SLC4A4/NBCe1 INHIBITORS

FIELD OF THE INVENTION

The disclosure relates to the compounds selectively inhibiting Solute Carrier Family 4 member 4/ Electrogenic Sodium Bicarbonate Cotransporter 1 (SLC4A4/NBCe1). More in particular, said compounds are SLC4A4/NBCe1-inhibiting single domain antibodies, compounds comprising at least one CDR polypeptide of a SLC4A4/NBCe1-inhibiting single domain antibody, and compounds comprising a SLC4A4/NBCe1-inhibiting single domain antibody. Applications of compounds inhibiting SLC4A4/NBCe1 are likewise included.

BACKGROUND OF THE INVENTION

Solute carrier proteins (SLCs) form a very diverse group of membrane transporters with over 400 members classified in 65 families.

One group of SLCs are the bicarbonate transporters which can be subdivided further according to whether an individual bicarbonate transporter is sodium independent or sodium driven, and according to whether an individual bicarbonate transporter is an acid loader, an acid extruder, or whether its acidification effect is variable or unclear. SLC4A4 is one of the several sodium driven, acid extruding bicarbonate transporters (others including SLC4A6, SLC4A7, SLC4A8, SLC4A9 and SLC4A10) (Figure 1A of McIntyre et al. 2015, Cancer Res 76:3744-3755). SLCs are reviewed in e.g. Parker & Boron 2013 (Physiol Rev 93:803-959).

SLC4A4 (Solute Carrier Family 4 member 4, also known as Electrogenic Sodium Bicarbonate Cotransporter 1 or NBCe1; US6096517) is protecting cells against intracellular acidosis (low intracellular pH, pHi). S0859 was developed as inhibitor of Na-driven bicarbonate transporters, but is not specific (Heidtmann et al. 2015, Eur J Pharmacol 762:344-349). DIDS (4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid) is another non-specific inhibitor. Polyclonal antibody preparations (Abs; including inhibitory and excitatory IgG preps) have been described (De Giusti et al. 2011, Br J Pharmacol 164:1976-1989; Khandoudi et al. 2001, Cardiovasc Res 52:387-396). More in particular, the polyclonal Abs against the putative extracellular loop domains 3 (EL3) and 4 (EL4) of human NBCe1 were raised with the polyclonal antibodies to EL3 being inhibitory and the polyclonal antibody to EL4 being excitatory (De Giusti et al. 2011, Br J Pharmacol 164:1976-1989). The cryoEM structure of hSLC4A4 has been determined (Huynh et al. 2018, Nat Commun 9:900).

shRNA knockdown (kd) of SLC4A4 in cancer cell lines (MDA-MB-231 breast cancer, expressing high levels of SLC4A4) resulted in strong impact on cell proliferation, migration and invasion (Parks & Pouyssegur 2015, J Cell Physiol 230:1954-1963). SLC4A4 was suggested as one of four prognostic

markers/therapeutic targets of colorectal cancer (Bian et al. 2019, Oncol Lett 18:5043-5054). SLC4A4 kd and SLC4A9 kd interfere with spheroid growth of LS174 (colorectal cancer cells) and knockdown of SLC4A9 dramatically reduces tumor xenograft formation (McIntyre et al 2016 – Cancer Res 76:3744-3755). Genetic disruption of NBCn1 (SLC4A7) delayed breast cancer development: tumor latency was ~ 50% increased while tumor growth rate was ~ 65% reduced in NBCn1 KO compared with wild-type (WT) mice. Breast cancer histopathology in NBCn1 KO mice differed from that in WT mice and included less aggressive tumor types (Lee et al. 2016 – Oncogene 35:2112-2122). In addition, Cappellesso et al. 2022 (Nature Cancer 3:1464-1483) and WO2022175392A1 reported tumor growth regression upon SLC4A4/NBCe1 inhibition and anti-tumor synergism between SLC4A4/NBCe1 inhibition in tumor cells (CRISPR knock-out) and different immune checkpoint inhibitors in multiple tumor models.

Inhibition of SLC4A4/NBCe1was reported as potential novel therapeutic approach for treating cardiac ischemia, ischemic heart disease (Khandoudi et al. 2001, Cardiovasc Res 52:387-396; De Giusti et al. 2011, Br J Pharmacol 164:1976), for treating reperfusion injury (Fantinelli et al. 2014, Cardiovascular Pathol 23:224-230), as well as for treating diabetes mellitus (Brown et al. 2021, J Clin Invest 131:e142365).

What remains lacking are potent and specific inhibitors of SLC4A4/NBCe1.

SUMMARY OF THE INVENTION

The current disclosure relates to compounds specifically inhibiting Electrogenic Sodium Bicarbonate Cotransporter 1 (NBCe1)/solute carrier family member 4A4 (SLC4A4), wherein the compounds are binding to and inhibiting the activity of human NBCe1-B, are binding to human NBCe1-B and human NBCe1-B(Δ2-85) expressed by a wild-type cell, and are not binding to amino acids 629-644 of human NBCe1-B.

The current disclosure further relates to compounds specifically inhibiting NBCe1/SLC4A4, wherein the compounds are selected from NBCe1/SLC4A4-inhibiting immunoglobulin single variable domain antibody (ISVD) polypeptide, compounds comprising at least one CDR polypeptide of a NBCe1/SLC4A4-inhibiting ISVD polypeptide, and compounds comprising a NBCe1/SLC4A4-inhibiting ISVD polypeptide, wherein the ISVD polypeptide is chosen from the ISVD polypeptides defined by SEQ ID NOs:14-22, 135, and 138-147. In one embodiment thereto, the at least one CDR polypeptide is chosen from the CDR1 polypeptides defined by SEQ ID NOs: 23-31, from the CDR2 polypeptides defined by SEQ ID NOs: 32-44 and 153-155, and/or from the CDR3 polypeptides defined by SEQ ID NOs: 45-55, 136-137, and 148-152. In a further embodiment thereto, such compounds are inhibiting human NBCe-1B, inhibiting NBCe1-B(Δ2-85), and are not inhibiting human NBC1e-A. More in particular, such compounds are binding to extracellular loop 3 of human NBCe1-B exposed by a cell and are not binding to amino acids 629-644 of

human NBCe1-B. Furthermore in particular, such compounds are binding to human NBCe-1B and to human NBCe-1B(D2-85) expressed or exposed by a cell, and optionally binding to human NBC1e-A expressed or exposed by such cell. Such compounds are not competing for binding to human NBCe1-B with a polyclonal antibody raised against a peptide spanning amino acids 609-685 of human NBCe1-B.

More in particular, such compounds are comprising an ISVD polypeptide chosen from the ISVD polypeptides defined by SEQ ID NOs:19-21, 138, and 144-147, or comprising at least one CDR polypeptide chosen from the CDR1 polypeptide defined by SEQ ID NO: 23, from the CDR2 polypeptides defined by SEQ ID Nos: 32-38, and 153-155, and/or from the CDR3 polypeptides defined by SEQ ID Nos: 45-50, 148-149, and 152.

In another embodiment, such compounds are inhibiting human NBCe-1B, inhibiting NBCe1-B(Δ2-85), and inhibiting human NBC1e-A. More in particular, such compounds are binding to amino acid glutamate 775 in extracellular loop 4 (amino acid position 775 of human NBCe-1B) of human NBCe1-B exposed by a cell. More in particular, such compounds are binding to extracellular loop 2 and extracellular loop 4 of human NBCe1 exposed by a cell. Furthermore in particular, such compounds are comprising an ISVD polypeptide chosen from the ISVD polypeptides defined by SEQ ID NOs:14-18, 135, and 139-143, or comprising at least one CDR polypeptide chosen from the CDR1 polypeptide defined by SEQ ID Nos: 24-31, from the CDR2 polypeptides defined by SEQ ID Nos: 39-44, and/or from the CDR3 polypeptides defined by SEQ ID Nos: 51-55, 136-137, and 150-151.

The current disclosure further relates to isolated nucleic acids encoding a CDR polypeptide or ISVD polypeptide as described hereinabove; and to vectors including such a nucleic acid .

The current disclosure further relates to host cells expressing a CDR polypeptide or ISVD polypeptide as described hereinabove, to host cells comprising a nucleic acid as described hereinabove, and to host cells comprising a vector as described hereinabove.

The current disclosure further relates to compounds specifically inhibiting NBCe1/SLC4A4 as described hereinabove, to isolated nucleic acid as described hereinabove, to vectors as described hereinabove, and to host cells as described hereinabove for use as a medicament; more in particular for use in treating or inhibiting a disease or disorder, or for use in inhibiting progression or relapse of a disease or disorder, wherein the disease or disorder is cancer, diabetes, ischemic heart disease, cardiac ischemia, or reperfusion injury.

The current disclosure further relates to pharmaceutical compositions comprising a compound specifically inhibiting NBCe1/SLC4A4 as described hereinabove, an isolated nucleic acid as described hereinabove, a vector as described hereinabove, and/or a host cell as described hereinabove.

The current disclosure further relates to methods for producing a compound specifically inhibiting NBCe1/SLC4A4, such compound comprising a NBCe1/SLC4A4-inhibiting immunoglobulin single variable domain antibody (ISVD) polypeptide, or at least one CDR polypeptide of a NBCe1/SLC4A4-inhibiting ISVD polypeptide, the methods comprising:

- expressing a CDR polypeptide or ISVD polypeptide as described hereinabove in a host cell as described hereinabove, or synthetic manufacture of the polypeptide;
- purifying the expressed or manufactured CDR polypeptide or ISVD polypeptide.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1. A. Human SLC4A4/NBCe1-A to -E isoforms. "NTDv": variable part of N-terminal domain ("1": auto-stimulatory domain; "2": auto-inhibitory domain). "NTDcore": core part of N-terminal domain. "TMD": transmembrane domain. "CTD": C-terminal domain. **B.** Alignment of human NBCe1-B ("h-B"), murine NBCe1-B ("m-B") and human NBCe1-A ("h-A") isoform amino acid sequences, with, boxed, indication of extracellular loops 2, 3, and 4 ("EL2",""EL3", "EL4"). Arrow indicates amino acid glutamate (human NBCe1) or glycine (murine NBCe1) at position 775 relative to the NBCe1-B sequences, this amino acid position being the sole difference between human EL4 and murine EL4.

FIGURE 2. Principle of pHi measurements in acid shock recovery assay with subsequent data normalization. "NaAc": sodium acetate.

FIGURE 3. Dose-dependent binding, expressed as mean fluorescence intensity (MFI), of selected SLC4A4/NBCe1-blocking VHHs (VHHs P01400040, P01400023 and P01400014) and an irrelevant VHH (IRR00004) on SLC4A4 endogenously expressed in different human and mouse adenocarcinoma cell lines: pancreatic cancer (PDA) cell lines SUIT-2 and CFPAC-1; prostate cancer (PC) cell lines PC-3 and LNCaP; and triple negative breast cancer (TNBC) cell line MDA-MB-231. ND: not determined.

FIGURE 4. Binding, expressed as mean fluorescence intensity (MFI), of selected SLC4A4/NBCe1-blocking VHHs (VHHs P01400014, P01400020, P01400023, P01400024 and P01400040) on NBCe1-B ("pancreas") and NBCe1-A ("kidney") overexpressing HEK293 Flp-In T-Rex cell lines.

FIGURE 5. Dose-dependent inhibition, expressed as normalized AUC, by selected SLC4A4/NBCe1-blocking VHHs (VHHs P01400014, P01400020, P01400023, P01400024 and P01400040) of the activity of human NBCe1-B ("pancreas") and human NBCe1-A ("kidney") isoform of SLC4A4.

FIGURE 6. Dose-dependent binding, expressed as mean fluorescence intensity (MFI), of selected SLC4A4/NBCe1-blocking VHHs (VHHs P01400040 and P01400014) and an irrelevant VHH (IRR00004) to NBCe1-B, NBCe1-B(Δ2-85) and NBCe1-A overexpressing HEK293 Flp-In T-Rex cell lines.

FIGURE 7. Flow-cytometric determination of binding (expressed as normalized MFI) of human-mouse SCL4A4 chimeras to selected SLC4A4/NBCe1-blocking VHHs (VHHs P01400014, P01400020, P01400023,

P01400024 and P01400040)."human NCB1e + mELx": human NCB1e-B in which extracellular loop x (Elx) is replaced by the corresponding Elx of mouse NCB1e-B (mELx).

FIGURE 8. Flow-cytometric determination of binding (expressed as normalized MFI) of mouse-human SCL4A4 chimeras to selected SLC4A4/NBCe1-blocking VHHs (VHHs P01400014, P01400020, P01400023, P01400024 and P01400040). "mouse NCB1e + mELx": mouse NCB1e-B in which extracellular loop x (Elx) is replaced by the corresponding Elx of human NCB1e-B (hELx).

FIGURE 9. Cryo-EM model of VHH P01400040 (A) and P01400014 (B) in complex with dimeric NBCe1-B. VHHs are located in the circled areas.

FIGURE 10. Inhibition of human NBCe1-B activity by VHHs, expressed as normalized AUC. (A) monovalent P01400014 and bivalent P01400014 (=P01400082); (B) monovalent P01400020 and bivalent P01400020 (=P01400081); (C) monovalent P01400023 and bivalent P01400023 (=P01400083). "IRR0011" or "IRR11": irrelevant VHH.

FIGURE 11. Inhibition of human NBCe1-B activity (A; , expressed as normalized AUC) or mouse NBCe1-B activity (B; expressed as difference between AUC values determined at 80 and 60 minutes after start of the assay, see Figure 2) by VHHs: VHH P01400086 (biparatopic combination of P01400014- P01400040) and VHH P01400073 (biparatopic combination of P01400040- P01400014; reverse order compared to P01400086). "IRR0011": irrelevant VHH.

FIGURE 12. In vivo treatment of orthotopic pancreatic cancer (KPC mouse model). VHH P01400113 (a bivalent half-life extended version of mouse x-reactive P01400040) was compared to IRR00002 (a matching irrelevant control VHH) in the treatment in an orthotopic PDAC KPC mouse model. The treatment with P01400113 resulted in significant reductions of both primary tumor weight (A), as well as number of mesenteric metastasis (B).

FIGURE 13. Alignment of the amino acid sequences of VHHs P01400023 ("23"), P01400014 ("14"), P01400012 ("12"), P01400020 ("20"), P01400021 ("21"), P01400024 ("24"), P01400040 ("40"), P01400041 ("41") and P01400046 ("46"), with indication of the framework regions (FRs) surrounding the complementarity determining regions (CDRs, boxed). CDR's are as defined by Kabat.

FIGURE 14. Amino acid sequences of the CDR regions of VHHs P01400014, P01400020, P01400023, P01400040 as determined by Kabat ("CDR# Kabat"), by IMGT ("CDR# IMGT"), and by AbM ("CDR AbM"), wherein # is 1, 2, or 3.

FIGURE 15. Inhibition of human NBCe1-B by the indicated VHHs, expressed as normalized AUC.

FIGURE 16. Inhibition of NBCe1-B by the multivalent VHHs, expressed as normalized AUC. **(A)** Inhibition of human NBCe1-B by monovalent VHHs P01400014 and P01400040; by P01400073: bivalent/biparatopic, fusion of P01400040 and P01400014; by P01400086: bivalent/biparatopic, fusion of P01400014 and P01400040; by P01400105: bivalent, fusion of 2 P01400040 moieties. **(B)** Inhibition of

human NBCe1-B (filled circles) and of murine NBCe1-B (filled squares) by monovalent irrelevant VHH IRR00010 and monovalent VHH P01400040, and by bivalent P01400118 being an Fc-moiety fused with 2 P01400040 moieties (one per Fc-arm). **(C)** Inhibition of human NBCe1-A (kidney isoform) and human NBCe1-B (pancreatic isoform) by monovalent irrelevant VHH IRR00004, monovalent VHH P01400014, and monovalent VHH P01400040; by trivalent/triparatopic P01400109 (P01400040 + P01400014 + half-life extension VHH); and by trivalent/biparatopic P01400113 (P01400040 + P01400040 + half-life extension VHH).

FIGURE 17. Interaction of VHH P01400040 with EL3 of human NBCe1B. Indicated is an overview (A) of VHH P01400040 ("Nb40"; black, boxed, on top) relative to the stretch of NBCe1B amino acids C629 to D633 (the well resolved residues in the map of the stretch C629-T644 of hNBCe1B; lower left part of NBCe1B). In (B) a close up view of the stretch 629-633 of hNBCe1B ("hSLC4A4"; grey, upper left) and the VHH ("Nb40"; black, lower right) is depicted with indication of distances in support of a lack of true interaction. These data support a coincidental proximity of VHH P01400040 and the hSLC4A4 stretch 629-633 without true interactions as part of a paratope/epitope interface. (C) hSLC4A4 amino acid residues 634-644 are part of a disordered loop that turns away from VHH P01400040 ("VHH") towards the second VHH P01400040 ("VHH' ") to connect with Ser654. ECL3 and ECL3' are the two extracellular loops 3 of the 2 hSLC4A4 monomers complexed in a dimer.

FIGURE 18. HEK cells induced to express human NBCe1-B ("Flp-In T-Rex human NBCe1-B"), human NBCe1-B(Δ2-85) ("Flp-In T-Rex human NBCe1-B(D2-85)"), and human NBCe1-A ("Flp-In T-Rex human NBCe1-A") were pre-incubated for 30 minutes with different concentrations of VHH P01400040 (comprising HIS6-tag), unlabeled polyclonal antibody NBP2-32021 ("anti-EL3"), and with an unlabeled irrelevant VHH ("anti-Hint1", ProteinTech, 10717-1-AP). Subsequently, cells were incubated for 30 minutes with P01400040 (carrying a FLAG3-HIS6 tag), after which binding of P01400040 (with FLAG3-HIS6 tag) to the cells was detected using anti-FLAG antibody (Sigma, F1804) in flow cytometry (FACS analysis). Binding is expressed as mean fluorescence intensity (MFI).

FIGURE 19. Blocking of mouse NBCe1-B activity expressed in AUC by VHH P01400040, VHH P01400195, and by P01400014, at a VHH concentration of 500 nM.

FIGURE 20. In vivo treatment of subcutaneous pancreatic cancer (Pan3 402 mouse model) as outlined in Example 14. "P01400113": a bivalent half-life extended version of mouse x-reactive SLC4A4 blocking VHH P01400040); "IRR00002": an irrelevant control VHH matching VHH P01400113; "anti-PD1": a PD-1 inhibiting antibody. Compared to the control VHH, VHH P01400113 significantly increases the anti-tumor activities of anti-PD1 and of gemcitabine. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ****: $P < 0.0001$ (Tukey's multiple comparisons test). The dosing window is detailed in Table 13B.

FIGURE 21. (A) Alignment of amino acid sequences of the human IGHV3/JH germline consensus sequence relevant to the VHH context, and of the amino acid sequences of VHHs P01400040, P01400119, P01400024, P01400046, and P01400041. (B) Alignment of amino acid sequences of the human IGHV3/JH germline consensus sequence relevant to the VHH context, and of the amino acid sequences of VHHs P01400014 and P01400131. CDR regions determined according to the Kabat method are boxed (cfr. Figures 13/14) and VHH hallmark residues are shaded. Numbering on top of the alignments is according to the IMGT method.

FIGURE 22. Binding comparison of rabbit anti-EL3 polyclonal antibodies to P01400108 on the indicated cell lines overexpressing human NBCe1-B, NBCe1-B(D2-85), NBCe1-A, mouse NBCe1-B and on HEK293T wild-type cells with endogenous expression levels of human NBCe1.

DETAILED DESCRIPTION

Different isoforms of human SLC4A4 (solute carrier family 4 member 4) or NBCe1 (Electrogenic Sodium Bicarbonate Cotransporter 1) have been identified, these are outlined in Figure 1. NBCe1-A is exclusively expressed in the proximal tubule of the kidney. Isoform NBCe1-B is more globally expressed, with high levels in the pancreas and generally the digestive tract, and male and female reproductive tissues. NBCe1-B is hereinafter generally referred to, or interchangeably referred to as the "pancreatic isoform of SLC4A4 (or NBCe1)" or, simply, as "pancreatic SLC4A4 (or NBCe1)"; and NBCe1-A is hereinafter generally referred to, or interchangeably referred to as the "kidney isoform of SLC4A4 (or NBCe1)" or, simply, as "kidney SLC4A4 (or NBCe1)". NBCe1-C is a brain-specific isoform with a C-terminal PDZ domain. The relevance of NBCe1-D and E is unclear. For simplicity, the same naming scheme is used for the corresponding mouse isoforms. Hereinafter SLC4A4 and NBCe1 are used interchangeably. Amino acid sequences of NBCe1/SLC4A4 isoforms are included in Table 15 and some are aligned in Figure 1B.

Whereas it has been reported that inhibition of SLC4A4/NBCe1 can be beneficial in the treatment of cancer, cardiac ischemia, ischemic heart disease, reperfusion injury and type 2 diabetes (WO2022175392A1; Cappellesso et al. 2022, Nature Cancer 3:1464-1483; Khandoudi et al. 2001, Cardiovasc Res 52:387-396; De Giusti et al. 2011, Br J Pharmacol 164:1976; Fantinelli et al. 2014, Cardiovascular Pathol 23:224-230; Brown et al. 2021, J Clin Invest 131:e142365), pharmacological compounds specifically, selectively or exclusively inhibiting SLC4A4/NBCe1 and suitable for entry into preclinical or clinical development at present appear to be lacking. The only SLC4A4/NBCe1-inhibiting antibodies described in the art appear to be polyclonal antibody preparations to extracellular loop 3 (EL3) of the human NBCe1-B isoform, whereas a polyclonal antibody preparation to EL4 was reported as being excitatory (Khandoudi et al. 2001, Cardiovasc Res 52:387-396; De Giusti et al. 2011, Br J Pharmacol 164:1976-1989; see also Examples 15 and 16 hereinafter).

In work leading to the current invention as explained in the Examples and Figures, a set of immunoglobulin (single) variable domain (I(S)VD) molecules, herein also referred to a single domain antibodies (sdAb) or as the variable domain of heavy chain antibodies (HCAbs) or shortly VHHs (the terms sdAb, I(S)VD and VHH are used interchangeably herein), binding with high specificity to human, murine and/or cynomolgous SLC4A4/NBCe1, or inhibiting human pancreatic SLC4A4/NBCe1 or human pancreatic and human kidney SLC4A4/NBCe1, were identified and further characterized. *In vivo* biological activity of such SLC4A4/NBCe1 inhibitors alone or in combination with a further anti-cancer agent was demonstrated in pancreatic cancer models. SLC4A4/NBCe1-inhibiting ISVDs binding to EL3 of NBCe1-B were demonstrated to bind to human NBCe1-B and human NBCe1-B(Δ2-85) expressed by a wild-type cell, are not binding to amino acids 629-644 of human NBCe1-B; and not to compete for binding to human NBCe1-B with a polyclonal antibody raised against a peptide spanning amino acids 609-685 (EL3) of human NBCe1-B. The combined characteristics of the identified SLC4A4/NBCe1-inhibiting ISVDs render them particularly well-suited for entry into preclinical or clinical development. As indicated, some preclinical experiments were already performed, the outcome of which is reported hereinafter.

Based hereon, the invention is defined in the following aspects and embodiments, and described in more detail hereafter. As the invention relates to compounds or polypeptides comprising complementarity determining regions (CDRs) and, optionally framework regions (FRs), some explanation is first provided on how such CDRs/FRs are determined. The determination of the CDR regions in an antibody/immunoglobulin sequence generally depends on the algorithm/methodology applied, such as Kabat-, Chothia-, Martin (enhanced Chothia)-, IMGT (ImMunoGeneTics information system)-, AbM-, Gelfand- or Honneger- numbering schemes. See, e.g. Kabat et al. 1979, Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, books.google.com/books?id= OpW8- ibqyvcC; www.bioinf.org.Uk/abs/index.html#kabatnum; Chothia & Lesk 1987, J Mol Biol 4:901-917; the AbM definition is a compromise between Kabat and Chothia as used by Oxford Molecular's *AbM* antibody modelling software, see also Martin and Allen 2007, Bioinformatics tools for antibody engineering, In Handbook of therapeutic antibodies vol 1, Technologies Ed. Duebel, S. (Wiley-VCH, Weinheim), pp95-118, ISBN-10: 3-527-31453-9; Therapeutic Antibody Engineering, Current and Future Advances Driving the Strongest Growth Area in the Pharmaceutical Industry, Woodhead Publishing Series in Biomedicine, 2012, chapter 3: Antibody structure–function relationships, pp. 37-56; www.imgt.org/IMGTScientificChart/Numbering/IMGTnumbering.html; Dondelinger et al. 2018, Front Immunol 9:2278).

Applying different amino acid numbering methods to the same antibody/immunoglobulin amino acid sequence may give rise to different CDR amino acid sequences wherein the differences may reside in CDR sequence length and/or –delineation within the antibody/immunoglobulin/I(S)VD sequence, see e.g. Figure 14 applying the Kabat-, IMGT- and AbM-schemes to exemplary SLC4A4/NBCe1-inhibiting ISVDs subject of the current description (a further example of applying the Kabat, MacCallum, IMGT, AbM, or Chothia numbering to antibodies unrelated to the present invention can be found in e.g. Figure 64 of WO2022175532A1). The CDRs of the SLC4A4/NBCe1-inhibiting compounds or polypeptides of the invention can therefore be described as the CDR sequences as present in the single variable domain SLC4A4/NBCe1 antibodies characterized herein, or alternatively as determined or delineated according to a well-known methodology such as according to the Kabat-, Chothia-, Martin (enhanced Chothia), IMGT-, or AbM-numbering scheme or -method (or other, *supra*). The CDR sequences defined/given in SEQ ID Nos: 23, 26-28, 30-31, 35-38, 40-41, 43-44, 47-50, 52-53, 55, 136-137 for instance, have, been delineated from the SLC4A4/NBCe1 single domain antibodies defined by SEQ ID Nos: 14-22 and 135 by means of the Kabat method (e.g. Figures 13 and 14). CDR sequences defined/given in SEQ ID Nos: 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, and 112 for instance, have, been delineated from the SLC4A4/NBCe1 single domain antibodies defined by SEQ ID Nos: 14, 15, 17, 20 by means of the IMGT method (e.g. Figure 14). CDR sequences defined/given in SEQ ID Nos: 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, and 113 for instance, have, been delineated from the SLC4A4/NBCe1 single domain antibodies defined by SEQ ID Nos: 14, 15, 17, 20 by means of the AbM method (e.g. Figure 14). Applying another method may result in CDR sequences (slightly) different from those derived with the Kabat-method as defined in SEQ ID Nos: 23, 26-28, 30-31, 35-38, 40-41, 43-44, 47-50, 52-53, 55, 136-137. The delineation of the CDRs determines the delineation of the framework regions (FRs). A canonical I(S)VD antibody comprises 4 FR regions: FR1 preceding CDR1; FR2 interspersed between CDR1 and CDR2, or following CDR1 and preceding CDR2; FR3 interspersed between CDR2 and CDR3, or following CDR2 and preceding CDR3; FR4 following CDR3; wherein the relative positioning referred to is from the amino- to carboxy-terminus of the I(S)VD antibody.

A first aspect of this disclosure relates to SLC4A4/NBCe1-blocking or -inhibiting compounds (or, interchangeably, to compounds blocking or inhibiting SLC4A4/NBCe1, compounds blocking or inhibiting SLC4A4, SLC4A4-blocking or -inhibiting compounds, compounds blocking or inhibiting NBCe1, or NBCe1-blocking or -inhibiting compounds) wherein said compounds are blocking or inhibiting the activity of human NBCe1-B (human pancreatic SLC4A4; defined by SEQ ID NO:114 in Table 15).
In one embodiment thereto, such SLC4A4/NBCe1-blocking or -inhibiting compounds are binding to and blocking or inhibiting the activity of human NBCe1-B.

In a further embodiment to the above aspect or embodiment, such SLC4A4/NBCe1-blocking or -inhibiting compounds are further binding to and/or blocking or inhibiting the activity of human NBCe1-B from which amino acids 2 to 85 are deleted (this NBCe1-B variant further referred to as NBCe1-B(D2-85) or NBCe1-B(Δ2-85), defined by SEQ ID NO:127 in Table 15).

In a further embodiment to the above aspect or embodiments, such SLC4A4/NBCe1-blocking or -inhibiting compounds are binding to binding to human NBCe-1B and to human NBCe-1B(D2-85) expressed or exposed by a cell (a suitable cell/eukaryotic cell expressing human NBCe1-B; in particular a wild-type cells such as a HEK293 cell; further in particular a living or non-fixed wild-type cell), and optionally to human NBC1e-A expressed or exposed by such cell. Furthermore in particular, although binding to EL3 of human NBCe1-B expressed by a cell, the binding is not to amino acids 629-644 of human NBCe1-B. In addition, the SLC4A4/NBCe1-blocking or -inhibiting compounds of this embodiment are not competing for binding to human NBCe1-B (and not competing for binding to human NBCe1-A and human NBCe1-B(Δ2-85)) with a polyclonal antibody raised against a peptide spanning amino acids 609-685 of human NBCe1-B. In particular, this lack of binding and competition for binding relates to binding to human SLC4A4/NBCe1 expressed/exposed by any suitable (eukaryotic) cell. Such suitable (eukaryotic) cell in particular is a (eukaryotic) cell the (in vitro) culture of which is not significantly altered by the expression or overexpression of human SLC4A4/NBCe1, such suitable eukaryotic cells include e.g. HEK cells.

