the sequence GYFDV (SEQ ID NO: 3), light chain CDR1 comprising the sequence KSSQSLNLSGSQKNYLA (SEQ ID NO: 11), light chain CDR2 comprising the sequence GASTRES (SEQ ID NO: 5), and light chain CDR3 comprising the sequence QNDHSYPYT (SEQ ID NO: 6), or analogs thereof comprising no more than 5% amino acid substitution, deletion and/or insertion in the hypervariable region (HVR) sequence.

15 According to some specific embodiments the antibody or fragment comprises a set of six CDR sequences consisting of:

- i. heavy chain CDR1 having a sequence set forth in SEQ ID NO: 9 or 7;
- ii. heavy chain CDR2 having a sequence set forth in SEQ ID NO: 10;
- iii. heavy chain CDR3 having a sequence set forth in SEQ ID NO: 3;
- 20 iv. light chain CDR1 having a sequence set forth in SEQ ID NO: 11;
- v. light chain CDR2 having a sequence set forth in SEQ ID NO: 5; and
- vi. light chain CDR3 having a sequence set forth in SEQ ID NO: 6.

According to some embodiments, the light chain CDR1 sequence comprising the sequence GYNFTSY (SEQ ID NO: 9). According to other embodiments, the light chain CDR1 25 sequence comprising the sequence SYWIN (SEQ ID NO: 7).

According to some embodiments, the heavy chain CDR2 sequence comprising the sequence DIFPGSYSPNKNKKFKR (SEQ ID NO: 12).

According to some embodiments, the CDRs are determined by KABAT and set forth in SEQ ID NOs: 7, 8, 3, 4, 5, and 6. According to some embodiments, the CDRs are determined by KABAT and set forth in SEQ ID NOs: 7, 12, 3, 11, 5, and 6.

According to some embodiments, the CDRs are determined by Chothia and set forth in
5 SEQ ID NOs: 1, 2, 3, 4, 5, and 6. According to some embodiments, the CDRs are determined by Chothia and set forth in SEQ ID NOs: 9, 10, 3, 11, 5, and 6.

According to some embodiments, the antibody or fragment thereof comprises heavy chain variable region set forth in SEQ ID NO: 21, or an analog or derivative thereof having at least 90% sequence identity with the heavy chain variable region sequence.

10 According to some embodiments, the antibody or fragment thereof comprises light chain variable region set forth in SEQ ID NO: 23, or an analog thereof having at least 90% sequence identity with the light chain variable region sequence.

According to a specific embodiment, the antibody or fragment thereof comprises a heavy chain variable region having a sequence set forth in SEQ ID NO: 21, and a light chain variable
15 region having a sequence set forth in SEQ ID NO: 23, or an analog thereof having at least 90% sequence identity with the light and/or heavy chain sequence.

According to a specific embodiment, the antibody or fragment thereof comprises a heavy chain variable region having a sequence set forth in SEQ ID NO: 21, and a light chain variable region having a sequence set forth in SEQ ID NO: 23.

20 According to some embodiments, the antibody or fragment thereof recognizes human CD112R with affinity of at least 10^{-10} M. According to other embodiments, the antibody or antibody fragment binds with affinity of 5×10^{-11} M, or even higher, to human CD112R. According to some embodiments, the antibody or antibody fragment binds to human CD112R with affinity at the range of 0.5×10^{-9} M to 10^{-11} M. According to some embodiments, the
25 antibody or antibody fragment binds to human CD112R with affinity at the range of 10^{-10} M to 10^{-11} M. Each possibility represents a separate embodiment of the invention.

Analogous and derivatives of the antibody and the fragments described above, are also within the scope of the invention.

According to some embodiments, the antibody or antibody fragment analog have at least 95% sequence identity with the hypervariable region of the reference antibody sequence.

According to certain embodiments, the analog or derivative of the isolated antibody or fragment thereof has at least 91, 92, 93, 94, 95, 96, 97, 98 or 99% sequence identity with a variable region of the reference antibody sequence. Each possibility represents a separate embodiment of the invention.

According to some embodiments, the antibody or antibody fragment according to the invention comprises a heavy chain variable region set forth in SEQ ID NO: 28 or SEQ ID NO: 21, or an analog having at least 95% sequence similarity with said sequence.

According to some embodiments, the antibody or antibody fragment comprises a light chain variable region set forth in SEQ ID NO: 26 or SEQ ID NO: 23, or an analog having at least 95% sequence similarity with said sequence.

According to some embodiments, the antibody or antibody fragment comprises a heavy chain and a light chain, wherein: (i) the heavy chain comprises SEQ ID NO: 28 and the light chain comprises SEQ ID NO: 26; or (ii) the heavy chain comprises SEQ ID NO: 21 and the light chain comprises SEQ ID NO: 23. Analogs of the antibodies or fragments, having at least 95% sequence similarity with said heavy or light chains are also included.

According to some embodiments, the analog has at least 96, 97, 98 or 99% sequence similarity or identity with an antibody light or heavy chain variable regions described above.

According to some embodiments, the analog comprises no more than one amino acid substitution, deletion or addition to one or more CDR sequences of the hypervariable region, namely, any one of the CDR sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15. Each possibility represents a separate embodiment of the present invention. According to some embodiments, the amino acid substitution is a conservative substitution.

According to some embodiments, the antibody or antibody fragment comprises a hypervariable region (HVR) having light and heavy chain regions defined above, in which 1, 2, 3, 4, or 5 amino acids were substituted, deleted and/or added. Each possibility represents a separate embodiment of the invention.

According to some embodiments, the antibody or antibody fragment comprises a HVR having light and heavy chain regions defined above, in which one amino acid was substituted. According to specific embodiments, the antibody or antibody fragment comprises a CDR as defined above, in which one amino acid was substituted.

5 According to some embodiments, the antibody or the antibody fragment comprises a CDR set selected from the group consisting of:

- i. a set of six CDRs wherein: heavy-chain CDR1 is GYX₁FX₂SY (SEQ ID NO: 1) wherein X₁ denotes N, D, A, Q, S, or T; and X₂ denotes T or A; heavy-chain CDR2 is YPGSYIP (SEQ ID NO: 2); heavy-chain CDR3 is GYFDV (SEQ ID NO: 3); light-chain CDR1 is KSSQSLLXSGNQKNYLA (SEQ ID NO: 4) wherein X denotes N or S; light-chain CDR2 is GASTRES (SEQ ID NO: 5); and light-chain CDR3 is QNDHSYPYT (SEQ ID NO: 6);
- 10 ii. a set of six CDRs wherein: heavy-chain CDR1 is SYWIN (SEQ ID NO: 7); heavy-chain CDR2 is YPGSYIP (SEQ ID NO: 2); heavy-chain CDR3 is GYFDV (SEQ ID NO: 3); light-chain CDR1 is KSSQSLLXSGNQKNYLA (SEQ ID NO: 4) wherein X denotes N or S; light-chain CDR2 is GASTRES (SEQ ID NO: 5); and light-chain CDR3 is QNDHSYPYT (SEQ ID NO: 6); and
- 15 iii. a set of six CDRs wherein: heavy-chain CDR1 sequence is GYNFTSY (SEQ ID NO: 9) or SYWIN (SEQ ID NO: 7); heavy-chain CDR2 is FPGSYS (SEQ ID NO: 10); heavy-chain CDR3 is GYFDV (SEQ ID NO: 3); light-chain CDR1 is KSSQSLLNSGSQKNYLA (SEQ ID NO: 11); light-chain CDR2 is GASTRES (SEQ ID NO: 5); and light-chain CDR3 is QNDHSYPYT (SEQ ID NO: 6).
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The present invention also provides antibodies and binding fragments thereof, comprising a heavy chain and a light chain, wherein said chains comprise a set of heavy chain variable region sequence and light chain variable region sequence, said set is selected from the group consisting of:

- i. SEQ ID NOS: 17 and 19; and
- ii. SEQ ID NOS: 21 and 23.

30 The present invention also provides antibodies and binding fragments thereof, comprising a heavy chain and a light chain, wherein said chains comprises a set of heavy chain

variable region sequence set forth in SEQ ID NO: 28 and light chain variable region sequence set forth in SEQ ID NO: 26.

According to some embodiments, the antibody is an isolated monoclonal antibody.

According to specific embodiments, the mAb is a chimeric mAb.

5 According to some embodiments, the mAb or the chimeric mAb comprises a constant region selected from the group consisting of: mouse IgG1, mouse IgG2a, mouse IgG2b, mouse IgG3, human IgG1, human IgG2, human IgG3 and human IgG4. Each possibility represents a separate embodiment of the present invention.

10 According to yet other embodiments, the chimeric mAb comprised of human-derived constant region.

According to some embodiments the human constant regions of the chimeric mAb are selected from the group consisting of: human IgG1, human IgG2, human IgG3, and human IgG4.

15 According to some embodiments the human constant region of the chimeric mAb is human IgG1.

20 According to specific embodiments, the antibody is an antibody fragment. According to a specific embodiment, the antibody fragment is selected from the group consisting of: Fab, Fab', F(ab')₂, Fd, Fd', Fv, dAb, isolated CDR region, single chain variable fragment (scFv), single chain antibody (scab), "diabodies", and "linear antibodies". Each possibility represents a separate embodiment of the present invention.

The present invention also provides humanized antibodies comprising a set of six CDRs of any of the mAbs described herein.

25 According to some embodiments, the humanized antibody or the antibody fragment comprises a set of six CDRs wherein: heavy-chain CDR1 is GYX₁FX₂SY (SEQ ID NO: 1), wherein X₁ denotes N, D, A, Q, S, or T; and X₂ is T or A, or SYWIN (SEQ ID NO: 7); heavy-chain CDR2 is YPGSYIP (SEQ ID NO: 2); heavy-chain CDR3 is GYFDV (SEQ ID NO: 3); light-chain CDR1 is KSSQSLLXSGNQKNYLA (SEQ ID NO: 4), wherein X is N or S; light-

chain CDR2 is GASTRES (SEQ ID NO: 5); and light-chain CDR3 is QNDHSYPYT (SEQ ID NO: 6).

According to some embodiments, the humanized antibody comprises heavy-chain CDR1 GYTFTSY (SEQ ID NO: 13).

5 According to specific embodiments, the humanized antibody comprises light-chain CDR1 KSSQSLSSGNQKNYLA (SEQ ID NO: 15).

According to certain embodiments, the humanized antibody or the antibody fragment comprises a set of six CDRs wherein: heavy-chain CDR1 is GYTFTSY (SEQ ID NO: 13); heavy-chain CDR2 is YPGSYIP (SEQ ID NO: 2); heavy-chain CDR3 is GYFDV (SEQ ID NO: 3); light-chain CDR1 is KSSQSLSSGNQKNYLA (SEQ ID NO: 15); light-chain CDR2
10 is GASTRES (SEQ ID NO: 5); and light-chain CDR3 is QNDHSYPYT (SEQ ID NO: 6).

According to some embodiments, the humanized antibody comprises a heavy chain variable region sequence selected from the group consisting of: SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38 and SEQ ID NO: 39, or an analog or derivative thereof having at least 90% sequence identity with the heavy chain variable region sequence.
15 Each possibility represents a separate embodiment of the invention.

According to some embodiments, the humanized antibody comprises a light chain variable region sequence selected from the group consisting of: SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42, or an analog or derivative thereof having at least 90% sequence identity with the heavy chain variable region sequence. Each possibility represents a separate
20 embodiment of the invention.

According to some embodiments, the humanized antibody comprises a heavy chain variable region sequence selected from the group consisting of: SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38 and SEQ ID NO: 39, and a light chain variable region sequence selected from the group consisting of: SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42, or an analog or derivative thereof having at least 95% sequence identity with the variable region sequences.
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According to some embodiments, the humanized antibody comprises a heavy chain and a light chain, wherein said chains comprises a set of a heavy chain variable region sequence and a light chain variable region sequence, said set is selected from the group consisting of:

- i. SEQ ID NOs: 39 and 40 (denoted antibody H11K1);
- 5 ii. SEQ ID NOs: 39 and 41 (denoted antibody H11K2); and
- iii. SEQ ID NOs: 39 and 42 (denoted antibody H11K3).

According to some embodiments, a conjugate comprising the antibody or fragment thereof as described herein is provided.

10 A conjugate according to some embodiments of the present invention comprises an antibody or fragment thereof defined above, attached directly or through a spacer or a linker to a moiety including but not limited to, a radioactive moiety, a labeling tag and a cytotoxic moiety.

Polynucleotide sequences encoding antibodies, having high affinity and specificity for human CD112R, as well as vectors and host cells carrying these polynucleotide sequences, are
15 provided according to another aspect of the present invention.

According to some embodiments, polynucleotide sequences encoding the amino acid sequences of heavy chain variable region and light chain variable region described above are provided.

According to some embodiments, the polynucleotide sequence encodes an antibody or
20 antibody fragment comprising the sequence set forth in a sequence selected from the group consisting of: (i) SEQ ID NO: 16 and SEQ ID NO: 18; (ii) SEQ ID NO: 20 and SEQ ID NO: 22; and (iii) SEQ ID NO: 24 and SEQ ID NO: 25, or an analog or derivative thereof having at least 90% sequence identity with the variable region sequences. Each possibility represents a separate embodiment of the present invention.

25 According to yet some embodiments, the polynucleotide sequence according to the invention encodes an antibody or antibody fragment or chain comprising a set of six CDRs wherein: heavy-chain CDR1 is GYX₁FX₂SY (SEQ ID NO: 1) wherein X₁ denotes N, D, A, Q, S, or T; and X₂ is T or A or SYWIN (SEQ ID NO: 7); heavy-chain CDR2 is YPGSYIP (SEQ ID NO: 2); heavy-chain CDR3 is GYFDV (SEQ ID NO: 3); light-chain CDR1 is

KSSQSLLXSGNQKNYLA (SEQ ID NO: 4) wherein X denotes N or S; light-chain CDR2 is GASTRES (SEQ ID NO: 5); and light-chain CDR3 is QNDHSYPYT (SEQ ID NO: 6).

According to yet some embodiments, the polynucleotide sequence according to the invention encodes an antibody or antibody fragment comprising a set of six CDRs wherein:
5 heavy chain CDR1 sequence comprising the sequence GYTFTSY (SEQ ID NO: 13), heavy chain CDR2 comprising the sequence YPGSYIP (SEQ ID NO: 2), heavy chain CDR3 comprising the sequence GYFDV (SEQ ID NO: 3), light chain CDR1 comprising the sequence KSSQSLLSSGNQKNYLA (SEQ ID NO: 15), light chain CDR2 comprising the sequence GASTRES (SEQ ID NO: 5), and light chain CDR3 comprising the sequence QNDHSYPYT
10 (SEQ ID NO: 6).

According to some embodiments, the polynucleotide sequences defined above encode a molecule selected from the group consisting of: an antibody, an antibody fragment comprising at least an antigen-binding portion, an antibody chain, and an antibody conjugate comprising said antibody or antibody fragment. Each possibility represents a separate embodiment of the
15 present invention.

According to some embodiments, the polynucleotide sequence encodes a monoclonal antibody heavy chain variable region comprising a sequence set forth in SEQ ID NO: 16 or a variant thereof having at least 90% sequence identity.

According to some embodiments, the polynucleotide sequence encodes a monoclonal
20 antibody heavy chain variable region, comprising a sequence set forth in SEQ ID NO: 20, or a variant thereof having at least 90% sequence identity.

According to some embodiments, the polynucleotide sequence encodes a monoclonal antibody heavy chain variable region, comprising a sequence set forth in SEQ ID NO: 24, or a variant thereof having at least 90% sequence identity.

25 According to some embodiments, the polynucleotide sequence encodes a monoclonal antibody light chain variable region comprising a sequence set forth in SEQ ID NO: 18 or a variant thereof having at least 90% sequence identity.

According to some embodiments, the polynucleotide sequence encodes a monoclonal antibody light chain variable region comprising a sequence set forth in SEQ ID NO: 22, or a variant thereof having at least 90% sequence identity.

