mediated killing[4]. These receptors mostly recognize MHC class 1 and MHC class 1- related molecules and include the KIR (killer cell immunoglobulin-like receptors) and LIR (leukocyte immunoglobulin-like receptors) families, the Ly49 family in mice and CD94/NKG2 heterodimers in both species.

An emerging group of immunoglobulin superfamily members that interact with ligands of the nectin and nectin-like (necl) family has recently been described to influence NK cell and T cell functions[5]. These include CD226 (DNAM-l) [6], CD96 (TACTILE) [7], TIGIT (T cell immunoglobulin and ITIM domain) [8,9] and CRTAM (class I restricted T cell-associated molecule) [10]. DNAM-1 and TIGIT are the most extensively studied members of this family and they share a common ligand, CD155 (necl-5; PVR) and CD112 (nectin-2; PVRL2) [8,11]. TIGIT also bind an additional ligand CD113 (PVRL3) [8]. The functions of DNAM-l and TIGIT on NK cells are reportedly counter-balancing [12]. *In vitro* DNAM-1 potentiates the cytotoxicity of NK cells against a wide range of tumor cells [13,14] and is critical for tumor immunosurveillance *in vivo* [13.,I5,16]. In contrast, TIGIT bear an ITIM motif and has been proposed prevent self-tissue damage similar to inhibitory Ly49 or KIR interactions with MHC class I [17]. Indeed, engagement of TIGIT by CD155 has been shown to limit IFNγ production and cytotoxicity NK cells *in vitro* [18,19]. However, the role of TIGIT in NK cell biology relative to the other nectin receptors DNAM-1 and CD96 remains to be assessed *in vivo*.

Despite being cloned 20 years ago [7], little is known about CD96, the other Ig family member that shares CD155 ligand with DNAM-1 and TIGIT [20,21]. In humans, CD96 expression is largely confined to NK cells, CD8 T cells, and CD4 T cells [7]. The major ligand of CD96 is CD155, but CD96 has also been reported to associate with CD111 (nectin-l) and play a role in promoting NK and T cell adhesion [21,22].

SUMMARY

Surprisingly, the present inventors have discovered that CD96 acts as a negative regulator of T cell and NK cell anti-tumor functions. Accordingly, the invention is broadly directed to use of agents that at least partly block or inhibit CD96 to thereby reduce or relieve CD96-mediated immune inhibition to enhance or restore immune surveillance in the mammal. In certain examples, this may

facilitate treatment of diseases or conditions responsive at least partial blocking or inhibition of CD96, such as cancers and/or viral infections.

In one aspect, the present invention refers to use of a CD96-inhibitory agent in the manufacture of a medicament for reducing or relieving immune inhibition in a mammal by at least partly inhibiting or reducing CD96 activity in one or more cells of the mammal to thereby relieve immune inhibition and/or enhance or restore immune surveillance in the mammal, wherein at least partly inhibiting or reducing CD96 activity in the mammal does not include, or at least depend upon killing of CD96-expressing cells in the mammal. In one example, there is disclosed a method of reducing or relieving immune inhibition in a mammal, said method including the step of at least partly inhibiting or reducing CD96 activity in one or more cells of the mammal to thereby relieve immune inhibition and/or enhance or restore immune surveillance in the mammal.

Suitably, the step of inhibiting or reducing CD96 activity in the mammal does not include, or at least depend upon, killing of CD96-expressing cells in the mammal. In some examples, the step of inhibiting or reducing CD96 activity in the mammal includes inhibiting or reducing CD96 binding to CD155 and/or intracellular signaling in one or more cells of the mammal that express CD96. In some examples, the step of inhibiting or reducing CD96 activity in the mammal includes removing and/or down-regulating cell surface expression of CD96.

In one example, the step of inhibiting or reducing CD96 activity in the mammal includes increasing or enhancing expression, production and/or secretion of one or more cytokines or chemokines. Preferably, the cytokine is interferon γ (IFN-γ). Typically, the one or more cells of the mammal are T cells, inclusive of $CD4^+$ and $CD8^+$ T cells, γδT cells, NKT cells, and natural killer (NK) cells.

In another example, the method relieves immune inhibition and/or enhances or restores immune surveillance in the mammal to thereby treat or prevent cancer or cancer metastasis in the mammal.

In other examples, the method relieves immune inhibition and/or enhances or restores immune surveillance in the mammal to thereby treat or prevent a viral infection in the mammal.

In one aspect, the present invention refers to a method of screening, designing, engineering or otherwise producing a CD96-inhibitory agent, said method including the step of determining whether a candidate molecule is capable of at least partly inhibiting or reducing CD96 activity to thereby relieve immune inhibition and/or enhance or restore immune surveillance in a mammal, wherein the CD96-inhibitory agent binds or interacts with one or a plurality of external immunoglobulin-like domains of CD96 and wherein at least partly inhibiting or reducing CD96 activity in the mammal does not include, or at least depend upon killing of CD96-expressing cells in the mammal. In another example, there is described a method of screening, designing, engineering or otherwise producing a CD96-inhibitory agent, said method including the step of determining whether a candidate molecule is capable of at least partly inhibiting or reducing CD96 activity to thereby relieve immune inhibition and/or enhance or restore immune surveillance in a mammal.

In another example, there is disclosed a CD96-inhibitory agent screened, designed, engineered or otherwise produced according to the method of the second aspect.

In one example, the CD96-inhibitory agent is an antibody or antibody fragment.

In one another example, the CD96-inhibitory agent is an anti-cancer agent.

In yet another example, the CD96-inhibitory agent is anti-viral agent.

In another example, disclosed herein is a CD96-inhibitory agent according to the use as disclosed herein according to the method as disclosed herein.