A second aspect of this disclosure relates to SLC4A4/NBCe1-blocking or -inhibiting compounds, such as SLC4A4/NBCe1-blocking or -inhibiting compounds of the first aspect, wherein said compounds are selected from SLC4A4/NBCe1-inhibiting ISVDs or single domain antibodies or SLC4A4/NBCe1-inhibiting ISVD polypeptides, compounds comprising at least one CDR/CDR region/CDR polypeptide of a SLC4A4/NBCe1-inhibiting ISVD or SLC4A4/NBCe1-inhibiting ISVD polypeptide (also referred to as fusion proteins, chimeric constructs or molecules; fully or partly of proteinaceous nature, see further), and compounds comprising a SLC4A4/NBCe1-inhibiting ISVD or SLC4A4/NBCe1-inhibiting ISVD polypeptide (likewise also referred to as chimeric constructs or molecules). Herein, the moiety or part of the compound unrelated to the SLC4A4/NBCe1-inhibiting ISVD or unrelated to the at least one CDR of a SLC4A4/NBCe1-inhibiting ISVD, may be fully or partly of proteinaceous nature or be fully of non-proteinaceous nature. Just a few examples of such compounds fully of proteinaceous nature are e.g. classical monoclonal antibodies comprising at least one CDR/CDR region/CDR polypeptide of a SLC4A4/NBCe1-inhibiting ISVD, or antigen-binding fragments of such monoclonal antibodies (e.g. scFv, Fab), and e.g. Fc fragments of classical monoclonal antibodies to which one or more SLC4A4/NBCe1-inhibiting ISVD is linked or coupled. The moiety or part of the compound unrelated to the SLC4A4/NBCe1-

inhibiting ISVD or unrelated to the at least one CDR of a SLC4A4/NBCe1-inhibiting ISVD may in itself be an (alternative) SLC4A4/NBCe1-inhibitor and/or may in itself be a SLC4A4/NBCe1-binding moiety; alternatively, said moiety is (specifically or selectively) binding to SLC4A4/NBCe1 but not inhibiting SLC4A4/NBCe1 activity (such as one of the SLC4A4/NBCe1-binding ISVDs as referred to in the Examples); further alternatively, said moiety is not binding to SLC4A4/NBCe1 and not inhibiting SLC4A4/NBCe1 activity. Thus, the SLC4A4/NBCe1-inhibiting activity of compounds comprising at least one CDR of a SLC4A4/NBCe1-inhibiting ISVD, and of compounds comprising a SLC4A4/NBCe1-inhibiting ISVD (polypeptide) is either at least or solely effectuated by the at least one CDR or by the ISVD being present in the compound, or either at least or solely resulting from the at least CDR moiety or from the ISVD moiety being present in the compound. In particular, the SLC4A4/NBCe1-blocking or -inhibiting compounds are isolated SLC4A4/NBCe1-blocking or -inhibiting compounds. Further in particular, the at least one CDR is at least one CDR of any of the SLC4A4/NBCe1 single domain antibodies defined by SEQ ID Nos: 14-22, 135, and 138-147 (Table 14); more in particular the at least one CDR is a CDR1, a CDR2, a CDR3, a CDR1 and a CDR2, a CDR1 and a CDR3, a CDR2 and a CDR3, or a CDR1 and a CDR2 and a CDR3; more in particular the at least one CDR is as defined by the Kabat, Chothia, Martin, IMGT, or AbM method (or other method, see *supra*). More in particular, the at least one CDR region/polypeptide of any of the SLC4A4/NBCe1 single domain antibodies is chosen from the CDRs as defined in SEQ ID Nos: 23-55, 136-137, and 148-155 (Table 14); this as defined by or using the Kabat method. More in particular, the at least one CDR region/polypeptide of any of the SLC4A4/NBCe1 single domain antibodies is chosen from the CDR1 regions/polypeptides as defined in SEQ ID Nos:23-31, the CDR2 regions/polypeptides as defined in SEQ ID Nos:32-44 and 153-155, and/or the CDR3 regions/polypeptides as defined in SEQ ID Nos:45-55, 136-137, and 148-152. Furthermore in particular, said at least one CDR region/polypeptide is a CDR1 region/polypeptide chosen from SEQ ID Nos:23-31, a CDR2 region/polypeptide chosen from SEQ ID Nos:32-44 and 153-155, and a CDR3 region/polypeptide chosen from SEQ ID Nos:45-55, 136-137, and 148-152.

Related to this second aspect, the SLC4A4/NBCe1-inhibiting ISVDs, whether or not comprised in an SLC4A4-inhibiting compound, can be defined as comprising a CDR1, a CDR2 and a CDR3, and as optionally comprising one or more of a FR1, a FR2, a FR3 and/or a FR4, combinations of CDRs and the optional FRs being listed in Table A.

For clarification of Table A, e.g. the first row (#1) is to be read as an SLC4A4/NBCe1-inhibiting ISVD or SLC4A4/NBCe1-inhibiting ISVD polypeptide defined as comprising a CDR1, a CDR2 and a CDR3, and as optionally comprising one or more of a FR1, a FR2, a FR3 and/or a FR4, wherein CDR1 is defined by SEQ ID NO:23, CDR2 is defined by SEQ ID NO:32, and CDR3 is defined by SEQ ID NO:45; wherein the optional

FR1 is chosen from the FR1s defined by SEQ ID NOs:56-61 (or having at least 85, 90, 95, 98 or 99% sequence identity with one of SEQ ID Nos:56-61), wherein the optional FR2 is chosen from the FR2s defined by SEQ ID NOs:69-72 (or having at least 85, 90, 95, 98 or 99% sequence identity with one of SEQ ID Nos:69-72), wherein the optional FR3 is chosen from the FR3s defined by SEQ ID NOs:74-79 (or having at least 85, 90, 95, 98 or 99% sequence identity with one of SEQ ID Nos:74-79), and wherein the optional FR4 is chosen from the FR4s defined by SEQ ID NOs:86-88 (or having at least 85, 90, 95, 98 or 99% sequence identity with one of SEQ ID Nos:86-88); and wherein the SEQ ID NOs are listed in Table 14.

For further clarification of Table A, e.g. the tenth row (#10) is to be read as an SLC4A4/NBCe1-inhibiting ISVD or SLC4A4/NBCe1-inhibiting ISVD polypeptide defined as comprising a CDR1, a CDR2 and a CDR3, and as optionally comprising one or more of a FR1, a FR2, a FR3 and/or a FR4, wherein CDR1 is defined by SEQ ID NO:23, CDR2 is chosen from the CDRs defined by SEQ ID NOs:35-38, and CDR3 is chosen from the CDRs defined by SEQ ID NOs:47-50; wherein the optional FR1 is chosen from the FR1s defined by SEQ ID NOs:56-61 (or having at least 85, 90, 95, 98 or 99% sequence identity with one of SEQ ID Nos:56-61), wherein the optional FR2 is chosen from the FR2s defined by SEQ ID NOs:69-72 (or having at least 85, 90, 95, 98 or 99% sequence identity with one of SEQ ID Nos:69-72), wherein the optional FR3 is chosen from the FR3s defined by SEQ ID NOs:74-79 (or having at least 85, 90, 95, 98 or 99% sequence identity with one of SEQ ID Nos:74-79), and wherein the optional FR4 is chosen from the FR4s defined by SEQ ID NOs:86-88 (or having at least 85, 90, 95, 98 or 99% sequence identity with one of SEQ ID Nos:86-88), and wherein the SEQ ID NOs are listed in Table 14.

Table A. SLC4A4/NBCe1-inhibiting ISVDs or SLC4A4/NBCe1-inhibiting ISVD polypeptides can be defined as comprising a CDR1, a CDR2 and a CDR3, and as optionally comprising one or more of a FR1, a FR2, a FR3 and/or a FR4 as listed in a single same row; or as comprising a combination of the CDRs and the FRs as listed in a single same row. Numbers in the CDR and FR columns refer to SEQ ID NOs as listed in Table 14, as determined with/using the Kabat-method.

#	CDR1	CDR2	CDR3	FR1*	FR2*	FR3*	FR4*
1	23	32	45	56-61	69-72	74-79	86-88
2	24	39	51	56, 62-65	69, 73	74, 80-82	86, 88
3	29	42	54	56, 66-68	69, 73	74, 83-85	86, 88
4	29	42	55	56, 66-68	69, 73	74, 83-85	86, 88
5	25	41	53	56, 62, 64, 65	69, 73	74, 80-82	86, 88
6	23	33	45	56-59	69-71	74-77	86-87
7	23	33	46	56-59	69-71	74-77	86-87
8	23	34	45	56, 57, 60, 61	69, 71, 72	74, 75, 78, 79	86, 88
9	23	34	46	56, 57, 60, 61	69, 71, 72	74, 75, 78, 79	86, 88
10	23	35-38	47-50	56-61	69-72	74-79	86-88
11	24	40-41	52-53	56, 62-65	69, 73	74, 80-82	86, 88
12	29	43-44	54-55	56, 66-68	69, 73	74, 83-85	86, 88

#	CDR1	CDR2	CDR3	FR1*	FR2*	FR3*	FR4*
13	23	35	47	58	70	76	87
14	23	36	48	59	71	77	87
15	23	37	49	60	71	78	88
16	23	38	50	61	72	79	88
17	26	40	52	63	73	81	88
18	27	41	53	64	73	81	88
19	28	41	53	65	73	82	88
20	30	43	54	67	73	84	88
21	30	43	136	67	73	84	88
22	30	43	137	67	73	84	88
23	31	44	55	68	73	85	88
24	23	32	45	57	69-72	160	86-88
25	23	32	45	57	69	160	86
26	23	36	48, 148-149	56-61	69-72	74-79, 160	86-88
27	24	39	51	56, 62-65	69, 73	74, 80-82, 160	86, 88
28	24	39	51	62	69	160	86
29	29	42	55	56, 66-68	69, 73	74, 83-85, 160	86, 88
30	29	42	55	66	69	160	86
31	25	39	51	56, 62-64, 65	69, 73	74, 80-82, 160	86, 88
32	25	39	51	62	69	160	86
33	23	33	46	56-59	69-71	160	86-87
34	23	33	46	57	69	160	86
35	23	34	45	56, 57, 60, 61	69, 71, 72	160	86, 88
36	23	34	46	56, 57, 60, 61	69, 71, 72	160	86, 88
37	23	35-38	47-50	56-61	69-72	160	86-88
38	24	40-41	52-53	56, 62-65	69, 73	160	86, 88
39	29	43-44	54-55	56, 66-68	69, 73	160	86, 88
40	23	155	152	56-61	69-72	74-79, 160	86-88
41	23	153-154	48, 148-149	56-61, 156-157	69-72	74-79, 158-160	86-88
42	23	153	48, 148-149	56-61, 156-157	69-72	74-79, 158-160	86-88
43	23	153	148	157	71	158	88
44	23	154	148	157	71	159	88
45	23	155	149	157	69	160	86
46	29	42-44	54-55, 136-137, 150-151	56, 66-68	69, 73	74, 83-85, 160-163	86, 88
47	29	42-44	151	66	73	74	86,88
48	30	42-43	54-55, 136-137, 150-151	56, 66-68	69, 73	74, 83-85, 160-163	86, 88
49	30	43	151	67	73	84, 161-163	86,88
50	30	43	136, 137, 150	67	73	84, 161-163	86,88
51	30	43	136	67	73	161	86,88
52	30	43	136	67	73	161	88
53	30	43	150	67	73	162-163	86-88
54	30	43	150	67	73	162	88
55	30	43	150	67	73	163	88

#	CDR1	CDR2	CDR3	FR1*	FR2*	FR3*	FR4*
56	23	32-33, 36, 155	45, 48, 149, 152	57, 156	69-72	158, 160	86-88
57	23	36	48	156	69-72	158, 160	86-88
58	23	36	48	156	71	158	87

* FR region with an amino acid sequence defined by the indicated SEQ ID NO or with an amino acid sequence having at least 85, 90, 95, 98 or 99% sequence identity to the amino acid sequence defined by the indicated SEQ ID NO

In particular, the SLC4A4/NBCe1-inhibiting ISVDs or SLC4A4/NBCe1-inhibiting ISVD polypeptides, whether or not comprised in an SLC4A4/NBCe1-inhibiting compound, can be any one of ISVDs P01400023, P01400014, P01400012, P01400020, P01400021, P01400024, P01400040, P01400041, P01400046, and P01400195 defined by SEQ ID NOs:14 to 22, and 135, respectively, as listed in Table 14, and as aligned in Figure 13 (except for P01400195 which has an Arg amino acid at position 103 relative to a Thr amino acid in P01400014); or can be any one of ISVD P01400119 (defined by SEQ ID NO:138, Table 14), ISVD P01400131 (defined by SEQ ID NO:139, Table 14), ISVD P01400134 or variants thereof (defined by SEQ ID NO:140, or 141-143, respectively, Table 14), or ISVD P01400161 or variants thereof (defined by SEQ ID NO:144, or 145-147, respectively, Table 14). The amino acid sequences of ISVDs P01400040, P01400119, P01400024, P01400046, and P01400041 are aligned in Figure 21A; the amino acid sequences of ISVDs P01400014, and P01400131 are aligned in Figure 21B.

Related to this second aspect, SLC4A4/NBCe1-inhibiting compounds comprising at least one CDR of a SLC4A4/NBCe1-inhibiting ISVD or of a SLC4A4/NBCe1-inhibiting ISVD polypeptide, can be defined as comprising a CDR1, a CDR2, aCDR3, a CDR1 and a CDR2, a CDR1 and aCDR3, a CDR2 and CDR3, or a CDR1 and a CDR2 and a CDR3.

Herein, the CDR1 region can be defined as:

- the consensus CDR1 of, or the CDR1 region shared by, ISVDs P01400041, P01400040, P01400024 and P01400046, defined by SEQ ID NO:23 as listed in Table 14; or
- the consensus CDR1 of, or the CDR1 region shared by, ISVDs P01400021, P01400020 and P01400012, defined by SEQ ID NO:24 as listed in Table 14; or
- the consensus CDR1 of, or the CDR1 region shared by, ISVDs P01400012 and P01400020, defined by SEQ ID NO:25 as listed in Table 14; or
- the CDR1 region of ISVD P01400021, defined by SEQ ID NO:26 as listed in Table 14; or
- the CDR1 region of ISVD P01400020, defined by SEQ ID NO:27 as listed in Table 14; or
- the CDR1 region of ISVD P01400012, defined by SEQ ID NO:28 as listed in Table 14; or

- the consensus CDR1 of, or the CDR1 region shared by, ISVDs P01400014 and P01400023, defined by SEQ ID NO:29 as listed in Table 14; or
- the CDR1 region of ISVD P01400014, defined by SEQ ID NO:30 as listed in Table 14; or
- the CDR1 region of ISVD P01400023, defined by SEQ ID NO:31 as listed in Table 14.

Herein, the CDR2 region can be defined as:

- the consensus CDR2 of, or the CDR2 region shared by, ISVDs P01400041, P01400040, P01400024 and P01400046, defined by SEQ ID NO:32 as listed in Table 14; or
- the consensus CDR2 of, or the CDR2 region shared by, ISVDs P01400041 and P01400040, defined by SEQ ID NO:33 as listed in Table 14; or
- the consensus CDR2 of, or the CDR2 region shared by, ISVDs P01400024 and P01400046, defined by SEQ ID NO:34 as listed in Table 14; or
- the CDR2 region of ISVD P01400041, defined by SEQ ID NO:35 as listed in Table 14; or
- the CDR2 region of ISVD P01400040, defined by SEQ ID NO:36 as listed in Table 14; or
- the CDR2 region of ISVD P01400024, defined by SEQ ID NO:37 as listed in Table 14; or
- the CDR2 region of ISVD P01400046, defined by SEQ ID NO:38 as listed in Table 14; or
- the CDR2 region of ISVD P01400161, defined by SEQ ID NO:153 as listed in Table 14; or
- the consensus CDR2 of, or the CDR2 region shared by, ISVD P01400161 and variants of ISVD P01400161 defined by SEQ ID NOs:146 or 147; the CDR2 being defined by SEQ ID NO:154 as listed in Table 14; or
- the consensus CDR2 region defined by SEQ ID NO:155 as listed in Table 14, the consensus CDR2 regions being based on SEQ ID NOs:32 and 154; or
- the consensus CDR2 of, or the CDR2 region shared by, ISVDs P01400021, P01400020 and P01400012, defined by SEQ ID NO:39 as listed in Table 14; or
- the CDR2 region of ISVD P01400021, defined by SEQ ID NO:40 as listed in Table 14; or
- the consensus CDR2 of, or the CDR2 region shared by, ISVDs P01400020 and P01400012, defined by SEQ ID NO:41 as listed in Table 14; or
- the consensus CDR2 of, or the CDR2 region shared by, ISVDs P01400014 and P01400023, defined by SEQ ID NO:42 as listed in Table 14; or
- the CDR2 region of ISVD P01400014, defined by SEQ ID NO:43 as listed in Table 14; or
- the CDR2 region of ISVD P01400023, defined by SEQ ID NO:44 as listed in Table 14.

Herein, the CDR3 region can be defined as:

- the consensus CDR3 of, or the CDR3 region shared by, ISVDs P01400041, P01400040, P01400024 and P01400046, defined by SEQ ID NO:45 as listed in Table 14; or

- the consensus CDR3 of, or the CDR3 region shared by, ISVDs P01400041, P01400040, P01400024, P01400046, P01400195, P01400119 and P01400161, defined by SEQ ID NO:152 as listed in Table 14; or
- the consensus CDR3 of, or the CDR3 region shared by, ISVDs P01400041, P01400024 and P01400046, defined by SEQ ID NO:46 as listed in Table 14; or
- the CDR3 region of ISVD P01400041, defined by SEQ ID NO:47 as listed in Table 14; or
- the CDR3 region of ISVD P01400040, defined by SEQ ID NO:48 as listed in Table 14; or
- the CDR3 region of ISVD P01400024, defined by SEQ ID NO:49 as listed in Table 14; or
- the CDR3 region of ISVD P01400046, defined by SEQ ID NO:50 as listed in Table 14; or
- the CDR3 region of ISVD P014000161, defined by SEQ ID NO:148 as listed in Table 14; or
- the CDR3 region shared by ISVD P01400119 and P01400161, defined by SEQ ID NO:149 as listed in Table 14; or
- the consensus CDR3 of, or the CDR3 region shared by, ISVDs P01400021, P01400020 and P01400012, defined by SEQ ID NO:51 as listed in Table 14; or
- the CDR3 region of ISVD P01400021, defined by SEQ ID NO:52 as listed in Table 14; or
- the consensus CDR3 of, or the CDR3 region shared by, ISVDs P01400020 and P01400012, defined by SEQ ID NO:53 as listed in Table 14; or
- the consensus CDR3 of, or the CDR3 region shared by, ISVD P01400014 and P01400195, defined by SEQ ID NO:54 as listed in Table 14; or
- the CDR3 region of ISVD P01400014, defined by SEQ ID NO:136 as listed in Table 14; or
- the CDR3 region of the variants of ISVD P01400134 defined by SEQ ID NOs 141 or 143, the CDR3 region being defined by SEQ ID NO:150 as listed in Table 14; or
- the CDR3 region defined by SEQ ID NO:151 as listed in Table 14, being the consensus of the CDR3 regions defined by SEQ ID NOs: 54 and 150; or
- the CDR3 region of ISVD P01400195, defined by SEQ ID NO:137 as listed in Table 14; or
- the CDR3 region of ISVD P01400023, defined by SEQ ID NO:55 as listed in Table 14.

In addition, in the above, the at least one CDR of a SLC4A4/NBCe1-inhibiting ISVD or of a SLC4A4/NBCe1-inhibiting ISVD polypeptide, can be chosen from:

- a CDR1 region comprising or consisting of a polypeptide sequence having a difference of 3, 2 or 1 amino acids with a CDR1 region defined by any of SEQ ID NOs:23, 26-28, 30-31; and/or
– a CDR2 region comprising or consisting of a polypeptide sequence having a difference of 3, 2 or 1 amino acids with a CDR2 region defined by any of SEQ ID NOs:35-38, 40-41, 43-44; and/or
- a CDR3 region comprising or consisting of a polypeptide sequence having a difference of 3, 2 or 1 amino acids with a CDR3 region defined by any of SEQ ID NOs:47-50, 52-53, 55, 136-137.

Further related to this second aspect, when said SLC4A4/NBCe1-inhibiting compound is comprising a CDR1, the CDR1 can be preceded by a FR1 region and/or followed by a FR2 region; when said compound is comprising a CDR2, the CDR2 can be preceded by a FR2 region and/or followed by a FR3 region; when said compound is comprising a CDR3, the CDR3 can be preceded by a FR3 region and/or followed by a FR4 region.

Herein, the FR1 region can be defined as:

- the consensus FR1 of, or the FR1 region shared by, ISVDs P01400041, P01400040, P01400024, P01400046, P01400021, P01400020, P01400012, P01400014, P01400023, P01400195, P01400131, and P01400134 and its variants, the FR1 region defined by SEQ ID NO:56 as listed in Table 14; or
- the consensus FR1 of, or the FR1 region shared by, ISVDs P01400041, P01400040, P01400024, P01400046, P01400119, and P01400161 and its variants, the FR1 region defined by SEQ ID NO:57 as listed in Table 14; or
- the FR1 region of ISVD P01400041, defined by SEQ ID NO:58 as listed in Table 14; or
- the FR1 region of ISVD P01400040, defined by SEQ ID NO:59 as listed in Table 14; or
- the FR1 region of ISVD P01400024, defined by SEQ ID NO:60 as listed in Table 14; or
- the FR1 region of ISVD P01400046, defined by SEQ ID NO:61 as listed in Table 14; or
- the FR1 region of ISVD P01400119, defined by SEQ ID NO:156 as listed in Table 14; or
- the FR1 region of ISVD P01400161, defined by SEQ ID NO:157 as listed in Table 14; or
- the consensus FR1 of, or the FR1 region shared by, ISVDs P01400021, P01400020 and P01400012, defined by SEQ ID NO:62 as listed in Table 14; or
- the FR1 region of ISVD P01400021, defined by SEQ ID NO:63 as listed in Table 14; or
- the FR1 region of ISVD P01400020, defined by SEQ ID NO:64 as listed in Table 14; or
- the FR1 region of ISVD P01400012, defined by SEQ ID NO:65 as listed in Table 14; or
- the consensus FR1 of, or the FR1 region shared by, ISVDs P01400014, P01400195 and P01400023, defined by SEQ ID NO:66 as listed in Table 14; or
- the FR1 region of ISVD P01400014 and P01400195, defined by SEQ ID NO:67 as listed in Table 14; or
- the FR1 region of ISVD P01400023, defined by SEQ ID NO:68 as listed in Table 14.

In addition, in the above, in the at least one FR1 of a SLC4A4/NBCe1-inhibiting ISVD or of a SLC4A4/NBCe1-inhibiting ISVD polypeptide, the amino-terminal amino acid Glu (E) can be substituted for the amino acid Asp (D); this substitution is in particular advantageous when this amino-terminal amino acid remains free, e.g. as part of an ISVD or as part of a fusion protein, chimeric construct or molecule (compounds comprising at least one SLC4A4/NBCe1-inhibiting ISVD or ISVD polypeptide, supra).

Herein, the FR2 region can be defined as:

- the consensus FR2 of, or the FR2 region shared by, ISVDs P01400041, P01400040, P01400024, P01400046, P01400021, P01400020, P01400012, P01400014, P01400195, P01400023, P01400131, P01400134 and its variants, P01400119, and P01400161 and its variants, the FR2 region defined by SEQ ID NO:69 as listed in Table 14; or
- the FR2 region of ISVD P01400041, defined by SEQ ID NO:70 as listed in Table 14; or
- the consensus FR2 of, or the FR2 region shared by, ISVDs P01400040 and P01400024, defined by SEQ ID NO:71 as listed in Table 14; or
- the FR2 region of ISVD P01400046, defined by SEQ ID NO:72 as listed in Table 14; or
- the consensus FR2 of, or the FR2 region shared by, ISVDs P01400021, P01400020, P01400012, P01400014, P01400195 and P01400023 defined by SEQ ID NO:73 as listed in Table 14.

Herein, the FR3 region can be defined as:

- the consensus FR3 of, or the FR3 region shared by, ISVDs P01400041, P01400040, P01400024, P01400046, P01400021, P01400020, P01400012, P01400014, P01400195, P01400023, P01400131, and P01400134 and its variants, the FR3 region defined by SEQ ID NO:74 as listed in Table 14; or
- the consensus FR3 of, or the FR3 region shared by, ISVDs P01400041, P01400040, P01400024 and P01400046, defined by SEQ ID NO:75 as listed in Table 14; or
- the FR3 region of ISVD P01400041, defined by SEQ ID NO:76 as listed in Table 14; or
- the FR3 region of ISVD P01400040, defined by SEQ ID NO:77 as listed in Table 14; or
- the FR3 region of ISVD P01400024, defined by SEQ ID NO:78 as listed in Table 14; or
- the FR3 region of ISVD P01400046, defined by SEQ ID NO:79 as listed in Table 14; or
- the FR3 region of ISVD P01400195 and ISVD P01400131, defined by SEQ ID NO:161 as listed in Table 14; or
- the FR3 region of ISVD P01400119, defined by SEQ ID NO:158 as listed in Table 14; or
- the FR3 region of ISVD P01400161, defined by SEQ ID NO:159 as listed in Table 14; or
- the consensus FR3 region defined by SEQ ID NO:160 as listed in Table 14, the consensus FR3 regions being based on SEQ ID NOs:74 and 159; or
- the consensus FR3 of, or the FR3 region shared by, ISVDs P01400021, P01400020 and P01400012, defined by SEQ ID NO:80 as listed in Table 14; or
- the consensus FR3 of, or the FR3 region shared by, ISVDs P01400021 and P01400020, defined by SEQ ID NO:81 as listed in Table 14; or
- the FR3 region of ISVD P01400012, defined by SEQ ID NO:82 as listed in Table 14; or
- the consensus FR3 of, or the FR3 region shared by, ISVDs P01400014, P01400195 and P01400023, defined by SEQ ID NO:83 as listed in Table 14; or
- the FR3 region of ISVD P01400014 and P01400195, defined by SEQ ID NO:84 as listed in Table 14; or

- the FR3 region of ISVD P01400023, defined by SEQ ID NO:85 as listed in Table 14; or
- the FR3 region of ISVD P01400134, defined by SEQ ID NO:162 as listed in Table 14; or
- the consensus FR3 of, or the FR3 region shared by, ISVD P01400134 and variants of ISVD P01400134 defined by SEQ ID NOs:142 or 143; the FR3 being defined by SEQ ID NO:163 as listed in Table 14.

Herein, the FR4 region can be defined as:

- the consensus FR4 of, or the FR4 region shared by, ISVDs P01400041, P01400040, P01400024, P01400046, P01400021, P01400020, P01400012, P01400014, P01400195, P01400023, P01400131, P01400119, P01400134 and its variants, and P01400161 and its variants, the FR4 region defined by SEQ ID NO:86 as listed in Table 14; or
- the consensus FR4 of, or the FR4 region shared by, ISVDs P01400041 and P01400040, defined by SEQ ID NO:87 as listed in Table 14; or
- the consensus FR4 of, or the FR4 region shared by, ISVDs P01400024, P01400046, P01400021, P01400020, P01400012, P01400014, P01400195 and P01400023, defined by SEQ ID NO:88 as listed in Table 14.

Furthermore included as possible SLC4A4/NBCe1-inhibiting compounds are SLC4A4/NBCe1-inhibiting ISVDs or SLC4A4/NBCe1-inhibiting ISVD polypeptides comprising a CDR1, CDR2 and CDR3 as defined in any of the above and further comprising:

- FRs defined by SEQ ID NOs:58, 70, 76 and 87 or variants of these FRs such that the variants overall have at least 85, 90, 95, 98 or 99% sequence identify to the four framework regions (FR);
- FRs defined by SEQ ID NOs:59, 71, 77 and 87 or variants of these FRs such that the variants overall have at least 85, 90, 95, 98 or 99% sequence identify to the four framework regions (FR);
- FRs defined by SEQ ID NOs:60, 71, 78 and 88 or variants of these FRs such that the variants overall have at least 85, 90, 95, 98 or 99% sequence identify to the four framework regions (FR);
- FRs defined by SEQ ID NOs:61, 72, 79 and 88 or variants of these FRs such that the variants overall have at least 85, 90, 95, 98 or 99% sequence identify to the four framework regions (FR);
- FRs defined by SEQ ID NOs:63, 73, 81 and 88 or variants of these FRs such that the variants overall have at least 85, 90, 95, 98 or 99% sequence identify to the four framework regions (FR);
- FRs defined by SEQ ID NOs:64, 73, 81 and 88 or variants of these FRs such that the variants overall have at least 85, 90, 95, 98 or 99% sequence identify to the four framework regions (FR);
- FRs defined by SEQ ID NOs:65, 73, 82 and 88 or variants of these FRs such that the variants overall have at least 85, 90, 95, 98 or 99% sequence identify to the four framework regions (FR);
- FRs defined by SEQ ID NOs:67, 73, 84 and 88 or variants of these FRs such that the variants overall have at least 85, 90, 95, 98 or 99% sequence identify to the four framework regions (FR); or

- FRs defined by SEQ ID NOs:68, 73, 85 and 88 or variants of these FRs such that the variants overall have at least 85, 90, 95, 98 or 99% sequence identify to the four framework regions (FR); or
- FRs defined by SEQ ID NOs:67, 73, 161 (or 162 or 163) and 88 or variants of these FRs such that the variants overall have at least 85, 90, 95, 98 or 99% sequence identify to the four framework regions (FR); or
- FRs defined by SEQ ID NOs:156, 71, 158 and 87 or variants of these FRs such that the variants overall have at least 85, 90, 95, 98 or 99% sequence identify to the four framework regions (FR); or
- FRs defined by SEQ ID NOs:157, 71, 159 and 88 or variants of these FRs such that the variants overall have at least 85, 90, 95, 98 or 99% sequence identify to the four framework regions (FR).

Herein, "variants overall have at least 85, 90, 95, 98 or 99% sequence identify to the four framework regions (FR)" is meant to refer to the % amino acid sequence identity over the total sequence of the combined FRs defined by the listed SEQ ID Nos. In case of a FR1 region with a length of 25 amino acids, a FR2 region with a length of 14 amino acids, a FR3 region with a length of 33 amino acids, and a FR4 region with a length of 11 amino acids, this amounts to an overall or combined FR length of 83 amino acids. A variant of these four FRs as referred to above is then a variant overall having e.g. 90% sequence identity over the 83 amino acid overall or combined FR length, i.e., meaning that 8 of these 83 amino acids can be varied compared/relative to the 83 amino acids of the combined FRs as defined by the SEQ ID Nos herein.

All above referrals to SEQ ID NOs of CDRs and FRs are based on determining the CDR boundaries according to the Kabat method, i.e. "Kabat CDRs" (except when explicitly stated otherwise). In this respect, relating to the CDRs (and following therefrom the FRs) any of the Kabat CDRs referred to can in any of the foregoing or following be substituted by CDRs as determined with another method as described herein. In particular, examples of "non-Kabat" CDR are the "IMGT CDRs" and "AbM CDRs" as given in Figure 14 for the SLC4A4/NBCe1-inhibiting ISVDs P01400014, P01400020, P01400023, P01400040, which gives sufficient guidance for delineating alternative "non-Kabat" CDRs in the other SLC4A4/NBCe1-inhibiting ISVDs as disclosed herein.

In one embodiment to the second aspect, the SLC4A4/NBCe1-blocking or -inhibiting compounds are inhibiting human pancreatic SLC4A4/NBCe1 or human NBCe-1B (defined by SEQ ID NO:114 in Table 15) and are surprisingly not inhibiting human kidney SLC4A4/NBCe1 or human NBCe-1A (defined by SEQ ID NO:118 in Table 15). More in particular, when such SLC4A4/NBCe1-blocking or -inhibiting compounds are comprising at least one CDR (and, optionally, at least one FR), the at least one CDR (and optional FR) comprised in this type of SLC4A4/NBCe1-inhibiting compounds is one of the CDRs (and optional FRs) as

listed in Table A, lines #1, 6-10, 13-16, 24-26, 33-37, 40-45, and 56-58 (CDR1 region/polypeptide defined by SEQ ID NO:23, CDR2 region chosen from CDR2 polypeptide defined by SEQ ID NOs:32-38 and 153-155, and/or CDR3 region chosen from CDR3 polypeptide defined by SEQ ID NOs:45-50, 148-149 and 152). When comprising an ISVD, this type of SLC4A4/NBCe1-inhibiting compounds ISVDs is comprising an ISVD chosen from the ISVDs defined by any of SEQ ID NOs:19-21, 138 and 144-147.

