

studied ligand of the lipid-binding CD1d in NKT cell activation in vitro and in vivo. Other ligands comprise isoglobotrihexosylceramide, (microbial-derived) glycuronosylceramides, alpha-C-galactosylceramides, threitol ceramide, and a variety of (human and non-human) glycolipids such as lysophosphatidylcholine and lysosphingomyelin (see, for example, Fox et al (2009) PLOS Biology 7:10:e1000228) .

[006] Important roles of NKT cells have now been demonstrated in the regulation of autoimmune, allergic, antimicrobial, and antitumor immune responses (van der Vliet et al. (2004) Clinical Immunology 112(1): 8 – 23). Physiologically, the NKT-cells can augment or inhibit immune responses, including antitumor, autoimmune, and anti-pathogen responses, through a variety of mechanisms depending on context (Yue et al. (2010) The Journal of Immunology 184: 268–276), including induction of cell death in multiple myeloma cells. Conditions in which NKT-cells may be involved include autoimmune or inflammatory diseases, including myasthenia gravis, psoriasis, ulcerative colitis, primary biliary cirrhosis, colitis, autoimmune hepatitis, atherosclerosis, and asthma. In addition to cytokine release, NKT cell effector functions which result in cell lysis, such as perforin release and granzyme release and cell death, may also be relevant in conditions in which NKT cells are implicated, such as in cancer. Modulation of CD1d-mediated effects is therefore of potential therapeutic benefit.

[007] There is an ongoing need for compounds that can bind and/or interact with CD1d as specific as possible, i.e. while minimally or not binding to other family members of the CD1-family, both in vitro and in vivo. In particular there is need for such compounds that bind and/or modulate (activate or inhibit) biological functions that involve CD1d such as, but not limited to, NKT-cell activation. Such compounds may, for example, show benefit in the various diseases in which CD1d-mediated functions play a role.

SUMMARY OF THE INVENTION

[007a] In one aspect, the present invention provides a compound comprising at least one single-domain antibody which binds to human CD1d, wherein the single-domain antibody comprises complementarity determining regions CDR1, CDR2 and CDR3, wherein CDR1 comprises an amino acid sequence that has at least 60% sequence identity with SEQ ID NO: 22, CDR2 comprises an amino acid sequence that has at least 60% sequence identity with SEQ ID NO: 43 and CDR3 comprises the amino acid sequence set forth in SEQ ID NO: 75 or a conservative sequence variant of SEQ ID NO: 75.

[007b] In another aspect, the present invention provides a compound comprising at least one single-domain antibody which binds to human CD1d, wherein the single-domain antibody has complementarity determining regions CDR1, CDR2 and CDR3 as set forth in SEQ ID

NO: 33, SEQ ID NO: 54 and SEQ ID NO: 75, respectively, or conservative sequence variants thereof.

[007c] In yet another aspect, the present invention provides a compound comprising at least one single-domain antibody which binds to human CD1d, wherein the single-domain
5 antibody has complementarity determining regions CDR1, CDR2 and CDR3 as set forth in SEQ ID NO: 33, SEQ ID NO: 54 and SEQ ID NO: 75, respectively.

[007d] In yet another aspect, the present invention provides use of a compound disclosed herein, wherein the compound is used *in vitro* or wherein the compound is used in an *in vitro* diagnostic method.

10 [007e] In yet another aspect, the present invention provides pharmaceutical composition comprising a compound disclosed herein.

[007f] In yet another aspect, the present invention provides a nucleotide sequence that encodes a compound disclosed herein.

[007g] In yet another aspect, the present invention provides a host cell or non-human
15 organism comprising a nucleotide sequence disclosed herein.

[007h] In yet another aspect, the present invention provides a method for preparing a compound disclosed herein, wherein the method comprises allowing a host cell disclosed herein to express the compound or part thereof; and obtaining the compound.

[007i] In yet another aspect, the present invention provides an antibody that comprises a CDR1, CDR2 and CDR3, wherein the CDR1 has the amino acid sequence set forth in SEQ ID NO: 33, or conservative sequence variants thereof, the CDR2 has the amino acid sequence set forth in SEQ ID NO: 54, or conservative sequence variants thereof, and the CDR3 has the amino acid sequence set forth in SEQ ID NO: 75, or conservative sequence variants thereof, preferably
20 wherein CDR1, CDR2 and CDR3 have an amino acid sequence that has at least 80%, 90%, 95% or 100% amino acid sequence identity to the amino acid sequence of respectively CDR1, CDR2 and CDR3 as shown for VHH nr 12 in Table 1.
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[008] The present disclosure provides a compound comprising at least one single-domain antibody. The single-domain antibody binds to human CD1d. The single-domain antibody that binds to human CD1d comprises a CDR1, CDR2 and CDR3 region with an amino acid
30 sequence as disclosed herein, and conservative sequence variants thereof.

