

Claims

1. A nanobody which binds to human CD4, the nanobody comprising
 - a) the amino acid sequences (i) SGFTFSKL (SEQ ID NO: 1) as CDR1, (ii) IDSSGDTTDYLA (SEQ ID NO: 2) as CDR2 and (iii) REDPPG (SEQ ID NO: 3) as CDR3; or
 - b) the amino acid sequences (i) SGFDVDYY (SEQ ID NO: 4) as CDR1, (ii) IASSDGSTYYAD (SEQ ID NO: 5) as CDR2 and (iii) DA-TCPYYCSGSVCYLETGMD (SEQ ID NO: 6) as CDR3; or
 - c) the amino acid sequences (i) SGFALEY (SEQ ID NO: 7) as CDR1, (ii) MSASGGVINYSE (SEQ ID NO: 8) as CDR2 and (iii) EKAYYGSSWAECYLMMD (SEQ ID NO: 9) as CDR3.
2. The nanobody of claim 1, wherein the nanobody binds to domain D1 or to domain D3 of human CD4.
3. The nanobody of claim 1 or 2, comprising four framework regions (FR1 to FR4) and three complementarity determining regions (CDR1 to CDR3), the three complementarity determining regions consisting of one of the amino acid sequences a) to c),

wherein preferably, the nanobody comprises an amino acid sequence selected from the group consisting of the amino acid sequences SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, SEQ ID No. 156, SEQ ID No. 157, or an amino acid sequence that has at least 90% sequence homology with one of the amino acid sequences SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, SEQ ID No. 156 or SEQ ID No. 157.

4. The nanobody of any of claims 1 to 3, wherein the nanobody is associated with at least one of a detectable label, a viral particle, and/or a therapeutically or pharmacologically active agent.
5. The nanobody of claim 4, wherein the detectable label is selected from detectable moieties and tracers for immuno-histochemistry, optical imaging, near infrared imaging (NIR), positron emission tomography (PET), single photon emission computed tomography (SPECT) or magnetic resonance imaging (MRI), and preferably which detectable label is selected from fluorophores, radionuclides or magnetic particles.
6. The nanobody of claim 4, wherein the viral particle is an Adenovirus-associated Virus (AAV) particle, an Adenovirus particle, or an lentiviral particle, and wherein preferably the AAV particle is selected from an AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, or an AAV9 particle.
7. The nanobody of claim 4, wherein the therapeutically active agent is an active gene transfer agent.
8. A nucleic acid comprising or consisting of a nucleic acid sequence coding for the nanobody as claimed in any of claims 1 to 7, optionally linked to another nucleic acid sequence.
9. The nanobody of any of claims 1 to 6, or the nucleic acid of claim 8, for use in diagnostic or prognostic methods, or for use as medicament, preferably for modulating CD4⁺ T cell function and/or cell depletion.
10. The nanobody of any of claims 1 to 7 for use as contrast agent in non-invasive medical imaging *in vivo*.
11. The nanobody of any of claims 1 to 7 for the *in vitro* detection of CD4 in a sample.

12. The nanobody of any of claims 1 to 7, or the nucleic acid as claimed in claim 8, for use in retargeting AAV, Adenovirus or lentiviral vectors or for retargeting gene transfer vectors, or for use in targeting virus-like particles (VLP) or lipid nanoparticles (LNP).
13. The nanobody of any of claims 1 to 7, or the nucleic acid as claimed in claim 8, wherein the nanobody or a nucleic acid coding for the nanobody is used for genetic labelling of AAV capsid proteins, preferably for genetic labeling of the AAC capsid proteins VP1 and/or VP2.
14. A biparatopic, bivalent or trivalent construct comprising at least one nanobody as claimed in any of claims 1 to 7 or a combination thereof.
15. A method for determining and/or imaging and/or monitoring the presence and/or amount and/or activity or differentiation state of CD4⁺ cells in a biological sample suspected of comprising CD4⁺ cells, preferably CD4⁺ T cells, the method comprising the steps of:
 - a) combining said sample with at least one of the nanobodies of any of claims 1 to 7, or with at least one construct of claim 14, and optionally with at least one second compound conjugated with a detectable label, the second compound binding to another marker of the CD4⁺ cells, preferably CD4⁺ T cells,
 - b) measuring, via the detectable label, imaging signals from said nanobody that has bound to CD4⁺ cells, preferably CD4⁺ T cells, in said sample, and, if a second compound has additionally been used in step a), from said second compound that has bound to another marker; and
 - c) imaging, determining and/or monitoring the presence and/or amount and/or activity of CD4⁺ cells, preferably CD4⁺ T cells, in said sample via the imaging signals,

wherein the determination and/or monitoring and/or imaging is preferably for patient stratification and for monitoring individual immune responses during personalized immunotherapy.

16. Use of at least one of the nanobodies of any of claims 1 to 7, or at least one construct of claim 14, as a diagnostic agent, preferably wherein the diagnostic agent is used in a diagnosis which is selected from diagnostic classification of a disease-associated immune status of a patient, and susceptibility to immunotherapies of a patient.
17. A method for diagnosing and/or monitoring the immune status and/or susceptibility to immunotherapies of a patient, wherein at least one nanobody as claimed in any of claims 1 to 7, or at least one construct as claimed in claim 14, is used to assess and/or monitor CD4⁺ T cells in a biological sample of the patient, wherein the nanobody is used alone or in combination with a second compound binding to a T-cell specific marker, wherein the T cell-specific marker is selected from at least one of CD2, CD3, CD7, CD8, CD27, CD28, CD127, HLA-DR, CD38, CD69, CCR4, CCR5, CCR6, CCR7, CTLA-4, LAG3, TIM-3, OX40, ICOS, CXCR4, PD-1, PD-L1, PD-L2, CD40L (synonym CD154), CD122, CD137, GITR, CD25, CD278, SIGLEC-7, SIGLEC-9, BTLA (synonym: CD272), TIGIT, VISTA, B7-H4 (synonym: VTCN1), CD276 (synonym: B7-H3), A2AR, CEACAM1, LAIR-3, HVEM, CD160, CD200, CD200R.
18. A pharmaceutical composition comprising at least one of the nanobodies as claimed in any of claims 1 to 7 and/or at least one construct as claimed in claim 14, in association with a pharmaceutically acceptable carrier or excipient.