In a further embodiment to the second aspect, the SLC4A4/NBCe1-blocking or -inhibiting compounds are inhibiting both human pancreatic SLC4A4/NBCe1 or human NBCe-1B (defined by SEQ ID NO:114 in Table 15) and human kidney SLC4A4/NBCe1or human NBCe-1A (defined by SEQ ID NO:118 in Table 15). More in particular, when such SLC4A4/NBCe1-blocking or -inhibiting compounds are comprising at least one CDR (and, optionally, at least one FR), the at least one CDR (and optional FR) comprised in this type of SLC4A4/NBCe1-inhibiting compounds is one of the CDRs (and optional FRs) as listed in Table A, lines #2-5, 11-12, 17-23, 27-32, 38-39, and 46-55 (CDR1 region chosen from CDR1 polypeptides defined by SEQ ID NOs:24-31, CDR2 region chosen from CDR2 polypeptide defined by SEQ ID NOs:39-44, and/or CDR3 region chosen from CDR3 polypeptide defined by SEQ ID NOs:51-55, 136-137 and 150-151). When comprising an ISVD, this type of SLC4A4/NBCe1-inhibiting compounds ISVDs is comprising an ISVD chosen from the ISVDs defined by any of SEQ ID NOs:14-18, 135, and 139-143.

In a further embodiment to the second aspect, the SLC4A4/NBCe1-blocking or -inhibiting compounds are binding to extracellular loop 3 (EL3) of human pancreatic SLC4A4/NBCe1 or human NBCe-1B, more in particular binding to EL3 of human NBCe1-B exposed by a cell (a suitable cell/eukaryotic cell expressing human NBCe1-B), further more in particular binding to human NBCe-1B and to human NBCe-1B(D2-85) expressed or exposed by a cell (a suitable cell/eukaryotic cell expressing human NBCe1-B; in particular a wild-type cells such as a HEK293 cell; further in particular a living or non-fixed wild-type cell), and optionally to human NBC1e-A expressed or exposed by such cell; herein the binding to the cell is at a significant level or the binding is significant binding (significantly higher compared to binding level of a control antibody). Furthermore in particular, although binding to EL3 of human NBCe1-B expressed by a cell, such binding does surprisingly not involve binding to amino acids 629-644 of human NBCe1-B. Furthermore in particular, further surprisingly, a polyclonal antibody raised against a peptide spanning amino acids 609-685 of human NBCe1-B is not competing for binding to EL3 of human NBCe1-B. More in particular, when such SLC4A4/NBCe1-blocking or -inhibiting compounds are comprising at least one CDR (and, optionally, at least one FR), the at least one CDR (and optional FR) comprised in this type of SLC4A4/NBCe1-inhibiting compounds is one of the CDRs (and optional FR) as listed in Table A, lines #1, 6-10, 13-16, 24-26, 33-37, 40-45, and 56-58 . When comprising an ISVD, this type of SLC4A4/NBCe1-

inhibiting compounds is comprising an ISVD chosen from the ISVDs defined by any of SEQ ID NOs:19-21, 138 and 144-147. This embodiment can be combined in any way with (parts of) a previous embodiment related to SLC4A4/NBCe1-blocking or -inhibiting compounds inhibiting NBCe1-B but not NBCe1-A.

In a further embodiment to the second aspect, the SLC4A4/NBCe1-blocking or -inhibiting compounds are surprisingly binding to the amino acid glutamate in extracellular loop 4 (EL4)/at amino acid position 775 of human pancreatic SLC4A4/NBCe1 or human NBCe-1B (this glutamate amino acid is referred to as E775; see Figure 1B and Example 9), more in particular binding to amino acid glutamate in extracellular loop 4 (EL4)/at amino acid position 775 of human NBCe-1B exposed by a cell (a suitable cell/eukaryotic cell expressing human NBCe1-B). More in particular, when such SLC4A4/NBCe1-blocking or -inhibiting compounds are comprising at least one CDR (and, optionally, at least one FR), the at least one CDR (and optional FR) comprised in this type of SLC4A4/NBCe1-inhibiting compounds is one of the CDRs (and optional FR) as listed in Table A, lines #2-5, 11-12, 17-23, 27-32, 38-39, and 46-55. When comprising an ISVD, this type of SLC4A4/NBCe1-inhibiting compounds is comprising an ISVD chosen from the ISVDs defined by any of SEQ ID NOs:14-18, 135 and 139-143. This embodiment can be combined in any way with (parts of) a previous embodiment related to SLC4A4/NBCe1-blocking or -inhibiting compounds inhibiting NBCe1-B and NBCe1-A.

In a further embodiment to the second aspect, the SLC4A4/NBCe1-blocking or -inhibiting compounds are surprisingly binding to extracellular loop 2 (EL2) and extracellular loop 4 (EL4) of human pancreatic SLC4A4/NBCe1 or human NBCe-1B, more in particular binding to EL2 and EL4 of human NBCe1-B exposed by a cell (a suitable cell/eukaryotic cell expressing human NBCe1-B). More in particular, when such SLC4A4/NBCe1-blocking or -inhibiting compounds are comprising at least one CDR (and, optionally, at least one FR), the at least one CDR (and optional FR) comprised in this type of SLC4A4/NBCe1-inhibiting compounds is one of the CDRs (and optional FR) as listed in Table A, lines #2-5, 11-12, 17-23, 27-32, 38-39, and 46-55. When comprising an ISVD, this type of SLC4A4/NBCe1-inhibiting compounds is comprising an ISVD chosen from the ISVDs defined by any of SEQ ID NOs:14-18, 135 and 139-143. This embodiment can be combined in any way with (parts of) a previous embodiment related to SLC4A4/NBCe1-blocking or -inhibiting compounds inhibiting NBCe1-B and NBCe1-A and/or with (parts of) a previous embodiment related to SLC4A4/NBCe1-blocking or -inhibiting compounds binding to Glu775 of EL4.

In a further embodiment to the second aspect, the SLC4A4/NBCe1-blocking or -inhibiting compounds in particular are any of the individual ISVDs as listed in Table A, lines #13-19 and 21-23.

Herein, the ISVD as listed in line #14 is cross-reactive (binding) with human NBCe1-B, human NBCe1-A, human NBCe1(D2-85), cynomolgus NCBe1-B, and mouse NBCe1-B; and is blocking or inhibiting activity of human NBCe1-B, human NBCe1(D2-85), and mouse NBCe1-B, and is not blocking or inhibiting activity of human NBCe1-A.

Herein, the ISVD as listed in line #15 is cross-reactive (binding) with human NBCe1-B, human NBCe1-A, human NBCe1(D2-85), and cynomolgus NCBe1-B; and is blocking or inhibiting activity of human NBCe1-B, and human NBCe1(D2-85), and is not blocking or inhibiting activity of human NBCe1-A and mouse NBCe1-B.

Herein, the ISVD as listed in line #21 is cross-reactive (binding) with human NBCe1-B, human NBCe1-A, human NBCe1(D2-85), cynomolgus NCBe1-B, and mouse NBCe1-B; and is blocking or inhibiting activity of human NBCe1-B, human NBCe1(D2-85), and human NBCe1-A, and is not blocking or inhibiting activity of mouse NBCe1-B. The closely related variant ISVD as listed in line #22 is in addition blocking or inhibiting activity of mouse NBCe1-B.

Herein, the ISVD as listed in line #23 is cross-reactive (binding) with human NBCe1-B, human NBCe1-A, and human NBCe1(D2-85); and is blocking or inhibiting activity of human NBCe1-B, human NBCe1(D2-85), and human NBCe1-A.

Herein, the ISVD as listed in line #18 is cross-reactive (binding) with human NBCe1-B, human NBCe1-A, human NBCe1(D2-85), and mouse NBCe1-B; and is blocking or inhibiting activity of human NBCe1-B, human NBCe1(D2-85), and human NBCe1-A.

In a further embodiment to any of the above, SLC4A4/NBCe1 binding in particular is *in vitro* binding to SLC4A4/NBCe1 expressed and exposed by a cell (a suitable cell/eukaryotic cell). More in particular, said binding is determined by e.g. flow cytometry, or by another method known in the art. More in particular, a labeled or tagged version of any of the above-defined SLC4A4/NBCe1-blocking or -inhibiting compounds is used in the binding assay.

In a further embodiment to any of the above, SLC4A4/NBCe1 blockage or inhibition in particular is blockage or inhibition as determined *in vitro* in a cell-based acid shock recovery assay. More in particular, the cells are expressing or overexpressing SLC4A4/NBCe1. More in particular, the SLC4A4/NBCe1-(over)expressing cells are sensitized to SLC4A4/NBCe1 inhibition by prior inhibition of the sodium/hydrogen exchanger 1 (Na/H Exchanger 1, NHE1, also known as Solute Carrier Family 9 Member A1 or SLC9A1). More in particular, the NHE1 inhibitor is cariporide (CAS No 159138-80-4; InChi Key IWXNYAIICFKCTM-UHFFFAOYSA-N computed by InChI 1.0.6–- PubChem release 2021.05.07).

The SLC4A4/NBCe1-blocking or -inhibiting ISVDs or SLC4A4/NBCe1-inhibiting ISVD polypeptides as described hereinabove are flexible for being incorporated in compounds (such as combined in fusion proteins, chimeric constructs or molecules) without loss or drastic loss of SLC4A4/NBCe1-blocking activity as evidenced by the experiments with bi-/multi-valent and biparatopic VHHs as described in the Examples including linker-separated fusions of different ISVDs (including a serum albumin binding ISVD for purposes of extending in vivo pharmacological half-life) and fusion of SLC4A4/NBCe1-blocking ISVDs on Fc arms or fragments of conventional antibodies. Therewith, the concept of different formats of SLC4A4/NBCe1-blocking or -inhibiting compounds comprising different SLC4A4/NBCe1-inhibiting ISVDs is widely supported. As mentioned hereinabove, SLC4A4/NBCe1-blocking or -inhibiting compounds are selected from SLC4A4/NBCe1-inhibiting ISVD, compounds comprising at least one CDR of a SLC4A4/NBCe1-inhibiting ISVD, and compounds comprising a SLC4A4/NBCe1-inhibiting ISVD; herein, the said compounds can, except for the CDR or ISVD, themselves be fully or partly of proteinaceous nature or be fully of non-proteinaceous nature. The at least one CDR of a SLC4A4/NBCe1-inhibiting ISVD and SLC4A4/NBCe1-inhibiting ISVD are, in the further explanation of the SLC4A4/NBCe1-blocking or -inhibiting compounds hereinafter simply referred to a the CDR and ISVD, and moieties of SLC4A4/NBCe1-blocking or -inhibiting compounds different from the CDR or ISVD are referred to as “non-CDR moieties” or “non-ISVD moieties”.

As already referred to hereinabove, non-CDR or non-ISVD moieties that can be included in/comprised in/part of these SLC4A4/NBCe1-blocking compounds include moieties themselves binding to and/or inhibiting SCL4A4/NBCe1 in as far as different from the CDRs or ISVDs as described herein.

Non-CDR or non-ISVD moieties that can be included in/comprised in/part of these SLC4A4/NBCe1-blocking compounds include, but are not limited to, a polyethylene glycol polymer (pegylated SLC4A4/NBCe1-inhibiting compound; e.g. WO2017/059397), one or more further polypeptide(s) or polypeptide domain(s) such as e.g. a His-tag, or a peptide ligase motif such as a sortag motif (sortase peptide ligase amino acid substrate motif LPXTG (SEQ ID NO:132), e.g. LPETG (SEQ ID NO:133); Mao et al. 2004, J Am Chem Soc 126:2670–2671) or a peptide asparaginyl ligase motif (recognized by e.g. butelase 1 or VyPAL2; motif sequence being NXL wherein X can be e.g. Gly, Ser, Ala, Gln; Zhang et al. 2022, Int J Mol Sci 23:458; Hu et al. 2022, Plant Cell 34: 4936-4949). Further possible proteinaceous labels or tags include affinity tags, such as chitin binding protein (CBP), maltose binding protein (MBP), glutathione-S-transferase (GST), poly(His) (e.g., 6x His or His6); solubilizing tags, such as thioredoxin (TRX), poly(NANP) and SUMO; chromatography tags, such as a FLAG-tag; epitope tags, such as V5-tag, myc-tag and HA-tag; fluorescent labels or tags, such as fluorescent proteins (e.g., GFP, YFP, RFP etc.); luminescent labels or tags, such as luciferase; and (other) enzymatic labels (e.g., peroxidase, alkaline phosphatase, beta-galactosidase, urease or glucose oxidase). In another instance, a further polypeptide

or polypeptide domain (such as connected through a flexible linker such as a linker based on Gly-Pro repeats, Pro-Ala repeats, Gly-Ser repeats, or combinations thereof, to a SLC4A4/NBCe1-blocking ISVD or SLC4A4/NBCe1-inhibiting ISVD polypeptide; or included in a fusion protein with a SLC4A4/NBCe1-blocking ISVD) may confer increased serum half-life. Such half-life extending moiety can for instance be a serum albumin binding I(S)VD (e.g. WO2012/175400 and WO2019/016237), or albumin itself. Other half-life extension modalities include PEGylation (or any modification such as glycol-PEGylation, biotinylated PEG), attaching (whether or not in the form of a fusion protein) peptides such as XTEN, PAS ("Pro Ala Ser"), ELP (elastin-like polypeptide), GLK (gelatin-like protein), HAPylation, and adding a polysaccharide moiety (reviewed in e.g. Zaman et al. 2019, J Controlled Release 301:176–189). In one instance, an SLC4A4/NBCe1-blocking or -inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide itself may be duplicated or multiplicated (wherein the monomers are e.g. connected through a flexible linker such as a linker based on Gly-Pro repeats, Pro-Ala repeats, Gly-Ser repeats, or combinations thereof; or by coupling on one or both arms of a Fc-protein to form an Fc-fusion protein) to form a multivalent (though monospecific) SLC4A4/NBCe1-inhibiting compound. In another instance, different SLC4A4/NBCe1-blocking or -inhibiting ISVD themselves may be linked directly (wherein the monomers are e.g. connected through a flexible linker such as a linker based on Gly-Pro repeats, Pro-Ala repeats, Gly-Ser repeats, or combinations thereof; or by coupling on one or both arms of a Fc-protein to form an Fc-fusion protein) to form a multivalent and multispecific/multiparatopic SLC4A4/NBCe1-inhibiting compound. More in general, multiple identical ISVDs can be fused directly or via a linker, as to provide for bi- or multivalent VHH variants, such as in tandem repeat fashion or in head-to-tail fusion fashion. Monovalent and/or multivalent ISVDs may alternatively or additionally be fused to an Fc tail, such as to a human IgG1 Fc. More in general, multiple different ISVDs can be fused directly or via a linker, as to provide for multiparatopic VHH variants, such as in tandem repeat fashion or in head-to-tail fusion fashion. Monoparatopic and/or multiparatopic ISVDs may alternatively or additionally be fused to an Fc tail, such as to a human IgG1 Fc.

Non-CDR or non-ISVD moieties that can be included in/comprised in/part of these SLC4A4/NBCe1-blocking compounds further include (non-proteinaceous) affinity tags (e.g. biotin or streptavidin), detectable moieties (e.g. fluorescent dyes, chemiluminescent compounds, phosphorescent labels, radioisotopes) or chelators of detectable moieties. A "detectable moiety" in general refers to a moiety that emits a signal or is capable of emitting a signal upon adequate stimulation, and is detectable by any means, such as by a non-invasive means once inside the body of subject. Furthermore, the detectable moiety may allow for computerized composition of an image, as such the detectable moiety may be called an imaging agent. Detectable moieties include fluorescence emitters, positron emitters, radioemitters, etc. If the detectable moiety is a radionuclide having a metallic nature a chelators may be

needed as non-CDR or non-ISVD moiety of a SLC4A4/NBCe1-blocking compound, i.e. multidentate ligands, which form non-covalent compounds, called chelates, with the metal ions/radionuclide. Chelators include polyaminopolycarboxylate-type chelators which can be macrocyclic or acyclic.

Non-CDR or non-ISVD moieties that can be included in/comprised in/part of these SLC4A4/NBCe1-blocking compounds further include moieties which induce antibody-dependent cellular activity (ADCC), induce antibody-dependent cellular phagocytosis (ADCP), induce complement-dependent cytotoxicity (CDC), bind to and activate T-cells, or comprise a cytotoxic payload. Antibody-dependent cellular cytotoxicity (ADCC) refers to a cell-mediated reaction in which non-specific cytotoxic cells that express Fc receptors recognize binders on a target cell and subsequently cause lysis of the target cell. Examples of non-specific cytotoxic cells that express Fc receptors include natural killer cells, neutrophils, monocytes and macrophages. Complement-dependent cytotoxicity (CDC) refers to the lysis of a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a binder complexed with a cognate antigen. Antibody-dependent cellular phagocytosis (ADCP) refers to a cell-mediated reaction in which phagocytes (such as macrophages) that express Fc receptors recognize binders on a target cell and thereby lead to phagocytosis of the target cell. CDC, ADCC and ADCP can be measured using assays that are known in the art (Vafa et al. Methods 2014 Jan 1;65(1):114-26 (2013)).

Non-CDR or non-ISVD moieties that can be included in/comprised in/part of these SLC4A4/NBCe1-blocking compounds further include a fragment crystallisable (Fc) region moiety or an antibody or antigen-binding part thereof that specifically binds to an FcγR. Such Fc region moiety can be engineered to increase ADCC, ADCP and/or CDC activity. ADCC may be increased by methods that reduce or eliminate the fucose moiety from the Fc moiety glycan and/or through introduction of specific mutations on the Fc region of an immunoglobulin, such as IgG1 (e.g. S298A/E333/K334A, S239D/I332E/A330L or G236A/S239D/A330L/I332E) (Lazar et al. 2006, Proc Natl Acad Sci USA 103:2005-2010; Smith et al. 2012, Proc Natl Acad Sci USA 209:6181-6). ADCP may also be increased by the introduction of specific mutations on the Fc portion of human IgG (Richards et al. 2008, Mol Cancer Ther 7:2517-27). Methods for engineering increased ADCC, CDC and ADCP activity have further been described in Saunders 2019 (Frontiers in Immunology 10:1296) and Wang et al. 2019 (Protein Cell 9:63-73).

Non-CDR or non-ISVD moieties that can be included in/comprised in/part of these SLC4A4/NBCe1-blocking compounds (such as by covalent or non-covalent linkage) further include therapeutic moieties. In an alternative, a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein is not covalently or non-covalently linked with a further therapeutic moiety but combined in any way with a further therapeutic moiety. Such exemplary therapeutic moieties are described in the “Combinations” section

hereinafter, and may be adapted to the disease or disorder that is the target of the therapy. A "therapeutic moiety" is any molecule or composition of molecules that has or may have a therapeutic effect (i.e. curative or prophylactic effect) in the context of treatment of a disease. Preferably, a therapeutically active agent is a disease-modifying agent, which can be a cytotoxic agent, such as a toxin, a cytotoxic drug, an enzyme capable of converting a prodrug into a drug, a radionuclide, or a cytotoxic cell; or which can be a non-cytotoxic agent. In particular, the therapeutic moiety is a moiety helpful in treating, curing or preventing a disease or disorder associated with SLC4A4/NBCe1. Diseases or disorders associated with SLC4A4/NBCe1 include diseases and disorders associated with aberrant SLC4A4/NBCe1 activity, and diseases and disorders which can be treated, cured or prevented by a treatment scheme including administering an SLC4A4/NBCe1-blocking or -inhibiting compound as described herein to a subject having the disease or disorder. Known such diseases and disorders include cancer, diabetes (more in particular type 2 diabetes or type 2 diabetes mellitus), cardiac ischemia, ischemic heart disease, or reperfusion injury.

In any of the above, any of the CDR regions and/or FR regions of the SLC4A4/NBCe1-blocking or -inhibiting I(S)VDs or SLC4A4/NBCe1-blocking ISVD polypeptides may be humanized (humanization process) and/or their amino acid sequence may be optimized. Humanized CDRs and/or FRs and/or I(S)VDs can be obtained in any suitable manner known. Humanized immunoglobulin single variable domains may have several advantages, such as a reduced immunogenicity, compared to the corresponding naturally occurring VHH domains. Amino acid sequence optimization may introduce one or more advantages, such as increased melting temperature and/or increased chemical stability, compared to the starting amino acid sequence. Such humanization generally involves replacing one or more amino acid residues in the sequence of a naturally occurring CDR and/or framework region (FR) with the amino acid residues that occur at the same position in a human VH domain, such as a human VH3 domain. The humanizing and/or amino acid sequence optimizing substitutions should be chosen such that the resulting immunoglobulin domains still retain the favourable properties of the originator immunoglobulin (or further improved by e.g. affinity maturation). The skilled person will be able to select humanizing and/or amino acid sequence optimizing substitutions or suitable combinations of humanizing and/or amino acid sequence optimizing substitutions, which optimize or achieve a suitable balance between the favourable properties provided by the substitutions on the one hand and the favourable properties of naturally occurring VHH domains on the other hand. Humanized and/or functional variants can be obtained such as based on primary sequence alignment with the human IGHv3 coding sequence, to substitute one or more key residues of the alpaca/llama-derived framework regions of the VHHs, followed by biophysical analysis of the resulting VHHs after recombinant production.

Specifically, the stability and SLC4A4/NBCe1-inhibiting properties of any of the resulting VHHs are analysed. Moreover, said original and/or variant sequence can be fused directly or via a linker, as to provide for (humanized and/or optimized) bivalent VHH variants, as tandem repeats, or head-to-tail fusion, as interchangeably used herein. Alternatively, they may be additionally fused to an Fc tail, more specifically a human IgG1 Fc. In general, the specificity of binding to the target is not significantly (negatively) affected by the humanization/optimization process and, in general, the affinity and/or avidity of binding to the target is not significantly (negatively) affected by the humanization/optimization process. More in particular, the SLC4A4/NBCe1-inhibiting activity is not significantly (negatively) affected by the humanization/optimization process. Example 19 provides the experimental layout and results of such humanization/optimization process as applied to and starting from some of the SLC4A4/NBCe1-inhibiting or -blocking ISVDs as characterized herein.

A further aspect of this disclosure relates to isolated nucleic acids encoding at least one CDR and, optionally, at least one FR of a SLC4A4/NBCe1-inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide as described herein; or encoding a SLC4A4/NBCe1-inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide as described herein; and relates to vectors comprising such nucleic acid; and relates to host cells comprising such nucleic acid or vector, to host cells expressing at least one CDR and, optionally, at least one FR of a SLC4A4/NBCe1-inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide as described herein, and to host cells expressing a SLC4A4/NBCe1-inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide as described herein.

A further aspect of this disclosure relates to compositions, in particular pharmaceutical compositions, comprising a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein as active ingredient. Such pharmaceutical compositions comprise a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein formulated in a carrier (a substrate for delivery of the active ingredient such as to improve selectivity, effectiveness and/or safety of the medicament or composition comprising the active ingredient), diluent (such as a solvent used for dissolution if required; any composition for diluting a concentrated medicament or composition comprising the active ingredient) or excipient (constituent of a medicament or composition other than the active ingredient), in particular a “pharmaceutically acceptable carrier”, “pharmaceutically acceptable diluent” or “pharmaceutically acceptable excipient”. The carrier, diluent, or excipient is a suitable one, meaning it being chemically compatible with the active ingredient and biologically compatible with administration to a subject, e.g. is not toxic or not eliciting an immune or other unwanted response in the subject. On the other hand, the carrier, diluent, or

excipient may function in e.g. stabilizing or solubilizing the SLC4A4/NBCe1-blocking or -inhibiting compound as described herein.

In a further aspect, this disclosure relates to methods for producing a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein, such methods comprising the steps of:

- expressing the SLC4A4/NBCe1-blocking or -inhibiting protein moiety (comprising or consisting of at least one CDR and, optionally, at least one FR of a SLC4A4/NBCe1-inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide described herein; or comprising or consisting of a SLC4A4/NBCe1-inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide described herein) in a suitable host cell (such as comprising a nucleic acid or vector as described herein, or synthetic manufacture of the SLC4A4/NBCe1-blocking or -inhibiting protein moiety; and
- purifying the expressed or synthesized/manufactured SLC4A4/NBCe1-blocking or -inhibiting protein moiety.

Such methods may further comprise a step of coupling, incorporating, binding, ligating, bonding, complexing, chelating, conjugating (e.g. site-specifically conjugating) or otherwise linking, covalently or non-covalently, a further moiety to the SLC4A4/NBCe1-blocking or -inhibiting protein moiety, such as to produce the SLC4A4/NBCe1-blocking or -inhibiting compound comprising at least one CDR and, optionally, at least one FR of a SLC4A4/NBCe1-inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide as described herein; or comprising a SLC4A4/NBCe1-inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide as described herein.

A further aspect of this disclosure relates to a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein for use as medicament, or for use in the manufacture of a medicament. Alternatively, this disclosure relates to methods of treating or inhibiting a disease or disorder, or of inhibiting progression or relapse of a disease or disorder, in a subject, individual or patient (in particular a mammalian subject or mammal, such as a human subject or human), such methods comprising administering a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein to the subject or individual. The administration of the SLC4A4/NBCe1-blocking or -inhibiting compound as described herein, such as a therapeutically effective amount of such SLC4A4/NBCe1-blocking or -inhibiting compound, to the subject, individual or patient results in the treatment or inhibition of the disease or disorder, or in inhibition of the progression or relapse of the disease or disorder.

In one embodiment thereto, the SLC4A4/NBCe1-blocking or -inhibiting compound as described herein is, or is for use in, combined therapy; or is used in, or is for use in (a) combination therapy together with

another therapeutic moiety useful in treating or inhibiting the disease or disorder, or useful in inhibiting progression or relapse of the disease or disorder.

In one further embodiment to the above aspect and embodiment, the disease or disorder is cancer, cardiac ischemia, ischemic heart disease, reperfusion injury, or diabetes.

In one further embodiment to the above aspect and embodiments, the SLC4A4/NBCe1-blocking or -inhibiting compound is more in particular a composition comprising the SLC4A4/NBCe1-blocking or -inhibiting compound. In particular, such composition is a pharmaceutical composition.

In one further embodiment to the above aspect and embodiments, the SLC4A4/NBCe1-blocking or -inhibiting compound is more in particular an isolated nucleic acid as defined hereinabove, a vector as defined hereinabove, or a host cell as defined hereinabove.

A further aspect of this disclosure relates to a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein for use in treating or inhibiting a disease or disorder associated with SLC4A4/NBCe1, or for use in inhibiting progression or relapse of a disease or disorder associated with SLC4A4/NBCe1. Alternatively, the disclosure relates to use of a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein in the manufacture of a medicine or medicament for treating or inhibiting a disease or disorder associated with SLC4A4/NBCe1, or for use in inhibiting progression or relapse a disease or disorder associated with SLC4A4/NBCe1. Further alternatively, this disclosure relates to methods of treating or inhibiting a disease or disorder associated with SLC4A4/NBCe1, or of inhibiting progression or relapse of a disease or disorder associated with SLC4A4/NBCe1, in a subject, individual or patient (in particular a mammalian subject or mammal, such as a human subject or human), such methods comprising administering a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein to the subject or individual. The administration of the SLC4A4/NBCe1-blocking or -inhibiting compound as described herein, such as a therapeutically effective amount of such SLC4A4/NBCe1-blocking or -inhibiting compound, to the subject, individual or patient results in the treatment or inhibition of the disease or disorder associated with SLC4A4/NBCe1, or in inhibition of the progression or relapse of the disease or disorder associated with SLC4A4/NBCe1.

In one embodiment thereto, the SLC4A4/NBCe1-blocking or -inhibiting compound as described herein is combined or used in (a) combination therapy together with another therapeutic moiety useful in treating or inhibiting the disease or disorder associated with SLC4A4/NBCe1, or useful in inhibiting progression or relapse of the disease or disorder associated with SLC4A4/NBCe1.

In one further embodiment to the above aspect and embodiment, the disease or disorder is cancer, cardiac ischemia, ischemic heart disease, reperfusion injury, or diabetes.

In one further embodiment to the above aspect and embodiments, the SLC4A4/NBCe1-blocking or -inhibiting compound is more in particular a composition comprising the SLC4A4/NBCe1-blocking or -inhibiting compound. In particular, such composition is a pharmaceutical composition.

In one further embodiment to the above aspect and embodiments, the SLC4A4/NBCe1-blocking or -inhibiting compound is more in particular an isolated nucleic acid as defined hereinabove, a vector as defined hereinabove, or a host cell as defined hereinabove.

A further aspect of this disclosure relates to a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein for use in treating or inhibiting cancer or a tumor, or for use in inhibiting progression, relapse or metastasis of cancer or of a tumor. Alternatively, the disclosure relates to use of a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein in the manufacture of a medicine or medicament for treating or inhibiting cancer or a tumor, or for use in inhibiting progression, relapse or metastasis of cancer or a tumor. Further alternatively, the invention relates to methods of treating or inhibiting cancer or a tumor, or of inhibiting progression, relapse or metastasis of cancer or a tumor, in a subject, individual or patient (in particular a mammalian subject or mammal, such as a human subject or human), such methods comprising administering a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein to the subject or individual. The administration of the SLC4A4/NBCe1-blocking or -inhibiting compound as described herein, such as a therapeutically effective amount of such SLC4A4/NBCe1-blocking or -inhibiting compound, to the subject, individual or patient results in the treatment or inhibition of cancer or tumor growth, or in inhibition of the progression, relapse or metastasis of cancer or tumor growth.

In one embodiment to this aspect, the SLC4A4/NBCe1-blocking or -inhibiting compound as described herein is, or is for use in, combined therapy; or is used in, or is for use in (a) combination therapy together with another therapeutic moiety useful in treating or inhibiting cancer or a tumor, or for use in inhibiting progression, relapse or metastasis of cancer or of a tumor. In particular, the other or further therapeutic moiety is a chemotherapeutic moiety (see further hereinafter under the section “Combinations”).