Suitably, accordingly to the aforementioned examples the mammal is a human.

Unless the context requires otherwise, the terms "*comprise*", "*comprises*", and "*comprising*", or similar terms are intended to mean a non-exclusive inclusion, such that a recited list of elements or features does not include those stated or listed elements solely, but may include other elements or features that are not listed or stated.

The indefinite articles '*a*' and '*an*' are used here to refer to or encompass singular or plural elements or features and should not be taken as meaning or defining "one" or a "single" element or feature.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: CD96 competes with DNAM-1 for CD155 binding **a, b** The expression of CD96 was analyzed by flow cytometry on the indicated spleen lymphocyte populations from C57BL/6 WT (light grey) and $CD96^{+}$ mice (dark grey). The representative FACS Histrograms (a) and the mean ± SD (b) of 3 mice from one representative experiment out of 3 are shown. **c, d** The expression of CD96, DNAM-1 and TIGIT was determined on WT spleen NK cells freshly isolated or activated for 48 hrs with IL-2 (1000 U/ml). **e.** The binding of mouse CD155-Fc coupled with AF-647 to purified NK cells freshly isolated from WT, $CD96^{-/-}$, $DNAM\text{-}1^{-/-}$ or $DNAM\text{-}1^{-/-}$, $CD96^{-/-}$ mice was assessed at the indicated concentrations by flow cytometry. **f.** The binding of CD155-Fc coupled with AF-647 (10 μg/ml) was analyzed on purified WT NK cells in the presence of anti-CD96 and or anti-DNAM-1 mAbs. **g.** The binding of

CLAIMS

1. Use of a CD96-inhibitory agent in the manufacture of a medicament for reducing or relieving immune inhibition in a mammal by at least partly inhibiting or reducing CD96 activity in one or more cells of the mammal to thereby relieve immune inhibition and/or enhance or restore immune surveillance in the mammal,

wherein at least partly inhibiting or reducing CD96 activity in the mammal does not include, or at least depend upon killing of CD96-expressing cells in the mammal.

2. The use of Claim 1, wherein the CD96-inhibitory agent binds or interacts with one or a plurality of external immunoglobulin-like domains of CD96.

3. The use of Claim 2, wherein the CD96-inhibitory agent binds or interacts with: one or a plurality of external immunoglobulin-like domains of CD96 selected from the group consisting of; domain 1; domain 2; domain 3; domain 1 and domain 2; domain 1 and domain 3: domain 2 and domain 3; and domain 1, domain 2 and domain 3.

4. The use of Claim 2 or Claim 3, wherein the CD96-inhibitory agent binds or interacts with one or a plurality of external immunoglobulin-like domains of human CD96 isoform 2 (SEQ ID NO:2).

5. The use of any one or Claims 1-4, wherein the CD96-inhibitory agent at least partly blocks or inhibits CD96 binding to CD 155 and/or intracellular signalling by CD96.

6. The use of Claim 5, wherein the CD96-inhibkory agent is an anti-CD96 antibody or antibody fragment.

7. The use of any preceding claim, wherein the medicament includes one or more other therapeutic agents to be administered.

8. The use of Claim 7, wherein the one or more other therapeutic agents include a chemotherapeutic agent and one or more antibodies or antibody fragments that bind PD1 and/or CTLA4.

9. The use of any preceding claim, wherein the medicament is capable of increasing or enhancing cytokine and/or chemokine expression and/or secretion by one or more cells in the mammal.

10. The use of Claim 9, wherein the cytokine and/or chemokines include MIP-1α, MIP-1β, RANTES, TNF-α and IFN-γ.

11. The use of Claim 10, wherein the cytokine is interferon γ (IFN-γ).

12. The use of Claim 9, Claim 10 or Claim 11, wherein the one or more cells are T cells, inclusive of $CD4^+$ and $CD8^+$ T cells, γδ T cells and NK T cells and natural killer (NK.) cells.

13. The use of any preceding claim, wherein the medicament is capable of treating or preventing cancer or cancer metastasis in the mammal.

14. The use of any preceding claim, wherein the medicament is capable of treating or preventing viral infection in the mammal.

15. The use of any preceding claim, wherein the mammal is a human.

16. A method of screening, designing, engineering or otherwise producing a CD96-inhibitory agent, said method including the step of determining whether a candidate molecule is capable of at least partly inhibiting or reducing CD96 activity to thereby relieve immune inhibition and/or enhance or restore immune surveillance in a mammal, wherein the CD96-inhibitory agent binds or interacts with one or a plurality of external immunoglobulin-like domains of CD96 and wherein at least partly inhibiting or reducing CD96 activity in the mammal does not include, or at least depend upon killing of CD96-expressing cells in the mammal.

17. The method of Claim 16, wherein the CD96-inhibitory agent binds or interacts with one or a plurality of external immunoglobulin-like domains of CD96 selected from the group consisting of; domain 1; domain 2; domain 3; domain 1 and domain 2; domain 1 and domain 3: domain 2 and domain 3; and domain 1, domain 2 and domain 3.

18. The method of Claim 16 or Claim 17 wherein the CD96-inhibitory agent binds or interacts with one or a plurality of external immunoglobulin-like domains of human CD96 isoform 2 (SEQ ID NO:2).

19. The method of any one of Claims 16-18, wherein the CD96-inhibitory agent is an antibody or antibody fragment.

20. The method of any one of Claims 16-18, wherein the CD96-inhibitory agent is an anti-cancer agent.

21. The method of any one of Claims 16-18, wherein the CD96-inhibitory agent is an anti-viral agent.

22. The method of any one of Claims 16-21, wherein the mammal is a human.