[009] Preferably the single-domain antibody has a CDR1, CDR2 and CDR3 region in the combination as disclosed herein, for example as shown in Table 1.

[010] Even more preferably, the single domain antibody has an amino acid sequence selected from the group of SEQ ID NO: 1 – SEQ ID NO: 21.

35 [011] The compound according to the disclosure may be any kind of compound, for example a complex, as long as the single-domain antibody that binds to human CD1d is comprised in the compound. Preferably the compound is a polypeptide. In certain examples the

compound may consist of only the single-domain antibody that binds to human CD1d. In other examples the compounds consists of the single-domain antibody that binds to human CD1d and a label. In even further examples the compound may comprise the single-domain antibody that binds to human CD1d linked to a pharmaceutical active agent and/or other antibodies.

[012] Also provided is use of the compound according to the disclosure in medical treatment and/or as a diagnostic agent.

[013] Also provided is a pharmaceutical composition that comprises a compound as disclosed herein and nucleotide sequences and host cells comprising such nucleotide sequences that encode for the compounds according to the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[014] Figure 1: CD1d-specificity of individual selected nanobodies. Flow-cytometry was used to detect binding of isotype control mAb (IgG2b), anti-CD1d 51.1 mAb, R2 negative control VHH, and individual CD1d-specific VHH. Data demonstrate binding to CD1a, CD1b, CD1c, and CD1d transfected tumor cell lines (n=3).

[015] Figure 2: Induction of moDC maturation and cytokine production by CD1d-specific nanobodies. Immature moDC were cultured with CD1d-specific nanobodies. After 24 hours, supernatants were harvested for detection of cytokine production (ELISA). After 72h, moDC were analyzed for cell surface expression of the maturation marker CD83 using flow cytometry. NC=negative control, PC=positive control, IgG2b=isotype control mAb, 51.1= anti-CD1d 51.1 mAb, LPS=lipopolysaccharide, LPS-PMB= lipo-polysaccharide with polymyxin B. Data represent mean + SEM, n=3.

[016] Figure 3: Inhibition of α -GalCer induced iNKT cell activation. CD1d-transfected HeLa cells were pulsed overnight with vehicle control (veh) or α -GalCer (all other conditions). After washing, vehicle or α -GalCer pulsed HeLa-CD1d were cultured for 2 hours with IgG2b isotype control mAb, anti-CD1d 51.1 mAb, negative control VHH R2, or a neutralizing anti-CD1d VHH (VHH 24 (18-29c)) after which time iNKT cells were added. After 24 hr iNKT cell activation (CD25 expression) was determined using flow cytometry. Data indicate mean + SEM of 3 experiments. Superior neutralization of iNKT cell activation by anti-CD1d VHH.

[017] Figure 4: Induction of iNKT cell activation. CD1d-transfected C1R cells were pulsed overnight with vehicle control (veh) or α -GalCer as indicated. After washing, vehicle or α -GalCer pulsed C1R-CD1d were cultured for 2 hours with or without a specific anti-CD1d VHH after which iNKT cells were added. After 24 hr iNKT cell activation (CD25 expression) was determined using flow cytometry. Representative flow cytometric dotplots demonstrating activation of iNKT cells by α -GalCer, but more strikingly after co-culture with