In another embodiment to this aspect, the cancer or tumor is poorly responding to or is resistant to immunotherapy, or is poorly responding to, or is resistant to treatment or therapy comprising immunotherapy. In particular, the immunotherapy is a treatment or therapy with an immune checkpoint inhibitor, or a treatment or therapy comprising an immune checkpoint inhibitor. Poor response or resistance to immunotherapy (such as checkpoint inhibitor therapy) is herewith understood as either non-response (NR) or partial response (PR) to the immunotherapy (such as immune checkpoint inhibitor therapy), in particular to a therapy consisting of administration of immunotherapy only, or in particular to a therapy comprising administration of an immunotherapeutic compound or agent. When such

therapy is comprising administration of an immunotherapeutic compound or agent, it is in particular not comprising, or is excluding, administration of the SLC4A4/NBCe1-blocking or -inhibiting compound as described herein. The poor response, resistance, non-response or partial response in particular may be based on clinical experience or observation and/or may be based on analysis of biomarkers that are predictive or prognostic for efficacy of immune therapy (such as immune checkpoint inhibitor treatment or therapy). Such biomarkers may be analyzed in tumor tissue (e.g. tumor biopsy) or in a liquid biopsy taken e.g. from the patient's circulation (e.g. cfDNA, ctDNA, circulating cancer cells, exosomes, serum proteins...). Cancer immunotherapy has provided patients with a promising treatment option. Therapeutic regimens such as adoptive T cell transfer (ACT), cancer vaccines and immune checkpoint inhibitors (*e.g.* anti-PD-1, anti-PDL1 or anti-CTLA-4 antibodies), harness the ability of the immune system to recognize and reject the tumor (Smyth et al. 2015, Nat Rev Clin Oncol 13:143-158). However, despite high response rates, with prolonged survival in a subset of melanoma (e.g. Schadendorf et al. 2015, J Clin Oncol 33:1889-189), lung (e.g. Borghaei et al. 2015, N Engl J Med 373:1627-1639), and renal cancer patients (e.g. Motzer et al. 2015, N Engl J Med 373:1803-1813), for several other tumors such as mismatch repair (MMR)-proficient colorectal cancer (CRC) (e.g. Le et al. 2015, N Engl J Med 372: 1509-2520) and pancreatic ductal adenocarcinoma (PDAC) (e.g. Sarantis et al. 2020, World J Gastrointest Oncol 12: 173-181) immunotherapy fails to show any clinical benefit.

In one further embodiment to the above aspect and embodiments, the SLC4A4/NBCe1-blocking or -inhibiting compound is more in particular an isolated nucleic acid as defined hereinabove, a vector as defined hereinabove, or a host cell as defined hereinabove.

In yet a further aspect, this disclosure relates to a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein for use in treating or inhibiting cancer or for use in inhibiting progression, relapse or metastasis of cancer, in combination with immunotherapy; or wherein the treatment or inhibition further comprises therapy with or administration of an immunotherapy/ immunotherapeutic compound or agent; or wherein the treatment or inhibition is combined with a therapy comprising an immunotherapy or with administration of an immunotherapy/ immunotherapeutic compound or agent (to the subject, individual or patient having the cancer or tumor).

Alternatively, this disclosure relates to use of a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein in the manufacture of a medicament for use in combination with (administration of) an immunotherapy/ immunotherapeutic compound or agent for treating or inhibiting cancer or for inhibiting progression, relapse or metastasis of cancer (in a subject, individual or patient having the cancer or tumor); or wherein the treatment or inhibition further comprises therapy with or administration of an immunotherapy/ immunotherapeutic compound or agent; or wherein the

treatment or inhibition is combined with a therapy comprising an immunotherapy or with administration of an immunotherapy/ immunotherapeutic compound or agent (to the subject, individual or patient having the cancer or tumor).

Alternatively, this disclosure relates to use of a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein in the manufacture of a medicament for treating or inhibiting cancer or for inhibiting progression, relapse or metastasis of cancer (in a subject, individual or patient having the cancer) in combination with an immunotherapy (for treating or inhibiting cancer or for inhibiting progression, relapse or metastasis of cancer); or in combination with administering an immunotherapy/ immunotherapeutic compound or agent to the subject, individual or patient; or wherein the treatment or inhibition further comprises therapy with or administration of an immunotherapy/ immunotherapeutic compound or agent; or wherein the treatment or inhibition is combined with a therapy comprising an immunotherapy or with administration of an immunotherapy/ immunotherapeutic compound or agent (to the subject, individual or patient having the cancer or tumor).

In one further embodiment to the above aspect and embodiments, the SLC4A4/NBCe1-blocking or -inhibiting compound is more in particular an isolated nucleic acid as defined hereinabove, a vector as defined hereinabove, or a host cell as defined hereinabove.

In an alternative aspect, this disclosure relates to an immunotherapy for use in treating or inhibiting cancer or for use in inhibiting progression, relapse or metastasis of cancer, in combination with (administration of) a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein; or wherein the treatment or inhibition further comprises therapy with or administration of an inhibitor of SLC4A4/NBCe1; or wherein the treatment or inhibition is combined with a therapy comprising a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein or with administration of a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein (to the subject, individual or patient having the cancer or tumor).

Alternatively, this disclosure relates to use of an immunotherapeutic compound or agent in the manufacture of a medicament for treating or inhibiting cancer or for inhibiting progression, relapse or metastasis of cancer (in a subject, individual or patient having the cancer) in combination with a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein (for treating or inhibiting cancer or for inhibiting progression, relapse or metastasis of cancer); or in combination with administering a SLC4A4-blocking or -inhibiting compound as described herein to the subject, individual or patient; or wherein the treatment or inhibition further comprises therapy with or administration of a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein; or wherein the treatment or

inhibition is combined with a therapy comprising a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein or with administration of a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein (to the subject, individual or patient having the cancer or tumor).

In one further embodiment to the above aspect and embodiments, the SLC4A4/NBCe1-blocking or -inhibiting compound is more in particular an isolated nucleic acid as defined hereinabove, a vector as defined hereinabove, or a host cell as defined hereinabove.

In another alternative aspect, this disclosure relates to a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein and an immunotherapy for use in treating or inhibiting cancer or for use in inhibiting progression, relapse or metastasis of cancer. Alternatively, the invention relates to use of a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein and (use of) an immunotherapy/ immunotherapeutic compound or agent in the manufacture of a medicament for use in treating or inhibiting cancer or for inhibiting progression, relapse or metastasis of cancer (in a subject, individual or patient having the cancer).

In one further embodiment to the above aspect and embodiments, the SLC4A4/NBCe1-blocking or -inhibiting compound is more in particular an isolated nucleic acid as defined hereinabove, a vector as defined hereinabove, or a host cell as defined hereinabove.

A further aspect of this disclosure relates to a method for treating or inhibiting cancer, or a method for inhibiting progression, relapse or metastasis of cancer, in a subject, individual or patient (in particular a mammalian subject or mammal, such as a human subject or human), the methods comprising administering a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein and administering an immunotherapy/ immunotherapeutic compound or agent to the subject, individual or patient. By administering the SLC4A4/NBCe1-blocking or -inhibiting compound as described herein and the immunotherapy/ immunotherapeutic compound or agent, the cancer is treated or inhibited, or the progression, relapse or metastasis of the cancer is inhibited. In particular, an effective amount of the SLC4A4/NBCe1-blocking or -inhibiting compound as described herein and of the immunotherapy/immunotherapeutic compound or agent is administered to the subject, individual or patient; or an effective amount of a combination (in any way) of the SLC4A4/NBCe1-blocking or -inhibiting compound as described herein and of the immunotherapy/immunotherapeutic compound or agent is administered to the subject, individual or patient.

In one further embodiment to the above aspect and embodiments, the SLC4A4/NBCe1-blocking or -inhibiting compound is more in particular an isolated nucleic acid as defined hereinabove, a vector as defined hereinabove, or a host cell as defined hereinabove.

In any of the above tumor- or cancer-related aspects and embodiments, the tumor or cancer may be carcinoma, lymphoma, leukemia, blastoma, and sarcoma. More particular examples of such cancers include squamous cell carcinoma, myeloma, small-cell lung cancer, non-small cell lung cancer, glioma, hepatocellular carcinoma (HCC), Hodgkin's lymphoma, non-Hodgkin's lymphoma, acute myeloid leukemia (AML), anaplastic large cell lymphoma (ALCL), cutaneous T-cell lymphoma (CTCL), Adult T-cell leukemia/lymphoma (ATLL), multiple myeloma, gastrointestinal (tract) cancer, renal cancer, ovarian cancer, liver cancer, lymphoblastic leukemia, lymphocytic leukemia, colorectal cancer, endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, melanoma, chondrosarcoma, neuroblastoma, pancreatic cancer, glioblastoma multiforme, cervical cancer, brain cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma and head and neck cancer. In particular, the tumor or cancer may be a solid tumour. Examples of solid tumours are sarcomas (including cancers arising from transformed cells of mesenchymal origin in tissues such as cancellous bone, cartilage, fat, muscle, vascular, hematopoietic, or fibrous connective tissues), carcinomas (including tumours arising from epithelial cells), mesothelioma, neuroblastoma, retinoblastoma, etc. Tumours involving solid tumours include, without limitations, brain cancer, lung cancer, stomach cancer, duodenal cancer, esophagus cancer, breast cancer, colon and rectal cancer, renal cancer, bladder cancer, kidney cancer, pancreatic cancer, prostate cancer, ovarian cancer, melanoma, mouth cancer, sarcoma, eye cancer, thyroid cancer, urethral cancer, vaginal cancer, neck cancer, lymphoma, and the like. Furthermore in particular, the tumor or cancer may be a pancreatic tumor or cancer, a lung tumor or cancer, glioblastoma, or a colorectal tumor or cancer.

In any of the above tumor- or cancer-related aspects and embodiments, the immunotherapy in one embodiment is a treatment or therapy with an immune checkpoint inhibitor or a treatment or therapy comprising an immune checkpoint inhibitor (the immunotherapeutic compound or agent in this case thus is an immune checkpoint inhibitor). In a further embodiment, the immunotherapy is a treatment or therapy with two immune checkpoint inhibitors or a treatment or therapy comprising two immune checkpoint inhibitors (the immunotherapeutic compound or agent in this case thus is a combination of two immune checkpoint inhibitors). In a particular embodiment, said two immune checkpoint inhibitors are then chosen such that each of the two inhibitors is inhibiting a different immune checkpoint protein or a different immune checkpoint protein-ligand interaction.

In any of the above aspects and embodiments, the combination (such as in combination therapy) is in particular a combination in any way or in any appropriate way (explained in more detail hereinafter).

In any of the above aspects and embodiments, the SLC4A4/NBCe1-blocking or -inhibiting compounds (or, interchangeably, to compounds blocking or inhibiting SLC4A4/NBCe1, compounds blocking or inhibiting SLC4A4, SLC4A4-blocking or -inhibiting compounds, compounds blocking or inhibiting NBCe1, or NBCe1-blocking or -inhibiting compounds) in particular are compounds selectively, specifically, or exclusively blocking or inhibiting SLC4A4/NBCe1 activity, in particular SLC4A4/NBCe1 proton transport activity. The terms "blocking", "inhibiting", "antagonist", "blocker" or "inhibitor" of a target are used interchangeably herein when referring to the SLC4A4/NBCe1-blocking compounds as described herein. Further interchangeable alternatives for blocker etc. include repressor, suppressor, and inactivator. A blocker etc. thus refers to a molecule that decreases, blocks, inhibits, abrogates, or interferes with the function or activity of SLC4A4/NBCe1.

In general, pharmacological inhibition of a target of interest (such as inhibition of SLC4A4/NBCe1 activity) by means of an agent is inhibiting at least one of the biological activities (if more than one is known) of a target protein of interest. In particular, such pharmacological inhibitor is binding, such as specifically, selectively and/or exclusively binding to a target protein or protein of interest, or is specifically, selectively and/or exclusively inhibiting the targeted biological activity of the a target protein of interest.

Such binding may occur with high affinity although this is not an absolute requirement. The pharmacological inhibitor of a target protein or protein of interest may for instance have a binding affinity (dissociation constant) to (one of) its target of about 1000 nM or less, a binding affinity of about 100 nM or less, a binding affinity of about 50 nM or less, a binding affinity of about 10 nM or less, or a binding affinity of about 1 nM or less. Cross-reactivity of a pharmacological inhibitor with more than one protein is possible; for clinical development it can e.g. be desired to be able to test a pharmacological inhibitor in a suitable *in vitro* model or *in vivo* animal model before starting clinical testing with the same pharmacological inhibitor in a human population, which may require the pharmacological inhibitor to cross-react with the animal (or other non-human) target protein and with the orthologous human target protein (orthologous proteins are homologous proteins separated by a speciation event).

Specificity or selectivity of binding refers to the situation in which a pharmacological inhibitor is, at a certain concentration (sufficient to inhibit the target protein or protein of interest) binding to the target protein with higher affinity (e.g. at least 2-fold, 5-fold, or at least 10-fold higher affinity, e.g. at least 20-, 50- or 100-fold or more higher affinity) than the affinity with which it is possibly (if at all) binding to other proteins (proteins not of interest). Such specificity or selectivity of binding is in particular determined within the setting of the target subject (e.g. human patient, or animal model) and thus can encompass/does not exclude binding to (at least one) orthologous target proteins. Exclusivity of binding

refers to the situation in which a pharmacological inhibitor is binding only to the target protein of interest (and possibly to (at least one) orthologous target protein).

Alternatively, the pharmacological inhibitor may exert the desired level of inhibition of the targeted biological activity or biological activity of interest of a target protein or protein of interest with an IC50 of 1000 nM or less, with an IC50 of 500 nM or less, with an IC50 of 100 nM or less, with an IC50 of 50 nM or less, with an IC50 of 10 nM or less, or with an IC50 of 1 nM or less.

Cross-inhibition by a pharmacological inhibitor of more than one protein is possible; for clinical development it can e.g. be desired to be able to test a pharmacological inhibitor in a suitable *in vitro* model or *in vivo* animal model before starting clinical testing with the same pharmacological inhibitor in a human population, which may require the pharmacological inhibitor to cross-inhibit the animal (or other non-human) target protein and the orthologous human target protein.

Specificity or selectivity of inhibition refers to the situation in which a pharmacological inhibitor is, at a certain concentration (sufficient to inhibit the target protein or protein of interest) inhibiting the target protein with higher efficacy (e.g. with an at least 2-fold, 5-fold, or 10-fold lower IC50, e.g. at least 20-, 50- or 100-fold or more lower IC50) than the efficacy with which it is possibly (if at all) inhibiting other proteins (proteins not of interest). Such specificity or selectivity of inhibition is in particular determined within the setting of the target subject (e.g. human patient, or animal model) and thus can encompass/does not exclude inhibition of (at least one) orthologous target proteins. Exclusivity of inhibition refers to the situation in which a pharmacological inhibitor is inhibiting only the target protein of interest (or (at least one) orthologous target protein).

Specificity or selectivity of inhibition may refer to inhibition of a single biological activity of a protein of interest (and possibly of (at least one) orthologue) if the protein of interest is known to have more than one biological activity; or may refer to inhibition of the protein of interest (and possibly of (at least one) orthologue) as such, independent of it possibly having multiple biological activities.

Exclusivity of inhibition refers to the situation in which a pharmacological inhibitor is inhibiting only a single biological activity of a protein of interest (and possibly of (at least one) orthologue) if the protein of interest is known to have more than one biological activity; or may refer to inhibition of only the protein of interest (and possibly of (at least one) orthologue) as such, independent of it possibly having multiple biological activities.

Inactivation of a process as envisaged in the current disclosure refers to different possible levels of inactivation, e.g., at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or even 100% or more if inactivation (compared to a normal situation).

Thus, in any of the above aspects and embodiments, the SLC4A4/NBCe1-blocking or -inhibiting compounds (or, interchangeably, to compounds blocking or inhibiting SLC4A4/NBCe1, compounds blocking or inhibiting SLC4A4, SLC4A4-blocking or -inhibiting compounds, compounds blocking or inhibiting NBCe1, or NBCe1-blocking or -inhibiting compounds) in particular are compounds selectively, specifically, or exclusively blocking or inhibiting SLC4A4/NBCe1 activity, in particular SLC4A4/NBCe1 proton transport activity.

In any of the above aspects and embodiments, the compound inhibiting NBCe1/SLC4A4 is selected from NBCe1/SLC4A4-inhibiting immunoglobulin single variable domain antibodies (ISVDs) or SLC4A4/NBCe1-blocking ISVD polypeptide, compounds comprising at least one CDR polypeptide of a NBCe1/SLC4A4-inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide, and compounds comprising a NBCe1/SLC4A4-inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide, wherein the ISVD or ISVD polypeptide is chosen from the ISVD polypeptides defined by SEQ ID NOs:14 to 22, 135 and 138-147 (such as for instance defined by SEQ ID NO:15, SEQ ID NO:135, SEQ ID NO:140, SEQ ID NO:141 SEQ ID NO:142, or SEQ ID NO:143, or such as for instance defined by SEQ ID NO:20, SEQ ID NO: 138, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146 or SEQ ID NO:147). In an embodiment thereto, the compounds inhibiting NBCe1/SLC4A4 are selected from nucleic acids encoding a NBCe1/SLC4A4-inhibiting immunoglobulin single variable domain antibody (ISVD) or or SLC4A4/NBCe1-blocking ISVD polypeptide, a nucleic acid encoding a compound comprising at least one CDR polypeptide of a NBCe1/SLC4A4-inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide, and nucleic acids encoding a compound comprising a NBCe1/SLC4A4-inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide; wherein the ISVD or ISVD polypeptide is chosen from the ISVD polypeptides defined by SEQ ID NOs:14 to 22, 135 and 138-147 (such as for instance defined by SEQ ID NO:15, SEQ ID NO:135, SEQ ID NO:140, SEQ ID NO:141 SEQ ID NO:142, or SEQ ID NO:143, or such as for instance defined by SEQ ID NO:20, SEQ ID NO:138, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146 or SEQ ID NO:147). More in particular, the compounds encoded by such nucleic acids are proteinaceous compounds.

In another embodiment thereto, the compounds inhibiting NBCe1/SLC4A4 are host cells comprising a nucleic acid encoding a NBCe1/SLC4A4-inhibiting immunoglobulin single variable domain antibody (ISVD) or or SLC4A4/NBCe1-blocking ISVD polypeptide, a nucleic acid encoding a compound comprising at least one CDR polypeptide of a NBCe1/SLC4A4-inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide, or a nucleic acid encoding a compound comprising a NBCe1/SLC4A4-inhibiting ISVD or SLC4A4/NBCe1-blocking ISVD polypeptide; wherein the ISVD or ISVD polypeptide is chosen from the ISVD polypeptides defined by SEQ ID NOs:14 to 22, 135 and 138-147 (such as for instance defined by SEQ ID NO:15, SEQ ID NO:135, SEQ ID NO:140, SEQ ID NO:141 SEQ ID NO:142, or SEQ ID NO:143, or such as

for instance defined by SEQ ID NO:20, SEQ ID NO:138, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146 or SEQ ID NO:147).

Methods for administering nucleic acids include methods applying non-viral (DNA or RNA) or viral nucleic acids (DNA or RNA viral vectors). Methods for non-viral gene therapy include the injection of naked DNA (circular or linear), electroporation, the gene gun, sonoporation, magnetofection, the use of oligonucleotides, lipoplexes (e.g. complexes of nucleic acid with DOTAP or DOPE or combinations thereof, complexes with other cationic lipids), dendrimers, viral-like particles, inorganic nanoparticles, hydrodynamic delivery, photochemical internalization (Berg et al. 2010, Methods Mol Biol 635:133-145) or combinations thereof.

Many different vectors have been used in human nucleic acid therapy trials and a listing can be found on http://www.abedia.com/wiley/vectors.php. Currently the major groups are adenovirus or adeno-associated virus vectors (in about 21% and 7% of the clinical trials), retrovirus vectors (about 19% of clinical trials), naked or plasmid DNA (about 17% of clinical trials), and lentivirus vectors (about 6% of clinical trials). Combinations are also possible, e.g. naked or plasmid DNA combined with adenovirus, or RNA combined with naked or plasmid DNA to list just a few. Other viruses (e.g. alphaviruses, vaccinia viruses such as vaccinia virus Ankara) are used in nucleic acid therapy and are not excluded in the context of the current invention.

Administration may be aided by specific formulation of the nucleic acid e.g. in liposomes (lipoplexes) or polymersomes (synthetic variants of liposomes), as polyplexes (nucleic acid complexed with polymers), carried on dendrimers, in inorganic (nano)particles (e.g. containing iron oxide in case of magnetofection), or combined with a cell penetrating peptide (CPP) to increase cellular uptake. Organ- or cellular-targeting strategies may also be applied to the nucleic acid (nucleic acid combined with organ- or cell-targeting moiety); these include passive targeting (mostly achieved by adapted formulation) or active targeting (e.g. by coupling a nucleic acid-comprising nanoparticle with any compound (e.g. an aptamer or antibody or antigen binding molecule) binding to a target organ- or cell-specific antigen) (e.g. Steichen et al. 2013, Eur J Pharm Sci 48:416-427).

In a further embodiment to any of the above aspects and embodiments referring to SLC4A4/NBCe1-blocking or -inhibiting ISVDs, such ISVDs are in particular obtained by immunization with DNA encoding the SLC4A4/NBCe1, more in particular with DNA encoding the SLC4A4/NBCe1 as outlined in the Examples.

Antibodies

The term "antibody" as used herein, refers to an immunoglobulin (Ig) molecule, which specifically or selectively binds with an antigen. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. Antibodies are typically tetramers of immunoglobulin molecules. The term "immunoglobulin domain" as used herein refers to a globular region of an antibody chain (such as e.g., a chain of a conventional 4-chain antibody or a chain of a heavy chain antibody), or to a polypeptide that essentially consists of such a globular region/immunoglobulin domain. Immunoglobulin domains are characterized in that they retain the immunoglobulin fold characteristic of antibody molecules, which consists of a two-layer sandwich of about seven antiparallel β-strands arranged in two β-sheets, optionally stabilized by a conserved disulphide bond.

The specificity or selectivity of an antibody/immunoglobulin/immunoglobulin domain/immunoglobulin variable domain (IVD) for an antigen is defined by the composition of the antigen-binding domains in the antibody/immunoglobulin/IVD (usually one or more of the CDRs, the particular amino acids of the antibody/immunoglobulin/IVD interacting with the antigen, and forming the paratope or antigen-binding site) and the composition of the antigen (the parts of the antigen interacting with the antibody/immunoglobulin/IVD and forming the epitope or antibody binding site). Specificity or selectivity of binding is understood to refer to a binding between an antibody/immunoglobulin/IVD with a single target molecule or with a limited number of target molecules that (happen to) share an epitope recognized by the antibody/immunoglobulin/IVD.

Affinity of an antibody/immunoglobulin/IVD for its target is a measure for the strength of interaction between an epitope on the target (antigen) and an epitope/antigen binding site in the antibody/immunoglobulin/IVD. It can be defined as:

$$K_A = \frac{[Ab\text{-}Ag]}{[Ab]\,[Ag]}$$

Wherein KA is the affinity constant, [Ab] is the molar concentration of unoccupied binding sites on the antibody/immunoglobulin/IVD, [Ag] is the molar concentration of unoccupied binding sites on the antigen, and [Ab-Ag] is the molar concentration of the antibody-antigen complex. Avidity provides information on the overall strength of an antibody/immunoglobulin/IVD-antigen complex, and generally depends on the above-described affinity, the valency of antibody/immunoglobulin/IVD and of antigen, and the structural interaction of the binding partners.

The term "immunoglobulin variable domain" (abbreviated as "IVD") as used herein means an immunoglobulin domain essentially consisting of four "framework regions" which are referred to in the

art and herein below as "framework region 1" or "FR1"; as "framework region 2" or "FR2"; as "framework region 3" or "FR3"; and as "framework region 4" or "FR4", respectively; which framework regions are interrupted by three "complementarity determining regions" or "CDRs", which are referred to in the art and herein below as "complementarity determining region 1" or "CDR1"; as "complementarity determining region 2" or "CDR2"; and as "complementarity determining region 3" or "CDR3", respectively. Thus, the general structure or sequence of an immunoglobulin variable domain can be indicated as follows: FR1–- CDR1–- FR2–- CDR2–- FR3–- CDR3–- FR4. It is the immunoglobulin variable domain(s) (IVDs) that confer specificity or selectivity to an antibody for the antigen by carrying the antigen-binding site. Methods for delineating/confining a CDR in an antibody/immunoglobulin/immunoglobulin domain/IVD have been described in the art and include the Kabat, Chothia, IMTG, Martin, Gelfand, and Honneger systems (see Dondelinger et al. 2018, Front Immunol 9:2278).

The term "immunoglobulin single variable domain" (abbreviated as "ISVD"), equivalent to the term "single variable domain", defines molecules wherein the antigen binding site is present on, and formed by, a single immunoglobulin domain. This sets immunoglobulin single variable domains apart from "conventional" immunoglobulins or their fragments, wherein two immunoglobulin domains, in particular two variable domains, interact to form an antigen binding site. Typically, in conventional immunoglobulins, a heavy chain variable domain (VH) and a light chain variable domain (VL) interact to form an antigen binding site. In this case, the complementarity determining regions (CDRs) of both VH and VL will contribute to the antigen binding site, i.e. a total of 6 CDRs will be involved in antigen binding site formation. In view of the above definition, the antigen-binding domain of a conventional 4-chain antibody (such as an IgG, IgM, IgA, IgD or IgE molecule; known in the art) or of a Fab fragment, a F(a'')2 fragment, an Fv fragment such as a disulphide linked Fv or a scFv fragment, or a diabody (all known in the art) derived from such conventional 4-chain antibody, would normally not be regarded as an immunoglobulin single variable domain, as, in these cases, binding to the respective epitope of an antigen would normally not occur by one (single) immunoglobulin domain but by a pair of (associated) immunoglobulin domains such as light and heavy chain variable domains, i.e., by a VH-VL pair of immunoglobulin domains, which jointly bind to an epitope of the respective antigen. In contrast, immunoglobulin single variable domains are capable of specifically or selectively binding to an epitope of the antigen without pairing with an additional immunoglobulin variable domain. The binding site of an immunoglobulin single variable domain is formed by a single VH/VHH or VL domain. Hence, the antigen binding site of an immunoglobulin single variable domain is formed by no more than three CDRs. As such, the single variable domain may be a light chain variable domain sequence (e.g., a VL-sequence) or a suitable fragment thereof; or a heavy chain variable domain sequence (e.g., a VH-sequence or VHH

sequence) or a suitable fragment thereof; as long as it is capable of forming a single antigen binding unit (i.e., a functional antigen binding unit that essentially consists of the single variable domain, such that the single antigen binding domain does not need to interact with another variable domain to form a functional antigen binding unit). In one embodiment of the invention, the immunoglobulin single variable domains are heavy chain variable domain sequences (e.g., a VH-sequence); more specifically, the immunoglobulin single variable domains can be heavy chain variable domain sequences that are derived from a conventional four-chain antibody or heavy chain variable domain sequences that are derived from a heavy chain antibody. For example, the immunoglobulin single variable domain may be a (single) domain antibody (or an amino acid sequence that is suitable for use as a (single) domain antibody), a "dAb" or dAb (or an amino acid sequence that is suitable for use as a dAb) or a Nanobody® (as defined herein, and including but not limited to a VHH); other single variable domains, or any suitable fragment of any one thereof. In particular, the immunoglobulin single variable domain may be a Nanobody® (as defined herein) or a suitable fragment thereof. *Note:* Nanobody®, Nanobodies® and Nanoclone® are registered trademarks of Ablynx (now part of Sanofi). For a general description of Nanobodies®, reference is made to the further description below, as well as to e.g. WO2008/020079.

"VHH domains", also known as VHHs, VHH domains, VHH antibody fragments, and VHH antibodies, have originally been described as the antigen binding immunoglobulin (variable) domain of "heavy chain antibodies" (i.e., of "antibodies devoid of light chains"; Hamers-Casterman et al. 1993, Nature 363:446-448). The term "VHH domain" has been chosen to distinguish these variable domains from the heavy chain variable domains that are present in conventional 4-chain antibodies (which are referred to herein as "VH domains") and from the light chain variable domains that are present in conventional 4-chain antibodies (which are referred to herein as "VL domains"). For a further description of VHHs and Nanobody®, reference is made to the review article by Muyldermans 2001 (Rev Mol Biotechnol 74:277-302), as well as to the following patent applications, which are mentioned as general background art: WO 94/04678, WO 95/04079, WO 96/34103; WO 94/25591, WO 99/37681, WO 00/40968, WO 00/43507, WO 00/65057, WO 01/40310, WO 01/44301, EP 1134231 and WO 02/48193; WO 97/49805, WO 01/21817, WO 03/035694, WO 03/054016 and WO 03/055527; WO 03/050531; WO 01/90190; WO 03/025020; WO 04/041867, WO 04/041862, WO 04/041865, WO 04/041863, WO 04/062551, WO 05/044858, WO 06/40153, WO 06/079372, WO 06/122786, WO 06/122787 and WO 06/122825. As described in these references, Nanobody® (in particular VHH sequences and partially humanized Nanobody®) can in particular be characterized by the presence of one or more "hallmark residues" in one or more of the framework sequences. A further description of the Nanobody®, including humanization and/or camelization of Nanobody®, as well as other modifications, parts or fragments, derivatives or "Nanobody® fusions", multivalent constructs (including some non-limiting examples of

linker sequences) and different modifications to increase the half-life of the Nanobody® and their preparations can be found in e.g. WO 08/101985 and WO 08/142164.

"Domain antibodies", also known as "Dabs" (the terms "Domain Antibodies" and "dAbs" being used as trademarks by the GlaxoSmithKline group of companies) have been described in e.g., EP 0368684, Ward et al. 1989 (Nature 341:544-546), Holt et al. 2003 (Trends in Biotechnology 21:484-490) and WO 03/002609, WO 04/068820, WO 06/030220, and WO 06/003388. Domain antibodies essentially correspond to the VH or VL domains of non-camelid mammalians, in particular human 4-chain antibodies. In order to bind an epitope as a single antigen binding domain, i.e., without being paired with a VL or VH domain, respectively, specific selection for such antigen binding properties is required, e.g. by using libraries of human single VH or VL domain sequences. Domain antibodies have, like VHHs, a molecular weight of approximately 13 to approximately 16 kDa and, if derived from fully human sequences, do not require humanization for e.g. therapeutic use in humans. It should also be noted that single variable domains can be derived from certain species of shark (for example, the so-called "IgNAR domains", see e.g. WO 05/18629).

When an Fc-region is present in an antibody (any format; the Fc-region either naturally present or introduced by means of genetic engineering), antibody-dependent cellular cytotoxicity (ADCC) can be part of the antibody's action in that when the Fc-region is capable of binding to an Fcγ receptor (FcγR or FCGR) on the surface of an immune effector cell, the cell carrying the antibody's target can be killed or destroyed. When an antibody comprises a (naturally occurring or engineered) C1q binding site, complement-dependent cytotoxicity (CDC) can be part of the antibody's action. When an antibody comprises a (naturally occurring or engineered) Fc domain capable of binding to a specific receptor on phagocytic cells, antibody-dependent cellular phagocytosis (ADCP) can be part of the antibody's action. ADCC-, CDC-, and ADCP-inducing antibodies thus are included herein as means of effectuating pharmacological inhibition of a target of interest.