According to some embodiments, the polynucleotide sequence encodes a monoclonal antibody light chain variable region comprising a sequence set forth in SEQ ID NO: 25, or a variant thereof having at least 90% sequence identity.

The present invention provides, according to some embodiments, a polypeptide comprising at least one sequence encoded by at least one polynucleotide sequence disclosed above.

In a further aspect, the present invention provides a nucleic acid construct comprising a nucleic acid molecule encoding at least one antibody chain or fragment thereof as described herein. According to some embodiments the nucleic acid construct is a plasmid.

According to some embodiments the plasmid comprises at least one polynucleotide sequence set forth in a sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24 and SEQ ID NO: 25. Each possibility represents a separate embodiment of the present invention.

In still another aspect the present invention provides a cell capable of producing an antibody or an antibody fragment comprising the specific CDR sequences and/or specific heavy and light chain variable regions described herein.

According to some embodiments, a cell, or a population of cells is provided comprising at least one polynucleotide sequence disclosed above.

According to some embodiments, the cell producing the monoclonal antibody is a hybridoma cell.

The present invention provides, according to another aspect, a pharmaceutical composition comprising as an active ingredient, at least one antibody, antibody fragment or conjugates thereof, as described herein, and optionally at least one pharmaceutical acceptable excipient, diluent, salt, or carrier.

According to some embodiments, the pharmaceutical composition comprises at least one antibody comprising a set of six CDRs wherein: heavy-chain CDR1 is GYX₁FX₂SY (SEQ ID NO: 1) wherein X₁ denotes N, D, A, Q, S, or T; and X₂ is T or A or SYWIN (SEQ ID NO: 7); heavy-chain CDR2 is YPGSYIP (SEQ ID NO: 2); heavy-chain CDR3 is GYFDV (SEQ ID NO: 3); light-chain CDR1 is KSSQSLLXSGNQKNYLA (SEQ ID NO: 4) wherein X denotes N or S; light-chain CDR2 is GASTRES (SEQ ID NO: 5); and light-chain CDR3 is QNDHSYPYT (SEQ ID NO: 6).

According to some embodiments, the pharmaceutical composition comprises an antibody or fragment thereof, comprising a heavy-chain CDR1 sequence GYX₁FX₂SY (SEQ ID NO: 1), wherein X₂ is T. According to some embodiments, the pharmaceutical composition comprises an antibody or fragment thereof, comprising a heavy-chain CDR1 sequence GYTFTSY (SEQ ID NO: 13). According to other embodiments, the pharmaceutical composition comprises an antibody or fragment thereof, comprising a heavy-chain CDR1 sequence GYNFASY (SEQ ID NO: 14). According to certain embodiments, the pharmaceutical composition comprises an antibody or fragment thereof, comprising a heavy-chain CDR1 sequence SYWIN (SEQ ID NO: 7).

According to some embodiments, the pharmaceutical composition comprises an antibody or fragment thereof, comprising a light-chain CDR1 sequence KSSQSLLSSGNQKNYLA (SEQ ID NO: 15).

According to some embodiments, the pharmaceutical composition comprises an antibody or antibody fragment comprising a set of six CDRs wherein: heavy chain CDR1 sequence comprising the sequence GYTFTSY (SEQ ID NO: 13), heavy chain CDR2 comprising the sequence YPGSYIP (SEQ ID NO: 2), heavy chain CDR3 comprising the sequence GYFDV (SEQ ID NO: 3), light chain CDR1 comprising the sequence KSSQSLLSSGNQKNYLA (SEQ ID NO: 15), light chain CDR2 comprising the sequence GASTRES (SEQ ID NO: 5), and light chain CDR3 comprising the sequence QNDHSYPYT (SEQ ID NO: 6).

According to some embodiments, the pharmaceutical composition comprises an antibody or fragment thereof comprising a heavy chain set forth in SEQ ID NO: 39.

According to some embodiments, the pharmaceutical composition comprises an antibody or fragment thereof comprising a light chain variable region having a sequence selected from

the group consisting of SEQ ID NO: 40, SEQ ID NO: 41 and SEQ ID NO: 42. Each possibility represents a separate embodiment of the invention.

According to a specific embodiment, the pharmaceutical composition comprises an antibody or fragment thereof comprising a heavy chain variable region having the sequence set forth in SEQ ID NO: 39 and a light chain variable region having the sequence selected from the group consisting of SEQ ID NO: 40, SEQ ID NO: 41 and SEQ ID NO: 42.

Single chain variable fragment (scFv) molecules of the antibodies of the present invention are also provided. The scFv molecules comprise the antigen binding site of the antibody expressed in one polypeptide chain. According to some embodiments, the invention provides scFv molecules comprising a heavy chain and a light chain variable regions of the anti-CD112R antibodies. According to certain embodiments, the scFv comprises a hinge region between the two variable regions. According to specific embodiments, the scFv comprises the heavy and light chains of the humanized antibodies described herein.

According to some embodiments, the scFv comprises a CD112R binding site comprising six CDR sequences selected from the group consisting of:

- i. three complementarity determining regions (CDRs) of a heavy-chain variable region comprising SEQ ID NO: 28 and three CDRs of a light-chain variable comprising SEQ ID NO: 26, or an analog or derivative thereof having at least 90% sequence identity with said antibody or fragment sequence; and
- ii. three CDRs of a heavy-chain variable region comprising SEQ ID NO: 17 and three CDRs of a light-chain variable region comprising SEQ ID NO: 19, or an analog or derivative thereof having at least 90% sequence identity with said antibody or fragment sequence.

According to some embodiments, the scFv comprises a CD112R binding site comprising a set of six CDRs wherein: heavy-chain CDR1 is GYX₁FX₂SY (SEQ ID NO: 1) wherein X₁ denotes N, D, A, Q, S, or T; and X₂ is T or A or SYWIN (SEQ ID NO: 7); heavy-chain CDR2 is YPGSYIP (SEQ ID NO: 2); heavy-chain CDR3 is GYFDV (SEQ ID NO: 3); light-chain CDR1 is KSSQSLLXSGNQKNYLA (SEQ ID NO: 4) wherein X denotes N or S; light-chain CDR2 is GASTRES (SEQ ID NO: 5); and light-chain CDR3 is QNDHSYPYT (SEQ ID NO: 6).

According to some embodiments, the scFv comprises a heavy-chain CDR1 sequence GYX₁FX₂SY (SEQ ID NO: 1), wherein X₂ is T. According to some embodiments, the scFv comprises heavy-chain CDR1 sequence GYTFTSY (SEQ ID NO: 13). According to other embodiments, the scFv comprises a heavy-chain CDR1 sequence GYNFASY (SEQ ID NO: 14). According to certain embodiments, the scFv comprises a heavy-chain CDR1 sequence SYWIN (SEQ ID NO: 7).

According to some embodiments, the scFv comprises a light-chain CDR1 sequence KSSQSLSSGNQKNYLA (SEQ ID NO: 15).

According to some embodiments, the scFv comprises a set of six CDRs wherein: heavy chain CDR1 sequence comprising the sequence GYTFTSY (SEQ ID NO: 13), heavy chain CDR2 comprising the sequence YPGSYIP (SEQ ID NO: 2), heavy chain CDR3 comprising the sequence GYFDV (SEQ ID NO: 3), light chain CDR1 comprising the sequence KSSQSLSSGNQKNYLA (SEQ ID NO: 15), light chain CDR2 comprising the sequence GASTRES (SEQ ID NO: 5), and light chain CDR3 comprising the sequence QNDHSYPYT (SEQ ID NO: 6).

According to some embodiments, the scFv comprises a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 29-39.

According to some embodiments, the scFv comprises a light chain variable region sequence selected from the group consisting of SEQ ID NOs: 40-42.

According to some embodiments, the scFv comprises a linker connecting the heavy chain and light chain variable regions. According to some embodiments, the linker is a glycine-serine (GlySer) linker. According to some embodiments, the linker comprises (GS)_n, (GSGGS)_n, (GGGS)_n or (GGGGS)_n, where n is an integer selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. According to some embodiments, the linker comprises 2-6 repeats of the sequence GGGS. According to certain exemplary embodiments, a linker comprising three or four repeats of the sequence GGGS connects the two variable regions within the scFv. According to some embodiments, the heavy chain variable region is on the N-terminal side of the scFv and is connected by the linker (GGGS)₃ to the light chain variable region. According to some embodiments, the light chain variable region is on the N-terminal side of the scFv and is connected by the linker (GGGS)₄ to the heavy chain variable region.

According to some embodiments, the scFv comprises a sequence selected from the group consisting of SEQ ID NOs: 43 to 54, or an analog or derivative thereof having at least 90% sequence identity with said antibody. Each possibility represents a separate embodiment of the invention.

- 5 According to some embodiments, the scFv comprises a sequence selected from the group consisting of SEQ ID NOs: 43 to 54. According to certain embodiments, the scFv consist of a sequence selected from the group consisting of SEQ ID NOs: 43 to 54.

According to some embodiments, the scFv is connected to a human Fc region to form scFv-Fc. According to some embodiments, the Fc is of human IgG1.

- 10 Also provided are pharmaceutical compositions, comprising at least one antibody, antibody fragment, or antibody conjugate according to the invention, for use in restoring NK cytotoxicity by inhibiting binding of CD112R, expressed on NK cells, to Nectin-2 (CD112).

According to other embodiments, the antibody or antibody fragment is capable of inhibiting binding of human CD112R, expressed on T-cells, to Nectin-2.

- 15 According to some embodiments, the pharmaceutical composition comprises at least one antibody, antibody fragment, or antibody conjugate according to the invention and an antibody against PD-1. According to some embodiments, the pharmaceutical composition comprises at least one antibody, antibody fragment, or antibody conjugate according to the invention and an antibody against TIGIT. According to some embodiments, the pharmaceutical composition
20 comprises at least one antibody, antibody fragment, or antibody conjugate according to the invention and an antibody against PVR. According to some embodiments, the pharmaceutical composition comprises at least one antibody, antibody fragment, or antibody conjugate according to the invention and an antibody against CTLA-4.

- 25 According to some embodiments, the pharmaceutical composition according to the present invention is for use in cancer immunotherapy or in enhancing immune responses.

According to some embodiments of the invention, the cancer is selected from the group consisting of a lung cancer, a breast cancer, a colorectal cancer, a melanoma, an ovarian cancer, a pancreatic cancer, a colon cancer, a cervical cancer, a kidney cancer, a thyroid cancer, a prostate cancer, a brain cancer, a renal cancer, a throat cancer, a laryngeal carcinoma, a bladder

cancer, a hepatic cancer, a fibrosarcoma, an endometrial cells cancer, a glioblastoma, sarcoma, a myeloid, a leukemia and a lymphoma. Each possibility represents a separate embodiment of the invention.

According to some embodiments, the cancer is a solid cancer. According to some specific
5 embodiments, the solid cancer is selected from the group consisting of breast cancer, lung cancer, bladder cancer, pancreatic cancer and ovarian cancer.

According to some embodiments, the cancer is lung adenocarcinoma. According to some embodiments, the cancer is breast adenocarcinoma. According to some embodiments, the cancer is a colorectal cancer.

10 According to certain embodiments, the cancer is selected from the group consisting of: breast cancer, colorectal cancer, lung cancer, kidney cancer, melanoma, prostate cancer, and brain cancer. Each possibility represents a separate embodiment of the invention.

According to other embodiments, the cancer is hematologic cancer. According to some embodiments, the hematological cancer is selected from leukemia, lymphoma and multiple
15 myeloma. Each possibility represents a separate embodiment of the invention.

According to some embodiments, the pharmaceutical composition is for use of treating cancer, together with human lymphocytes.

According to some embodiments, the human lymphocytes are killer cells selected from the group consisting of: T cells, NK cells and NKT cells. Each possibility represents a separate
20 embodiment of the invention.

According to some embodiments, the killer cells are autologous or allogenic.

According to some embodiments, the pharmaceutical composition is for use in treating a viral infection.

According to certain embodiments, the pharmaceutical composition is for use in treating
25 chronic infections. According to exemplary embodiments, the pharmaceutical composition is for use in treating a viral infection selected from the group consisting of hepatitis C viral infection (HCV), polyomavirus (JCV) infection and BK-virus (BKV) infection.

According to yet another aspect, the present invention provides a method of inhibiting binding of human CD112R to Nectin-2 by using a monoclonal antibody or antibody fragment defined herein.

5 According to an additional aspect, the present invention provides a method for enhancing immune response in a subject in need thereof comprising administering to said subject a therapeutically effective amount of an antibody or antibody fragment or as described herein.

According to yet another aspect, the present invention provides a method of treating cancer comprising administering to a subject in need thereof, a pharmaceutical composition comprising a therapeutically effective amount of the antibody or antibody fragment thereof
10 described herein.

According to yet another aspect, the present invention provides a method of treating cancer comprising administering to a subject in need thereof, a therapeutically effective amount of the antibody or antibody fragment thereof against CD112R as described herein; and a therapeutically effective amount of an antibody against PD-1, TIGIT, or PVR. Each possibility
15 represents a separate embodiment of the invention. The administration of the anti-CD112R antibody and the anti PD-1, TIGIT or PVR may be combined administration or sequential administration in any order of administration.

According to some embodiments of the invention, the therapeutically effective amount results in a decrease in tumor size or in the number of metastases in the subject.

20 According to some embodiments, the method of treating cancer comprises administering or performing at least one additional anti-cancer therapy. According to certain embodiments, the additional anticancer therapy is surgery, chemotherapy, radiotherapy, or immunotherapy.

According to some embodiments, the method of treating cancer comprises administration of the antibody and an additional anti-cancer agent. According to some embodiments, the
25 additional anti-cancer agent is selected from the group consisting of: immune-modulator, agent that inhibits immune co-inhibitory receptor, activated lymphocyte cells, kinase inhibitor, and chemotherapeutic agent.

According to other embodiments, the additional immune-modulator is an antibody, antibody fragment or antibody conjugate that binds to human Nectin-2.

According to some embodiments, the additional immune-modulator is an antibody against an immune checkpoint molecule. According to some embodiments, the additional immune modulator is an antibody against an immune checkpoint molecule selected from the group consisting of human programmed cell death protein 1 (PD-1), PD-L1 and PD-L2, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), lymphocyte activation gene 3 (LAG3), CD137, OX40 (also referred to as CD134), killer cell immunoglobulin-like receptors (KIR), TIGIT, PVR, CTLA-4, NKG2A, GITR, and any other checkpoint molecule or a combination thereof. Each possibility represents a separate embodiment of the invention.

10 According to certain embodiments, the additional immune modulator is an antibody against PD-1. According to certain embodiments, the additional immune modulator is an antibody against TIGIT. According to certain embodiments, the additional immune modulator is an antibody against PVR. According to some embodiments, the additional immune modulator is an antibody against CTLA-4.

15 According to some embodiments of the invention, the immune co-inhibitory receptor is selected from the group consisting of PD-1, TIGIT, PVR, CTLA-4, LAG3, TIM3, BTLA, VISTA, B7H4, CD96, BY55 (CD 160), LAIR1, SIGLEC10, and 2B4. Each possibility represents a separate embodiment of the invention.

According to some embodiments, the anti-cancer agent is selected from the group consisting of: erbitux, cytarabine, fludarabine, fluorouracil, mercaptopurine, methotrexate, thioguanine, gemcitabine, vincristine, vinblastine, vinorelbine, carmustine, lomustine, chlorambucil, cyclophosphamide, cisplatin, carboplatin, ifosfamide, mechlorethamine, melphalan, thiotepa, dacarbazine, bleomycin, dactinomycin, daunorubicin, doxorubicin, idarubicin, mitomycin, mitoxantrone, plicamycin, etoposide, teniposide and any combination thereof. Each possibility represents a separate embodiment of the invention.

20 According to some embodiments, the anti-cancer agent is epidermal growth factor receptor (EGFR) inhibitor. According to some embodiments, the EGFR inhibitor is selected from the group consisting of: Cetuximab (Erbix®), Panitumumab (Vectibix®), and necitumumab (Portrazza®). Each possibility represents a separate embodiment of the invention.