CLAIMS

1. A compound comprising at least one single-domain antibody which binds to human CD1d, wherein the single-domain antibody comprises complementarity determining regions CDR1, CDR2 and CDR3, wherein CDR1 comprises an amino acid sequence that has at least 60% sequence identity with SEQ ID NO: 22, CDR2 comprises an amino acid sequence that has at least 60% sequence identity with SEQ ID NO: 43 and CDR3 comprises the amino acid sequence set forth in SEQ ID NO: 75 or a conservative sequence variant of SEQ ID NO: 75.
2. A compound comprising at least one single-domain antibody which binds to human CD1d, wherein the single-domain antibody has complementarity determining regions CDR1, CDR2 and CDR3 as set forth in SEQ ID NO: 33, SEQ ID NO: 54 and SEQ ID NO: 75, respectively, or conservative sequence variants thereof.
3. A compound comprising at least one single-domain antibody which binds to human CD1d, wherein the single-domain antibody has complementarity determining regions CDR1, CDR2 and CDR3 as set forth in SEQ ID NO: 33, SEQ ID NO: 54 and SEQ ID NO: 75, respectively.
4. A compound according to any of the previous claims wherein the compound comprises the amino acid sequence set forth in SEQ ID NO: 12, or a conservative sequence variant thereof.
5. A compound according to any of the previous claims wherein the compound comprises further single domain antibodies.
6. A compound according to any of the previous claims wherein the compound is a bispecific or multispecific compound.
7. A compound according to any of the previous claims, wherein the compound comprises a label, wherein an pharmaceutical active agent is linked to the compound, wherein the single-domain antibody is humanized, wherein the compound is a bivalent or multivalent compound, wherein the compound is fused to an antigen, a peptide or a nucleotide sequence, wherein the compound is a liposome, wherein the compound is a virus, and/or wherein the compound is a nanoparticle.

8. A compound according to any of the previous claims wherein the single domain antibody, and preferably the compound, binds to human CD1d but not to human CD1a, human CD1b and/or human CD1c.

5 9. A compound according to any of the previous claims, wherein
the compound is capable of inducing activation of CD1d-restricted T cells,
including invariant natural killer T-cells, and/or stimulating glycolipid-induced, for
example alpha-galactosyl ceramide-induced, activation of CD1d-restricted T
cells, including invariant natural killer T-cells.

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10. A compound according to any of the previous claims wherein the compound is a single
domain antibody, and wherein the compound is a single domain antibody that has
complementarity determining regions CDR1, CDR2 and CDR3 as set forth in SEQ ID NO:
33, SEQ ID NO: 54 and SEQ ID NO: 75, respectively, or conservative sequence variants
15 thereof, or wherein the single domain antibody has the amino acid sequence set forth in
SEQ ID NO: 12, or conservative sequence variants thereof.

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11. A compound according to any of the previous claims wherein the single domain antibody
is a humanized single domain antibody, preferably wherein the compound is a humanized
20 single domain antibody.

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12. A compound according to any of the previous claims comprising an antibody, preferably a
single-domain antibody which binds to human CD1d, wherein the antibody, preferably
single-domain antibody, comprises complementarity determining regions CDR1, CDR2
25 and CDR3 wherein CDR1, CDR2 and CDR3 have an amino acid sequence that has at
least 80%, 90%, 95% or 100% amino acid sequence identity to the amino acid sequence
of respectively CDR1, CDR2 and CDR3 as shows for VHH nr 12 as shown in Table 1.

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13. A compound according to any of the previous claims for use in medical treatment, or for *in*
30 *vivo* use as a diagnostic agent.

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14. Use of a compound according to any of the previous claims, wherein the compound is
used *in vitro* of wherein the compound is used in an *in vitro* diagnostic method.

35 15. Pharmaceutical composition comprising a compound according to any of the claims 1 –
12.

16. A nucleotide sequence that encodes a compound according to any of the claims 1 - 12.

17. A nucleotide sequence according to claim 14 that encodes for a compound comprising the amino acid sequence set forth in SEQ ID NO: 33, or conservative sequence variants thereof, and the amino acid sequence set forth in SEQ ID NO: 54, or conservative sequence variants thereof, and the amino acid sequence set forth in SEQ ID NO: 75, or conservative sequence variants thereof.

18. A host cell or non-human organism comprising a nucleotide sequence according any of claim 16 - 17.

19. The host cell according to claim 18 that produces a compound or part thereof according to any one of claims 1 - 12.

20. A method for preparing a compound according to any of the claims 1 - 12, wherein the method comprises allowing a host cell according to claim 18 to express the compound or part thereof; and obtaining the compound.

21. An antibody that comprises a CDR1, CDR2 and CDR3, wherein the CDR1 has the amino acid sequence set forth in SEQ ID NO: 33, or conservative sequence variants thereof, the CDR2 has the amino acid sequence set forth in SEQ ID NO: 54, or conservative sequence variants thereof, and the CDR3 has the amino acid sequence set forth in SEQ ID NO: 75, or conservative sequence variants thereof, preferably wherein CDR1, CDR2 and CDR3 have an amino acid sequence that has at least 80%, 90%, 95% or 100% amino acid sequence identity to the amino acid sequence of respectively CDR1, CDR2 and CDR3 as shown for VHH nr 12 in Table 1.