Therapeutic moiety /treatment / therapeutically effective amount

The terms therapeutic moiety, therapeutic modality, therapeutic agent, and agent are used interchangeably herein. All refer to a therapeutically active compound, to a combination of therapeutically active compounds, or to a therapeutically active composition (comprising one or more therapeutically active compounds). In particular, the therapeutic moiety is different from the SLC4A4/NBCe1-blocking or -inhibiting compounds as described herein. "Treatment"/"treating" refers to any rate of reduction, delaying or retardation of the progress of the disease or disorder, or a single symptom thereof, compared to the progress or expected progress of the disease or disorder, or single symptom thereof, when left untreated. This implies that a therapeutic modality on its own may not result

in a complete or partial response (or may even not result in any response), but may, in particular when combined with other therapeutic modalities (such as, but not limited thereto: surgery, radiation, etc.), contribute to a complete or partial response (e.g. by rendering the disease or disorder more sensitive to therapy). More desirable, the treatment results In no/zero progress of the disease or disorder, or single symptom thereof (i.e. "inhibition" or "inhibition of progression"), or even in any rate of regression of the already developed disease or disorder, or single symptom thereof. "Suppression/suppressing" can in this context be used as alternative for "treatment/treating". Treatment/treating also refers to achieving a significant amelioration of one or more clinical symptoms associated with a disease or disorder, or of any single symptom thereof. Depending on the situation, the significant amelioration may be scored quantitatively or qualitatively. Qualitative criteria may e.g. by patient well-being. In the case of quantitative evaluation, the significant amelioration is typically a 10% or more, a 20% or more, a 25% or more, a 30% or more, a 40% or more, a 50% or more, a 60% or more, a 70% or more, a 75% or more, a 80% or more, a 95% or more, or a 100% improvement over the situation prior to treatment. The time-frame over which the improvement is evaluated will depend on the type of criteria/disease observed and can be determined by the person skilled in the art.

A "therapeutically effective amount" refers to an amount of a therapeutic agent to treat or prevent a disease or disorder in a subject (such as a mammal). In the case of cancers, the therapeutically effective amount of the therapeutic agent may reduce the number of cancer cells; reduce the primary tumor size; inhibit (i.e., slow down to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow down 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 any therapy, efficacy *in vivo* can, e.g., be measured by assessing the duration of survival (e.g. overall survival), time to disease progression (TTP), response rates (e.g., complete response and partial response, stable disease), length of progression-free survival (PFS), duration of response, and/or quality of life.

The term "effective amount" or "therapeutically effective amount" may depend on the dosing regimen of the agent/therapeutic agent or composition comprising the agent/therapeutic agent (e.g. medicament or pharmaceutical composition). The effective amount will generally depend on and/or will need adjustment to the mode of contacting or administration. The effective amount of the agent or composition comprising the agent is the amount required to obtain the desired clinical outcome or therapeutic effect without causing significant or unnecessary toxic effects (often expressed as maximum tolerable dose, MTD). To obtain or maintain the effective amount, the agent or composition comprising the agent may be administered as a single dose or in multiple doses. The effective amount may further

vary depending on the severity of the condition that needs to be treated; this may depend on the overall health and physical condition of the subject or patient and usually the treating doctor's or physician's assessment will be required to establish what is the effective amount. The effective amount may further be obtained by a combination of different types of contacting or administration.

The aspects and embodiments described above in general may comprise the administration of one or more therapeutic compounds to a subject (such as a mammal) in need thereof, i.e., harboring a tumor, cancer or neoplasm in need of treatment. In general a (therapeutically) effective amount of (a) therapeutic compound(s) is administered to the mammal in need thereof in order to obtain the described clinical response(s).

"Administering" means any mode of contacting that results in interaction between an agent (e.g. a therapeutic compound or immunotherapeutic compound or agent) or composition comprising the agent (such as a medicament or pharmaceutical composition) and an object (e.g. cell, tissue, organ, body lumen) with which said agent or composition is contacted. The interaction between the agent or composition and the object can occur starting immediately or nearly immediately with the administration of the agent or composition, can occur over an extended time period (starting immediately or nearly immediately with the administration of the agent or composition), or can be delayed relative to the time of administration of the agent or composition. More specifically the "contacting" results in delivering an effective amount of the agent or composition comprising the agent to the object.

Combinations

The invention further relates to combinations of a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein and a further therapeutic moiety. Alternatively, the invention relates to a combination of a composition, such as a pharmaceutically acceptable composition, comprising a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein; and of a composition, such as a pharmaceutically acceptable composition, comprising a further therapeutic moiety. In one embodiment thereto, the invention relates to a combination of a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein and a further therapeutic moiety which is an immunotherapeutic compound or agent.

The invention further relates to any composition comprising a combination of a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein and a further therapeutic moiety for use as a medicine or medicament. Alternatively, the invention relates to a medicine or medicament comprising a combination of a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein and a further therapeutic moiety. In one embodiment thereto, these combinations, compositions, medicines or medicaments are for use in treating or inhibiting a disease or disorder, or for use in inhibiting progression or relapse of a

disease or disorder. In one embodiment, the disease or disorder is cancer, diabetes, cardiac ischemia, ischemic heart disease, or reperfusion injury. In a further embodiment, the cancer is poorly responding to or resistant to immunotherapy or to therapy comprising an immunotherapeutic compound or agent. In a further embodiment, the immunotherapeutic compound or agent is an immune checkpoint inhibitor.

"Combination", "combination in any way" or "combination in any appropriate way" as referred to herein is meant to refer to any sequence of administration of two (or more) therapeutic modalities, i.e. the administration of the two (or more) therapeutic modalities can occur concurrently in time or separated from each other by any amount of time; and/or "combination", "combination in any way" or "combination in any appropriate way" as referred to herein can refer to the combined or separate formulation of the two (or more) therapeutic modalities, i.e. the two (or more) therapeutic modalities can be individually provided in separate vials or (other suitable) containers, or can be provided combined in the same vial or (other suitable) container. When combined in the same vial or (other suitable) container, the two (or more) therapeutic modalities can each be provided in the same vial/container chamber of a single-chamber vial/container or in the same vial/container chamber of a multi-chamber vial/container; or can each be provided in a separate vial/container chamber of a multi-chamber vial/container.

Further therapeutic moieties for treating pancreatic cancer include standard-of-care first line therapies such as gemcitabine and gemcitabine + Nab paclitaxel (Mohammad 2018, Oncol Rev 12:370).

Further therapeutic moieties for treating lung cancer include standard-of-care first line therapies such as cisplatin or carboplatin in combination with radiotherapy (stage III non-small-cell lung cancer; Eberhardt et al. 2015, Annals of Oncology 26: 1573–1588) and irinotecan + platinum or etoposide + platinum (small-cell lung cancer; Stahel et al. 2011, Annals of Oncology 22: 1973–1980).

Further therapeutic moieties for treating glioblastoma include therapies such as maximal surgical resection, followed by radiotherapy plus concomitant and maintenance temozolomide (Fernandes et al. 2017, Current Standards of Care in Glioblastoma Therapy. In: De Vleeschouwer S, editor. Glioblastoma [Internet]. Brisbane (AU): Codon Publications; 2017 Sep 27. Chapter 11. Available from: https://www.ncbi.nlm.nih.gov/books/NBK469987/ doi: 10.15586/codon.glioblastoma.2017.ch11).

Further therapeutic moieties for treating colorectal cancer include standard-of-care first line therapies such as capecitabine + bevacizumab, capecitabine + oxaliplatin (CAPOX), fluorouracil (plus leucovorin) + irinotecan (FOLFIRI), fluorouracil (plus leucovorin) + oxaliplatin (FOLFOX), and fluorouracil (plus leucovorin) + oxaliplatin + irinotecan (FOLFOXIRI) (metastatic colorectal cancer; De Falco et al. 2020, ESMO Open 4:e000813 and Loupakis et al. 2014, N Engl J Med 371:1609-1618).

Further therapeutic moieties for treating cardiac ischemia or ischemic heart disease include standard-of-care therapies such as anti-platelet agents, beta blockers, renin-angiotensin-aldosterone blockers, lipid-lowering drugs, nitrates, beta blockers, and calcium channel blockers (Dababneh and Goldstein 2023, Chronic Ischemic Heart Disease Selection of Treatment Modality. [Updated 2023 Feb 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK507703/).

Further therapeutic moieties for treating diabetes, in particular type 2 diabetes, include therapies such as metformin, statins, insulins (Standards of Care in Diabetes—2023, *Clin Diabetes* 2023;41:4–31), sulfonylureas (e.g. glyburide, glipizide and glimepiride), glinides (e.g. repaglinide and nateglinide), thiazolidinediones (e.g. pioglitazone), DPP-4 inhibitors (e.g. sitagliptin, saxagliptin and linagliptin), GLP-1 receptor agonists (e.g. exenatide, liraglutide, semaglutide), and SGLT2 inhibitors (e.g. canagliflozin, dapagliflozin, empagliflozin) (www.mayoclinic.org/diseases-conditions/type-2-diabetes/diagnosis-treatment/drc-20351199).

Kits

The invention further relates to kits comprising a container or vial (any suitable container or vial, such as a pharmaceutically acceptable container or vial) comprising a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein or comprising a composition comprising a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein; and optionally comprising a container or vial (any suitable container or vial, such as a pharmaceutically acceptable container or vial) comprising a further therapeutic moiety.

Alternatively, such kits are comprising a container or vial (any suitable container or vial, such as a pharmaceutically acceptable container or vial) comprising a combination of a SLC4A4/NBCe1-blocking or -inhibiting compound as described herein and a further therapeutic moiety (such as one or two immune checkpoint inhibitors) (see discussion on "combination in any way" on how such combination in a single container, e.g., vial can be defined). Other optional components of such kit include one or more diagnostic agents capable of predicting, prognosing, or determining the success of a therapy comprising one of the therapies according to the invention; use instructions; one or more containers with sterile pharmaceutically acceptable carriers, excipients or diluents [such as for producing or formulating a (pharmaceutically acceptable) composition of the invention]; one or more syringes; one or more needles; etc. In particular, such kits may be pharmaceutical kits.

Immune checkpoint inhibitors

Immune checkpoints antagonists or inhibitors as referred to herein include the cell surface protein cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed cell death protein-1 (PD-1) and their respective ligands. CTLA-4 binds to its co-receptor B7-1 (CD80) or B7-2 (CD86); PD-1 binds to its ligands PD-L1 (B7-H10) and PD-L2 (B7-DC). Other immune checkpoint inhibitors include the adenosine A2A receptor (A2AR), B7-H3 (or CD276), B7-H4 (or VTCN1), BTLA (or CD272), IDO (indoleamine 2,3-10 dioxygenase), KIR (killer-cell immunoglobulin-like receptor), LAG3 (lymphocyte activation gene-3), NOX2 (nicotinamide adenine dinucleotide phosphate (NADPH) oxidase isoform 2), TIM3 (T-cell immunoglobulin domain and mucin domain 3), VISTA (V-domain Ig suppressor of T cell activation), SIGLEC7 (sialic acid-binding immunoglobulin-type lectin 7, or CD328) and SIGLEC9 (sialic acid-binding immunoglobulin-type lectin 9, or CD329).

In any of the above aspects, embodiments, and kits, the further therapeutic moiety can in particular be a combination in any way of two immune checkpoint inhibitors. In one embodiment these are each inhibiting a different immune checkpoint or a different immune checkpoint-ligand interaction. For instance, when an inhibitor of PD1 is selected as a first immune checkpoint inhibitor, the second immune checkpoint inhibitor could be an inhibitor of PDL1 or an inhibitor of PDL2. Such first and second immune checkpoint inhibitor are each inhibiting a different immune checkpoint protein. In a further non-limiting example, an inhibitor of PD1 is selected as a first immune checkpoint inhibitor, and as second immune checkpoint inhibitor an inhibitor different from an inhibitor of PDL1 and different from an inhibitor of PDL2 is selected, e.g. an inhibitor of CTLA-4 is selected. In this latter example, the first and second immune checkpoint inhibitor are not only each inhibiting a different immune checkpoint, but also each inhibiting a different immune checkpoint-ligand interaction.

An overview of clinical developments in the field of immune checkpoint therapy is given by Fan et al. 2019 (Oncology Reports 41:3-14). Immune checkpoint inhibitors include, but are not limited to anti-PD-1, anti-PD-L1 or anti-CTLA-4 antibodies.

PD1

Aliases of PD1 provided in GeneCards® include PDCD1; Programmed Cell Death 1; Systemic Lupus Erythematosus Susceptibility 2; PD-1; CD279; HPD-1; SLEB2; and HPD-L. The genomic locations for the PDCD1 gene are *chr2:241,849,881-241,858,908 (in* GRCh38/hg38) and *chr2:242,792,033-242,801,060 (in* GRCh37/hg19). The GenBank reference PD1 mRNA sequence is known under accession no. NM_005018.3. Approved PD1-inhibiting antibodies include nivolumab, pembrolizumab, and cemiplimab; PD1-inhibiting antibodies under development include CT-011 (pidilizumab) and therapy

with PD1-inhibiting antibodies is referred to herein as α-PD-1 therapy or α-PD1 therapy. PD1 siRNA and shRNA products are available through e.g. Origene.

PD-L1

Aliases of PD-L1 provided in GeneCards® include CD274, Programmed Cell Death 1 Ligand 1, B7 Homolog 1, B7H1, PDL1, PDCD1 Ligand 1, PDCD1LG1, PDCD1L1, HPD-L1, B7-H1, B7-H, and Programmed Death Ligand 1. The genomic locations for the PDCD1 gene are chr9:5,450,503-5,470,567 (in GRCh38/hg38) and chr9:5,450,503-5,470,567 (in GRCh37/hg19). The GenBank reference PD1 mRNA sequence is known under accession no. NM_001267706.1, NM_001314029.2 and NM_014143.4. Approved PD-L1-inhibiting antibodies include atezolizumab, avelumab, and durvalumab. PD-L1 siRNA and shRNA products are available through e.g. Origene.

CTLA4

Aliases of CTLA4 provided in GeneCards® include Cytotoxic T-Lymphocyte Associated Protein 4; CTLA-4; CD152; Insulin-Dependent Diabetes Mellitus 12; Cytotoxic T-Lymphocyte Protein 4; Celiac Disease 3; GSE; Ligand And Transmembrane Spliced Cytotoxic T Lymphocyte Associated Antigen 4; Cytotoxic T Lymphocyte Associated Antigen 4 Short Spliced Form; Cytotoxic T-Lymphocyte-Associated Serine Esterase-4; Cytotoxic T-Lymphocyte-Associated Antigen 4; CELIAC3; IDDM12; ALPS5; and GRD4.

The genomic locations for the CTLA4 gene are *chr2:203,867,771-203,873,965 (in* GRCh38/hg38) and *chr2:204,732,509-204,738,683 (in* GRCh37/hg19). The GenBank reference CTLA4 mRNA sequences are known under accession nos. NM_001037631.3 and NM_005214.5. Approved CTLA4-inhibiting antibodies include ipilumab; CTLA4-inhibiting antibodies under development include tremelimumab; therapy with CTLA4-inhibiting antibodies is referred to herein as α-CTLA4 therapy. CTLA4 siRNA and shRNA products are available through e.g. Origene.

Immunotherapy / immunotherapeutic compound or agent

Immunotherapy in general is defined as a treatment comprising administration of an immunotherapeutic compound or agent that supports (including activation or reactivation) the body's own immune system to help fight a disease, more specifically cancer in the context of the current invention. Immunotherapeutic treatment as used herein refers to the reactivation and/or stimulation and/or reconstitution of the immune response of a mammal towards a condition such as a tumor, cancer or neoplasm evading and/or escaping and/or suppressing normal immune surveillance. The reactivation and/or stimulation and/or reconstitution of the immune response of a mammal in turn in part results in an Increase in elimination of tumorous, cancerous or neoplastic cells by the mammal's immune system (anticancer, antitumor or anti-neoplasm immune response; adaptive immune response to the tumor, cancer or neoplasm).

Immunotherapeutic agents include antibodies, in particular monoclonal antibodies, employed as (targeted) anti-cancer agents include alemtuzumab (chronic lymphocytic leukemia), bevacizumab (colorectal cancer), cetuximab (colorectal cancer, head and neck cancer), denosumab (solid tumor`s bony metastases), gemtuzumab (acute myelogenous leukemia), ipilumab (melanoma), ofatumumab (chronic lymphocytic leukemia), panitumumab (colorectal cancer), rituximab (Non-Hodgkin lymphoma), tositumomab (Non-Hodgkin lymphoma) and trastuzumab (breast cancer). Other antibodies include for instance abagovomab (ovarian cancer), adecatumumab (prostate and breast cancer), afutuzumab (lymphoma), amatuximab, apolizumab (hematological cancers), blinatumomab, cixutumumab (solid tumors), dacetuzumab (hematologic cancers), elotuzumab (multiple myeloma), farletuzumab (ovarian cancer), intetumumab (solid tumors), muatuzumab (colorectal, lung and stomach cancer), onartuzumab, parsatuzumab, pritumumab (brain cancer), tremelimumab, ublituximab, veltuzumab (non-Hodgkin's lymphoma), votumumab (colorectal tumors), zatuximab and anti-placental growth factor antibodies such as described in WO 2006/099698.

Immunotherapeutic agents of particular interest further include immune checkpoint inhibitors (such as anti-PD-1, anti-PD-L1 or anti-CTLA-4 antibodies; detailed hereinafter), bispecific antibodies bridging a cancer cell and an immune cell, dendritic cell vaccines, CAR-T cells, oncolytic viruses, RNA vaccines, and so on. Immunotherapy is a promising new area of cancer therapeutics and several immunotherapies are being evaluated preclinically as well as in clinical trials and have demonstrated promising activity (Callahan et al. 2013, J Leukoc Biol 94:41-53; Page et al. 2014, Annu Rev Med 65:185-202). However, not all the patients are sensitive to immune checkpoint blockade and sometimes PD-1 or PD-L1 blocking antibodies accelerate tumor progression. An overview of clinical developments in the field of immune checkpoint therapy is given by Fan et al. 2019 (Oncology Reports 41:3-14). Combinatorial cancer treatments that include chemotherapies can achieve higher rates of disease control by impinging on distinct elements of tumor biology to obtain synergistic antitumor effects. It is now accepted that certain chemotherapies can increase tumor immunity by inducing immunogenic cell death and by promoting escape in cancer immunoediting, such therapies are therefore called immunogenic therapies as they provoke an immunogenic response. Drug moieties known to induce immunogenic cell death include bleomycin, bortezomib, cyclophosphamide, doxorubicin, epirubicin, idarubicin, mafosfamide, mitoxantrone, oxaliplatin, and patupilone (Bezu et al. 2015, Front Immunol 6:187). Other forms of immunotherapy include chimeric antigen receptor (CAR) T-cell therapy in which allogenic T-cells are adapted to recognize a tumoral neo-antigen and oncolytic viruses preferentially infecting and killing cancer cells. Treatment with RNA, e.g. encoding MLKL, is a further means of provoking an immunogenic response (Van Hoecke et al. 2018, Nat Commun 9:3417), as well as vaccination with neo-epitopes (Brennick et al. 2017, Immunotherapy 9:361-371).

Further anti-tumor agents include those described in general terms in the sections “Inhibition of a target of interest”, and “Pharmacological knock-down of a protein of interest” included herein, and wherein the target or protein of interest can be any known anti-cancer target or protein.

SLC4A4

Aliases of SLC4A4 provided in GeneCards® include Solute Carrier Family 4 Member 4; NBC1; Solute Carrier Family 4 (Sodium Bicarbonate Cotransporter), Member 4; Electrogenic Sodium Bicarbonate Cotransporter 1; Na(+)/HCO3(-) Cotransporter; HNBC1; HhNMC; KNBC1; PNBC; NBCe1-A; NBCE1; KNBC; and NBC. The genomic locations for the SLC4A4 gene are *chr4:71,062,646-71,572,087 (in* GRCh38/hg38) and *chr4:72,053,003-72,437,804 in* GRCh37/hg19). The GenBank reference SLC4A4 mRNA sequences are known under accession nos. NM_001098484.3, NM_001134742.2, and NM_003759.4. SLC4A4 human shRNA lentiviral particles are offered for sale by e.g. Origene. Other SLC4A4 siRNA and shRNA products are available through e.g. Santa Cruz Biotechnology.

Other Definitions

The present invention is described with respect to particular embodiments and with reference to certain drawings but the invention is not limited thereto but only by the claims. Any reference signs in the claims shall not be construed as limiting the scope. The drawings described are only schematic and are non-limiting. In the drawings, the size of some of the elements may be exaggerated and not drawn on scale for illustrative purposes. Where the term “comprising” is used in the present description and claims, it does not exclude other elements or steps. Where an indefinite or definite article is used when referring to a singular noun e.g. “a" or "an", "the”, this includes a plural of that noun unless something else is specifically stated. Furthermore, the terms first, second, third and the like in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein. Unless specifically defined herein, all terms used herein have the same meaning as they would to one skilled in the art of the present invention. Practitioners are particularly directed to Sambrook et al., Molecular Cloning: A Laboratory Manual, ^{4t}h ed., Cold Spring Harbor Press, Plainsview, New York (2012); and Ausubel et al., current Protocols in Molecular Biology (Supplement 100), John Wiley & Sons, New York (2012), for definitions and terms of the art. The definitions provided herein should not be construed to have a scope less than understood by a person of ordinary skill in the art.

The term "defined by SEQ ID NO:X" as used herein refers to a biological sequence consisting of the sequence of amino acids or nucleotides given in the SEQ ID NO:X. For instance, an polypeptide defined in/by SEQ ID NO:X consists of the amino acid sequence given in SEQ ID NO:X. A further example is an amino acid sequence comprising SEQ ID NO:X, which refers to an amino acid sequence longer than the amino acid sequence given in SEQ ID NO:X but entirely comprising the amino acid sequence given in SEQ ID NO:X (wherein the amino acid sequence given in SEQ ID NO:X can be located N-terminally or C-terminally in the longer amino acid sequence, or can be embedded in the longer amino acid sequence), or to an amino acid sequence consisting of the amino acid sequence given in SEQ ID NO:X.

It is to be understood that although particular embodiments, specific configurations as well as materials and/or molecules, have been discussed herein for cells and methods according to the present invention, various changes or modifications in form and detail may be made without departing from the scope and spirit of this invention. The following examples are provided to better illustrate particular embodiments, and they should not be considered limiting the application. The application is limited only by the claims

In referring to genes or proteins herein, no distinction is made in the annotation. Thus, whereas for example the human Slc4A4 gene would be referred to as the *SLC4A4 or NBCe1* gene, the mRNA as *SLC4A4 or NBCe1* mRNA, and the protein as SLC4A4 or NBCe1, such distinction is not, or not always, made hereinabove or hereinafter.

The content of the documents cited herein are incorporated by reference.

EXAMPLES

EXAMPLE 1. Generation of stable SLC4A4 cell lines

1.1. Constitutively expressing HEK cell lines

The human cell line HEK-293T (ATCC CRL-3216) was cultured at 37 °C and 5% CO_2 in DMEM (Thermo Scientific 11965092) supplemented with 10% heat-inactivated FBS (Thermo Scientific 10500064), 1 mM sodium pyruvate (Thermo Scientific 11360070), 1 mM Glutamax (Thermo Scientific 35050-061) and 100 U/mL penicillin and streptomycin (Sigma Aldrich P0781). This medium will be referred to as 'full DMEM' further herein. Before transfection, cells were seeded at a density of 5 x 10^5 cells per well in 6-well plates and cultured overnight. The next day, cells were transfected with 2 µg of either the human or mouse pancreatic isoform of SLC4A4 in the pCDNA3.1 Hygro vector (constructs # 1 and 2 in Table 1), using FUGENE HD transfection reagent (Promega E2312).

Table 1.

#	Construct	Vector	Remark
1	3x HA-Human pancreatic SLC4A4	pCDNA3.1 Hygro	Triple HA-tagged human NBCe1-B; GenBank accession no. NP_001091954
2	3x HA-Mouse pancreatic SLC4A4	pCDNA3.1 Hygro	Triple HA-tagged mouse NBCe1-B; GenBank accession no. NP_061230

SEQ ID NO:	identifier	amino acid sequence
1	3xHA	GYPYDVPDYAGYPYDVPDYAGYPYDVPDYA
2	HIS6	HHHHHH

After 6 hours, supernatants were removed and replaced by fresh complete DMEM. After 48 hours, culture medium was replaced to include 125 µg/ml Hygromycin B (Merck 10843555001) to select for cells harboring the expression cassette. Medium was changed every 2-3 days and after approximately 3 weeks, limiting dilutions were made to obtain monoclonal lines, which were maintained at 80 µg/ml Hygromycin.

Identification of human or mouse SLC4A4-overexpressing monoclonal lines was based on acquiring at least 10^4 cells in flow cytometry (Attune NxT, Thermo Scientific) using anti-HA detection (no suitable anti-SLC4A4 commercial antibody was identified) on fixed and permeabilized cells. Prior to the primary antibody incubation, cells were fixed and permeabilized (or not, for the control population) following the manufacturer's instructions for the Cell Permeabilization Kit (Thermo Fisher GAS003), thereby allowing (or not) detection of the N-terminal, intracellular HA-tag on the expression constructs.

In total, 11 human SLC4A4 expressing clones and 7 mouse SLC4A4 expressing clones were analyzed. Based on histogram shape and median fluorescence intensity (MFI) values, human clone 9 (MFI value of 24974) and mouse clones 1 (MFI value of 17599) and 4 (MFI value of 19061) were selected for further experiments (background MFI values are about 150).

1.2. Inducible HEK cell lines

The human cell line HEK293 Flp-In T-Rex (Life Technologies K6500-01) was cultured at 37 °C and 5% CO_2 in full medium containing 100 µg/mL zeocin and 15 µg/mL blasticidin.

Cells were seeded at a density of 4 x 10^5 cells per well in 6-well plates in medium without zeocin, and allowed to attach for 4-6 hours. Cells were then co-transfected with one of the constructs # 5 to #9 (Table 2) and the flp recombinase vector pOG44, using FUGENE HD transfection reagent.

Table 2.

#	Construct	Vector	Remark
5	Rhesus pancreatic SLC4A4 (SEQ ID NO:116)	pcDNA5_FRT_TO	Macaca mulatta NBCe1-B; GenBank accession no. XP_014994048 ; tet-inducible*

#	Construct	Vector	Remark
6	Cyno pancreatic SLC4A4 (SEQ ID NO:117)	pcDNA5_FRT_TO	Macaca fascicularis NBCe1- B; GenBank accession no. XP_00555516 8; tet-inducible*
7	Human pancreatic SLC4A4 (SEQ ID NO:114)	pcDNA5_FRT_TO	Human NBCe1-B; tet-inducible*
8	Human kidney SLC4A4 (SEQ ID NO:118)	pcDNA5_FRT_TO	Human NBCe1-A; GenBank accession no. NP_003750; tet-inducible*
9	Mouse pancreatic SLC4A4 (SEQ ID NO:115)	pcDNA5_FRT_TO	Mouse NBCe1-B; tet-inducible*

*tet-inducible – tetracycline inducible; SEQ ID NOs referred to are listed in Table 15

The next day, supernatants were removed and replaced by fresh medium. After 48 hours, culture medium was replaced with selection medium containing 15 µg/mL blasticidin and 150 µg/ml Hygromycin B. Cells were transferred to larger cell culture vessels as needed until a sufficient number of cells for cryo preservation was obtained.

From dose response experiments, it was determined that 100 ng/ml tetracycline induced SLC4A4 expression in >80% of the cells. For quality control, binding of select VHHs at 100 nM to induced cells was determined by flow cytometry by anti-FLAG detection.

EXAMPLE 2. Immunizations

SLC4A4-targeting VHHs were generated through immunization of two llamas and one alpaca with SLC4A4 DNA, essentially as described elsewhere (Pardon *et al.* 2014, Nat Protocol 9:674-669; Henry and MacKenzie 2018, Single-Domain Antibodies: Biology, Engineering and Emerging Applications, Frontiers Media SA). In the primary immunization round, animals were immunized four times at two week intervals with human SLC4A4-encoding DNA (#3, Table 3), and once after another four weeks with mouse SLC4A4-encoding DNA (#4, Table 3) by intradermal injection followed by electroporation.

Table 3.

#	Construct	Vector	Remark
3	Human pancreatic SLC4A4 (SEQ ID NO:114)	pVAX1	Human NBCe1-B, for immunization
4	Mouse pancreatic SLC4A4 (SEQ ID NO:115)	pVAX1	Mouse NBCe1-B , for immunization

SEQ ID NOs referred to are listed in Table 15

Immune reaction was boosted with mouse SLC4A4-encoding DNA (two treatments) after four weeks, and finally again with mouse SLC4A4-encoding DNA after another four weeks (one treatment) for a total

of three immunization rounds (human/mouse – mouse – mouse). Prior to the first immunization, and three and seven days after each immunization round, blood was drawn from each animal for the isolation of peripheral blood lymphocytes (PBLs).

EXAMPLE 3. Phage display selections

Phage display libraries derived from PBLs were essentially prepared and used as previously described (Pardon *et al.* 2014, Nat Protocol 9:674-669; Henry and MacKenzie 2018, Single-Domain Antibodies: Biology, Engineering and Emerging Applications, Frontiers Media SA).Three VHH libraries, one per vaccinated animal per immunization round, were constructed from PBLs to screen for the presence of antigen specific VHHs. To this end, for each animal, total RNA from PBLs three days post last immunization was mixed 1:1 with total RNA from PBLs taken seven days post last immunization. This RNA mix was used as template for first strand cDNA synthesis with an oligo(dT) primer. Using this cDNA, the VHH encoding sequences were amplified by PCR, digested with Pst1 and Not1, and cloned into the phagemid vector pVDS107. All libraries were panned on HEK293T cells expressing either human or mouse SLC4A4 for two rounds, and the number of phagemid particles eluted from the cells was determined for each panning round. Polyclonal phagemid DNA was prepared from *E. coli* TG1 cells that were infected with the eluted phages from the second rounds. The VHH fragments were amplified by means of PCR from these samples and subcloned into an *E. coli* expression vector pVDS100, in frame with N-terminal PelB signal peptide and C-terminal FLAG3 and HIS6 tags. Electrocompetent *E. coli* TG1 cells were transformed with the resulting VHH-expression plasmid ligation mixture and individual colonies were grown in 96-deep-well plates. Monoclonal VHHs were expressed essentially as described before (Pardon *et al.* 2014, Nat Protocol 9:674-669). The crude periplasmic extracts containing the VHHs were prepared by freezing the bacterial pellets overnight, followed by resuspension in PBS and centrifugation to remove cell debris.

EXAMPLE 4. Cloning, mutagenesis, expression and purification of VHHs

Synthetic DNA fragments encoding VHHs were ordered (Integrated DNA Technologies (IDT), Leuven, Belgium) and subcloned into an *E. coli* expression vector under control of an IPTG-inducible *lac* promoter, in frame with N-terminal PelB signal peptide and C-terminal FLAG3 and HIS6 tags. Electrocompetent *E. coli* TG1 cells were transformed and the resulting clones were sequence verified. VHH proteins were purified from these clones by means of IMAC chromatography followed by desalting according to established procedures (Pardon *et al.* 2014, Nat Protocol 9:674-669).