30 According to some embodiments of the invention, the subject is a human subject.

According to some embodiments of the invention, the immune cell is a T cell.

According to an aspect, the present invention provides a method for modulating immune system function and/or activity comprising modulating the binding of CD112R to Nectin-2 using an antibody according to the invention.

5 According to some embodiments, the method of treating cancer involves preventing or reducing formation, growth or spread of metastases in a subject.

According to some embodiments, the method of treating cancer comprises administering to a subject in need thereof a pharmaceutical composition comprising an antibody or antibody fragment thereof, capable of inhibiting the binding of human CD112R to human Nectin-2, and
10 further administering to said subject human lymphocytes.

According to some embodiments, the human lymphocytes are killer cells selected from the group consisting of: T cells, NK cells and NKT cells.

According to some embodiments, the killer cells are autologous or allogenic.

The present invention also provides a method of preventing or treating a viral infection
15 comprising administering to a subject the antibody or a fragment thereof comprising at least the antigen binding domain described herein, wherein said mAb or fragment thereof is capable of inhibiting binding of CD112R to Nectin-2.

The present invention further comprises, according to another aspect, a method of determining or quantifying CD112R in a sample, the method comprising contacting a
20 biological sample with an antibody or antibody fragment, and measuring the level of complex formation, wherein the antibody or antibody fragment comprises:

i. a set of six CDRs wherein: heavy-chain CDR1 is GYX₁FX₂SY (SEQ ID NO: 1) wherein X₁ denotes N, D, A, Q, S, or T; and X₂ denotes T or A or SYWIN (SEQ ID NO: 7); heavy-chain CDR2 is YPGSYIP (SEQ ID NO: 2); heavy-
25 chain CDR3 is GYFDV (SEQ ID NO: 3); light-chain CDR1 is KSSQSLLXSGNQKNYLA (SEQ ID NO: 4) wherein X denotes N or S; light-chain CDR2 is GASTRES (SEQ ID NO: 5); and light-chain CDR3 is QNDHSYPYT (SEQ ID NO: 6); or

- 5 ii. a set of six CDRs wherein: heavy-chain CDR1 sequence is GYNFTSY (SEQ ID NO: 9) or SYWIN (SEQ ID NO: 7); heavy-chain CDR2 is FPGSYS (SEQ ID NO: 10); heavy-chain CDR3 is GYFDV (SEQ ID NO: 3); light-chain CDR1 is KSSQSLLNSGSQKNYLA (SEQ ID NO: 11); light-chain CDR2 is GASTRES (SEQ ID NO: 5); and light-chain CDR3 is QNDHSYPYT (SEQ ID NO: 6).

10 According to some embodiments, the method comprising contacting a biological sample with an antibody or antibody fragment comprising a set of six CDRs wherein: heavy chain CDR1 sequence comprising the sequence GYTFTSY (SEQ ID NO: 13), heavy chain CDR2 comprising the sequence YPGSYIP (SEQ ID NO: 2), heavy chain CDR3 comprising the sequence GYFDV (SEQ ID NO: 3), light chain CDR1 comprising the sequence KSSQSLLSSGNQKNYLA (SEQ ID NO: 15), light chain CDR2 comprising the sequence GASTRES (SEQ ID NO: 5), and light chain CDR3 comprising the sequence QNDHSYPYT (SEQ ID NO: 6).

15 According to some embodiments, the method for detecting or quantifying the expression of CD112R comprises the steps of:

- i. incubating a sample with an antibody specific to CD112R or an antibody fragment thereof comprising at least an antigen-binding portion; and
- ii. detecting the bound CD112R using a detectable probe.

20 According to some embodiments, the method further comprises the steps of:

- iii. comparing the amount of (ii) to a standard curve obtained from a reference sample containing a known amount of CD112R; and
- iv. calculating the amount of the CD112R in the sample from the standard curve.

25 The antibodies according to the present invention may also be used to configure screening methods. For example, an enzyme-linked immunosorbent assay (ELISA), or a radioimmunoassay (RIA), as well as methods such as immunohistochemistry (IHC) or fluorescence-activated cell sorting (FACS), can be constructed for measuring levels of secreted or cell-associated CD112R in a biological sample, using the antibodies and methods known in the art.

According to some embodiments, the biological sample is a body fluid or tissue.
According to additional embodiment, the sample is body tissue sample.

According to some embodiments, the method is performed in-vitro or ex-vivo.

According to an aspect, the present invention provides a method of diagnosing or
5 prognosing cancer in a subject, the method comprising determining the expression level of
CD112R in a biological sample of said subject using at least one antibody as described herein.

A kit for measuring the expression or presence of CD112R in biological sample is also
provided comprising at least one antibody or antibody fragment according to the present
invention. According to some embodiments, the kit comprises an antibody or antibody
10 fragment comprising:

- i. a set of six CDRs wherein: heavy-chain CDR1 is GYX₁FX₂SY (SEQ ID NO: 1) wherein X₁ denotes N, D, A, Q, S, or T; and X₂ denotes T or A or SYWIN (SEQ ID NO: 7); heavy-chain CDR2 is YPGSYIP (SEQ ID NO: 2); heavy-chain CDR3 is GYFDV (SEQ ID NO: 3); light-chain CDR1 is
15 KSSQSLLXSGNQKNYLA (SEQ ID NO: 4) wherein X denotes N or S; light-chain CDR2 is GASTRES (SEQ ID NO: 5); and light-chain CDR3 is QNDHSYPYT (SEQ ID NO: 6); or
- ii. a set of six CDRs wherein: heavy-chain CDR1 sequence is GYNFTSY (SEQ ID NO: 9) or SYWIN (SEQ ID NO: 7); heavy-chain CDR2 is FPGSYS (SEQ
20 ID NO: 10); heavy-chain CDR3 is GYFDV (SEQ ID NO: 3); light-chain CDR1 is KSSQSLLNSGSQKNYLA (SEQ ID NO: 11); light-chain CDR2 is GASTRES (SEQ ID NO: 5); and light-chain CDR3 is QNDHSYPYT (SEQ ID NO: 6).

25 According to some embodiments, the kit comprises an antibody or antibody fragment comprising a set of six CDRs wherein: heavy chain CDR1 sequence comprising the sequence GYTFTSY (SEQ ID NO: 13), heavy chain CDR2 comprising the sequence YPGSYIP (SEQ ID NO: 2), heavy chain CDR3 comprising the sequence GYFDV (SEQ ID NO: 3), light chain CDR1 comprising the sequence KSSQSLLSSGNQKNYLA (SEQ ID NO: 15), light chain
30 CDR2 comprising the sequence GASTRES (SEQ ID NO: 5), and light chain CDR3 comprising the sequence QNDHSYPYT (SEQ ID NO: 6).

Any embodiment disclosed herein above can optionally be combined with the subject matter of one or any combination of another embodiment disclosed herein. Further embodiments and the full scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

10 BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D depict the expression levels of CD112R on various immune cells. **Fig. 1A** - PVRIG transcript levels from Database of Immune Cell Expression (DICE). **Figures 1B-1D** show FACS analysis of staining of various immune cells by anti-CD112R mAbs of the present invention (hybridoma supernatants). **Fig. 1B** - resting PBMCs had no detectable levels of CD112R; **Fig. 1C** - robust expression of CD112R was seen on T cells following 96 hours activation of PBMCs; **Fig. 1D** - robust expression of CD112R by activated NK cells. Filled histograms depict background staining. The two empty histograms, at each graph, represent staining by two anti-CD112R mAb clones (#13 and #15). Shown are results obtained for two independent healthy donors.

Figure 2 is a schematic illustration of receptors expressed on NK/CD8 T cells (TIGIT, DNAM-1, CD112R) and their respective affinities to Nectin-2 expressed by tumors or by antigen presenting cells (APCs).

Figures 3A-3C provide analysis of binding and blocking of some of the anti CD112R mAbs of the present invention. **Fig. 3A** illustrates specific binding, determined by FACS analysis, of anti-CD112R mAb clones to 293T cells that overexpress CD112R and not to parental cells. **Fig. 3B** - Calculated EC-50 values: CD112R chimeric mAbs were used in concentration from 99-0.4 nM in series of three-fold dilutions and incubated with HEK 293T cells (Human embryonic kidney 293 cells) that overexpress CD112R. For detection, anti-human-APC antibody (Ab) was used at 1:200 dilution, and cell binding was analyzed by FACS. **Fig. 3C** - 8866 cells (chronic myelogenous leukemia cells) overexpressing human Nectin-2 were incubated with 10 µg/ml of CD112R-Fc in the presence of isotype control Ab, or with the

indicated clones at 4 µg/ml. Bound CD112R-Fc was detected by anti-mouse-Dy-647 1:250 dilution and analyzed by FACS. The blocking of CD112R binding exceeded 95% by all the variants.

Figures 4A-4C illustrate blocking of CD112R by the anti-CD112R mAbs and its effect on NK cell activation. NK activation was measured by the induction of surface expression of CD107a, which is expressed as fold change over control IgG (Y axis). Results are shown for the human cancer cell lines A549 (lung adenocarcinoma; **Figs. 4A and 4B**) and MDA-MB-231 (breast adenocarcinoma; **Fig. 4C**). **Fig. 4A** - The activity of anti-CD112R with murine IgG1 Fc (mIgG1, inert Fc with no detectable binding to human CD16) was compared to a chimeric clone with the same Fabs, and to the chimeric clone VH0K0 comprising N31S (also denoted herein N27dS according to the Kabat numbering), both having human IgG1 Fc (hIgG1, effector Fc capable of activating human CD16). **Fig. 4B-4C** - The anti-CD112R mAbs activity was compared to that of a combination of the antibody with anti-immune check point inhibitor (ICI) antibodies, namely, anti-TIGIT (VSIG#9, described in WO2017037707) and anti-PVR (named NTX-1088) mAbs, as indicated. * $p < 0.03$, ** $p < 0.001$, *** $p < 0.0001$ by two tailed student t-test. Representative data for two out of five donors is shown.

Figures 5A-5B illustrate effect of blocking of CD112R by the anti-CD112R mAbs (Clone 13 N31S) on T cell-mediated target cell killing. The effect of blocking of CD112R was further examined in the presence of additional ICIs, such as TIGIT and PD-1. Target cell killing was normalized to that observed when no Ab was added (Y axis). Results are shown for the human cancer cell lines A549 (lung adenocarcinoma, **Fig. 5A**) and RKO (colorectal cancer, **Fig. 5B**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0005$ by two tailed student t-test.

Figure 6 shows activation effect of the humanized anti CD112R antibodies on NK cells. NK cell activation was assessed by the induction of surface expression of CD107a, as measured by FACS analysis, and was expressed as fold change over control IgG (Y axis). Results are shown for NK cells, isolated from a healthy human volunteer, that were co-incubated with the human cancer cell line A549 (lung adenocarcinoma). Incubation was done in the presence of various anti-CD112R humanized clones, or with an isotype IgG control, as depicted at the X axis, with or without additional anti-TIGIT mAbs. All Abs were added at 3 µg/ml.

Figures 7A-7B show the effect of the selected humanized anti-CD112R, alone and in combination with anti-PVR, on NK cell activation. NK cell activation was measured by the induction of surface CD107a and was expressed as fold-change over no Ab (Y axis). **Fig. 7A**

– treatment of NK cells, cultured with MDA-MB-231 (breast adenocarcinoma) cells with selected humanized anti-CD112R antibodies. **Fig. 7B** - a comparison of treatment of the above cell culture with various humanized anti-CD112R mAb and with anti-PVR mAb (NTX-1088) as standalone or in combination ($p < 0.03$; black bars).

5 **Figures 8A-8B** illustrate the effect of the selected humanized anti-CD112R antibodies on activation of T cells (IL-2 secretion). Jurkat cells overexpressing the human CD112R were incubated with A549 cells in the presence of anti-CD3, with or without the indicated anti-CD112R mAbs. After 24 hours the supernatants were collected, and IL-2 content was quantify using Human IL-2 ELISA MAX™ Deluxe by Biolegend. **Fig. 8A** - Dose response assay of all the humanized variants and a reference anti-CD112R Ab (Ref) from 4-0.0625 ug/ml. **Fig. 8B** 10 - Selected humanized variants were tested in a range of concentrations from 26.4-0.1 nM and the reference anti-CD112R Ab was added only at the highest 24.6 nM concentration. Comparison was done to the highest concentration of hIgG1.

Figures 9A-9B illustrate the effect of the humanized anti-CD112R mAb variant H11K2 15 (having HC variable region sequence set forth in SEQ ID NO:39, and LC variable region sequence set forth in SEQ ID NO: 41) on T cell activation. The blockade of CD112R effect was measured with blocking of additional ICIs, such as PVR (NTX-1088) and PD-1 (Keytruda), as indicated. Surface expression (MFI) of CD69 (**Fig. 9A**) or CD137 (4-1BB) (**Fig. 9B**) was normalized to that observed when no Ab was added (Y axis). Results are shown for the human cancer cell line A549 (lung adenocarcinoma). All changes are statistically significant ($p < 0.05$). 20

Figures 10A-10B show effect of CD112R blockade on NK cell activation when target cells express Nectin-2, but not PVR, and the effect of combining anti-CD112R with anti-PVR (NTX-1088) on NK activation, when target cells co-express Nectin-2 and PVR. NK cell 25 activation was measured by surface expression of CD137, which is expressed as fold induction over no antibody treatment (no Ab. Y axis). Results are shown for the human cancer cell lines JEG-3 (choriocarcinoma) (**Fig. 10A**), which is PVR negative, and JEG-3-hPVR (**Fig. 10B**), which express human PVR. The anti-CD112R Ab with hIgG1 Fc was compared to the anti-PVR Ab NTX-1088, or to the anti-TIGIT Ab -Tiragolumab (KEGG DRUG entry D11482). 30 Additionally, combinations of CD112R and the two mAbs were tested. * $p < 0.005$, ** $p < 0.001$, *** $p < 0.0001$ by two tailed student t-test. Representative data of two out of five NK cell donors is shown. Tira = Tiragolumab.

FIGURES 11A-11C demonstrate the effect of a combination of CD112R and other ICI blockade on NK cell activation when target cells express both Nectin-2 and PVR. Results are shown for the human non-small cell lung cancer cell line H1299 (**Fig 11A**), the lung adenocarcinoma A549 cell line (**Fig 11B**), and for the breast adenocarcinoma cell line MDA-MB-231 (**Fig. 11C**). The anti-CD112R mAbs activity was evaluated as a standalone or in combination with other ICI antibodies, as indicated. * $p < 0.005$, ** $p < 0.001$, *** $p < 0.0001$ by two tailed student t-test. Representative data of two out of four NK cell donors is shown. Tira = Tiragolumab.

FIGURE 12 demonstrates effect of in-vivo anti-CD112R mAb treatment on tumor growth in a mouse model. A549 cells were s.c. co-implanted with fresh human PBMCs at 1:5 E:T ratio to NSG-HLA-A2/HHD mice. Treatment started on day 3 post implantation. Mice were treated once weekly with either control IgG1, or with anti-CD112R mAb, or with anti PD-1 mAb (Pembrolizumab), all at 10 mg/kg for a total of 5 doses. $p=0.001$ by two-way ANOVA.

15 DETAILED DESCRIPTION OF THE INVENTION

The present invention provides effective antibodies, including chimeric and humanized mAbs and fragments thereof, specific to the human protein CD112R. The invention also provides production and use of the antibodies as therapeutic agents. In some embodiments, the invention provides antibodies specific to CD112R for efficient restoration of immune activity against cancer cells overexpressing Nectin-2, and for treatment of cancer as a stand-alone treatment or in combination with other therapies, e.g., with additional immune checkpoint inhibitors. In some embodiments, the antibodies described herein are for use in treating viral infections.

The present invention further provides humanized antibodies specific to the human CD112R. Advantageously, the antibodies of the invention are humanized, thus avoiding the risk of adverse immune response towards the antibodies and are therefore safe for in-vivo use in humans.