Single site-saturation libraries of every CDR residue of a given VHH were generated by site-directed mutagenesis using “22c-trick” primers (Kille *et al.* 2013, ACS Synth Biol 2:83–92) ordered at IDT (Leuven,

Belgium) in combination with KAPA Hifi DNA polymerase according to the manufacturer's instructions (Roche, Switzerland). Alternatively, such pooled single site-saturation libraries were ordered from Twist Bioscience (South San Francisco, CA). These repertoires were then pooled per CDR allowing for every possible CDR substitution to be systematically interrogated for its effect on binding. In a second wave, repertoires combining successful substitutions from the first wave were made by overlap extension PCR with KAPA Hifi DNA polymerase or ordered directly from Integrated DNA Technologies. This way, 100s of affinity maturation variants were interrogated as *E. coli*–derived periplasmic extracts for binding to the mouse SLC4A4 overexpression cell line.

Different versions of multivalent VHHs combining two anti-SLC4A4 building blocks, and optionally one anti-serum albumin building block (WO 2019/016237), separated by flexible G_4S linkers of different lengths (35 G_4S, 20 G_4S, 10 G_4S and 5 G_4S; see Table 4) were generated essentially as described before (De Tavernier et al. 2016, J Biol Chem 291:15243–15255; Maussang et al. 2013, J Biol Chem 288:29562–29572). Irrelevant control molecules were generated using the 13F07 anti-PcrV VHH (De Tavernier et al. 2016, J Biol Chem 291:15243–15255) to match different active (multivalent) anti-SLC4A4 leads. The anti-serum albumin binding VHH SA26h5 used in different constructs as an *in vivo* half-life extension moiety, is derived from SA1_S30K_h4 as described (WO 2019/016237).

Table 4.

SEQ ID NO:	identifier	amino acid sequence
3	5 GS / 5 G_4S	GGGGS
4	10 GS / 10 G_4S	GGGGSGGGGS
5	20 GS / 20 G_4S	GGGGSGGGGSGGGGSGGGGS
6	35 GS / 35 G_4S	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS

Some multivalent constructs were cloned into a *Pichia pastoris* expression vector under control of an AOX1 methanol-inducible promoter, in frame with an N-terminal *Saccharomyces cerevisiae* alpha mating factor signal peptide, which directs the expressed recombinant proteins to the extracellular environment. Transformation and expression in *Pichia pastoris* and purification by means of protein A purification were done essentially as described before (Lin-Cereghino et al. 2005, BioTechniques 38:44, 46, 48; Schotte et al. 2016, Microbial Cell Factories 15:1–9).

Different versions of Fc-fusion constructs, connecting the VHH moieties to the mouse IgG2a Fc domain by flexible GlySer linkers (10 G_4S) were generated. A full effector function dead version, having no ADCC or CDC activity, was obtained by introduction of the well-known LALAPG (SEQ ID NO:7) mutations in the lower hinge and CH2 domain of mouse IgG2a (Lo et al. 2017, J Biol Chem 292:3900–3908). These were cloned in a pcDNA3.1-derived mammalian expression vector, in frame with the mouse Ig heavy chain V

region 102 signal peptide to direct the expressed recombinant proteins to the extracellular environment. At day 1, Freestyle HEK 293F cells (ThermoFisher Scientific, R79007) are seeded out at a density of 4 x 10^5 cells/ml in a 1L flask in 400 ml freestyle expression medium (ThermoFisher Scientific, 12338018). The cells are incubated in at 37°C, 8% CO_2 at 125 rpm. At day 2, transfection and dilution of the cells were performed at a density of 1 x 10^6 cells/ml in a 1L flask in 400 ml freestyle medium. 400 µg DNA is added to 13 ml Opti-MEM (ThermoFisher Scientific, 31985070) and 800 µg PEI (PolySciences, 23966) is added to another 13 ml of Opti-MEM, after which both solutions are mixed together drop wise by vortexing. The resulting mixture was kept for 15 min at RT before drop wise addition to the cells while shaking. The cells are incubated in at 37°C, 8% CO_2 at 125 rpm. At day 3, 24 hours post-transfection: 20% Excell medium is added to the transfected cultures (100 ml Excell medium on 400 ml transfection mix). The cells are incubated in at 37°C, 8% CO_2 at 125 rpm. At day 5, cell cultures are harvested by centrifugation at 5000 rpm for 10 minutes. Protein A purification of the VHH-Fc fusions from the spent culture medium was done as described above.

VHHs (100 ug, 1mg/ml) were biotinylated using the EZ-Link NHS-PEG4-Biotin kit (ThermoFisher, 15921312), at a 2:1 molar ratio of biotin reagent:VHH.

EXAMPLE 5. Flow cytometry binding experiments

5.1. Direct binding of VHHs

Cells were recovered using cell dissociation non-enzymatic solution (Sigma Aldrich C5914) and suspended to a final concentration of 1 x 10^6 cells/ml in FACS buffer (PBS, 2% FBS). Dilutions (1:5 in FACS buffer) of crude periplasmic extracts containing FLAG3-tagged (Table 5) VHHs or different concentrations of purified FLAG3-tagged VHHs were incubated with mouse anti-FLAG biotinylated antibody (Sigma Aldrich F9291-1MG) at 5 µg/ml in FACS buffer for 30 min at RT.

Table 5.

SEQ ID NO:	identifier	amino acid sequence
165	FLAG3-tag	DYKDHDGDYKDHDIDYKDDDDK

After blocking for 20 min, cell suspensions were distributed into a 96-well v-bottom plate and incubated with the above described VHH/antibody mixture for 1 hour on ice without shaking. Alternatively, the incubation of cells with VHHs was done at different pH values in pH-adjusted Bis-Tris buffer. After washing the cells once by centrifugation (300 g for 5 min) and resuspending with ice cold FACS buffer, biotinylated VHHs binding to cells were detected with streptavidin R-PE (Invitrogen, cat. # SA10044) at 1:400 dilution (0.18 µg/ml) in FACS buffer, incubated for 30 minutes on ice with shaking and protection from light. Alternatively, binding of VHH-Fc fusions was detected using R-PE AffiniPure $F(a'')_2$ Fragment Goat Anti-Mouse IgG (polyclonal) (Jackson ImmunoResearch, 115-116-071). After washing the cells

twice by centrifugation (300 g for 5 min) and resuspending with ice cold FACS buffer, the cells were analyzed in 200 µl FACS buffer with the AttuneNXT flow cytometer.

The polyclonal antibody preparations as used by De Giusti et al. 2011 (Br J Pharmacol 164:1976-1989; referring to Alvarez et al.2003, Biochemistry 42:12321 for the antigen which is human EL3 peptide spanning amino acids 609-685 of human NBCe1-B) and by Khandoudi et al. 2001 (Cardiovasc Res 52:387-396; antigen described as amino acids 629-644 of "human heart electrogenic Na /HCO cotransporter (hhNBC)", GenBank Accession no. AF069510 = human NBCe1-B) are not available. Two commercially available polyclonal mAbs raised against EL3 were tested for binding to different types of cell expressing SLC4A4 via FACS. These polyclonal mAbs are i) polyclonal rabbit anti-rat SLC4A4 EL3 (Alomone Labs, ANT-075; peptide antigen used for immunization reported as spanning amino acid residues 672-685 of rat NBCe1 (Accession Q9JI66))) and ii) polyclonal rabbit anti-human SLC4A4 EL3 (Novus Biologicals, NBP2-32021; peptide antigen used for immunization reported as spanning amino acids 609-685 of human SLC4A4). The polyclonal rabbit anti-human SLC4A4 EL3 showed binding to human NBCe1-A and NBCe1-B(D2-85), albeit substantially weaker than the VHHs as disclosed herein; but no binding to human NBCe1-B or mouse NBCe1-B was measured; the polyclonal rabbit anti-rat SLC4A4 EL3 did not shown any meaningful binding to any of the NBCe1 isoforms tested. Results are summarized in Figure 22.

5.2. Binding competition

For binding competition experiments, cells were first incubated with different concentrations of a tagless VHH for 30-60 minutes at 4°C, followed by a 30 minutes incubation at 4°C of a fixed concentration of FLAG3-tagged VHHs, followed by washing and anti-FLAG detection as described above.

Alternatively, cells were incubated with a high (EC_{100}) concentration of SLC4A4 VHHs or an irrelevant control for 30-60 minutes at 4°C, followed by incubation at EC_{40} concentrations of biotinylated VHHs (30 minutes at 4°C) and detection by streptavidin-PE conjugate (eBioscience, 12-4317-87).

Under more stringent conditions, 3 washing steps with FACS buffer are included after the incubation of the tagless or FLAG3-tagged VHHS and before the addition of the FLAG3-tagged or biotinylated probe.

5.3. Binding to chimeric constructs

Selected VHHs were tested at a concentration of 500 nM in flow cytometry on transiently transfected (constructs #10 to #19, Table 6) HEK293T cells. Constructs #10 and #11 served as positive controls.

Table 6.

#	Construct	Vector	Remark
10	Human pancreatic SLC4A4 (SEQ ID NO:114)	pcDNA_FRT	Human NBCe1-B; chimeric constructs control
11	Mouse pancreatic SLC4A4 (SEQ ID NO:115)	pcDNA_FRT	Mouse NBCe1-B; chimeric constructs control
12	hSLC4A4(mEL2) (SEQ ID NO:119)	pcDNA_FRT	Human NBCe1-B with mouse EL2* variant
13	hSLC4A4(mEL3) (SEQ ID NO:120)	pcDNA_FRT	Human NBCe1-B with mouse EL3*
14	hSLC4A4(mEL4) (SEQ ID NO:121)	pcDNA_FRT	Human NBCe1-B with mouse EL4* variant
15	hSLC4A4(mEL2+4) (SEQ ID NO:122)	pcDNA_FRT	Human NBCe1-B with mouse EL2* and EL4* variants
16	mSLC4A4(hEL2) (SEQ ID NO:123)	pcDNA_FRT	Mouse NBCe1-B with human EL2* variant
17	mSLC4A4(hEL3) (SEQ ID NO:124)	pcDNA_FRT	Mouse NBCe1-B with human EL3*
18	mSLC4A4(hEL4) (SEQ ID NO:125)	pcDNA_FRT	Mouse NBCe1-B with human EL4* variant
19	mSLC4A4(hEL2+4) (SEQ ID NO:126)	pcDNA_FRT	Mouse NBCe1-B with human EL2* and EL4* variants
20	Human pancreatic SLC4A4 (D2-85) (SEQ ID NO:127)	pcDNA5_FRT_TO	Human NBCe1-B with N-terminal (residues 2-85) deletion; tet-inducible**

*EL#– extracellular loop number #; **tet-inducible – tetracycline inducible; SEQ ID NOs referred to are listed in Table 15

EXAMPLE 6. pHi measurements in acid shock recovery assay

To determine whether SLC4A4 binding VHHs can block transporter function, VHHs were tested in an intracellular pH (pH_i) acid shock recovery assay (modified from (Manning & Sontheimer 1999, J Neuroscience Methods 91:73–81) on SLC4A4 overexpressing HEK293T cell lines (see Example 1). Cells were plated at 50k per well in a volume of 100 µl in PDL-coated, transparent bottom, black 96 multi well plates (Greiner 655090) in full DMEM medium (see Example 1). The following day, medium was replaced with assay buffer (in mM: NaCl 145, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, Na-bicarbonate 4, HEPES 30; pH 7.4 /HEPES 30 was replaced by Bis-Tris 30 in case of experiments at pH 6 and pH 7) using several 'half volume' washes (adding/removing 100 µl to prevent cell loss) employing a Tecan robot, and stained by adding BCECF/AM (Thermo Fisher B1170) to a final concentration of 1 µM for 30 min at 25 °C in the dark. After dye washout (half volume washes on the Tecan) with assay buffer, VHHs and/or compounds diluted in assay buffer were added (25 µl, using the Tecan), and the measurement started. Specifically, the fluorescent signal at >530 nm (gain 1500-2000) from alternating excitations at 440 (pH insensitive) and 495 nm (pH

sensitive) was recorded on a BMG Clariostar, in bottom reading mode at 25 °C. To sensitize the cells towards SLC4A4 inhibition, a final concentration of 10 µM of the NHE1 blocker cariporide (CAS NO 159138-80-4; Sigma SML1360, final DMSO concentration = 0.1%) was added to each VHH treated well together with the VHH.

After 60 min the plate reader was paused, the plate removed from the reader and pH_i lowered by the addition of 25 µl of 300 mM sodium acetate in assay buffer to a final concentration of 50 mM using the Tecan robot. Afterwards, recording was resumed as quickly as possible for at least another hour. As a proxy for the pH_i, the ratio of the >530 nm emissions (495 over 440 nm excitation) was plotted over time.

To determine IC_{50} values, the area under the curve (AUC) was calculated from a ratio 495/450 nm baseline value equivalent to the lowest measure observed in a given experiment, for the first 20 min of recovery after sodium acetate addition. AUC values were plotted as a function of VHH concentration using GraphPad Prism (Figure 2). Alternatively, the AUC of the last 5 min of the compound addition stage was used to compare different blocking leads at a fixed concentration. In addition, the rate constant K (min^{-1}) and plateau values (ratio 495/450 nm at infinite times) of the recovery stage after sodium acetate addition were calculated from a nonlinear regression fitting ("Plateau followed by one phase association") in GraphPad Prism. To facilitate cross-experiment comparisons, AUC, K and plateau values were normalized to the irrelevant control VHH condition and expressed as the difference or ratio. Percentage (%) inhibition of SLC4A4 activity was derived from AUC values in the assay window defined by the irrelevant control condition (maximum SLC4A4 activity on HEK293 Flp-In T-Rex cell lines induced with tetracycline) and "non-induced" control condition (absence of SLC4A4 activity on HEK293 Flp-In T-Rex cell lines not induced with tetracycline): % inhibition = 100 * (AUC_{IRR}–- AUC_{VHH})/(AUC_{IRR} – $AUC_{non\text{-}induced}$).

For the human overexpressing cell line, clone 9 was consistently used. Since a difference in assay behavior between mouse SLC4A4 overexpressing clones 1 and 4 (with 4 being more responsive to inhibition) was noticed, most data points were obtained using clone 4 (for description of clones: see Example 1).

EXAMPLE 7. Monovalent VHHs on pancreas isoform NBCe1-B

VHH clones from the immunization campaign were screened as crude periplasmic extracts in flow cytometry for binding to HEK293T cells expressing the pancreas isoform (NBCe1-B) of human or mouse SLC4A4. From a total number of 12 plates screened (1109 clones), 46 unique clones were selected for detailed characterization as purified protein.

VHH P01400010 could not be produced in sufficient quantities, whereas the binding of VHHs P01400015, P01400028 and P01400033 could not be confirmed. In summary, 20/42 VHHs are human SLC4A4-selective binders, 19/42 are at least partially cross-reactive to mouse SLC4A4, and three are mouse SLC4A4-selective binders. At a sequence identity cut-off of 88% (Deschaght et al. 2017, Frontiers in Immunology 8:1–11) the 42 VHHs grouped into 18 clusters of related VHHs.

Of the 18 clusters, four contain VHHs that show inhibitory activity at 1 µM in the pH_i acid shock recovery assay (Table 7): cluster 3 (VHH P01400023), cluster 4 (VHH P01400014), cluster 5 (VHHs P01400012, P01400020 and P01400021) and cluster 19 (VHHs P01400024, P01400040, P01400041 and P01400046), wherein active members were, at 1 µM, characterized by a normalized AUC value of <= 0,8 in a functional inhibition assay on HEK293T expressing human SLC4A4.

Table 7.

Cluster ID	ISVD/VHH /sdAbB ID	Binding EC_{50} to HEK293T | human SLC4A4 (M)	Binding EC_{50} to HEK293T | mouse SLC4A4 (M)	Binding EC_{50} to HEK293 Flp-In T-Rex | cyno SLC4A4 (M)	Functional inhibition on HEK293T at 1 mM | human SLC4A4 (normalized AUC)	Functional inhibition IC_{50} on HEK293T | human SLC4A4 (M)	Functional inhibition IC_{50} on HEK293T | mouse SLC4A4 (M)
3	P01400023 (SEQ ID NO:14)	6.6E-09	no binding	no binding	0.56/0.56	1.9E-08	ND
4	P01400014 (SEQ ID NO:15)	4.0E-09	no binding	4.9E-09	0.61	3.7E-08	ND
5	P01400012 (SEQ ID NO:16)	8.2E-10	weak binding	ND	0.67/0.67	ND	ND
5	*P01400019* (SEQ ID NO:129)	*2.3E-07*	*ND*	*ND*	*0.91*	*ND*	*ND*
5	P01400020 (SEQ ID NO:17)	1.7E-09	weak binding	no binding	0.78	5.2E-08	ND
5	P01400021 (SEQ ID NO:18)	2.4E-09	ND	ND	0.83	ND	ND
19	P01400024 (SEQ ID NO:19)	5.8E-08	no binding	2.2E-08	0.81/0.80	ND	ND
19	*P01400038* (SEQ ID NO:130)	*2.1E-08*	*1.2E-08*	*ND*	*1.03*	*ND*	*ND*
19	P01400040 (SEQ ID NO:20)	1.9E-08	1.1E-08	2.5E-08	0.75	3.6E-08	3.9E-08

Cluster ID	ISVD/VHH /sdAbB ID	Binding EC_{50} to HEK293T | human SLC4A4 (M)	Binding EC_{50} to HEK293T | mouse SLC4A4 (M)	Binding EC_{50} to HEK293 Flp-In T-Rex | cyno SLC4A4 (M)	Functional inhibition on HEK293T at 1 mM | human SLC4A4 (normalized AUC)	Functional inhibition IC_{50} on HEK293T | human SLC4A4 (M)	Functional inhibition IC_{50} on HEK293T | mouse SLC4A4 (M)
19	P01400041 (SEQ ID NO:21)	6.3E-08	8.4E-08	ND	0.77	ND	ND
19	P01400046 (SEQ ID NO:22)	1.7E-08	7.6E-09	ND	0.80	ND	ND

ND: not determined; italics: normalized AUC value of > 0,8 in a functional inhibition assay on HEK293T expressing human SLC4A4; SEQ ID NOs referred to are listed in Table 14

Of the nine blocking VHHs, one representative per cluster was selected for further characterization: P01400014, P01400020, P01400023 and P01400040. In addition, P01400024 was included in certain assays as human selective member of cluster 19. We determined that VHHs P01400014, P01400024 and P01400040 (but not P01400020 and P01400023) are cross-reactive to NBCe1-B of *Macaca mulatta* (cynomolgus monkey) (Table 7) and M. *fascicularis* (rhesus macaque) (data not shown). In addition, P01400040, P01400041 and P01400046 are cross-reactive to mouse NBCe1-B (Table 7).

The selected blocking VHHs also recognized SLC4A4 endogenously expressed in different human and mouse adenocarcinoma cell lines: pancreatic cancer (PDA) cell lines SUIT-2 and CFPAC-1; prostate cancer (PC) cell lines PC-3 and LNCaP; and triple negative breast cancer (TNBC) cell line MDA-MB-231 (Figure 3). More so, they bound comparably well at pH 7 vs. at pH 6, conditions representative of the acidic tumor micro environment with pH_e values ranging from 6.5 to 7 (Webb et al. 2011, Nat Rev Cancer 11:671–677).

EXAMPLE 8. Monovalent VHHs on kidney isoform NBCe1-A

All SLC4A4 isoforms share identical transmembrane and extracellular domains but differ in their intracellular N- and C-termini (Figure 1) as well as their tissue distribution. NBCe1-A is exclusively expressed in the kidney and in a therapeutic setting its inhibition may cause unwanted side effects. Therefore, all monovalent SLC4A4 blockers were tested in flow cytometry (Figure 4) and in the pH_i acid shock recovery assay (Table 8, Figure 5) against the human pancreatic (NBCe1-B) and kidney (NBCe1-A) isoforms. NBCe1-B has lower activity under basal conditions due to an auto-inhibitory N-terminal domain, but can be fully de-repressed by interaction of the N-terminus with IRBIT (Su et al. 2021, J Physiol 599:1151–1172, and references therein)).

Despite binding comparably to both pancreatic and kidney NBCe1 isoforms, P01400040 as well as human-selective family member P01400024 do not block the kidney isoform NBCe1-A (Table 8, Figure

5). In contrast, P01400014, 20 and 23 functionally inhibit the kidney isoform, albeit with reduced efficacy compared to the pancreas isoform.

To evaluate whether the enhanced transport rate of NBCe1-A compared to NBCe1-B contributes to the isoform-selective profile of P01400040 and P01400024, an N-terminal deletion variant (deleting amino acids 2 to 85) of NBCe1-B was generated that lacks both the auto-inhibitory and IRBIT interaction domains (resulting in NBCe1-B (Δ2-85) or NBCe1-B (D2-85)) . In line with published results (Su et al. 2021, J Physiol 599:1151–1172), the removal of residues 2-85 from NBCe1-B brought its transport rate to the level of NBCe1-A, as evidenced by the enhanced/more alkaline baseline pH_i and the faster acid shock recovery.

Flow cytometry experiments revealed that P01400040 and P01400014 bind comparably well across the three isoforms NBCe1-B, NBCe1-B(D2-85) and NBCe1-A (Figure 6). Two independent experiments, compared the activity of different blocking VHHs at a high saturating concentration of 400 nM across the three isoforms NBCe1-B, NBCe1-B(D2-85) and NBCe1-A by looking at different parameters derived from the AUC values of the first 20 minutes of the acid shock recovery stage. Percentage (%) inhibition of SLC4A4 activity values were also obtained and are included in Tables 8A and 8B.

Table 8A.

NBCe1 isoform / VHH/ISVG/sdAb	% inhibition of SLC4A4 activity		
	NBCe1-B, pancreatic	NBCe1-A, kidney	NBCe1-B (Δ2-85)
IRR00004	0	0	0
P01400014	130	39	62
P01400023	123	49	77
P01400024	107	-3	40
P01400040	126	4	45
P01400020	58	19	31
non-induced	100	100	100

NBCe1-B (Δ2-85): NBCe1-B from which amino acids 2 to 85 were deleted, therewith removing the auto-inhibitory domain

Table 8B.

NBCe1 isoform / VHH/ISVD/sdAb	% inhibition of SLC4A4 activity		
	NBCe1-B, pancreatic	NBCe1-A, kidney	NBCe1-B (Δ2-85)
IRR00004	0	0	0
P01400024	93	-4	20
P01400041	93	3	21
P01400046	87	-1	21
P01400040	125	2	42
P01400014	136	41	55
P01400023	132	56	81
P01400020	74	25	30

NBCe1-B (Δ2-85): NBCe1-B from which amino acids 2 to 85 were deleted, therewith removing the auto-inhibitory domain

P01400041 and P01400046 were shown to inhibit human NBCe1-B to a similar extent as P01400024 and P01400040 (Figure 15). P01400012 and P01400021 were shown to inhibit human NBCe1-B to a similar extent as P01400020 and P01400023 (Figure 15).

EXAMPLE 9. Epitope mapping of monovalent VHHs

To determine which extracellular loop or loops (eLs) of SLC4A4 are sufficient and/or necessary for binding of the selected SLC4A4-blocking VHHs, we made use of the limited mouse cross-reactivity of the VHHs that block human SLC4A4. Several human/mouse chimeric SLC4A4 constructs were created (#10 to #19 including controls, see Table 6).

The NBCe1 extracellular loops are identical for all human NBCe1 isoforms (NBCe1-A to -E ; see Figure 1B for alignment of amino acid sequences of human NBCe1-A, human NBCe1-B, and murine NBCe1-B). In particular, the human NBCe1 (hNBCe1) EL2 is defined by the amino acid sequence SKDNNFDY (SEQ ID NO:8);

the human NBCe1 EL3 is defined by the amino acid sequence YYPINSNFKVGYNTLFSCTCVPPDPANISISNDTTLAPEYLPTMSSTDMYHNTTFDWAFLSKKECSKYGGNLVGNNCN FVPDI (SEQ ID NO:9);

and the human NBCe1 EL4 is defined by the amino acid sequence VGVDTPKLIVPSEFKPTSPNRGWFVPPFGENPWW (SEQ ID NO:10).

As murine NBCe1 extracellular loops, those from the murine NBCe1-B isoform were taken. In particular the murine NBCe1 (mNBCe1) EL2 is defined by the amino acid sequence SKDHNFDY (SEQ ID NO:11), which differs from the human NBCe1 EL2 at a single amino acid position, see sequence alignment hereinafter–- more in particular, human NBCe1 EL2 has a glutamine (N) at position 546 (relative to full-length human NBCe1-B, see Figure 1B), whereas murine NBCe1 EL2 has a histidine (H) at this position;

the murine NBCe1 EL3 is defined by the amino acid sequence YYPINSDFKVGYNTHFSCACLPPDPVNLSVSNDTTLAPEDLPTISSTDMYHNVTFDWAYLSKKECVKYGGKLVGNNCD FVPDI (SEQ ID NO:12) which differs from the human NBCe1 EL3 at 14 amino acid positions, see sequence alignment hereinafter;

and the murine NBCe1 EL4 is defined by the amino acid sequence VGVDTPKLIVPSEFKPTSPNRGWFVPPFGGNPWW (SEQ ID NO:13) which differs from the human NBCe1 EL4 at a single amino acid position, see sequence alignment hereinafter. More in particular, human NBCe1 EL4 has a glutamic acid (E) at position 775 (relative to full-length human NBCe1-B, see Figure 1B), whereas murine NBCe1 EL4 has a glycine (G) at this position.

The remaining four extracellular loops are conserved between human NBCe1 and murine NBCe1.

```
hNBCe1-EL2 SKDNNFDY (SEQ ID NO:8)
mNBCe1-EL2 SKDHNFDY (SEQ ID NO:11)
           ***:****

hNBCe1-EL3 YYPINSNFKVGYNTLFSCTCVPPDPANISISNDTTLAPEYLPTMSSTDMYH-
mNBCe1-EL3 YYPINSDFKVGYNTHFSCACLPPDPVNLSVSNDTTLAPEDLPTISSTDMYH-
           ******:******* ***:*:****.*:*:********* ***:*******
hNBCe1-EL3 NTTFDWAFLSKKECSKYGGNLVGNNCNFVPDI (SEQ ID NO:9)
mNBCe1-EL3 NVTFDWAYLSKKECVKYGGKLVGNNCDFVPDI (SEQ ID NO:12)
           *.*****:****** ****:******:*****

hNBCe1-EL4 VGVDTPKLIVPSEFKPTSPNRGWFVPPFGENPWW (SEQ ID NO:10)
mNBCe1-EL4 VGVDTPKLIVPSEFKPTSPNRGWFVPPFGGNPWW (SEQ ID NO:13)
           ***************************** ****
```

Next, the selected SLC4A4-blocking VHHs were compared for binding to the transiently transfected human-mouse NBCe1 chimeras by flow cytometry at a single high concentration of 500 nM. P01400040 served as the species cross-reactive control. P01400024 was included as a non-cross-reactive member of the same family as P01400040.

Summarizing the results, we were able to determine that residue E775 on EL4 is both necessary and sufficient for the binding of VHH P01400014, P01400020 and P01400023 to human SLC4A4 (Figures 7 and 8). When E775 is changed to G as in the murine isoform of SLC4A4, binding of these three VHH molecules to human SLC4A4 is abolished. Introducing the human G775E mutation to mouse SCL4A4 fully enables the binding of VHHs P01400014, P01400020 and P01400023.

Human EL3 is partially necessary and fully sufficient for binding of P01400024, indicative of an additional interaction with another loop. Human EL4 is partially sufficient for binding of P001400024, indicative of an additional interaction with another loop. P01400040 belongs the same sequence cluster as P01400024 (see above) and thus binds to a fully overlapping orthosteric footprint.

For P01400014, P01400020 and P01400023, human EL3 is not sufficient to restore binding to mouse SLC4A4, and neither does the opposite replacement (mouse EL3 in human SLC4A4) lead to a reduction in binding. In addition, the one residue difference on EL2 (N546H) has no influence on the binding of any of the tested VHHs.

EXAMPLE 10. Epitope binning of monovalent VHHs

We used flow cytometry binding competition experiments to determine whether the blocking VHHs compete with each other for binding on SLC4A4. To this end we incubated cells of the human SLC4A4 overexpressing HEK line with a high (EC_{100}) concentration of P01400014, P01400020, P01400023, P01400024, P01400040 or an irrelevant control, followed by incubation at EC_{40} concentrations of biotinylated P01400014, P01400020, P01400023 or P01400040 and detection.

This analysis showed that P01400014, P01400020 and P01400023 belong to the same epitope bin (also referred to as "bin A" herein), as these VHHs fully compete with each other for binding on SLC4A4. The VHHs P01400024 and P01400040 belong to a distinct bin (also referred to as "bin B" herein), competing with each other, as is expected for members of the same sequence cluster. Members of the two distinct bins appear to weakly interfere with each other's binding. This could be due to (i) a partially overlapping binding footprint, or (ii) steric clashes away from the binding site or (iii) allosteric interference between the two bins.

Biparatopic combinations of blocking and non-blocking VHHs can result in outspoken improvements of potency (Terryn et al. 2014, pLoS One 9:e109367; Desmyter et al. 2017, Frontiers in Immunology 8:884; Schütze et al. 2018, Frontiers in Immunology 9:2553). To this end, all binding non-blocking leads were competed against biotinylated P01400014 and P01400040 (top and bottom panel of Figure 19) in two separate experiments. In summary, many non-blocking VHHs fully or partially compete for binding with the two blocking VHHs. Of these, P01400032 of VHH cluster 17 emerged as a promising non-blocking human-mouse x-reactive biparatopic partner, binding to a non-competing epitope compared to those of P01400014 and P01400040. VHH cluster 17 is comprising a second VHH, i.e. VHH P01400003 (Table 9).

Table 9.

Cluster ID	VHH/ISVD /sdAb ID	Binding EC_{50} to HEK293T | human SLC4A4 (M)	Binding EC_{50} to HEK293T | mouse SLC4A4 (M)	Binding EC_{50} to HEK293 Flp-In T-Rex | cyno SLC4A4 (M)	Functional inhibition on HEK293T at 1 mM | human SLC4A4 (normalized AUC)	Functional inhibition IC_{50} on HEK293T | human SLC4A4 (M)	Functional inhibition IC_{50} on HEK293T | mouse SLC4A4 (M)
17	P01400003 (SEQ ID NO:128)	9.2E-09	ND	ND	0.94	ND	ND
17	P01400032 (SEQ ID NO:89)	ND*	2.2E-08	ND	0.97	ND	ND

* binding to human SLC4A4 determined during screening (Examples 3, 5, 7); SEQ ID NOs referred to are listed in Table 14

EXAMPLE 11. Structural elucidation of VHH-SLC4A4 complexes by cryo EM

The data on the different SLC4A4-blocking VHHs shown in the previous Examples can be summarized as follows:

- VHHs P01400014, P01400020 and P01400023 (bin A) bind to an epitope that includes human SLC4A4 EL4 and that is distinct from that of P01400040 and P01400024 (bin B)

- VHHs of the two epitope bins display some negative cooperativity for binding relative to each other, but are not mutually exclusive
- the VHH members of bin B fully inhibit the NBCe1-B (pancreas) isoform but remain inactive on the NBCe1-A (kidney) isoform, whereas the members of bin A also partially block the NBCe1-A (kidney) isoform

To gain more insights in the structural underpinnings of these functionally distinct blocking epitope bins, the structures of the complexes of P01400014 and P01400040 with NBCe1-B and NBCe1-A were resolved by means of cryo EM. The structural data corroborated the aforementioned summary: P01400014 binds to the outer edge of SLC4A4, mainly interacting with EL4 and EL2 whereas P01400040 binds to EL3 at the SLC4A4 dimer interface (Figure 9). Both VHHs simultaneously occupy epitopes on each of the two SLC4A4 protomers in the dimer, without sterically interfering with each other's binding. This sterical context allows for the possibility of bivalent and biparatopic avid binding to SLC4A4.

The paratope-epitope interface of the cryo-EM model of P01400014 on human NBCe1-B was analyzed in detail. Fourteen residues (numbering according to the IMGT nomenclature (Lefranc and Lefranc 2023; Antibody Sequence and Structure Analyses Using IMGT®: 30 Years of Immunoinformatics, in *Computer-Aided Antibody Design* (Tsumoto K, and Kuroda D eds) pp 3–59, Springer US, New York, NY) in the CDRs (see Figure 14) were considered to be important for the binding interaction,:

in CDR1: R28, S35 and Y37

in CDR2: S57, R58, G62, T63, R64 and T65

in CDR3: S110, T111, N111.1 and S112.

Likewise, the cryo-EM data of P01400040 bound to human NBCe1-B was analyzed. Nineteen residues (IMGT #) in the CDRs (see Figure 14) were considered to be involved in the binding interaction, and as such selected for full randomization to every possible amino acid:

in CDR1: S30 and Q37

in CDR2: S59, V62, S64,T65, A66 and Y67

in CDR3: K109, L110, T111, S111.1, S111.2, T112.2 and T112.3.

EXAMPLE 12. Key characteristics of monovalent SLC4A4-blocking VHHs

Table 10 summarizes the properties of the key NBCe1-B blocking VHHs. The main functional distinction between the EL3 and EL2/EL4 binding leads is that the former are totally inactive on the kidney NBCe1-A isoform whereas the latter partially block NBCe1-A.

Table 10.

VHH/ISVD /sdAb	binding						*in vitro* inhibition			
	h-B	h-B (Δ2-85)	h-A	c	m	epitope	h-B	h-B (Δ2-85)	h-A	m
P01400040	+	+	+	+	+	EL3	+	+/-	-	+
P01400024	+	+	+	+	-	EL3	+	+/-	-	-
P01400014	+	+	+	+	+/-	EL2/4	+	+	+/-	-
P01400023	+	+	+	-	-	EL2/4	+	+	+/-	-
P01400020	+	+	+	-	+/-	EL2/4	+/-	+/-	+/-	-

"h-B": human NBCe1-B; "h-B (Δ2-85)": human NBCe1-B from which amino acids 2-85 have been deleted; "h-A": human NBCe1-A; "c": cynomolgous NBCe1-B; "m": mouse NBCe1-B; "+", "+/-", "-": relative inhibition of NBCe1 isoform by VHH.

EXAMPLE 13. In vitro characterization of multivalent VHHs

To explore their combinatorial potential, a selection of 20 VHHs -7 blockers (P01400014, P01400020, P01400023, P01400024, P01400040, P01400041 and P01400046) and 13 mouse cross-reactive binders/non-blockers (P01400027, P01400030, P01400031, P01400032, P01400033, P01400034, P01400035, P01400036, P01400038, P01400039, P01400043, P01400044 and P01400045 – see Table 11) were subjected to a library screening approach. The VHHs were randomly combined into biparatopic (two different VHHs) or bivalent (twice the same VHH) constructs (for a total of 20 × 20 = 400 possible combinations) separated by a long and flexible 35GS linker (De Tavernier et al. 2016, J Biol Chem 291:15243–15255).

Table 11.

Cluster ID	VHH/ISVD /sdAb ID	Binding EC_{50} to HEK293T | human SLC4A4 (M)	Binding EC_{50} to HEK293T | mouse SLC4A4 (M)	Binding EC_{50} to HEK293 Flp-In T-Rex | cyno SLC4A4 (M)	Functional inhibition on HEK293T at 1 mM | human SLC4A4 (normalized AUC)	Functional inhibition IC_{50} on HEK293T | human SLC4A4 (M)	Functional inhibition IC_{50} on HEK293T | mouse SLC4A4 (M)
1	P01400027	2.0E-08	6.9E-08	ND	1.01	ND	ND
1	P01400034	ND*	7.6E-09	ND	1.00	ND	ND
1	P01400036	ND*	8.5E-09	ND	1.00	ND	ND
1	P01400039	ND*	9.7E-09	ND	1.00	ND	ND
12	P01400045	ND*	3.0E-09	ND	0.98	ND	ND
14	P01400043	ND*	1.6E-08	ND	1.00	ND	ND
14	P01400044	ND*	1.6E-08	ND	1.00	ND	ND
15	P01400030	6.1E-09	2.2E-07	ND	1.02	ND	ND
18	P01400031	ND*	5.1E-08	ND	1.00	ND	ND
	P01400033	*					
19	P01400038	2.1E-08	1.2E-08	ND	1.03	ND	ND
20	P01400035	ND*	3.0E-09	ND	1.03	ND	ND

* binding to human SLC4A4 determined during screening (Examples 3, 5, 7)

The library was sampled by screening crude periplasmic extracts of 360 clones in flow cytometry for binding to human and mouse SLC4A4 overexpressing lines. Clones yielding a binding signal substantially above the one of a strong monovalent binder (P01400044) serving as control were sequenced (data not shown).

In addition, specific sub-sets of bivalent/biparatopic combinations of VHHs with 35GS linkers were generated: (i) blockers combined with blockers (P01400014, P01400020, P01400023 and P01400040) and (ii) P01400040 combined with binders/non-blockers.

The best binders identified by the above approaches were tested as FLAG3-HIS6-tagged purified proteins at a single high concentration (500 nM) in the pH_i acid shock recovery assay on human and mouse SLC4A4 in two independent experiments. The three parameters (AUC, rate constant and plateau) that capture the blocking/inhibitory effect of the leads on the acid shock recovery stage respectively in terms of magnitude, kinetics and duration are congruent across the board. A number of trends emerged: (i) as a group, bivalent and biparatopic combinations of blockers exert a stronger inhibitory effect on human NBCe1-B in comparison to monovalent controls and biparatopic combinations of blockers and binders/non-blockers; (ii) combinations with P01400014, P01400023 and P01400040 bring about stronger effects in comparison to combinations with P01400020; (iii) blocking activity on mouse NBCe1-B is mainly determined by the presence of the mouse cross-reactive blocker P01400040.

The above mentioned observations were further explored in confirmatory experiments with full dose response curves of the multivalent leads.

Bivalent VHHs P01400082, P01400081, and P01400083 with a long 35GS linker demonstrate improved efficacy and potency -indicative of avid binding to dimeric SLC4A4 – when compared to their respective monovalent counterparts P01400014, P01400020 and P01400023, respectively in the acid shock recovery assay (Figure 10). More so, this was also observed with bivalent half-life extended or dimeric Fc-fusion versions of P01400014. This avidity effect was not observed when comparing bivalent half-life extended or dimeric Fc-fusion versions of P01400040 to their monovalent counterpart in functional or binding competition assays. These observations were further corroborated in a series of independent experiments comparing the blocking activity of bivalent VHHs P01400082, P01400083, and P01400113 (bivalent versions of P0140014, P01400023 and P01400040, respectively) to their monovalent counterparts on NBCe1-B, NBCe1-B(D2-85) and NBCe1-A (Table 12). The bivalent versions of P01400014 and P01400023 even achieve full inhibition of NBCe1-B(D2-85) but only partial inhibition of NBCe1-A.

Table 12

NBCe1 isoform / VHH/ISVD/sdAb	% inhibition of SLC4A4 activity		
	NBCe1-B, pancreatic	NBCe1-A, kidney	NBCe1-B (Δ2-85)
IRR00004	0	0	0
P01400014	130	39	62
P01400082	149	39	113
P01400023	123	49	77
P01400083	156	56	116
P01400040	126	4	45
P01400113	119	6	49

NBCe1-B (Δ2-85): NBCe1-B from which amino acids 2 to 85 were deleted, therewith removing the auto-inhibitory domain

Subsequent experiments with shorter linker length bivalent versions of P01400014 and P01400040 revealed that too short linkers (5GS and 10GS in comparison to 20GS) bring about potency and/or efficacy penalties in functional and binding competition assays. The latter findings support the contribution of avidity in functional inhibition by bivalents, also for P01400040.

For *biparatopic* VHHs the situation is more complex. As mentioned before, we found no combinations of binders/non-blockers and blockers with improved efficacy or potency compared to a single blocker, even with non-competing binder P01400032. Biparatopic combinations of blockers with blockers consistently ranked high for blocking activity.

We studied in more detail the combinations of P01400040 with P01400014 -two potent blockers with a non-overlapping binding footprint – in two orientations (from N- to C-terminus) P01400014- P01400040 (P01400086) and P01400040- P01400014 (P01400073) separated by a long 35GS linker. Functional (Figure 11A) and binding competition experiments revealed that on human SLC4A4 there is a clear orientation effect, with a preference for P01400014 in the N-terminal position. On mouse SLC4A4 (Figure 11B), there is a preference for the opposite orientation, with P01400040 in the N-terminal position.

Next, a series of biparatopics with P01400014- P01400040 orientation separated by linkers of different lengths (ranging from 35GS to 5GS) were constructed and compared in binding competition experiments on human SLC4A4. From this experiment, it could be concluded that (i) the shorter linkers (5G and 10GS) are not sufficiently long to convey full competition in comparison to the monovalent control P01400040 and the biparatopics with the longer linkers; and (ii) the longer linkers (20GS and 35GS) allow for concomitant/avid binding to SLC4A4 of the two VHH building blocks in the biparatopic as evidenced by the leftward potency shift compared to the monovalent control P01400040. Functional comparison of these biparatopics on human NBCe1-B, NBCe1-B(D2-85) and NBCe1-A paints a slightly more complex

picture. Here, the 20GS linker P01400014- P01400040 biparatopic (P01400181) is less active across the board in comparison to its 35GS counterparts (P01400086 and P01400182). The 35GS linker P01400014-P01400040 biparatopics (P01400086 and P01400182) are comparable to the bivalent version of P01400014 (P01400082) in terms of their inhibitory activity across the three isoforms. Finally, an interesting distinction can be made when comparing the 35GS linker P01400014- P01400040 biparatopics (P01400086 and P01400182) with the opposite orientation P01400040- P01400014 biparatopic (P01400073). Although the latter is less potent on human NBCe1-B, it achieves the comparable maximum effects on NBCe1-B and NBCe1-B(D2-85) at high concentrations, but consistently is the most active of all leads on NBCe1-A where it achieves near complete inhibition.

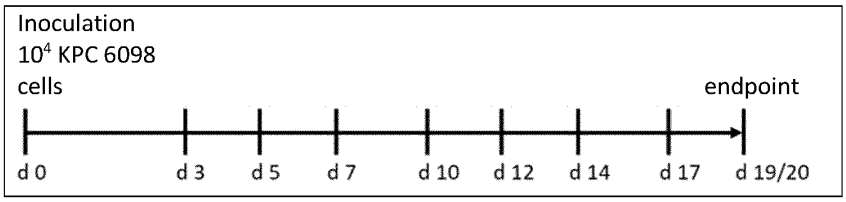
In toto, the experiments with bi-/multi-valent and biparatopic VHHs indicate that individual SLC4A4-blocking VHHs are flexible for being combined in fusion proteins without loss or drastic loss of SLC4A4-blocking activity. This is further illustrated in Figure 16 illustrating the SLC4A4-inhibiting activities of by P01400073: bivalent/biparatopic VHH, fusion of P01400040 and P01400014; by P01400086: bivalent/biparatopic VHH, fusion of P01400014 and P01400040; by P01400105: bivalent VHH, fusion of 2 P01400040 moieties (Figure 16A); by bivalent P01400118 being an Fc-moiety fused with 2 P01400040 moieties (one per Fc-arm) (Figure 16B); by trivalent/triparatopic P01400109 (P01400040 + P01400014 + half-life extension VHH) and by trivalent/biparatopic P01400113 (P01400040 + P01400040 + half-life extension VHH) (Figure 16C). Half-life extension ISVDs have been previously described and binding to human serum albumin (WO 2012/175400 and WO 2019/016237), the sequence of such VHH as used herein, SA26h5, in its monovalent form is defined by SEQ ID NO:131 in Table 15.

EXAMPLE 14. In vivo activity of SLC4A4-blocking VHHs

In an i*n vivo* study, P01400113 (a bivalent half-life extended version of mouse x-reactive P01400040) was compared to IRR00002 (a matching irrelevant control VHH), in the treatment in an orthotopic pancreatic cancer (PDAC) KPC mouse model according to the schedule depicted in (Table 13A, showing days on which VHH was administered = indicated days between inoculation and endpoint). The treatment with P01400113 resulted in significant reductions of both primary tumor weight, as well as number of mesenteric metastasis (Figure 12). This observation formally demonstrates that selective pharmacological blockade of SLC4A4 reproduces the findings with genetic knock down of SLC4A4 in the tumor cells (Cappellesso et al. 2022, Nature Cancer 3:1464-1483) in a high bar orthotopic model.

Table 13A.

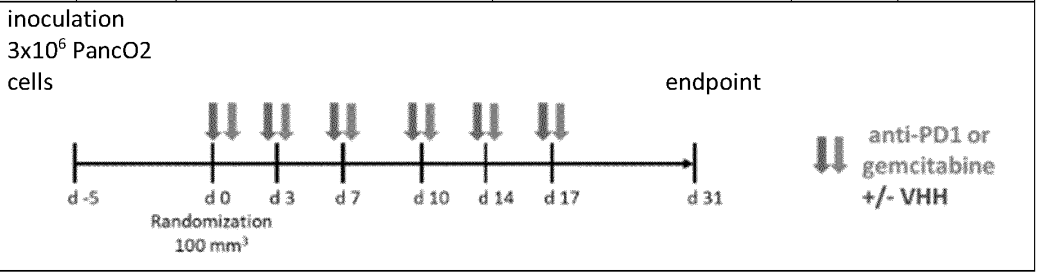
Group	# mice	treatment	dose (~10mg/kg)	route	schedule
1	8	IRR00002	200 µg/animal	ip	3x/week
2	8	P01400113	200 µg/animal	ip	3x/week



A further *in vivo* study, run at CrownBio (San Diego, CA), evaluated the combination of P01400113 with anti-PD1 immune checkpoint blockade or gemcitabine chemotherapy in the Pan02 subcutaneous PDAC model. The treatment schedule is shown in Table 13B. Monotherapy with anti-PD1 had a very limited effect on tumor growth whereas monotherapy with gemcitabine resulted in a more substantial inhibition of tumor growth (Figure 20). When either treatment was combined with P01400113, a substantial and significant improvement of the anti-tumor effect was observed (Figure 20). This study demonstrates that therapeutic blockade of SLC4A4 is a complementary treatment to both immune checkpoint blockade or chemotherapy.

Table 13B.

Group	# mice	treatment	dose (~10mg/kg)	route	schedule
1	8	IRR00002	200 µg/animal	ip	3x/week
2	8	anti-PD1+ IRR00002	200 + 200 µg/animal	ip	3x/week
3	8	anti-PD1+ P01400113	200 + 200 µg/animal	ip	3x/week
4	8	gemcitabine + IRR00002	1000 + 200 µg/animal	ip	3x/week
5	8	gemcitabine + P01400113	1000 + 200 µg/animal	Ip	3x/week



EXAMPLE 15. Interaction of VHH P01400040 with NBCe1 EL3 region spanning amino acids 629-644

The atomic structure of human NBCe1 in complex with VHH P01400040 obtained by single-particle electron microscopy has revealed the precise binding interface of the VHH with its antigen. VHH P01400040 binds a relatively large area of human SLC4A4 (hSLC4A4) making interactions with both extracellular loops 3 (EL3s) of the two hSLC4A4 part of the dimeric hSLC4A4 complex (Figure 17C). Mostly, these are polar interactions and involve hydrogen bonds (H-bonds) with distances of typically less than 3.5 Å.

In contrast to previously reported structures of NBCe1, EL3 (amino acid residues ~612-692, see e.g. Figure 1B) is to large part well resolved in our map and uncovers details of its fold and interactions. A

beta strand close to the beginning of EL3 (amino acid residues 626-629) is part of a short beta sheet together with a beta strand formed by amino acid residues 680-683 towards the end of EL3. The beta sheet and the parts of EL3 connecting it with transmembrane helices 5 and 6 are very well resolved. A 16 amino acid (aa) peptide of NBCe1 EL3 containing residues 629-644 was used to raise polyclonal antibodies (Khandoudi et al. 2001, Cardiovasc Res 52:387-396). The first five residues of the immunized 16-aa peptide, residues 629-633, could unambiguously be placed into the density of our map due to a high stiffness in the backbone in this area, also exemplified by the relatively low b-factors in that region. The stretch is starting as part of the above-mentioned beta-sheet (Cys629), and is followed by Val630, two consecutive prolines (amino acid residues 631-632) and Asp633. These residues are found in a distance of 4.7-8.5Å away from VHH P01400040 with a proline being closest (4.7 Å), making a contribution to VHH binding highly unlikely (also because no hydrogen-donors or acceptors would be provided due to the amino acids in this stretch) (Figure 17 A/B). Furthermore, other relevant interactions based on Van-der-Waals forces or cation-π interactions between the stretch 629-633 and VHH P01400040 could not be seen. The other eleven residues of the 16-aa peptide (amino acid residues 634-644) are part of a disordered loop that turns away from VHH P01400040 towards the second VHH P01400040 to connect with Ser654, and are not visible in our map. The absence of density in this region can be interpreted as lack of interaction with any other parts of the peptide (amino acid residues 634-644) and a high flexibility.

Based hereon, it is concluded that the NBCe1/SLC4A4 EL3 region spanning amino acids 629-644 is not part of the epitope truly bound by VHH P01400040.

EXAMPLE 16. Polyclonal SLC4A4 blocking antibodies compared with VHH P01400040

In another experiment, we evaluated the binding of two commercially available anti-SLC4A4 rabbit polyclonal antibodies raised against different EL3-derived peptides to a variant of EL3-binding P01400040 by flow cytometry on different cell lines overexpressing SLC4A4/NBCe1. P01400108 is a FLAG3-HIS6 variant of P01400040 with comparable functional activity that shares its VHH sequence with HIS6 tagged variant P01400119 (see Figure 21 and Table 14). The two polyclonal antibodies were compared at their recommended dilution (Novus Biologicals, NBP-32021 at 1/200 dilution of 0.3 mg/ml stock, raised in rabbits to the epitope spanning amino acids 609-685 of human NBCe1-B; and Alomone Labs, ANT-075 at 1/400 dilution of 0.8 mg/ml stock solution, raised in rabbits to the epitope spanning amino acids 672-685 of rat NBCe1) to P01400108 (at 500 nM) and a negative control anti-Hint1 rabbit polyclonal (ProteinTech, 10717-1-A9, 1/300 dilution of 0.1 mg/ml stock solution). The rabbit polyclonals were detected by Alexa Fluor 488 labelled goat anti-rabbit IgG (Invitrogen, A11034) and the FLAG3-tagged VHH was detected by mouse anti-FLAG M2 (Sigma-Aldrich F1804) followed by R-PE labelled Goat

Anti-Mouse F(ab')$_2$ fragment (Jackson ImmunoResearch, 115-116-071). The Alomone Labs polyclonal did not show any binding signal above the negative control polyclonal antibody, and this across all cell lines (Figure 22). In comparison to P01400118, the Novus Biologicals polyclonal antibody did not show any detectable binding on the cell lines overexpressing human and mouse NBCe1-B, nor was it able to detect the lower endogenous expression levels of human NBCe1 on HEK293T wild type cells (Figure 22). On the two highest expression cell lines (human NBCe1-A and human NBCe1-B(D2-85)) the Novus Biologicals polyclonal antibody showed binding signal above the negative control background, albeit at substantially lower levels than P01400108 (Figure 22).

The rabbit anti-human SLC4A4 EL3 polyclonal antibody preparation NBP2-32021 (Novus Biologicals) is a commercially available polyclonal antibody raised against amino acids 609-685 of human NBCe1-B, this is the closest available comparator to the SLC4A4 blocking polyclonal antibody reported by De Giusti et al. 2011 (Br J Pharmacol 164:1976-1989, referring to Alvarez et al.2003, Biochemistry 42:12321) and using amino acids 603-689 of human NBCe1-B as antigen.

HEK cells stably overexpressing human NBCe1-B, human NBCe1-B(Δ2-85), and human NBCe1-A (see Example 1.1 / Table 1; and Table 15) were pre-incubated for 30 minutes with different concentrations of VHH P01400040 (comprising HIS6-tag), unlabeled polyclonal antibody NBP2-32021, and with an unlabeled irrelevant VHH (anti-Hint1, ProteinTech, 10717-1-AP). Subsequently, cells were incubated for 30 minutes with VHH P01400040 (carrying a FLAG3-HIS6 tag), after which binding of VHH P01400040 (with FLAG3-HIS6 tag) to the cells was detected using anti-FLAG antibody (Sigma, F1804) in flow cytometry (FACS analysis).

From Figure 18 it can be appreciated that VHH P01400040 (with FLAG3-HIS6 tag) fully competed for the binding of P01400040 (with HIS6 tag) to all three SLC4A4/NBCe1 isoforms, whereas the anti-EL3 SLC4A4 NBP2-32021 and anti-Hint1 10717-1-AP polyclonal antibodies did not compete for binding. From this it can be concluded that VHH P01400040 binds to a different (non-overlapping) epitope than the anti-EL3 SLC4A4 NBP2-32021 polyclonal antibody.

A polyclonal antibody raised to the antigen spanning amino acids 609-685 of human NBCe1-B is thus not competing with VHH P01400040 for binding to human NBCe1-B, human NBCe1-B(Δ2-85), and human NBCe1-A.

EXAMPLE 17. Rendering VHH P01400014 cross-reactive with mouse NBCe1 and cross-blocking mouse NBCe1

From Table 10 it is clear that VHH P01400014 is not blocking mouse NBCe1, this despite exhibiting binding to mouse NBCe1. A variant of VHH P01400014 was produced, i.e. VHH P01400195, in which the

amino acid residue Thr at position 103 (in CDR3) was mutated to an Arg amino acid. This variant is, compared to parent VHH P01400014, very active in blocking mouse NBCe1, and is blocking mouse NBCe1 at a comparable level as

VHH P01400040 (Figure 19). For clarity the CDR3 sequence of VHH P01400014 (TTGSTNSPITDYAY, SEQ ID NO:136) was changed in VHH P01400195 into (TTGSRNSPITDYAY, SEQ ID NO:137). The full amino acid sequence of VHH P01400195 is defined by /given in SEQ ID NO:135 (Table 14).

EXAMPLE 18. Amino acid sequences

Amino acid sequences of the monovalent VHHs referred to hereinabove are given in Table 14, together with their CDR1, CDR2, CDR3, FR1, FR2, FR3, and FR4 amino acid sequences. All sequences are further shown in the sequence alignment of Figure 13, and in Figure 14.

Table 14.

SEQ ID NO:	VHH/ISVD/ sdAb ID or CDR ID or FR ID	Sequence (amino acids labeled "X" in 1-letter code are defined by the description "Xaa" in 3-letter code)
14	P01400023	EVQLVESGGGLVQPGGSLRLSCVASGRTFTGYAMGWFRQAPGKEREFVAAVSRS GTRVIYADSVKGRFTISKDNAQGTVYLQMNSLRPEDTAVYYCAADHKPSTAWVS ARGDMDFWGQGTLVTVSS
15	P01400014	EVQLVESGGGLVQPGGSLRLSCATSGRTFSGYAIGWFRQAPGKEREFVAAISRS GTRTVYADSVKGRFTIARDSAKSTTYLQMNSLRPEDTAVYYCAATTGSTNSPIT DYAYWGQGTLVTVSS
16	P01400012	EVQLVESGGGLVQPGGSLRLSCAASIATFSDYTVAWFRQAPGKEREFVAGIMRR AETTIYGDSVKGRFTVSRGNARNTVYLQMNSLRPEDTAVYYCAARPSMRIQTTA RQYDSWGQGTLVTVSS
17	P01400020	EVQLVESGGGLVQPGGSLRLSCAASTATFSDYTVGWFRQAPGKEREFVAGIMRR AETTIYGDSVKGRFTISRDNAKNTVYLQMNSLRPEDTAVYYCAARPSMRIQTTA RQYDSWGQGTLVTVSS
18	P01400021	EVQLVESGGGLVQPGGSLRLSCAASTATVSTYTMGWFRQAPGKEREFVAGIMRN ADTTLYGDSVKGRFTISRDNAKNTVYLQMNSLRPEDTAVYYCAARPSMRIQTTV RQYDSWGQGTLVTVSS
19	P01400024	EVQLVESGGGLVQPGGSLRLSCATSGRTSTIQTMAWFRQAPGKLREFVAAIRWS VGSTAYLNSVKGRFTISRDPLATTLYLQMNSLRPEDTAVYYCAADRTFSDSDSV TLQATYDYWGQGTLVTVSS
20	P01400040	EVQLVESGGGLVQAGGSLRLSCTTSGRTSRIQTMAWFRQAPGKLREFVAGIRWS VGSTAYLNSVKGRFTISRDPLATTLYLQMNNLKSEDTAVYYCAATTKLTSSDST TLQDTYDYWGRGTLVTVSS
21	P01400041	EVQLVESGGGLVQSGGSLRLSCVPSGRTSSIQTMAWFRQAPGKTREFVAGIRWS VGSTAYHNSVKGRFTISRDSYQTTLYLQMNNLKPEDTAVYYCAADRDLSSSDST TSQTTYDYWGRGTLVTVSS
22	P01400046	EVQLVESGGGLVQAGGSLRLSCVASGRTSSIQTMAWFRQAPGKSREFVAAIRWS VGSAAYLNSVKGRFTISRDYLKSTLYLQMNNLQPEDTAVYYCAADRRVSSSDST TLQATYDYWGQGTLVTVSS
23	CDR1-1	IQTMA

SEQ ID NO:	VHH/ISVD/ sdAb ID or CDR ID or FR ID	Sequence (amino acids labeled "X" in 1-letter code are defined by the description "Xaa" in 3-letter code)
24	CDR1-2	XYTXX wherein Xaa at position 1 is Thr or Asp; Xaa at position 4 is Met or Val; Xaa at position 5 is Gly or Ala
25	CDR1-21	DYTVX wherein Xaa at position 5 is Gly or Ala
26	CDR1-22	TYTMG
27	CDR1-23	DYTVG
28	CDR1-24	DYTVA
29	CDR1-3	GYAXG wherein Xaa at position 4 is Ile or Met
30	CDR1-31	GYAIG
31	CDR1-32	GYAMG
32	CDR2-1	XIRWSVGSXAYXNSVK wherein Xaa at position 1 is Gly or Ala; Xaa at position 9 is Thr or Ala; Xaa at position 12 is His or Leu
33	CDR2-11	GIRWSVGSTAYXNSVK wherein Xaa at position 12 is His or Leu
34	CDR2-12	AIRWSVGSXAYLNSVK wherein Xaa at position 9 is Thr or Ala
35	CDR2-13	GIRWSVGSTAYHNSVK
36	CDR2-14	GIRWSVGSTAYLNSVK
37	CDR2-15	AIRWSVGSTAYLNSVK
38	CDR2-16	AIRWSVGSAAYLNSVK
39	CDR2-2	GIMRXAXTTXYGDSVK wherein Xaa at position 5 is Asn or Arg; Xaa at position 7 is Asp or Glu; Xaa at position 10 is Leu or Ile
40	CDR2-21	GIMRNADTTLYGDSVK
41	CDR2-22	GIMRRAETTIYGDSVK
42	CDR2-3	AXSRSGTRXXYADSVK wherein Xaa at position 2 is Ile or Val; Xaa at position 9 is Thr or Val; Xaa at position 10 is Val or Ile
43	CDR2-31	AISRSGTRTVYADSVK
44	CDR2-32	AVSRSGTRVIYADSVK
45	CDR3-1	XXXXXXSDSXTXQXTYDY wherein Xaa at position 1 is Asp or Thr; Xaa at position 2 is Arg or Thr; Xaa at position 3 is Asp, Lys, Thr or Arg; Xaa at position 4 is Leu, Phe or Val; Xaa at position 5 is Ser or Thr; Xaa at position 6 is Ser or Asp; Xaa at position 10 is Thr or Val; Xaa at position 12 is Ser or Leu; X at position 14 is Thr, Asp or Ala
46	CDR3-11	DRXXSXSDSXTXQXTYDY wherein Xaa at position 3 is Asp, Thr or Arg; Xaa at position 4 is Leu, Phe or Val; Xaa at position 6 is Ser or Asp; Xaa at position 10 is Thr or Val; Xaa at position 12 is Ser or Leu; Xaa at position 14 is Thr or Ala
47	CDR3-12	DRDLSSSDSTTSQTTYDY
48	CDR3-13	TTKLTSSDSTTLQDTYDY
49	CDR3-14	DRTFSDSDSVTLQATYDY
50	CDR3-15	DRRVSSSDSTTLQATYDY