In the following description, certain specific details are set forth in order to provide a thorough understanding of various embodiments. However, one skilled in the art will understand that the embodiments provided may be practiced without these details. Unless the context requires otherwise, throughout the specification and claims which follow, the word

“comprise” and variations thereof, such as, “comprises” and “comprising” are to be construed in an open, inclusive sense, that is, as “including, but not limited to.” As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the content clearly dictates otherwise. It should also be noted that the term “or”
5 is generally employed in its sense including “and/or” unless the content clearly dictates otherwise. Further, headings provided herein are for convenience only and do not interpret the scope or meaning of the claimed embodiments. As used herein the term “about” refers to an amount that is near the stated amount by 10% or less.

The term “CD112R” or “PVRIG” as used herein refers to a human cell surface receptor
10 for Nectin-2, also known as C7orf15. PVRIG stands for Poliovirus receptor-related immunoglobulin domain-containing protein. The protein, following interaction with Nectin-2, inhibits T-cell proliferation. An exemplary CD112R protein or gene encoding to the protein according to the invention is set forth in SwissPort, UniPort and GenBank symbols or accession numbers: Gene ID: 79037, Q6DKI7, D6W5U9, Q9BVK3.

15 The antibodies or a fragment thereof according to the invention bind to an epitope in CD112R. Specifically, the antibodies bind to an epitope within the ectodomain (extracellular part) of the CD112R protein.

To generate the parent mAbs of the present invention, a recombinant fusion protein, comprising the extracellular part of human CD112R and a murine Fc region of an IgG protein
20 as a carrier, was produced and purified. The resulted fusion protein hCD112R–Fc was used as an immunogen. BALB/c mice were injected with 50 µg of the immunogen in complete Freund’s adjuvant, and 2 weeks later a boost injection was given using incomplete Freund’s adjuvant. After 2 weeks, mice sera were screened for the antibody titer using ELISA. The best responders were further boosted with the immunogen in PBS. Three days later, spleen cells
25 were collected, and after lysis of red blood cells, fused with SP2/0 myeloma cells. The cells were seeded in 20 % RPMI 1640 medium containing hypoxanthine, aminopterin, and thymidine for hybridoma selection and screened for mAbs secretion using ELISA. Positive cell lines, i.e., cells secreting mAbs that recognize hCD112R–Fc, were further selected to develop a product that will have the following differentiating characteristics: a) lack of cross-reactivity
30 to other ligands of related immune cell receptors (e.g., DNAM1 and TIGIT); b) strong binding to the native, mature human CD112R molecule expressed on the surface of living cells.

For the humanization of selected Ab clones, IGHV1-69*10 germline framework was selected for the heavy chain, and IGKV4-1*01 was selected for the light chain framework. Both are very well represented at the human germline, which is further supported by the high expression titer of the resulting humanized variants.

5 The CD112R serves as an antigen to the antibodies described herein. The term "antigen" as used herein refers to a molecule or a portion of a molecule capable of eliciting antibody formation and being specifically bound by an antibody. An antigen may have one or more than one epitope. The specific binding referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude
10 of other antibodies which may be evoked by other antigens. An antigen according to some embodiments of the present invention is a CD112R protein or an immunogenic portion thereof.

A binding affinity can be quantified using known methods such as, Surface Plasmon Resonance (SPR) (described in Scarano S, Mascini M, Turner AP, Minunni M. Surface plasmon resonance imaging for affinity-based biosensors. *Biosens Bioelectron.* 2010, 25: 957-66), and
15 can be calculated using, e.g., a dissociation constant, K_d , such that a lower K_d reflects higher affinity.

Antibodies, or immunoglobulins, comprise two heavy chains linked together by disulfide bonds and two light chains, each light chain being linked to a respective heavy chain by disulfide bonds in a "Y" shaped configuration. Proteolytic digestion of an antibody yields Fv
20 (Fragment variable) and Fc (Fragment crystallizable) domains. The antigen binding domains, Fab, include regions where the polypeptide sequence varies. The term $F(ab')_2$ represents two Fab' arms linked together by disulfide bonds. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains (C_H). Each light chain has a variable domain (V_L) at one end and a constant domain (C_L) at its other end, the light chain variable
25 domain being aligned with the variable domain of the heavy chain and the light chain constant domain being aligned with the first constant domain of the heavy chain ($CH1$). The variable domains of each pair of light and heavy chains form the antigen-binding site. The domains on the light and heavy chains have the same general structure and each domain comprises four framework regions, whose sequences are relatively conserved, joined by three hyper-variable
30 domains known as complementarity determining regions (CDRs 1-3). These domains contribute specificity and affinity of the antigen-binding site.

The isotype of the heavy chain (gamma, alpha, delta, epsilon or mu) determines immunoglobulin class (IgG, IgA, IgD, IgE or IgM, respectively). The light chain is either of two isotypes (kappa, κ or lambda, λ). Both isotopes are found in all antibody classes.

The term "antibody" is used in the broadest sense and includes monoclonal antibodies
5 (including full length or intact monoclonal antibodies), polyclonal antibodies, multivalent antibodies, chimeric antibodies, humanized antibodies and antibody fragments long enough to exhibit the desired biological activity, namely binding to human CD112R.

Antibody or antibodies according to the invention includes intact antibodies, such as polyclonal antibodies or monoclonal antibodies (mAbs), as well as proteolytic fragments
10 thereof, such as the Fab or F(ab')₂ fragments. Single chain antibodies (e.g. scFv) also fall within the scope of the present invention.

Antibody Fragments

"Antibody fragments" comprise only a portion of an intact antibody, generally including an antigen binding site of the intact antibody and thus retaining the ability to bind antigen.
15 Examples of antibody fragments encompassed by the present definition include: (i) the Fab fragment, having VL, CL, VH and CH1 domains; (ii) the Fab' fragment, which is a Fab fragment having one or more cysteine residues at the C-terminus of the CH1 domain; (iii) the Fd fragment having VH and CH1 domains; (iv) the Fd' fragment having VH and CH1 domains and one or more cysteine residues at the C-terminus of the CH1 domain; (v) the Fv fragment
20 having the VL and VH domains of a single arm of an antibody; (vi) the dAb fragment (Ward et al., Nature 1989, 341, 544-546) which consists of a VH domain; (vii) isolated CDR regions; (viii) F(ab')₂ fragments, a bivalent fragment including two Fab' fragments linked by a disulphide bridge at the hinge region; (ix) single chain antibody molecules (e.g. single chain Fv; scFv) (Bird et al., Science 1988, 242, 423-426; and Huston et al., Proc. Natl. Acad. Sci.
25 (USA) 1988, 85,5879-5883); (x) "diabodies" with two antigen binding sites, comprising a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (see, e.g., EP 404,097; WO 93/11161; and Hollinger et al., Proc. Natl. Acad. Sci. USA, 1993, 90, 6444-6448); (xi) "linear antibodies" comprising a pair of tandem Fd segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides,
30 form a pair of antigen binding regions (Zapata et al. Protein Eng., 1995, 8, 1057-1062; and U.S. Pat. No. 5,641,870).

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan et al., *Science*, 229:81 (1985)). However, these fragments can now be produced
5 directly by recombinant host cells. For example, the antibody fragments can be isolated from antibody phage libraries. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')₂ fragments (Carter et al., *Bio/Technology* 10:163-167 (1992)). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will
10 be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv).

Single chain antibodies can be single chain composite polypeptides having antigen binding capabilities and comprising amino acid sequences homologous or analogous to the variable regions of an immunoglobulin light and heavy chain i.e., linked V_H-V_L or single chain
15 Fv (scFv). Techniques for the production of single-chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce single-chain antibodies to CD112R.

The term "monoclonal antibody" (mAb) as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that
20 may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular
25 method. mAbs may be obtained by methods known to those skilled in the art. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al., *Nature* 1975, 256, 495, or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). Monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described,
30 for example, in Clackson et al., *Nature* 1991, 352, 624-628 or Marks et al., *J. Mol. Biol.*, 1991, 222:581-597.

The mAbs of the present invention may be of any immunoglobulin class including IgG, IgM, IgE, IgA, and IgD.

Anti-idiotypic antibodies specifically immunoreactive with the hypervariable regions of an antibody of the invention are also comprehended.

5 According to an aspect, the present invention provides an antibody or antibody fragment thereof comprising a set of six CDR sequences selected from the group consisting of:

- i. three complementarity determining regions (CDRs) of a heavy-chain variable region comprising SEQ ID NO: 28 and three CDRs of a light-chain variable comprising SEQ ID NO: 26, or an analog or derivative thereof having at least 90% sequence identity
10 with said antibody or fragment sequence; and
- ii. three CDRs of a heavy-chain variable region comprising SEQ ID NO: 21 and three CDRs of a light-chain variable region comprising SEQ ID NO: 23, or an analog or derivative thereof having at least 90% sequence identity with said antibody or fragment sequence.

15 Sequence identity is the percentage of amino acids or nucleotides which match exactly between two different sequences. Sequence similarity permits conservative substitution of amino acids to be determined as identical amino acids. The polynucleotide sequences described herein may be codon-optimized for expression in specific cells, such as human cells. Codon optimization does not change the encoded amino acid sequences of the antibody's chain but
20 may, for example, increase the expression in cells.

The invention also provides conservative amino acid variants of the antibody molecules according to the invention. Variants according to the invention also may be made that conserve the overall molecular structure of the encoded proteins. Given the properties of the individual amino acids comprising the disclosed protein products, some rational substitutions will be
25 recognized by the skilled worker. Amino acid substitutions, *i.e.*, "conservative substitutions," may be made, for instance, on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. The term "antibody analog" as used herein refers to an antibody derived from another antibody by one or more conservative amino acid substitutions.

The term “antibody variant” as used herein refers to any molecule comprising the antibody of the present invention. For example, fusion proteins in which the antibody or an antigen-binding-fragment thereof is linked to another chemical entity is considered an antibody variant.

5 Analog and variants of the antibody sequences are also within the scope of the present application. These include, but are not limited to, conservative and non-conservative substitution, insertion and deletion of amino acids within the sequence. Such modification and the resultant antibody analog or variant are within the scope of the present invention as long as they confer, or even improve the binding of the antibody to the human CD112R.

10 Conservative substitutions of amino acids as known to those skilled in the art are within the scope of the present invention. Conservative amino acid substitutions include replacement of one amino acid with another having the same type of functional group or side chain, e.g., aliphatic, aromatic, positively charged, negatively charged. These substitutions may enhance oral bioavailability, penetration, and targeting to specific cell populations, immunogenicity,
15 and the like. One of skill will recognize that individual substitutions, deletions or additions to a peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids
20 are well known in the art. For example, according to one table known in the art, the following six groups each contain amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Serine (S), Threonine (T);
- 2) Aspartic acid (D), Glutamic acid (E);
- 3) Asparagine (N), Glutamine (Q);
- 25 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

It should be emphasized that the variant chain sequences are determined by sequencing methods using specific primers. Different sequencing methods employed on the same sequence

may result in slightly different sequences due to technical issues and different primers, particularly in the sequence terminals.

CDR identification or determination from a given heavy or light chain variable sequence, is typically made using one of few methods known in the art. For example, such determination is made according to the Kabat (Wu T.T and Kabat E.A., *J Exp Med*, 1970; 132:211–50),
5 chothia and IMGT (Lefranc M-P, et al., *Dev Comp Immunol*, 2003, 27:55-77).

When the term “CDR having a sequence”, or a similar term is used, it includes options wherein the CDR comprises the specified sequences and also options wherein the CDR consists of the specified sequence.

10 The antigen specificity of an antibody is based on the hyper variable region (HVR), namely the unique CDR sequences of both light and heavy chains that together form the antigen-binding site.

The terms “complementarity determining region,” and “CDR,” which are synonymous with “hypervariable region” or “HVR,” are known in the art to refer to non-contiguous
15 sequences of amino acids within antibody variable regions, which confer antigen specificity and/or binding affinity. In general, there are three CDRs in each heavy chain variable region (CDR-H1, CDR-H2, CDR-H3) and three CDRs in each light chain variable region (CDR-L1, CDR-L2, CDR-L3). “Framework regions” and “FR” are known in the art to refer to the non-CDR portions of the variable regions of the heavy and light chains. In general, there are four
20 FRs in each full-length heavy chain variable region (FR-H1, FR-H2, FR-H3, and FR-H4), and four FRs in each full-length light chain variable region (FR-L1, FR-L2, FR-L3, and FR-L4). The precise amino acid sequence boundaries of a given CDR or FR can be readily determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), “Sequences of Proteins of Immunological Interest,” 5th Ed. Public Health Service,
25 National Institutes of Health, Bethesda, MD (“Kabat” numbering scheme), Al-Lazikani et al., (1997) *JMB* 273,927-948 (“Chothia” numbering scheme); MacCallum et al., *J. Mol. Biol.* 262:732-745 (1996), “Antibody-antigen interactions: Contact analysis and binding site topography,” *J. Mol. Biol.* 262, 732-745.” (“Contact” numbering scheme); Lefranc MP et al., “IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig
30 superfamily V-like domains,” *Dev Comp Immunol*, 2003 Jan;27(1):55-77 (“IMGT” numbering scheme); Honegger A and Plückthun A, “Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool,” *J Mol Biol*, 2001 Jun

8;309(3):657-70, ("Aho" numbering scheme); and Whitelegg NR and Rees AR, "WAM: an improved algorithm for modelling antibodies on the WEB," Protein Eng. 2000 Dec;13(12):819-24 ("AbM" numbering scheme. In certain embodiments the CDRs of the antibodies described herein can be defined by a method selected from Kabat, Chothia, IMGT, 5 Aho, AbM, or combinations thereof.

The boundaries of a given CDR or FR may vary depending on the scheme used for identification. For example, the Kabat scheme is based on structural alignments, while the Chothia scheme is based on structural information. Numbering for both the Kabat and Chothia schemes is based upon the most common antibody region sequence lengths, with insertions 10 accommodated by insertion letters, for example, "30a," and deletions appearing in some antibodies. The two schemes place certain insertions and deletions ("indels") at different positions, resulting in differential numbering. The Contact scheme is based on analysis of complex crystal structures and is similar in many respects to the Chothia numbering scheme.

Alterations (e.g., substitutions) may be made in CDRs, e.g., to improve antibody affinity. 15 Such alterations may be made in CDR encoding codons with a high mutation rate during somatic maturation (See e.g., Chowdhury, Methods Mol. Biol. 207:179-196 (2008)), and the resulting variant can be tested for binding affinity. Affinity maturation (e.g., using error-prone PCR, chain shuffling, randomization of CDRs, or oligonucleotide-directed mutagenesis) can be used to improve antibody affinity (See e.g., Hoogenboom et al. in Methods in Molecular 20 Biology 178:1-37 (2001)). CDR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling (See e.g., Cunningham and Wells Science, 244:1081-1085 (1989)). CDR-H3 and CDR-L3 in particular are often targeted. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring 25 residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

The terms "molecule having the antigen-binding portion of an antibody" and "antigen-binding-fragments" as used herein are intended to include not only intact immunoglobulin molecules of any isotype and generated by any animal cell line or microorganism, but also the 30 antigen-binding reactive fraction thereof, including, but not limited to, the Fab fragment, the Fab' fragment, the F(ab')₂ fragment, the variable portion of the heavy and/or light chains thereof, Fab mini-antibodies (see e.g., WO 93/15210, US patent application 08/256,790, WO

96/13583, US patent application 08/817,788, WO 96/37621, US patent application 08/999,554), and single-chain antibodies incorporating such reactive fraction, as well as any other type of molecule in which such antibody reactive fraction has been physically inserted. Such molecules may be provided by any known technique, including, but not limited to, enzymatic cleavage, peptide synthesis or recombinant techniques.

The antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species, or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)). In addition, complementarity determining region (CDR) grafting may be performed to alter certain properties of the antibody molecule including affinity or specificity. Antibodies that have variable region framework residues substantially from human antibody (termed an acceptor antibody) and CDRs substantially from a mouse antibody (termed a donor antibody) are also referred to as humanized antibodies. Chimeric antibodies are primarily used to reduce immunogenicity in application and to increase yields in production, for example, where murine mAbs have higher yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric mAbs are used. Chimeric antibodies and methods for their production are known in the art (for example PCT patent applications WO 86/01533, WO 90/07861, WO 92/22653 and US patents 5,693,762, 5,693,761, 5,585,089, 5,530,101 and 5,225,539).

According to some embodiments, the antibody is a monoclonal antibody.

According to some specific embodiments, the monoclonal antibody is a chimeric monoclonal antibody.

According to some embodiments, the chimeric antibody comprises human-derived constant regions.

According to some embodiments the human constant regions of the chimeric antibody are selected from the group consisting of: human IgG1, human IgG2, human IgG3, and human IgG4.

According to some embodiments the human constant regions of the chimeric antibody are selected from the group consisting of: human IgG1.

According to a particular embodiment, a chimeric monoclonal antibody which recognizes human CD112R is provided comprising:

- 5 i. a set of six CDRs wherein: heavy-chain CDR1 sequence is SEQ ID NO: 1 or SEQ ID NO: 7; heavy-chain CDR2 sequence is SEQ ID NO: 2 or SEQ ID NO: 8; heavy-chain CDR3 sequence is SEQ ID NO: 3; light-chain CDR1 sequence is SEQ ID NO: 4; light-chain CDR2 sequence is SEQ ID NO: 5; and light-chain CDR3 sequence is SEQ ID NO: 6; or
- 10 ii. a set of six CDRs wherein: heavy-chain CDR1 sequence is SEQ ID NO: 9 or SEQ ID NO: 7; heavy-chain CDR2 sequence is SEQ ID NO: 10 or SEQ ID NO: 12; heavy-chain CDR3 sequence is SEQ ID NO: 3; light-chain CDR1 sequence is SEQ ID NO: 11; light-chain CDR2 sequence is SEQ ID NO: 5; and light-chain CDR3 sequence is SEQ ID NO: 6.

15 In some embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known and available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, 20 polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n vinyl pyrrolidone)polyethylene glycol, polypropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol 25 propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if two or more polymers are attached, they can be the same or different molecules.

30 The antibodies described herein can be encoded by a nucleic acid. A nucleic acid is a type of polynucleotide comprising two or more nucleotide bases. In certain embodiments, the nucleic acid is a component of a vector that can be used to transfer the polypeptide encoding

polynucleotide into a cell. As used herein, the term “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a genomic integrated vector, or “integrated vector,” which can become integrated into the chromosomal DNA of the host cell. Another type of vector is an “episomal” vector, e.g., a
5 nucleic acid capable of extra-chromosomal replication. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as “expression vectors”. Suitable vectors comprise plasmids, bacterial artificial chromosomes, yeast artificial chromosomes, viral vectors and the like. In the expression vectors regulatory elements such as promoters, enhancers, polyadenylation signals for use in controlling transcription can be
10 derived from mammalian, microbial, viral or insect genes. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated. Vectors derived from viruses, such as lentiviruses, retroviruses, adenoviruses, adeno-associated viruses, and the like, may be employed. Plasmid vectors can be linearized for integration into a chromosomal location.
15 Vectors can comprise sequences that direct site-specific integration into a defined location or restricted set of sites in the genome (e.g., AttP-AttB recombination). Additionally, vectors can comprise sequences derived from transposable elements.

The nucleic acids encoding the antibodies described herein can be used to infect, transfect, transform, or otherwise render a suitable cell transgenic for the nucleic acid, thus
20 enabling the production of antibodies for commercial or therapeutic uses. Standard cell lines and methods for the production of antibodies from a large-scale cell culture are known in the art. In certain embodiments, the cell is a Eukaryotic cell. In certain embodiments, the Eukaryotic cell is a mammalian cell. In certain embodiments, the mammalian cell is a cell line useful for producing antibodies is a Chines Hamster Ovary cell (CHO) cell, an NS0 murine
25 myeloma cell, or a PER.C6® cell. In certain embodiments, the nucleic acid encoding the antibody is integrated into a genomic locus of a cell useful for producing antibodies. In certain embodiments, described herein is a method of making an antibody comprising culturing a cell comprising a nucleic acid encoding an antibody under conditions in vitro sufficient to allow production and secretion of said antibody.

30 Also described herein are methods of making the mAbs and fragments specific to CD112R of the present invention. Such methods comprise incubating a cell or cell-line comprising a nucleic acid encoding the antibody in a cell culture medium under conditions sufficient to allow for expression and secretion of the antibody, and further harvesting the

antibody from the cell culture medium. The harvesting can further comprise one or more purification steps to remove live cells, cellular debris, non-antibody proteins or polypeptides, undesired salts, buffers, and medium components. In certain embodiments, the additional purification step(s) include centrifugation, ultracentrifugation, protein A, protein G, protein A/G, or protein L purification, and/or ion exchange chromatography.

According to one aspect, the present invention provides a mAb, or a fragment thereof comprising at least the antigen binding portion, which specifically binds to human CD112R, said antibody or fragment thereof have an affinity to human CD112R of at least $0.5 \times 10^{-9} \text{M}$.

In certain embodiments, the EC₅₀ of a humanized mAb or antigen binding fragment thereof for binding human CD112R is less than about 0.5 nM, 0.1 nM, 0.05 nM, or 0.01 nM.

Half maximal effective concentration (EC₅₀) refers to the concentration of the antibody that induces a response halfway between the baseline and maximum after a specified exposure time.

According to some embodiments, the mAb or mAb fragment comprises a set of six CDR sequences selected from the group consisting of:

- i. three complementarity determining regions (CDRs) of a heavy-chain variable region comprising SEQ ID NO: 28 and three CDRs of a light-chain variable comprising SEQ ID NO: 26, or an analog or derivative thereof having at least 90% sequence identity with said antibody or fragment sequence; and
- ii. three CDRs of a heavy-chain variable region comprising SEQ ID NO: 21 and three CDRs of a light-chain variable region comprising SEQ ID NO: 23, or an analog or derivative thereof having at least 90% sequence identity with said antibody or fragment sequence.

According to some specific embodiments the mAb or fragment comprises heavy chain CDR1 sequence comprising the amino acid sequence SY, heavy chain CDR2 comprising the sequence YPGSYIP (SEQ ID NO: 2), heavy chain CDR3 comprising the sequence GYFDV (SEQ ID NO: 3), light chain CDR1 comprising the sequence KSSQSLLXSGNQKNYLA (SEQ ID NO: 4) wherein X denotes N or S, light chain CDR2 comprising the sequence GASTRES (SEQ ID NO: 5), and light chain CDR3 comprising the sequence QNDHSYPYT

(SEQ ID NO: 6), or analogs thereof comprising no more than 5% amino acid substitution, deletion and/or insertion in the hypervariable region (HVR) sequence.

According to some specific embodiments the mAb or fragment comprises heavy chain CDR1 sequence consists of the sequence GYX₁FX₂SY (SEQ ID NO: 1) wherein X₁ denotes
5 N, D, A, Q, S, or T; and X₂ denotes T or A or SYWIN (SEQ ID NO: 7), heavy chain CDR2 consists of the sequence YPGSYIP (SEQ ID NO: 2), heavy chain CDR3 consists of the sequence GYFDV (SEQ ID NO: 3), light chain CDR1 consists of the sequence KSSQSLLXSGNQKNYLA (SEQ ID NO: 4) wherein X denotes N or S, light chain CDR2 consists of the sequence GASTRES (SEQ ID NO: 5), and light chain CDR3 consists of the
10 sequence QNDHSYPYT (SEQ ID NO: 6).

According to some embodiments, the antibodies are humanized antibodies.

A “humanized” antibody is an antibody in which all or substantially all CDR amino acid residues are derived from non-human CDRs and all or substantially all FR amino acid residues are derived from human FRs. A humanized antibody optionally may include at least
15 a portion of an antibody constant region derived from a human antibody. A “humanized form” of a non-human antibody refers to a variant of the non-human antibody that has undergone humanization, typically to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. According to some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-
20 human antibody (e.g., the antibody from which the CDR residues are derived), e.g., to restore or improve antibody specificity or affinity.

A “human antibody” is an antibody with an amino acid sequence corresponding to that of an antibody produced by a human or a human cell, or non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences, including human antibody
25 libraries. The term excludes humanized forms of non-human antibodies comprising non-human antigen-binding regions, such as those in which all or substantially all CDRs are non-human.

Pharmacology and methods of treatments

In pharmaceutical and medicament formulations, the active agent is preferably utilized together with one or more pharmaceutically acceptable carrier(s) and optionally any other therapeutic ingredients. The carrier(s) must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not unduly deleterious to the recipient thereof. The active agent is provided in an amount effective to achieve the desired pharmacological effect, as described above, and in a quantity appropriate to achieve the desired exposure.

Typically, the antibodies and fragments and conjugates thereof of the present invention comprising the antigen binding portion of an antibody or comprising another polypeptide including a peptide-mimetic will be suspended in a sterile saline solution for therapeutic uses. The pharmaceutical compositions may alternatively be formulated to control release of active ingredient (molecule comprising the antigen binding portion of an antibody) or to prolong its presence in a patient's system. Numerous suitable drug delivery systems are known and include, e.g., implantable drug release systems, hydrogels, hydroxymethylcellulose, microcapsules, liposomes, microemulsions, microspheres, and the like. Controlled release preparations can be prepared through the use of polymers to complex or adsorb the molecule according to the present invention. For example, biocompatible polymers include matrices of poly(ethylene-co-vinyl acetate) and matrices of a polyanhydride copolymer of a stearic acid dimer and sebacic acid. The rate of release of the molecule according to the present invention, i.e., of an antibody or antibody fragment, from such a matrix depends upon the molecular weight of the molecule, the amount of the molecule within the matrix, and the size of dispersed particles.

The pharmaceutical composition of this invention may be administered by any suitable means, such as intravenously, orally, topically, intranasally, subcutaneously, intramuscularly, intra-arterially, intraarticular, intralesionally, intratumorally or parenterally. Ordinarily, intravenous (i.v.) administration is used for delivering antibodies.

According to an aspect, the present invention provides a method of treating cancer comprising administering to a subject in need thereof, a therapeutically effective amount of a pharmaceutical composition comprising the antibody or antibody fragment thereof described herein.

As used herein the term “subject”, “individual”, or “patient” refers to individuals diagnosed with, suspected of being afflicted with, or at-risk of developing at least one disease for which the described compositions and method are useful for treating. According to some embodiments the individual is a mammal. According to some embodiments, the mammal is a mouse, rat, rabbit, dog, cat, horse, cow, sheep, pig, goat, llama, alpaca, or yak. According to some embodiments, the individual is a human.

It will be apparent to those of ordinary skill in the art that the therapeutically effective amount of the molecule according to the present invention will depend, *inter alia* upon the administration schedule, the unit dose of molecule administered, whether the molecule is administered in combination with other therapeutic agents, the immune status and health of the patient, the therapeutic activity of the molecule administered, its persistence in the blood circulation, and the judgment of the treating physician.

As used herein the term "therapeutically effective amount" refers to an amount of a drug effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the disorder. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy in vivo can, for example, be measured by assessing the duration of survival, time to disease progression (TTP), the response rates (RR), duration of response, and/or quality of life.

The cancer amenable for treatment by the present invention includes, but is not limited to: carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer, lung cancer (including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer, as well as B-cell lymphoma (including low grade/follicular non-

Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high-grade immunoblastic NHL; high-grade lymphoblastic NHL; high-grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome. Preferably, the cancer is selected from the group consisting of breast cancer, colorectal cancer, rectal cancer, non-small cell lung cancer, non-Hodgkins lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, Kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, melanoma, ovarian cancer, mesothelioma, and multiple myeloma. The cancerous conditions amendable for treatment of the invention include metastatic cancers.

The molecules of the present invention as active ingredients are dissolved, dispersed or admixed in an excipient that is pharmaceutically acceptable and compatible with the active ingredient as is well known. Suitable excipients are, for example, water, saline, phosphate buffered saline (PBS), dextrose, glycerol, ethanol, or the like and combinations thereof. Other suitable carriers are well known to those skilled in the art. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents.

The pharmaceutical composition according to the present invention may be administered together or with combination with an anti-neoplastic composition.

As used herein the term "combination" or "combination treatment" can refer either to concurrent administration of the articles to be combined or sequential administration of the articles to be combined. As described herein, when the combination refers to sequential administration of the articles, the articles can be administered in any temporal order.

The term "treatment" as used herein refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

The term "cancer" refers to or describes the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not

limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include melanoma, lung, thyroid, breast, colon, prostate, hepatic, bladder, renal, cervical, pancreatic, leukemia, lymphoma, myeloid, ovarian, uterus, sarcoma, biliary, or endometrial cancer.

- 5 According to some embodiments, the method of treating cancer comprises administering the pharmaceutical composition as part of a treatment regimen comprising administration of at least one additional anti-cancer agent.

 According to some embodiments, the anti-cancer agent is selected from the group consisting of an antimetabolite, a mitotic inhibitor, a taxane, a topoisomerase inhibitor, a
10 topoisomerase II inhibitor, an asparaginase, an alkylating agent, an antitumor antibiotic, and combinations thereof. Each possibility represents a separate embodiment of the invention.

 According to some embodiments, the antimetabolite is selected from the group consisting of cytarabine, fludarabine, fluorouracil, mercaptopurine, methotrexate, thioguanine, gemcitabine, and hydroxyurea. According to some embodiments, the mitotic inhibitor is
15 selected from the group consisting of vincristine, vinblastine, and vinorelbine. According to some embodiments, the topoisomerase inhibitor is selected from the group consisting of topotecan and irinotecan. According to some embodiments, the alkylating agent is selected from the group consisting of busulfan, carmustine, lomustine, chlorambucil, cyclophosphamide, cisplatin, carboplatin, ifosfamide, mechlorethamine, melphalan, thiotepa,
20 dacarbazine, and procarbazine. According to some embodiments, the antitumor antibiotic is selected from the group consisting of bleomycin, dactinomycin, daunorubicin, doxorubicin, idarubicin, mitomycin, mitoxantrone, and plicamycin. According to some embodiments, the topoisomerase II is selected from the group consisting of etoposide and teniposide. Each possibility represents a separate embodiment of the present invention.

25 According to some particular embodiments, the additional anti-cancer agent is selected from the group consisting of bevacizumab, carboplatin, cyclophosphamide, doxorubicin hydrochloride, gemcitabine hydrochloride, topotecan hydrochloride, thiotepa, and combinations thereof. Each possibility represents a separate embodiment of the present invention.

30 Monoclonal antibodies according to the present invention may be used as part of combined therapy with at least one anti-cancer agent. According to some embodiments, the

additional anti-cancer agent is an immuno-modulator, an activated lymphocyte cell, a kinase inhibitor or a chemotherapeutic agent.

According to some embodiments, the anti-cancer agent is an immuno-modulator, whether agonist or antagonist, such as antibody against an immune checkpoint molecule.

5 Checkpoint immunotherapy blockade has proven to be an exciting new venue of cancer treatment. Immune checkpoint pathways consist of a range of co-stimulatory and inhibitory molecules which work in concert in order to maintain self-tolerance and protect tissues from damage by the immune system under physiological conditions. Tumors take advantage of certain checkpoint pathways in order to evade the immune system. Therefore, the inhibition of
10 such pathways has emerged as a promising anti-cancer treatment strategy.

The anti-cytotoxic T lymphocyte 4 (CTLA-4) antibody ipilimumab (approved in 2011) was the first immunotherapeutic agent that showed a benefit for the treatment of cancer patients. The antibody interferes with inhibitory signals during antigen presentation to T cells. Anti-programmed cell death 1 (PD-1) antibody pembrolizumab (approved in 2014) blocks
15 negative immune regulatory signaling of the PD-1 receptor expressed by T cells. An additional anti-PD-1 agent was filed for regulatory approval in 2014 for the treatment of non-small cell lung cancer (NSCLC). Active research is currently exploring many other immune checkpoints, among them: CEACAM1, NKG2A, B7-H3, B7-H4, VISTA, lymphocyte activation gene 3 (LAG3), CD137, OX40 (also referred to as CD134), and killer cell immunoglobulin-like
20 receptors (KIR).

According to some specific embodiments, the immuno-modulator is selected from the group consisting of: an antibody inhibiting CTLA-4, an anti-human programmed cell death protein 1 (PD-1), PD-L1 and PD-L2 antibody, an activated cytotoxic lymphocyte cell, a lymphocyte activating agent, an antibody against CEACAM, an antibody against TIGIT, and
25 a RAF/MEK pathway inhibitor. Each possibility represents a separate embodiment of the present invention. According to some specific embodiments, the additional immuno-modulator is selected from mAb to PD-1, mAb to PD-L1, mAb to PD-L2, mAb to CEACAM1, mAb to CTLA-4, mAb to TIGIT, mAb to PVR, Interleukin 2 (IL-2) or lymphokine-activated killer (LAK) cell.

30 In certain embodiments, the inhibitor of PD-1 signaling is an antibody or fragment thereof that binds to PD-1. In certain embodiments, the antibody or fragment thereof that binds to PD-

1 is Pembrolizumab, Nivolumab, AMP-514, Tislelizumab, Spartalizumab, or a PD-1 binding fragment thereof. In certain embodiments, the inhibitor of PD-1 signaling is an antibody that specifically binds PD-L1 or PD-L2. In certain embodiments, the antibody that specifically binds PD-L1 or PD-L2 comprises Durvalumab, Atezolizumab, Avelumab, BMS-936559, or FAZ053, or a PD-L1 or PD-L2 binding fragment thereof. In certain embodiments, the inhibitor of PD-1 signaling comprises an Fc-fusion protein that binds PD-1, PD-L1, or PD-L2. In certain embodiments, the Fc-fusion protein comprises AMP-224 or a PD-1 binding fragment thereof. In certain embodiments, the inhibitor of PD-1 signaling comprises a small molecule inhibitor of PD-1, PD-L1, or PD-L2. In certain embodiments, the small molecule inhibitor of PD-1, PD-L1, or PD-L2 signaling comprises one or more of: N-{2-[(2-methoxy-6-[(2-methyl[1,1'-biphenyl]-3-yl)methoxy]pyridin-3-yl)methyl]amino]ethyl}acetamide (BMS 202); (2-((3-cyanobenzyl)oxy)-4-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)-5-methylbenzyl)-D-serine hydrochloride; (2R,4R)-1-(5-chloro-2-((3-cyanobenzyl)oxy)-4-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)benzyl)-4-hydroxypyrrolidine-2-carboxylic acid; 3-(4,6-dichloro-1,3,5-triazin-2-yl)-1-phenylindole; 3-(4,6-dichloro-1,3,5-triazin-2-yl)-1-phenyl-1H-indole; L- α -Glutamine, N₂,N₆-bis(L-seryl-L-asparaginyl-L-threonyl-L-seryl-L- α -glutamyl-L-seryl-L-phenylalanyl)-L-lysyl-L-phenylalanyl-L-arginyl-L-valyl-L-threonyl-L-glutaminyl-L-leucyl-L-alanyl-L-prolyl-L-lysyl-L-alanyl-L-glutaminyl-L-isoleucyl-L-lysyl; (2S)-1-[[2,6-dimethoxy-4-[(2-methyl[1,1'-biphenyl]-3-yl)methoxy]phenyl]methyl]-2-piperidinecarboxylic acid; Glycinamide, N-(2-mercaptoacetyl)-L-phenylalanyl-N-methyl-L-alanyl-L-asparaginyl-L-prolyl-L-histidyl-L-leucyl-N-methylglycyl-L-tryptophyl-L-seryl-L-tryptophyl-N-methyl-L-norleucyl-N-methyl-L-norleucyl-L-arginyl-L-cysteinyl-, cyclic (1 $\rightarrow$ 14)-thioether; or a derivative or analog thereof.

According to other embodiments the additional anti-cancer agent is a chemotherapeutic agent. The chemotherapy agent, which could be administered together with the antibody according to the present invention, or separately, may comprise any such agent known in the art exhibiting anticancer activity, including but not limited to: mitoxantrone, topoisomerase inhibitors, spindle poison from vinca: vinblastine, vincristine, vinorelbine (taxol), paclitaxel, docetaxel; alkylating agents: mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide; methotrexate; 6-mercaptopurine; 5-fluorouracil, cytarabine, gemcitabine; podophyllotoxins: etoposide, irinotecan, topotecan, dacarbazine; antibiotics: doxorubicin (adriamycin), bleomycin, mitomycin; nitrosoureas: carmustine (BCNU), lomustine, epirubicin,

idarubicin, daunorubicin; inorganic ions: cisplatin, carboplatin; interferon, asparaginase; hormones: tamoxifen, leuprolide, flutamide, and megestrol acetate.

According to some embodiments, the chemotherapeutic agent is selected from alkylating agents, antimetabolites, folic acid analogs, pyrimidine analogs, purine analogs and related
5 inhibitors, vinca alkaloids, epipodophyllotoxins, antibiotics, L-asparaginase, topoisomerase inhibitor, interferons, platinum coordination complexes, anthracenedione substituted urea, methyl hydrazine derivatives, adrenocortical suppressant, adrenocorticosteroids, progestins, estrogens, antiestrogen, androgens, antiandrogen, and gonadotropin-releasing hormone analog. According to another embodiment, the chemotherapeutic agent is selected from the group
10 consisting of 5-fluorouracil (5-FU), leucovorin (LV), irinotecan, oxaliplatin, capecitabine, paclitaxel and docetaxel. One or more chemotherapeutic agents can be used.

In some embodiments, the pharmaceutical composition according to the present invention is for use in treating cancer or for use in enhancing the immune response.

The term "enhancing immune response" refers to increasing the responsiveness of the
15 immune system and inducing or prolonging its memory. The pharmaceutical composition according to the present invention may be used to stimulate immune system upon vaccination. Thus, in one embodiment the pharmaceutical composition can be used for improving vaccination.

In certain embodiments, the cancer is selected from lung, thyroid, breast, colon,
20 melanoma, prostate, hepatic, bladder, renal, cervical, pancreatic, leukemia, lymphoma, myeloid, ovarian, uterus, sarcoma, biliary, and endometrial cells cancer. Each possibility represents a separate embodiment of the invention.

According to some embodiments, a pharmaceutical composition, comprising at least one antibody or fragment thereof according to the present invention, and a pharmaceutical
25 composition, comprising an additional immuno-modulator or a kinase inhibitor, are used in treatment of cancer by separate administration.

According to still another aspect the present invention provides a method of treating cancer in a subject in need thereof comprising administering to said subject a therapeutically effective amount of an antibody or antibody fragment according to the present invention.

30 Toxicity and therapeutic efficacy of the compositions described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the IC₅₀ (the concentration which provides 50% inhibition) and the maximal

tolerated dose for a subject compound. The data obtained from these cell culture assays, and animal studies can be used in formulating a range of dosages for use in humans. The dosage may vary depending inter alia upon the dosage form employed, the dosing regimen chosen, the composition of the agents used for the treatment and the route of administration utilized, among
5 other relevant factors. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. Depending on the severity and responsiveness of the condition to be treated, dosing can also be a single administration of a slow-release composition, with course of treatment lasting from several days to several weeks or until cure is affected or diminution of the disease state is achieved. The amount of a
10 composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, and all other relevant factors.

The term "administering" or "administration of" a substance, a compound or an agent to a subject can be carried out using one of a variety of methods known to those skilled in the art.
15 For example, a compound or an agent can be administered enterally or parenterally. Enterally refers to administration via the gastrointestinal tract including *per os*, sublingually or rectally. Parenteral administration includes administration intravenously, intradermally, intramuscularly, intraperitoneally, subcutaneously, ocularly, sublingually, intranasally, by inhalation, intraspinally, intracerebrally, and transdermally (by absorption, e.g., through a skin
20 duct). A compound or agent can also appropriately be introduced by rechargeable or biodegradable polymeric devices or other devices, e.g., patches and pumps, or formulations, which provide for the extended, slow or controlled release of the compound or agent. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods. In some embodiments, the administration includes both direct
25 administration, including self-administration, and indirect administration, including the act of prescribing a drug. For example, as used herein, a physician who instructs a patient to self-administer a drug, or to have the drug administered by another and/or who provides a patient with a prescription for a drug is administering the drug to the patient.

Antibodies are generally administered in the range of about 0.1 to about 20 mg/kg of
30 patient weight, commonly about 0.5 to about 10 mg/kg, and often about 1 to about 5 mg/kg. In this regard, it is preferred to use antibodies having a circulating half-life of at least 12 hours, preferably at least 4 days, more preferably up to 21 days. Chimeric antibodies are expected to have circulatory half-lives of up to 14-21 days. In some cases, it may be advantageous to

administer a large loading dose followed by periodic (e.g., weekly) maintenance doses over the treatment period. Antibodies can also be delivered by slow-release delivery systems, pumps, and other known delivery systems for continuous infusion.

5 The term "about" means that an acceptable error range, e.g., up to 5% or 10%, for the particular value should be assumed.

The present invention further comprises, according to another aspect, a method of determining or quantifying CD112R in a sample, the method comprising contacting a biological sample with an antibody or antibody fragment according to the invention, and measuring the level of complex formation

10 The present invention further discloses methods for diagnosing and prognosing cancer.

According to an aspect, the present invention provides a diagnostic and/or prognostic method of cancer or infectious disease in a subject, the method comprises the step of determining the expression level of CD112R in a biological sample of said subject using at least one antibody as described herein.

15 The term "biological sample" encompasses a variety of sample types obtained from an organism that may be used in a diagnostic or monitoring assay. The term encompasses blood and other liquid samples of biological origin, solid tissue samples, such as a biopsy specimen, or tissue cultures or cells derived there from and the progeny thereof. Additionally, the term may encompass circulating tumor or other cells. The term specifically encompasses a clinical
20 sample, and further includes cells in cell culture, cell supernatants, cell lysates, serum, plasma, urine, amniotic fluid, biological fluids including aqueous humour and vitreous for eyes samples, and tissue samples. The term also encompasses samples that have been manipulated in any way after procurement, such as treatment with reagents, solubilization, or enrichment for certain components.

25 Determining the expression level of CD112R can be performed by a labeled anti-CD112R antibody as described herein. Determining the expression can be performed, for example, by ELISA.

The method of the invention can further comprise the step of comparing said level of expression to a control level.

The following examples are presented in order to more fully illustrate some embodiments of the invention. They should, in no way be construed as limiting the scope of the invention.

EXAMPLES

5 Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non-limiting fashion.

 Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological, immunological and recombinant DNA techniques. Such techniques are well known in the art. Other general
10 references referring to well-known procedures are provided throughout this document for the convenience of the reader.

Example 1. Expression of CD112R is increased after immune cells activation.

 CD112R mRNA expression in human immune cells as found at the Database of Immune Cell Expression (DICE, <https://dice-database.org/>) is depicted in Fig. 1A. It is shown
15 that CD112R is expressed mainly on NK cells, B cells and T cells. For CD112R detection on human immune cells (Figs. 1B-1D), 50K cells were stained using 30 µl/well of supernatants from hybridoma of clone 13 and 15. For detection, Goat anti-mouse-647 Ab (Jackson immunoresearch cat. 315-605-006) was used at 1:250 dilution. Peripheral blood mononuclear cell (PBMCs) from two healthy donors were used. While no detectable staining was depicted
20 on the surface of resting PBMCs (Fig. 1B), After 96 hours in NK medium (400 u/ml hIL-2) with 2 µg/ml PHA-L activation, significant and uniform expression of CD112R was observed (Fig. 1C). Both NK cell population's tested expressed CD112R, regardless of the activation status (Fig. 1D).

Example 2. CD112R is an inhibitory receptor predominantly engaged by Nectin-2 binding

25

 A schematic illustration of receptors expressed on immune cells and their respective affinities to Nectin-2 (CD112), an inhibitory ligand for immune cells, which is expressed by tumors or on antigen presenting cells (APCs), is shown in Fig. 2. TIGIT is a co-inhibitory receptor on immune cells such as T and NK cells; DNAM-1 (also termed CD226) is an
30 activating receptor on immune cells (e.g., T cells), and CD112R (also termed PVRIG) is a co-

inhibitory receptor on cytotoxic immune cells (e.g., CD8+ T and NK cells); According to some embodiments of the present invention, anti-CD112R mAbs block CD112R interactions with its ligand Nectin-2 and increase activation of immune cells, without interfering with DNAM-1 signaling.

5 **Example 3. Analysis of binding and blocking characteristics of the anti-CD112R mAbs**

Fig. 3A illustrates specific binding of anti-CD112R clones to 293T cells (highly transfectable derivative of human embryonic kidney 293 cells) engineered to overexpress CD112R. CD112R mAbs were used in a saturating dose (66nM) on either parental or on CD112R overexpressing 293T cells. Only the overexpressing cells showed a positive staining
 10 signal (with no significant difference between the clones tested), pointing to the high specificity of the mAbs to CD112R. EC-50 values are summarized in Fig. 3B. CD112R chimeric mAbs comprising human IgG1 with or without N31S substitution were used in concentration of 0.4-99 nM in series of three-fold dilutions and incubated with 293T cells that overexpress CD112R. For detection, goat anti-human labeled with allophycocyanin (APC) (Jackson immunoresearch
 15 109-136-170) Ab was used at 1:200 dilution. Binding was analyzed by FACS and used for calculation of EC-50 values. These results suggest that all tested mAbs bind cell surface expressed CD112R at sub-nM or at nM affinities. The N31S variant, engineered to remove an N-Linked glycosylation motif at the CDR1 of the heavy chain, had improved (sub-nM) binding to human CD112R in this cell-based assay. In addition, 8866 cells (chronic myelogenous
 20 leukemia cells) overexpressing human Nectin-2 were incubated with 10 ug/ml (156 nM) of CD112R-Fc in the presence of isotype control or with the indicated mAbs at 4 µg/ml (26.4 nM, meaning 6-fold excess of the CD112R-Fc). As can be seen in Fig. 3C, the blocking of CD112R-Fc binding exceeded 95% by all tested variants.

25 **Example 4. Blocking of CD112R by the hIgG1 anti-CD112R mAbs enhances NK cell activation**

NK cells from healthy donors were incubated in the presence of different mAbs and target cell lines at 2:1 E:T ratio for 2 hours at 37°C degrees. NK cell activation was measured by the induction of surface expression of CD107a, and is depicted in Fig. 4 as fold change over control IgG (Y axis). To assess whether Fc type affects the function of the CD112R mAbs, the
 30 activity of an anti-CD112R mAb (clone 13) comprising mouse IgG1 (inert Fc with no detectable binding to hCD16) was compared to two other anti-CD112R mAbs: the chimeric Clone-13-hIgG1, and chimeric clone-13(N31S)-hIgG1 (VH0K0) both having hIgG1 Fc

(effector Fc capable of activating hCD16). All Ab variants were tested at 2nM (0.3µg/ml). As seen in Fig. 4A, both hIgG-Fc mAbs exhibited significant superiority over the inert Fc Ab. Thus, further development was done using anti-CD112R mAbs with human IgG1. For assessment of additive and synergistic effects, CD112R blockade was combined with
5 additional ICIs and results are shown in Figs. 4B and 4C. Blockade of CD112R by the two mAb clones tested induced NK activation (CD107a cell surface expression), and also synergized with other immune checkpoint blockade, including blocking of TIGIT and PVR (NTX-1088). All were statistically significant ($p<0.05$) over control IgG. This data suggests that blocking of CD112R by specific mAbs may increase NK cell activity against cancer targets
10 and may have a synergistic effect on NK activations when combined with other immune checkpoint inhibitor (ICI) mAbs. All mAbs were used at 3 µg/ml (3+3 µg/ml for the combos). Results are shown for the human cancer cell lines A549 (Fig. 4A, 4B lung adenocarcinoma) and MDA-MB-231 (Fig. 4C, breast adenocarcinoma).

**Example 5. Blocking of CD112R by the anti-CD112R mAbs enhances T cell mediated
15 tumor cell killing**

PBMCs from healthy donors were used for T cell killing assay using human cancer cell lines A549 (lung adenocarcinoma, Fig. 5A) and RKO (colorectal cancer, Fig. 5B). All mAbs were used at 3 µg/ml (3+3 µg/ml were used for the combos, and 3+3+3 µg/ml for the triple). The anti-CD112R clone 13 was used in the assay. Target cell killing was normalized to effector
20 cells only (Y axis). The target cell killing was evaluated by the CellTiter-Glo® 2.0 Cell Viability Assay kit by Promega (Cat G9242) according to the manufacturer protocol. Results show that CD112R mAbs lead to significant target cells killing, synergies with anti-TIGIT and anti-PD-1 mAbs. Moreover, blocking all three receptors significantly increased target cells killing over the effect of the individual mAbs and of double mAb combination.

25 Example 6. Humanization of CD112R antibodies improved binding and developability properties of the mAbs

The heavy chain variable region sequence of anti-CD112R clone 13, contained several limitations, which were addressed during the humanization process.

A deamidation motif in the CDR1 of the light chain was addressed by replacing Asn
30 (N) by Ser (S) (named N31S).

Both the Clone-13-hIgG1 and the N31S (deamidation site abrogated) chimeric variants (hIgG1) exhibited exceptional high affinity and were significantly more potent (3-30 folds) in binding CD112R (PVRIG), than a reference (control) Ab that binds to the same antigen (an Ab disclosed in WO2016134333), (Table 1).

- 5 **Table 1:** affinities of the chimeric clone (clone 13) and clone N31S (in which the deamidation motif at amino acid No. 31 was deleted), and a reference Ab that is based on a sequence from WO2016134333 for binding of human CD112R (PVRIG).

Ligand	Analyte	K_a (1/Ms)	K_d (1/s)	K_D (M)
Human CD112R (PVRIG)	Clone 13	3.4E+05	2.96E-06	8.71-12*
	Clone 13 (N31S)	3.4E+05	3.26E-07	9.6-13*
	control	8.95E+04	2.19E-06	2.44-11*

* Limit of Biacore instrument

- 10 Next, variants were made to remove an N-linked glycosylation motif found at the CDR1 of the heavy chain (Chothia CDR definition). The variants contained substitution of the amino acid residue Asparagine (N) at position 28 with a residue selected from Aspartic acid (D), Alanine (A), Glutamine (Q), Serine (S), and Threonine (T), and substitution of the amino acid residue Threonine (T) at position 30 by Alanine (A). All variants were tested for productivity
- 15 (based on titer concentrations) and affinity as summarized in Table 2.

Table 2: productivity and affinity of the variants in which N-Linked glycosylation motif was removed

Antibody	Variant	Titer (ug/ml)	Kd (M)	Relative Kd
VH0Vko	WT	47.6	1.67E-10	1.00
VH0 N28D Vko	N28D	4.7	1.22E-10	0.73
VH0 N28A Vko	N28A	3.9	1.5E-10	0.9
VH0 N28Q Vko	VH0 N28Q Vko	3.0	1.16E-10	0.69
VH0 N28S Vko	N28S	4.0	1.16E-10	0.66
VH0 N28T Vko	VH0 N28T Vko	3.2	1.02E-10	0.61
VH0 T30A Vko	T30A	6.0	1.06E-10	0.63

Based on the relative affinity results, the variant N28T was selected. Removal of the N-linked glycosylation did not affect affinity, but significantly reduced productivity. To address this, 11 variants of humanized heavy chains, deleting the N-linked glycosylation motif, were designed as scFv molecules, on human IgG1 Fc, and tested for productivity (Table 3). The top three productive humanized scFv variants (#9-11) were tested for binding to the human CD112R (Table 4). Majority of the humanized scFv variants had significantly improved binding compared to the scFv of VH0K0 (Clone 13 N31S).

- 10 **Table 3:** shows productivity in 293EBNA cells of ten humanized variants that were designed based on N28T mutation, and that are co-expressed with the unmodified light chain as scFv.

ScFv-Fc	HEK Titer (μg/ml)
Variant 0	0.559
Variant 1	< 0.4
Variant 2	0.5353
Variant 3	< 0.4
Variant 4	Not received
Variant 5	< 0.4
Variant 6	0.524
Variant 7	< 0.4
Variant 8	< 0.4
Variant 9	6.61
Variant 10	1.26
Variant 11	8.23

The scFv molecules were connected to an Fc as a technical solution for a rapid production and screening of the variants.

Table 4: binding affinities to human CD112R of heavy chain variants (#9-11) and the non-humanized version (VH0), co-expressed with the non-modified light chain (VK0) and with three humanized variants of the light chain as indicated

Antibody	K_a (1/Ms)	K_d (1/s)	K_d (M)	Relative K_d
VH0K0	2.90E+5	8.89E-5	3.07E-10	1.00
VH10K2	6.23E+5	1.33E-4	2.14E-10	0.7
VH10K3	5.45E+5	1.07E-4	1.97E-10	0.64
VH11K1	6.3E+5	1.01E-4	1.65E-10	0.54
VH11K2	5.52E+5	1.05E-4	1.91E-10	0.62
VH11K3	5.30E+5	1.01E-4	1.90E-10	0.62

Based on proximity to human germline Abs and lack of potential MHC-2 binding motifs (predicted by iTopeTM), five variants were selected for further analysis. The affinity of selected variants is shown in Table 5. All variants had improved binding to human CD112R compared to the chimeric (clone N31S) Ab.

Table 5: properties of selected full antibody variants after purification

Antibody	K_a (1/Ms)	K_d (1/s)	K_d (M)	Relative K_d
VH0K0	2.10E+05	3.05E-05	1.45E-10	1.16
VH0K0 N28T	4.00E+05	5.02E-05	1.26E-10	1
VH0K1 N28T*	3.20E+05	8.30E-06	2.59E-11	0.21
VH0K2 N28T	3.98E+05	4.34E-05	1.09E-10	0.87
VH0K3 N28T*	3.30E+05	1.06E-05	3.21E-11	0.25
VH9K0 *	3.00E+05	3.09E-06	1.03E-11	0.08
VH9K1	3.50E+05	3.87E-05	1.11E-10	0.87
VH9K2 *	3.01E+05	1.30E-05	4.32E-11	0.34
VH9K3	3.30E+05	1.06E-05	3.21E-11	1.22
VH10K0	3.45E+05	4.66E-05	1.35E-10	1.07
VH10K1	3.51E+05	4.18E-05	1.19E-10	0.94
VH10K2	3.59E+05	4.27E-05	1.19E-10	0.94
VH10K3	3.61E+05	4.36E-05	1.21E-10	0.96
VH11K0	3.16E+05	3.19E-05	1.01E-10	0.8
VH11K1 *	2.87E+05	4.79E-06	1.67E-11	0.13
VH11K2	3.36E+05	3.77E-05	1.12E-10	0.89
VH11K3 *	3.07E+05	2.42E-05	7.88E-11	0.63

* Limit of the Biacore instrument sensitivity

Example 7. Similar binding of anti-CD112R mAbs to CD112R of human and cynomolgus monkey

5 The humanized mAbs were tested for binding of endogenous and overexpressed human and cynomolgus CD112R. The results show that the affinity of the humanized mAbs to the human and monkey CD112R is similar. Table 6 summarizes EC50 values calculated based on binding curves of selected humanized (Hu) mAbs, which were used at concentration of 99-0.05 nM in a series of three-fold dilutions to 293T cells expressing either human (protein id: NP_076975.2) or cynomolgus (Cyno) monkey (Macaca fascicularis) CD112R (protein id: XP_005549281.1). For detection, donkey anti-human-647 (Jackson immunoresearch cat-109-605-006) Ab was used at 1:250 dilution.

15 Table 7 summarizes EC50 values calculated based on the binding curves of selected humanized mAbs to cynomolgus CD8 T cells and human NK cells, both cells expressing endogenous CD112R of the relevant species. Assay was performed as in Table 6. An anti-CD112R mAb disclosed in WO2016134333 was used for comparison. The relative EC-50

values are also summarized in Tables 6 and 7. Max binding was assessed as well (human max binding – background / cyno max binding – background) and is summarized in Table 8. All the humanized variants have a complete cross-reactivity between the human and cynomolgus targets. Additionally, the superior binding of all the humanized variants to the antigen, compared to a control Ab is seen, with three of the five humanized variants having over two-fold improved binding compared to the reference Ab and the remaining two variants display about 50% improvement.

Table 6: assessment of binding characteristics (EC₅₀ and max binding) of selected humanized anti-CD112R mAbs to cell surface CD112R

Clone name	H0K0	H10K2	H10K3	H11K1	H11K2	H11K3
EC ₅₀ nM HuCD112R	0.55	0.40	0.30	0.30	0.28	0.31
EC ₅₀ nM CynoCD112R	0.97	0.42	0.50	0.72	0.71	0.60
Ratio Cyno/Hu	1.75	1.07	1.67	2.43	2.54	1.93

10

Table 7: assessment of binding of selected humanized anti-CD112R mAbs to cynomolgus CD8 T cells and to human NK cells which express endogenous CD112R of the relevant species.

Clone name	H10K2	H10K3	H11K1	H11K2	H11K3	Com
EC-50 nM Hu	0.86	0.77	0.91	1.06	1.11	1.87
EC-50 nM Cyno	1.45	1.39	1.46	1.29	1.27	2.05
Ratio Cyno/Hu	1.69	1.81	1.61	1.22	1.14	1.09

Table 8: a summary of absolute and relative Max binding assessment. of selected humanized anti-CD112R mAbs to cynomolgus CD8 T cells and to human NK cells which express endogenous CD112R of the relevant species. Relative binding (ratio) was calculated as (human max binding – background) / (cyno max binding – background)

Clone name	H10K2	H10K3	H11K1	H11K2	H11K3	Com
Max binding Hu (MFI a.u - background)	975	988	982	1018	1005	993
Max binding Cyno (MFI a.u - background)	914	933	820	839	833	1583
Ratio Hu/Cyno	1.07	1.06	1.20	1.21	1.21	0.63

Example 8. Humanized CD112R mAbs effectively block binding of CD112R to CD112

The humanized anti-CD112R antibodies are potent blockers of CD112R – Nectin-2 interactions. 293T cells overexpressing CD112R, were incubated (75k/well) with the fusion protein hNectin-2-mIgG2a at a set dose (125 nM) in the presence or absence of the humanized CD112R mAbs, on ice for 30 minutes. The antibodies were added in a range of concentrations of 40-0.018 nM in a series of three-fold dilutions. The amount of bound hNectin-2-mIgG2a was detected by anti-mouse antibodies fluorescently labeled with Dy-light 647 (115-606-071 Jackson immunoresearch) and analyzed by FACS. The EC-50 and EC-90 values were calculated from the resulting titration curves and are summarized in Table 9 below. The results show that the humanized CD112R antibodies are capable of blocking the CD112R-CD112 interactions at sub-nM concentrations.

Table 9: blocking of CD112R–Nectin-2 interactions by the humanized anti-CD112R antibodies

Antibody Clone	H11K1	H11K2	H11K3
EC-50 nM	0.29	0.32	0.26
EC-90 nM	10.7	9.9	9.6

Example 9. Blocking of CD112R by the humanized anti-CD112R mAbs enhances NK cell activation over the parental mAb and synergizes with blocking of TIGIT

To examine the blocking effect of the humanized anti-CD112R mAbs, NK cells from a healthy donor were incubated in presence of different mAbs with A549 cells (lung adenocarcinoma) at 2:1 E:T ratio for 2 hours at 37°C. NK cell activation was measured by the induction of surface expression of CD107a and is depicted in Fig. 6 as fold change over control IgG (Y axis). All mAbs were used at 3 µg/ml (3+3 for the combos). All the anti-CD112R mAbs (grey bars) significantly increased NK cell activation ($p < 0.0005$). Moreover, the humanized anti-CD112R antibodies were superior to the parental, non-humanized (H0K0) chimeric variants ($p < 0.05$). Additionally, all the humanized variants synergized with anti-TIGIT mAb (black bars), leading to significant increase of activity over each individual treatment ($p < 0.015$).

Overall, the data suggest that humanized anti-CD112R mAbs are potent inducers of NK cell activation, superior to the parental anti-CD112R and maintain the synergistic effect when combined with anti-TIGIT mAb.

Example 10. Blocking of CD112R by humanized anti-CD112R mAbs enhances NK cell activation in a dose dependent manner and synergizes with blocking of PVR

NK cells from a healthy donor were incubated in presence of different mAbs with MDA-MB-231 cells (breast adenocarcinoma) at 2:1 E:T ratio for 1.5 hours at 37°C. NK cell activation was measured by the induction of surface expression of CD107a as analyzed by FACS and is depicted as fold change over control IgG (Y axis). The humanized mAbs (H11K1, H11K2, and H11K3, having HC variable region sequence set forth in SEQ ID NO: 39, and LC variable region sequence set forth in SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, respectively) were used at a range of concentrations from 53- 0.05 nM in 4-fold dilutions (Fig. 7A). Additionally (Fig. 7B), a set dose of 13 nM of each mAb was tested in combination with 13 nM of anti-PVR Ab (NTX-1088). Fig. 7A shows that the lead humanized anti-CD112R mAbs are potent inducers of NK activation. All the anti-CD112R mAbs tested significantly ($p < 0.0172$) increased NK cells activation, up to a concentration of 0.05 nM, which is roughly 10-folds below EC-50. All the leading humanized variants (grey bars) synergized with the anti-PVR NTX-1088 Ab (black bars), leading to significant ($p < 0.03$) increase of activity over each individual treatment. Overall, the data suggest that humanized anti-CD112R mAbs are potent

activators of NK cells and that the selected variants synergize with anti-PVR mAb in NK cells activation.

Example 11. Humanized anti-CD112R mAbs are superior to the non-humanized ones and to a reference CD112R mAb in activation of T cells

5 To test the effect of the humanized anti-hCD112R antibodies on activation of T cells, Jurkat cells overexpressing the human CD112R (Jurkat hCD112R) were incubated with A549 cells (high Nectin-2 expression) in the presence of anti-CD3, with or without the indicated anti-CD112R mAbs. After 24 hours plates were centrifuged, and supernatants were collected. IL-2 quantification was done using Human IL-2 ELISA (MAX™ Deluxe by Biolegend), according to the manufacturer's protocol. Fig. 8A shows dose response assay of all the humanized variants and a reference anti-CD112R antibody (disclosed in WO2016134333), at concentration range of 4-0.0625 µg/ml. Most humanized clones were superior to the parental, non-humanized clone (H0K0), and all the anti-CD112R variants were superior to a reference Ab at all concentrations tested in inducing IL-2 secretion. Fig. 8B illustrates selected humanized variants tested as in Fig. 7A, in a range of concentrations from 26.4-0.1 nM. The reference Ab was only added at the highest concentration of 24.6 nM. All selected variants led to significant induction ($p < 0.0003$) of IL-2 secretion. Comparison was done to the highest concentration of hIgG1. Moreover, antibodies H11K2 and H11K3 were significantly ($p < 0.004$) more potent than the reference even at the lowest concentration tested, H11K1 was significantly superior to the reference starting from 0.41 nM ($p < 0.02$).

These results indicate that the anti-CD112R mAbs are potent inducers of T cell activation, with significant advantage over the existing reference.

Example 12. Blocking of CD112R by the anti-CD112R mAbs enhances T cell activation

PBMCs from healthy donors were used for T cell activation assay using human cancer cell line A549 (lung adenocarcinoma). The PBMCs were incubated for 48 hours with the target cells at 2:1 E:T ratio in the presence of 1 µg/ml of anti CD3 mAb (OKT3).

The effector cells were harvested and stained for the surface expression of (Fig. 9A) CD69 (BLG-310906) and (Fig. 9B) CD137 (BLG-309804, both from Biolegend). The surface expression (MFI) of the markers was normalized to the No mAb group.

30 As shown in Fig. 9. blocking of CD112R by the humanized anti-CD112R mAb H11K2 enhanced T cell activation. The blockade of CD112R had additive effect with blocking of

additional ICIs, such as PVR (NTX-1088) and PD-1 (Keytruda). All changes are statistically significant ($p < 0.05$).

Example 13. Effect of anti-CD112R on NK cell activation in the presence of target cells expressing Nectin-2, lacking PVR, and the impact of PVR expression the activity

5 NK cells from healthy donors were incubated in the presence of different mAbs and target cell lines at 1:1 E:T ratio for 20 hours at 37°C. NK cell activation was measured by the induction of surface expression of CD137, and is depicted in Fig. 10 as fold induction over no antibody (Y axis). The humanized anti-CD112R mAb (H11K3 comprising hIgG1 Fc) was compared to NTX-1088 (anti-PVR) or to the anti-TIGIT mAb Tiragolumab (KEGG DRUG
10 entry D11482) as standalone and in combinations. CD112R blockade by the lead humanized variant, was the most potent of all treatments when the target cells did not express membranal PVR (Fig. 10A). When PVR was expressed, CD112R synergized with other immune check point blockade, including blocking of TIGIT and blocking of PVR. The combination of NTX-1088 and anti-CD112R was significantly superior to all other interventions. All mAbs were
15 used at 3 µg/ml (3+3 µg/ml for the combos). Results are shown for the human cancer cell lines JEG3 choriocarcinoma, lacking endogenous PVR (Fig. 10A), and JEG3-hPVR (human PVR overexpression, Fig. 10B). * $p < 0.005$, ** $p < 0.001$, *** $p < 0.0001$ by two tailed student t-test. Representative data of two out of five donors is shown.

**Example 14. Effect of anti-CD112R on NK cell activation in the presence of cancer target
20 cells**

NK cells from healthy donors were incubated in the presence of different mAbs and the target cell lines: H1299 (NSCLC, Fig. 11A), A549 (lung adenocarcinoma, Fig. 11B), or MDA-MB-231 (triple negative breast cancer, Fig. 11C) at 1:1 E:T ratio for 48 hours at 37°C. NK cell activation was measured by the induction of surface expression of CD137, and is depicted in
25 Fig. 11 as fold induction over no antibody (Y axis). The humanized anti-CD112R mAb with hIgG1 Fc (H11K3) was evaluated as a standalone, or in combinations as in Example 1. In all settings, CD112R blockade as a standalone, had significant impact on activation of NK cells. Additionally, CD112R synergized with other immune check point blockades, including blocking of TIGIT (by Tirgaolumab KEGG DRUG entry D11482) and blocking of PVR (NTX-
30 1088). Of note, the combination of NTX-1088 and anti-CD112R mAb was significantly superior to all other interventions, across all cell line tested. All mAbs were used at 3 µg/ml

(3+3 µg/ml for the combos). * $p < 0.005$, ** $p < 0.001$, *** $p < 0.0001$ by two tailed student t-test. Representative data of two out of four NK donors is shown.

Example 15. CD112R mAbs inhibit tumor growth in vivo

The efficacy of the mAbs is demonstrated in vivo using animal models. Immune compromised mice (NSG, NOG or equivalent) are used as a model. Target cell lines that naturally express Nectin-2 (e.g., A549, MDA-MB-231, RKO) are injected subcutaneously (sc) into mice, in amounts sufficient to allow robust tumor growth (e.g., 5×10^6 cells per mouse). To provide immune cells, the tumor cells are mixed and injected with relevant human immune cells (e.g., whole PBMC, purified T cells, purified CD8+ T cells or NK cells).

The mice are then monitored for tumor growth until palpable tumors are formed. At this point, mice are randomized for different treatment groups that receive either control IgG, anti-CD112R mAbs, anti PD-1 mAb, or their combination. The effect of the individual and combined ICI treatments on tumor growth inhibition is evaluated over time against the control IgG group.

Additional approach is based on reconstitution of the immune cells after tumors are established. Here, same mice and tumor cells are used as above. However, the above mentioned human immune cells are injected intravenously (iv) and only after palpable tumors are established. A day later mice are randomized to different treatment groups and tumor growth inhibition is evaluated as above.

A third strategy is taken where human hematopoietic stem cells, rather than mature leukocytes, are used to reconstitute the missing immune cells at the immune-deficient mice. Once the hematopoietic cells give rise to mature human immune cells at the periphery the resulting “humanized” mice, are used for tumor implantation and testing of the effects of the different ICI mAbs as described above for the iv-reconstitution approach.

Example 16. Effect of anti-CD112R on tumor growth in mice

In vivo efficacy of anti-CD112R mAb treatment was evaluated in mice. A549 cells were co-implanted at 1:5 E:T ratio with PBMCs from a healthy male donor (1×10^6 PBMCs mixed with 5×10^6 A549 cells). Cell mixture was s.c. implanted at the rear flank of NSG-HLA-A2/HHd male mice at a 1:1 volume ratio cells-in-PBS:Matrigel (Matrigel® Matrix, Corning REF 356234), in a final volume of 100 µl/animal. On day 3 post implantation, treatment with either control IgG1 mAb (InVivoMAb human IgG1 isotype control BE0297 Bioxcell) or with

anti-CD112R mAb, or with anti-PD-1 mAb (Pembrolizumab), once weekly at 10 mg/kg for a total of 5 doses. Tumor volume was measured once weekly by a caliper. On day 35 the average tumor volume of the control IgG treated group was 641mm³ and the tumor volume of the anti PD-1 treated group was 840 mm³ (Fig. 12). These results were not significantly different by two-way-ANOVA. On the other hand, the average volume of the anti CD112R-treated tumors was 200 mm³, representing 68% tumor growth inhibition, and significant tumor reduction compared to IgG treated animals (***p=0.001 by two-way ANOVA).

Example 17. Antibody sequences

Table 10: details of CDR sequences, variable regions sequences of the different clones and variant thereof, and scFv sequences.

	Amino acid SEQ ID Nos:	Nucleotide SEQ ID Nos:
Clone 13 and variants		
HC CDR1	1, 7	
HC CDR2	2, 8	
HC CDR3	3	
LC CDR1	4	
LC CDR2	5	
LC CDR3	6	
HC variable region	17	16, 24
LC variable region	19	18, 25
LC CDR1 X= N (deamidation site) or S	26	
LC CDR1 N31S (deamidation removal)	27	
HC CDR1 N-Linked glycosylation site X ₁ = N (parental), D, A, Q, S, or T; X ₂ =T (parental) or A	28	
HC variant 1 (H1)	29	
HC variant 2 (H2)	30	
HC variant 3 (H3)	31	
HC variant 4 (H4)	32	
HC variant 5 (H5)	33	
HC variant 6 (H6)	34	
HC variant 7 (H7)	35	
HC variant 8 (H8)	36	
HC variant 9 (H9)	37	
HC variant 10 (H10)	38	
HC variant 11 (H11)	39	
LC variant 1 (K1)	40	
LC variant 2 (K2)	41	
LC variant 3 (K3)	42	
scFv variants connected to hIgG1		

Variant_0	43	
Variant 1 N28T LC	44	
Variants_2-11	45-54	
Clone 15		
HC CDR1	9, 7	
HC CDR2	10, 12	
HC CDR3	3	
LC CDR1	11	
LC CDR2	5	
LC CDR3	6	
HC variable region	20	21
LV variable region	22	23

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation.

CLAIMS

1. An antibody that binds to CD112R, or an antibody fragment thereof, comprising at least the antigen binding portion, wherein the antibody or antibody fragment comprises:
 - 5 (i) three complementarity determining regions (CDRs) of a heavy-chain (HC) variable region comprising SEQ ID NO: 16 and three CDRs of a light-chain (LC) variable comprising SEQ ID NO: 18, or an analog or derivative thereof having at least 90% sequence identity with said antibody or fragment sequence; or
 - 10 (ii) three CDRs of a heavy-chain variable region comprising SEQ ID NO: 21 and three CDRs of a light-chain variable region comprising SEQ ID NO: 23, or an analog or derivative thereof having at least 90% sequence identity with said antibody or fragment sequence.
2. The antibody or the antibody fragment according to claim 1, comprising a set of six
15 CDRs wherein: heavy chain CDR1 sequence comprising the sequence GYX₁FX₂SY (SEQ ID NO: 1), wherein X₁ denotes N, D, A, Q, S, or T, and X₂ is T or A, or the sequence SYWIN (SEQ ID NO: 7), heavy chain CDR2 comprising the sequence YPGSYIP (SEQ ID NO: 2), heavy chain CDR3 comprising the sequence GYFDV (SEQ ID NO: 3), light chain CDR1 comprising the sequence KSSQSLLXSGNQKNYLA (SEQ
20 ID NO: 4), wherein X denotes N or S, light chain CDR2 comprising the sequence GASTRES (SEQ ID NO: 5), and light chain CDR3 comprising the sequence QNDHSYPYT (SEQ ID NO: 6).
3. The antibody or the antibody fragment according to any one of claims 1 or 2, comprising a heavy chain variable region selected from SEQ ID NO: 28 and SEQ ID NO: 21, or an
25 analog having at least 95% sequence similarity with said heavy chain variable region sequence.
4. The antibody or the antibody fragment according to any one of claims 1 to 3, comprising a light chain variable sequence selected from SEQ ID NO: 26 and SEQ ID NO: 23, or an
30 analog having at least 95% sequence similarity with said slight chain variable region sequence.
5. The antibody or the antibody fragment according to any one of claims 1 to 4, comprising a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 28 and the light chain comprises SEQ ID NO: 26.

6. The antibody or the antibody fragment according to any one of claims 1 to 4 comprising a heavy chain and a light chain, wherein the heavy chain comprises SEQ ID NO: 21 and the light chain comprises SEQ ID NO: 23.
7. A variant of an antibody or antibody fragment according to any one of claims 5 or 6, having at least 95% identity with said antibody light chain or heavy chain.
8. An antibody fragment according to any one of claims 1 to 7, wherein the antibody fragment is a single chain Fv (scFv).
9. A polypeptide comprising the scFv according to claim 8 and a human IgG1 Fc region, wherein the polypeptide sequence comprises a sequence selected from the group consisting of SEQ ID NOs: 43 to 54, or an analog or derivative thereof having at least 90% sequence identity with said antibody.
10. The antibody or antibody fragment according to any one of claims 1 to 9, wherein the antibody binds to human CD112R with an affinity of $0.5 \times 10^{-9} \text{M}$ to 10^{-11}M .
11. The antibody or antibody fragment according to any one of claims 1 to 10, wherein the antibody comprises an Fc of hIgG1.
12. The antibody or antibody fragment according to any one of claims 1 to 11, wherein the antibody is a humanized antibody.
13. The antibody or antibody fragment according to claim 12, wherein heavy-chain CDR1 is GYTFTSY (SEQ ID NO: 13); or SYWIN (SEQ ID NO: 7).
14. The antibody or antibody fragment according to any one of claims 12 or 13, wherein light-chain CDR1 is KSSQSLSSGNQKNYLA (SEQ ID NO: 15).
15. The antibody or the antibody fragment according to any one of the preceding claims, comprising a set of six CDRs wherein: heavy chain CDR1 sequence comprising the sequence GYTFTSY (SEQ ID NO: 13), heavy chain CDR2 comprising the sequence YPGSYIP (SEQ ID NO: 2), heavy chain CDR3 comprising the sequence GYFDV (SEQ ID NO: 3), light chain CDR1 comprising the sequence KSSQSLSSGNQKNYLA (SEQ ID NO: 15), light chain CDR2 comprising the sequence GASTRES (SEQ ID NO: 5), and light chain CDR3 comprising the sequence QNDHSYPYT (SEQ ID NO: 6).
16. The antibody or antibody fragment according to any one of claims 12 to 15, wherein the humanized antibody comprises a heavy chain comprising a sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, and 39; or an analog having at least 95% sequence similarity with said heavy chain variable region sequence.

17. The antibody or antibody fragment according to any one of claims 12 to 16, wherein the humanized antibody comprises a light chain comprising a sequence selected from the group consisting of SEQ ID NOs: 40, 41, and 42; or an analog having at least 95% sequence similarity with said heavy chain variable region sequence.
- 5 18. The antibody or antibody fragment according to any one of claims 12 to 17, wherein the humanized antibody comprises heavy and light chains selected from the group consisting of:
- (i) heavy chain comprising SEQ ID NO: 39 and light chain comprising SEQ ID NO: 40;
 - 10 (ii) heavy chain comprising SEQ ID NO: 39 and light chain comprising SEQ ID NO: 41; and
 - (iii) heavy chain comprising SEQ ID NO: 39 and light chain comprising SEQ ID NO: 42.
19. A polynucleotide sequence encoding at least one sequence of a heavy chain or a light chain region of an antibody or antibody fragment according to any one of claims 1 to 18.
- 15 20. The polynucleotide sequence of claim 18, encoding an antibody heavy chain variable region, wherein the polynucleotide sequence comprises a sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 20, and SEQ ID NO: 24 or a variant thereof having at least 85% identity to said sequences.
- 20 21. The polynucleotide sequence of claim 19, encoding an antibody light chain variable region, wherein the polynucleotide sequence is selected from the group consisting of: SEQ ID NO: 18, SEQ ID NO: 22 and SEQ ID NO: 25, or a variant thereof having at least 85% identity to said sequences.
22. A plasmid comprising at least one polynucleotide sequence according to any one of claims 19 to 21.
- 25 23. A cell comprising a polynucleotide sequence according to any one of claims 19 to 21.
24. A cell capable of producing an antibody according to any one of claims 1 to 18.
25. A pharmaceutical composition comprising as an active ingredient, at least one antibody or fragment thereof, according to any one of claims 1 to 18, and a pharmaceutical acceptable excipient, diluent, salt or carrier.
- 30 26. The pharmaceutical composition of claim 25 for use in modulating the immune system by inhibiting binding of CD112R to Nectin-2.
27. The pharmaceutical composition of claim 25 for use in treating cancer in a subject.

28. The pharmaceutical composition of claim 25 for use in preventing or treating a viral infection in a subject.
29. A method of treating cancer comprising administering to a subject in need thereof, a therapeutically effective amount of the pharmaceutical composition according to claim 5 25.
30. The method of claim 29, further comprising an additional anti-cancer therapy selected from surgery, chemotherapy, radiotherapy, and immunotherapy.
31. The method of claim 29, further comprising administering to said subject an additional immuno-modulator, activated lymphocyte cell, kinase inhibitor, chemotherapeutic agent, 10 or any other anti-cancer agent.
32. The method of claim 31, wherein the additional immune-modulator is an antibody against an immune checkpoint molecule.
33. The method of claim 32, wherein the additional immune-modulator is an antibody selected from the group consisting of anti-PD-1, anti-TIGIT, anti-PVR and any combination thereof. 15
34. The method of any one of claims 29 to 33, wherein the cancer is a solid cancer.
35. The method of any one of claims 29 to 33, wherein the cancer is a hematologic cancer.
36. The method of any one of claims 29 to 33, wherein the cancer is selected from the group consisting of lung adenocarcinoma, breast adenocarcinoma, and colorectal cancer.
- 20 37. The method of any one of claims 29 to 33, wherein treating results in preventing or reducing metastases formation, growth or spread in a subject.
38. A method of determining or quantifying CD112R in a sample, the method comprising contacting a biological sample with an antibody or antibody fragment, and measuring the level of complex formation, wherein the antibody or antibody fragment is according to 25 any one of claims 1 to 18.
39. A method of diagnosing or prognosing cancer in a subject, the method comprises determining the expression level of CD112R in a biological sample of said subject using at least one antibody or antibody fragment is according to any one of claims 1 to 18.
40. A kit for measuring the expression or presence of CD112R in biological sample is also 30 provided comprising at least one antibody or antibody fragment according to any one of claims 1 to 18.