SEQ ID NO:	VHH/ISVD/ sdAb ID or CDR ID or FR ID	Sequence (amino acids labeled "X" in 1-letter code are defined by the description "Xaa" in 3-letter code)
51	CDR3-2	RPSMRIQTTXRQYDS wherein Xaa at position 10 is Val or Ala
52	CDR3-21	RPSMRIQTTVRQYDS
53	CDR3-22	RPSMRIQTTARQYDS
54	CDR3-3	TTGSXNSPITDYAY wherein Xaa at position 5 is Thr or Arg
55	CDR3-4	DHKPSTAWVSARGDMDF
56	FR1	EVQLVESGGGLVQXGGSLRLSCXXSXXTXX wherein Xaa at position 14 is Ser, Ala or Pro; Xaa at position 23 is Val, Thr, Ala; Xaa at position 24 is Pro, Thr, Ala; Xaa at position 26 is Gly, Thr or Ile; Xaa at position 27 is Arg, Ala; Xaa at position 29 is Ser, Val or Phe; Xaa at position 30 is Ser, Arg, Thr
57	FR1-11	EVQLVESGGGLVQXGGSLRLSCXXSGRTSX wherein Xaa at position 14 is Ser, Ala or Pro; Xaa at position 23 is Val, Thr or Ala; Xaa at position 24 is Pro, Thr or Ala; Xaa at position 30 is Ser, Arg, Thr
58	FR1-12	EVQLVESGGGLVQSGGSLRLSCVPSGRTSS
59	FR1-13	EVQLVESGGGLVQAGGSLRLSCTTSGRTSR
60	FR1-14	EVQLVESGGGLVQPGGSLRLSCATSGRTST
61	FR1-15	EVQLVESGGGLVQAGGSLRLSCVASGRTSS
62	FR1-21	EVQLVESGGGLVQPGGSLRLSCAASXATXS wherein Xaa at position 26 is Thr or Ile; Xaa at position 29 is Val or Phe
63	FR1-22	EVQLVESGGGLVQPGGSLRLSCAASTATVS
64	FR1-23	EVQLVESGGGLVQPGGSLRLSCAASTATFS
65	FR1-24	EVQLVESGGGLVQPGGSLRLSCAASIATFS
66	FR1-31	EVQLVESGGGLVQPGGSLRLSCXXSGRTFX wherein Xaa at position 23 is Ala or Val; Xaa at position 24 is Thr or Ala; Xaa at position 30 is Ser or Thr
67	FR1-32	EVQLVESGGGLVQPGGSLRLSCATSGRTFS
68	FR1-33	EVQLVESGGGLVQPGGSLRLSCVASGRTFT
69	FR2	WFRQAPGKXREFVA wherein Xaa at position 9 is Thr, Leu, Ser, Glu
70	FR2-11	WFRQAPGKTREFVA
71	FR2-12	WFRQAPGKLREFVA
72	FR2-13	WFRQAPGKSREFVA
73	FR2-21	WFRQAPGKEREFVA
74	FR3	GRFTXXXXXXXXTXYLQMNXLXXEDTAVYYCAA wherein Xaa at position 5 is Ile or Val; Xaa at position 6 is Ser or Ala; Xaa at position 7 is Arg or Lys; Xaa at position 8 is Asp or Gly; Xaa at position 9 is Ser, Pro, Tyr, Asn; Xaa at position 10 is Tyr, Leu, Ala; Xaa at position 11 is Gln, Ala, Lys, Arg; Xaa at position 12 is Thr, Ser, Asn, Gly; Xaa at position 14 is Leu, Val or Thr; Xaa at position 20 is Asn, Ser; Xaa at position 22 is Lys, Gln, Arg; Xaa at position 23 is Pro, Ser
75	FR3-11	GRFTISRDXXXXTLYLQMNXLXXEDTAVYYCAA wherein Xaa at position 9 is Ser, Pro or Tyr; Xaa at position 10 is Tyr or Leu; Xaa at position 11 is Gln, Ala or Lys; Xaa at position 12 is Thr or Ser; Xaa at position 20 is

SEQ ID NO:	VHH/ISVD/ sdAb ID or CDR ID or FR ID	Sequence (amino acids labeled "X" in 1-letter code are defined by the description "Xaa" in 3-letter code)
		Asn or Ser; Xaa at position 22 is Lys, Arg or Gln; Xaa at position 23 is Pro or Ser
76	FR3-12	GRFTISRDSYQTTLYLQMNNLKPEDTAVYYCAA
77	FR3-13	GRFTISRDPLATTLYLQMNNLKSEDTAVYYCAA
78	FR3-14	GRFTISRDPLATTLYLQMNSLRPEDTAVYYCAA
79	FR3-15	GRFTISRDYLKSTLYLQMNNLQPEDTAVYYCAA
80	FR3-21	GRFTXSRXNAXNTVYLQMNSLRPEDTAVYYCAA wherein Xaa at position 5 is Ile or Val; Xaa at position 8 is Asp or Gly; Xaa at position 11 is Lys or Arg
81	FR3-22	GRFTISRDNAKNTVYLQMNSLRPEDTAVYYCAA
82	FR3-23	GRFTVSRGNARNTVYLQMNSLRPEDTAVYYCAA
83	FR3-31	GRFTIXXDXAXXTXYLQMNSLRPEDTAVYYCAA wherein Xaa at position 6 is Ala or Ser; Xaa at position 7 is Arg or Lys; Xaa at position 9 is Ser or Asn; Xaa at position 11 is Lys or Gln; Xaa at position 12 is Ser or Gly; Xaa at position 14 is Thr or Val
84	FR3-32	GRFTIARDSAKSTTYLQMNSLRPEDTAVYYCAA
85	FR3-33	GRFTISKDNAQGTVYLQMNSLRPEDTAVYYCAA
86	FR4	WGXGTLVTVSS wherein Xaa at position 3 is Arg or Gln
87	FR4-11	WGRGTLVTVSS
88	FR4-12	WGQGTLVTVSS
89	P01400032	EVQLVESGGGLVQPGGSLRLSCAASGSISNTWPMGWYRQAPGKWREMVASISSS GSTNYAAPVKGRFTISREYDKNTVYLQLNSLKPEDTAVYYCNLLRTGQNYWGQG TLVTVSS
90	CDR1-312	GRTFSGYA
91	CDR1-313	GRTFSGYAIG
92	CDR2-312	ISRSGTRT
93	CDR2-312	AISRSGTRTV
94	CDR3-31	AATTGSTNSPITDYAY
95	CDR3-32	TTGSTNSPITDYAY
96	CDR1-231	TATFSDYT
97	CDR1-232	TATFSDYTVG
98	CDR2-221	IMRRAETT
99	CDR2-222	GIMRRAETTI
100	CDR3-221	AARPSMRIQTTARQYDS
101	CDR3-222	RPSMRIQTTARQYDS
102	CDR1-321	GRTFTGYA
103	CDR1-322	GRTFTGYAMG
104	CDR2-321	VSRSGTRV
105	CDR2-322	AVSRSGTRVI
106	CDR3-41	AADHKPSTAWVSARGDMDF
107	CDR3-42	DHKPSTAWVSARGDMDF
108	CDR1-12	GRTSRIQT
109	CDR1-13	GRTSRIQTMA
110	CDR2-142	IRWSVGST
111	CDR2-143	GIRWSVGSTA
112	CDR3-132	AATTKLTSSDSTTLQDTYDY

SEQ ID NO:	VHH/ISVD/ sdAb ID or CDR ID or FR ID	Sequence (amino acids labeled "X" in 1-letter code are defined by the description "Xaa" in 3-letter code)
113	CDR3-133	TTKLTSSDSTTLQDTYDY
128	P01400003	EVQLVESGGGLVQPGGSLRLSCAASGSISITWPMGWYRQAPGKQREMVATISTS LSTNYAAPVKGRFTISRENTKNTVYLQMNSLRPEDTAVYYCNLVMPGRTYWGQG TLVTVSS
129	P01400019	EVQLVESGGGLVQPGGSLRLSCAASTATFSSYTMGWFRQAPGKEREFVAGIMRS ADTTLYGDSVKGRFTISRDNAKNTVYLQMNSLRPEDTAVYYCAARPSMRIQTTV RQYDSWGQGTLVTVSS
130	P01400038	EVQLVESGGGLVQAGGSLRLSCVASGRTNSIQTMAWFRQTPGKSREFVAAIRWS VGSAAYLNSVKGRFTISRDYLKSTLYLQMNNLKPEDTATYYCAAVKTVSSSPST TLPATYDYWGRGTLVTVSS
135	P01400195	EVQLVESGGGLVQPGGSLRLSCATSGRTFSGYAIGWFRQAPGKEREFVAAISRS GTRTVYADSVKGRFTISRDSAKSTTYLQMNSLRPEDTAVYYCAATTGSRNSPIT DYAYWGQGTLVTVSS
136	CDR3-31	TTGSTNSPITDYAY
137	CDR3-32	TTGSRNSPITDYAY
138	P01400119	EVQLVESGGGLVQPGGSLRLSCTTSGRTSRIQTMAWFRQAPGKLREFVAGIRWS VGSTAYLNSVKGRFTISRDPLATTLYLQMNSLRPEDTAVYYCAATTKLTSSDST TLQDTYDYWGRGTLVTVSS
139	P01400131	EVQLVESGGGLVQPGGSLRLSCATSGRTFSGYAIGWFRQAPGKEREFVAAISRS GTRTVYADSVKGRFTISRDSAKSTTYLQMNSLRPEDTAVYYCAATTGSTNSPIT DYAYWGQGTLVTVSS
140	P01400134 with 1E/D	XVQLVESGGGLVQPGGSLRLSCATSGRTFSGYAIGWFRQAPGKEREFVAAISRS GTRTVYADSVKGRFTISRDSAKSTVYLQMNSLRPEDTAVYYCAATTGSTNSPIT DYAYWGQGTLVTVSS wherein Xaa at position 1 is Glu or Asp
141	P01400134 with 1E/D and/or 104N/G/S	XVQLVESGGGLVQPGGSLRLSCATSGRTFSGYAIGWFRQAPGKEREFVAAISRS GTRTVYADSVKGRFTISRDSAKSTVYLQMNSLRPEDTAVYYCAATTGSTXSPIT DYAYWGQGTLVTVSS wherein Xaa at position 1 is Glu or Asp; Xaa at position 104 is Asn, Gly or Ser
142	P01400134 with 1E/D and/or 74S/N and/or 77S/N	XVQLVESGGGLVQPGGSLRLSCATSGRTFSGYAIGWFRQAPGKEREFVAAISRS GTRTVYADSVKGRFTISRDXAKXTVYLQMNSLRPEDTAVYYCAATTGSTNSPIT DYAYWGQGTLVTVSS wherein Xaa at position 1 is Glu or Asp; Xaa at position 74 is Ser or Asn; Xaa at position 77 is Ser or Asn
143	P01400134 with 1E/D and/or 74S/N and/or 77S/N and/or 104N/G/S	XVQLVESGGGLVQPGGSLRLSCATSGRTFSGYAIGWFRQAPGKEREFVAAISRS GTRTVYADSVKGRFTISRDXAKXTVYLQMNSLRPEDTAVYYCAATTGSTXSPIT DYAYWGQGTLVTVSS wherein Xaa at position 1 is Glu or Asp; Xaa at position 74 is Ser or Asn; Xaa at position 77 is Ser or Asn; Xaa at position 104 is Asn, Gly or Ser
144	P01400161 with 1E/D	XVQLVESGGGLVQPGGSLRLSCATSGRTSRIQTMAWFRQAPGKLREFVAGIRWS VGSTAYANSVKGRFTISRDSYQTTLYLQMNSLRPEDTAVYYCAATTKLTSSGST TLQDTYDYWGQGTLVTVSS wherein Xaa at position 1 is Glu or Asp
145	P01400161	XVQLVESGGGLVQPGGSLRLSCATSGRTSRIQTMAWFRQAPGKLREFVAGIRWS VGSTAYANSVKGRFTISRDSYQTTLYLQMNSLRPEDTAVYYCAATTKLTSSXST

SEQ ID NO:	VHH/ISVD/ sdAb ID or CDR ID or FR ID	Sequence (amino acids labeled "X" in 1-letter code are defined by the description "Xaa" in 3-letter code)
	with 1E/D and/or 106D/G	TLQDTYDYWGQGTLVTVSS wherein Xaa at position 1 is Glu or Asp; Xaa at position 106 is Asp or Gly
146	P01400161 with 1E/D and/or 62N/D and/or 77T/N	XVQLVESGGGLVQPGGSLRLSCATSGRTSRIQTMAWFRQAPGKLREFVAGIRWS VGSTAYAXSVKGRFTISRDSYQXTLYLQMNSLRPEDTAVYYCAATTKLTSSGST TLQDTYDYWGQGTLVTVSS wherein Xaa at position 1 is Glu or Asp; Xaa at position 62 is Asn or Asp; Xaa at position 77 is Thr or Asn
147	P01400161 with 1E/D and/or 62N/D and/or 77T/N and/or 106D/G	XVQLVESGGGLVQPGGSLRLSCATSGRTSRIQTMAWFRQAPGKLREFVAGIRWS VGSTAYAXSVKGRFTISRDSYQXTLYLQMNSLRPEDTAVYYCAATTKLTSSXST TLQDTYDYWGQGTLVTVSS wherein Xaa at position 1 is Glu or Asp; Xaa at position 62 is Asn or Asp; Xaa at position 77 is Thr or Asn; Xaa at position 106 is Asp or Gly
148	CDR3-16	TTKLTSSGSTTLQDTYDY
149	CDR3-17	TTKLTSSXSTTLQDTYDY wherein Xaa at position 8 is Asp or Gly
150	CDR3-33	TTGSTXSPITDYAY wherein Xaa at position 6 is Asn, Gly or Ser
151	CDR3-34	TTGSXXSPITDYAY wherein Xaa at position 5 is Ser, Thr or Arg; Xaa at position 6 is Asn, Gly or Ser
152	CDR3-17	XXXXXXSXSXTXQXTYDY wherein Xaa at position 1 is Asp or Thr; Xaa at position 2 is Arg or Thr; Xaa at position 3 is Asp, Lys, Thr or Arg; Xaa at position 4 is Leu, Phe or Val; Xaa at position 5 is Ser, Thr or Arg; Xaa at position 6 is Ser, Asp, Asn or Gly; Xaa at position 8 is Asp or Gly;Xaa at position 10 is Thr or Val; Xaa at position 12 is Ser or Leu; X at position 14 is Thr, Asp or Ala
153	CDR2-17	GIRWSVGSTAYANSVK
154	CDR2-18	GIRWSVGSTAYXXSVK wherein Xaa at position 12 is Leu or Ala; Xaa at position 13 is Asp or Asn
155	CDR2-19	XIRWSVGSXAYXNSVK wherein Xaa at position 1 is Gly or Ala; Xaa at position 9 is Thr or Ala; Xaa at position 12 is His, Leu or Ala; Xaa at position 13 is Asn or Asp
156	FR1-16	EVQLVESGGGLVQPGGSLRLSCTTSGRTSR
157	FR1-17	EVQLVESGGGLVQPGGSLRLSCATSGRTSR
158	FR3-16	GRFTI SRDPL ATTLY LQMNS LRPED TAVYY CAA
159	FR3-17	GRFTI SRDSY QTTLY LQMNS LRPED TAVYY CAA
160	FR3-18	GRFTXXXXXXXXTXYLQMXXLXXEDTAVYYCAA wherein Xaa at position 5 is Ile or Val; Xaa at position 6 is Ser or Ala; Xaa at position 7 is Arg or Lys; Xaa at position 8 is Asp or Gly; Xaa at position 9 is Ser, Pro, Tyr, Asn; Xaa at position 10 is Tyr, Leu, Ala; Xaa at position 11 is Gln, Ala, Lys, Arg; Xaa at position 12

SEQ ID NO:	VHH/ISVD/ sdAb ID or CDR ID or FR ID	Sequence (amino acids labeled "X" in 1-letter code are defined by the description "Xaa" in 3-letter code)
		is Thr, Ser, Asn, Gly; Xaa at position 14 is Leu, Val or Thr; Xaa at position 19 is Ser or Asn; Xaa at position 20 is Asn, Ser; Xaa at position 22 is Lys, Gln, Arg; Xaa at position 23 is Pro, Ser
161	FR3-34	GRFTISRDSAKSTTYLQMNSLRPEDTAVYYCAA
162	FR3-35	GRFTISRDSAKSTVYLQMNSLRPEDTAVYYCAA
163	FR3-36	GRFTIXRDXAKXTXYLQMNSLRPEDTAVYYCAA Xaa at position 6 is Ser or Ala; Xaa at position 9 is Ser or Asn; Xaa at position 12 is Ser or Asn; Xaa at position 14 Thr or Val

Other sequences referred to herein are listed in Table 15.

Table 15. Other sequences

SEQ ID NO:	ID	Sequence (amino acids labeled "X" in 1-letter code are defined by the description "Xaa" in 3-letter code)
114	human NBCe1-B	MEDEAVLDRGASFLKHVCDEEEVEGHHTIYIGVHVPKSYRRRRRHKRKTGHK EKKEKERISENYSDKSDIENADESSSSILKPLISPAAERIRFILGEEDDSPA PPQLFTELDELLAVDGQEMEWKETARWIKFEEKVEQGGERWSKPHVATLSLH SLFELRTCMEKGSIMLDREASSLPQLVEMIVDHQIETGLLKPELKDKVTYTL LRKHRHQTKKSNLRSLADIGKTVSSASRMFTNPDNGSPAMTHRNLTSSSLND ISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFIAFVRLQQAVMLG ALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKAKDRH DLIAGIDEFLDEVIVLPPGEWDPAIRIEPPKSLPSSDKRKNMYSGGENVQMN GDTPHDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQ ALSAILFIYLATVTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAG QPLTILSSTGPVLVFERLLFNFSKDNNFDYLEFRLWIGLWSAFLCLILVATD ASFLVQYFTRFTEEGFSSLISFIFIYDAFKKMIKLADYYPINSNFKVGYNTL FSCTCVPPDPANISISNDTTLAPEYLPTMSSTDMYHNTTFDWAFLSKKECSK YGGNLVGNNCNFVPDITLMSFILFLGTYTSSMALKKFKTSPYFPTTARKLIS DFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGENPWWV CLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVI CSLMALPWYVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVF ILTGLSVFMAPILKFIPMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQ PDFIYLRHVPLRRVHLFTFLQVLCLALLWILKSTVAAIIFPVMILALVAVRK GMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKKKKKKGSLDSDNDDSDCPYSE KVPSIKIPMDIMEQQPFLSDSKPSDRERSPTFLERHTSC
115	Mouse NBCe1-B	MEDEAVLDRGASFLKHVCDEEEVEGHHTIYIGVHVPKSYRRRRRHKR KAGHKEKKEKERISENYSDKSDVENADESSSSILKPLISPAAERIRF ILGEEDDSPAPPQLFTELDELLAVDGQEMEWKETARWIKFEEKVEQG GERWSKPHVATLSLHSLFELRTCMEKGSIMLDREASSLPQLVEMIAD HQIETGLLKPDLKDKVTYTLLRKHRHQTKKSNLRSLADIGKTVSSAS RMFSNPDNGSPAMTHRNLTSSSLNDISDKPEKDQLKNKFMKKLPRDA EASNVLVGEVDFLDTPFIAFVRLQQAVMLGALTEVPVPTRFLFILLG PKGKAKSYHEIGRAIATLMSDEVFHDIAYKAKDRHDLIAGIDEFLDE VIVLPPGEWDPTIRIEPPKSLPSSDKRKNMYSGGENVQMNGDTPHDG GHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQAL

SEQ ID NO:	ID	Sequence (amino acids labeled "X" in 1-letter code are defined by the description "Xaa" in 3-letter code)
		SAILFIYLATVTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCL FAGQPLTILSSTGPVLVFERLLFNFSKDHNFDYLEFRLWIGLWSAFM CLVLVATDASFLVQYFTRFTEEGFSSLISFIFIYDAFKKMIKLADYY PINSDFKVGYNTHFSCACLPPDPVNLSVSNDTTLAPEDLPTISSTDM YHNVTFDWAYLSKKECVKYGGKLVGNNCDFVPDITLMSFILFLGTYT SSMAMKKFKTSRYFPTTARKLISDFAIILSILIFCVIDALVGVDTPK LIVPSEFKPTSPNRGWFVPPFGGNPWWVCLAAAIPALLVTILIFMDQ QITAVIVNRKEHKLKKGAGYHLDLFWVAILMVVCSFMALPWYVAATV ISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVFILTGLSVFM APILKFIPMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQPDFI YLRHVPLRRVHLFTFLQVLCLALLWILKSTVAAIIFPVMILALVAVR KGMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKKKKKGSLDSDNDDS DCPYSEKVPSIKIPMDIMEQQPFLSDNKPLDRERSSTFLERHTSC
116	Rhesus NBCe1-B	MEDEAVLDRGASFLKHVCDEEEVEGHHTIYIGVHVPKSYRRRRRHKRKAGHK EKKEKERISENYSDKSDIENADESSSSILKPLISPAAERIRFILGEEDDSPA PPQLFTELDELLAVDGQEMEWKETARWIKFEEKVEQGGERWSKPHVATLSLH SLFELRTCMEKGSIMLDREASSLPQLVEMIVDHQIETGLLKPELKDKVTYTL LRKHRHQTKKSNLRSLADIGKTVSSASRMFTNPDNGSPAMTHRNLTSSSLND ISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFIAFVRLQQAVMLG ALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKAKDRH DLIAGIDEFLDEVIVLPPGEWDPAIRIEPPKSLPSSDKRKNMYSGGENVQMN GDTPHDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQ ALSAILFIYLATVTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAG QPLTILSSTGPVLVFERLLFNFSKDNDFDYLEFRLWIGLWSAFLCLILVATD ASFLVQYFTRFTEEGFSSLISFIFIYDAFKKMIKLADYYPINSNFKVGYNTL FSCACVPPDPANISISNDTTLAPEYSPTISSTDMYHNTTFDWAFLSKKECLK HGGNLVGNNCNFVPDITLMSFILFLGTYTSSMALKKFKTSPYFPTTARKLIS DFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGENPWWV CLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVI CSLMALPWYVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVF ILTGLSVFMAPILKFIPMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQ PDFIYLRHVPLRRVHLFTFLQVLCLALLWILKSTVAAIIFPVMILALVAVRK GMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKKKKKGSLDSDNDDSDCPYSE KVPSIKIPMDIMEQQPFLSDSKPSDRERSPTFLERHTSC
117	Cyno NBCe1-B	MEDEAVLDRGASFLKHVCDEEEVEGHHTIYIGVHVPKSYRRRRRHKRKAGHK EKKEKERISENYSDKSDIENADESSSSILKPLISPAAERIRFILGEEDDSPA PPQLFTELDELLAVDGQEMEWKETARWIKFEEKVEQGGERWSKPHVATLSLH SLFELRTCMEKGSIMLDREASSLPQLVEMIVDHQIETGLLKPELKDKVTYTL LRKHRHQTKKSNLRSLADIGKTVSSASRMFTNPDNGSPAMTHRNLTSSSLND ISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFIAFVRLQQAVMLG ALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKAKDRH DLIAGIDEFLDEVIVLPPGEWDPAIRIEPPKSLPSSDKRKNMYSGGENVQMN GDTPHDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQ ALSAILFIYLATVTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAG QPLTILSSTGPVLVFERLLFNFSKDNDFDYLEFRLWIGLWSAFLCLILVATD ASFLVQYFTRFTEEGFSSLISFIFIYDAFKKMIKLADYYPINSNFKVGYNTL FSCACVPPDPANISISNDTTLAPEHSPTISSTDMYHNTTFDWAFLSKKECLK HGGNLVGNNCNFVPDITLMSFILFLGTYTSSMALKKFKTSPYFPTTARKLIS DFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGENPWWV CLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVI CSLMALPWYVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVF

SEQ ID NO:	ID	Sequence (amino acids labeled "X" in 1-letter code are defined by the description "Xaa" in 3-letter code)
		ILTGLSVFMAPILKFIPMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQ PDFIYLRHVPLRRVHLFTFLQVLCLALLWILKSTVAAIIFPVMILALVAVRK GMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKKKKKGSLDSDNDDSDCPYSE KVPSIKIPMDIMEQQPFLSDSKPSDRERSPTFLERHTSC
118	Human NBCe1-A	MSTENVEGKPSNLGERGRARSSTFLRVVQPMFNHSIFTSAVSPAAERIRFIL GEEDDSPAPPQLFTELDELLAVDGQEMEWKETARWIKFEEKVEQGGERWSKP HVATLSLHSLFELRTCMEKGSIMLDREASSLPQLVEMIVDHQIETGLLKPEL KDKVTYTLLRKHRHQTKKSNLRSLADIGKTVSSASRMFTNPDNGSPAMTHRN LTSSSLNDISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFIAFVR LQQAVMLGALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDI AYKAKDRHDLIAGIDEFLDEVIVLPPGEWDPAIRIEPPKSLPSSDKRKNMYS GGENVQMNGDTPHDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASD FYDALNIQALSAILFIYLATVTNAITFGGLLGDATDNMQGVLESFLGTAVSG AIFCLFAGQPLTILSSTGPVLVFERLLFNFSKDNNFDYLEFRLWIGLWSAFL CLILVATDASFLVQYFTRFTEEGFSSLISFIFIYDAFKKMIKLADYYPINSN FKVGYNTLFSCTCVPPDPANISISNDTTLAPEYLPTMSSTDMYHNTTFDWAF LSKKECSKYGGNLVGNNCNFVPDITLMSFILFLGTYTSSMALKKFKTSPYFP TTARKLISDFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPP FGENPWWVCLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLF WVAILMVICSLMALPWYVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQ RVTGTLVFILTGLSVFMAPILKFIPMPVLYGVFLYMGVASLNGVQFMDRLKL LLMPLKHQPDFIYLRHVPLRRVHLFTFLQVLCLALLWILKSTVAAIIFPVMI LALVAVRKGMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKKKKKGSLDSDND DSDCPYSEKVPSIKIPMDIMEQQPFLSDSKPSDRERSPTFLERHTSC
119	Human NBCe1-B (mouse EL2)	MEDEAVLDRGASFLKHVCDEEEVEGHHTIYIGVHVPKSYRRRRRHKRKTGHK EKKEKERISENYSDKSDIENADESSSSILKPLISPAAERIRFILGEEDDSPA PPQLFTELDELLAVDGQEMEWKETARWIKFEEKVEQGGERWSKPHVATLSLH SLFELRTCMEKGSIMLDREASSLPQLVEMIVDHQIETGLLKPELKDKVTYTL LRKHRHQTKKSNLRSLADIGKTVSSASRMFTNPDNGSPAMTHRNLTSSSLND ISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFIAFVRLQQAVMLG ALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKAKDRH DLIAGIDEFLDEVIVLPPGEWDPAIRIEPPKSLPSSDKRKNMYSGGENVQMN GDTPHDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQ ALSAILFIYLATVTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAG QPLTILSSTGPVLVFERLLFNFSKDHNFDYLEFRLWIGLWSAFLCLILVATD ASFLVQYFTRFTEEGFSSLISFIFIYDAFKKMIKLADYYPINSNFKVGYNTL FSCTCVPPDPANISISNDTTLAPEYLPTMSSTDMYHNTTFDWAFLSKKECSK YGGNLVGNNCNFVPDITLMSFILFLGTYTSSMALKKFKTSPYFPTTARKLIS DFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGENPWWV CLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVI CSLMALPWYVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVF ILTGLSVFMAPILKFIPMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQ PDFIYLRHVPLRRVHLFTFLQVLCLALLWILKSTVAAIIFPVMILALVAVRK GMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKKKKKGSLDSDNDDSDCPYSE KVPSIKIPMDIMEQQPFLSDSKPSDRERSPTFLERHTSC
120	Human NBCe1-B (mouse EL3)	MEDEAVLDRGASFLKHVCDEEEVEGHHTIYIGVHVPKSYRRRRRHKRKTGHK EKKEKERISENYSDKSDIENADESSSSILKPLISPAAERIRFILGEEDDSPA PPQLFTELDELLAVDGQEMEWKETARWIKFEEKVEQGGERWSKPHVATLSLH SLFELRTCMEKGSIMLDREASSLPQLVEMIVDHQIETGLLKPELKDKVTYTL LRKHRHQTKKSNLRSLADIGKTVSSASRMFTNPDNGSPAMTHRNLTSSSLND ISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFIAFVRLQQAVMLG ALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKAKDRH

SEQ ID NO:	ID	Sequence (amino acids labeled "X" in 1-letter code are defined by the description "Xaa" in 3-letter code)
		DLIAGIDEFLDEVIVLPPGEWDPAIRIEPPKSLPSSDKRKNMYSGGENVQMN GDTPHDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQ ALSAILFIYLATVTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAG QPLTILSSTGPVLVFERLLFNFSKDNNFDYLEFRLWIGLWSAFLCLILVATD ASFLVQYFTRFTEEGFSSLISFIFIYDAFKKMIKLADYYPINSDFKVGYNTH FSCACLPPDPVNLSVSNDTTLAPEDLPTISSTDMYHNVTFDWAYLSKKECVK YGGKLVGNNCDFVPDITLMSFILFLGTYTSSMALKKFKTSPYFPTTARKLIS DFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGENPWWV CLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVI CSLMALPWYVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVF ILTGLSVFMAPILKFIPMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQ PDFIYLRHVPLRRVHLFTFLQVLCLALLWILKSTVAAIIFPVMILALVAVRK GMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKKKKKGSLDSDNDDSDCPYSE KVPSIKIPMDIMEQQPFLSDSKPSDRERSPTFLERHTSC
121	Human NBCe1-B (mouse EL4)	MEDEAVLDRGASFLKHVCDEEEVEGHHTIYIGVHVPKSYRRRRRHKRKTGHK EKKEKERISENYSDKSDIENADESSSSILKPLISPAAERIRFILGEEDDSPA PPQLFTELDELLAVDGQEMEWKETARWIKFEEKVEQGGERWSKPHVATLSLH SLFELRTCMEKGSIMLDREASSLPQLVEMIVDHQIETGLLKPELKDKVTYTL LRKHRHQTKKSNLRSLADIGKTVSSASRMFTNPDNGSPAMTHRNLTSSSLND ISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFIAFVRLQQAVMLG ALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKAKDRH DLIAGIDEFLDEVIVLPPGEWDPAIRIEPPKSLPSSDKRKNMYSGGENVQMN GDTPHDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQ ALSAILFIYLATVTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAG QPLTILSSTGPVLVFERLLFNFSKDNNFDYLEFRLWIGLWSAFLCLILVATD ASFLVQYFTRFTEEGFSSLISFIFIYDAFKKMIKLADYYPINSNFKVGYNTL FSCTCVPPDPANISISNDTTLAPEYLPTMSSTDMYHNTTFDWAFLSKKECSK YGGNLVGNNCNFVPDITLMSFILFLGTYTSSMALKKFKTSPYFPTTARKLIS DFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGGNPWWV CLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVI CSLMALPWYVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVF ILTGLSVFMAPILKFIPMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQ PDFIYLRHVPLRRVHLFTFLQVLCLALLWILKSTVAAIIFPVMILALVAVRK GMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKKKKKGSLDSDNDDSDCPYSE KVPSIKIPMDIMEQQPFLSDSKPSDRERSPTFLERHTSC
122	Human NBCe1-B (mouse EL2+4)	MEDEAVLDRGASFLKHVCDEEEVEGHHTIYIGVHVPKSYRRRRRHKRKTGHK EKKEKERISENYSDKSDIENADESSSSILKPLISPAAERIRFILGEEDDSPA PPQLFTELDELLAVDGQEMEWKETARWIKFEEKVEQGGERWSKPHVATLSLH SLFELRTCMEKGSIMLDREASSLPQLVEMIVDHQIETGLLKPELKDKVTYTL LRKHRHQTKKSNLRSLADIGKTVSSASRMFTNPDNGSPAMTHRNLTSSSLND ISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFIAFVRLQQAVMLG ALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKAKDRH DLIAGIDEFLDEVIVLPPGEWDPAIRIEPPKSLPSSDKRKNMYSGGENVQMN GDTPHDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQ ALSAILFIYLATVTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAG QPLTILSSTGPVLVFERLLFNFSKDHNFDYLEFRLWIGLWSAFLCLILVATD ASFLVQYFTRFTEEGFSSLISFIFIYDAFKKMIKLADYYPINSNFKVGYNTL FSCTCVPPDPANISISNDTTLAPEYLPTMSSTDMYHNTTFDWAFLSKKECSK YGGNLVGNNCNFVPDITLMSFILFLGTYTSSMALKKFKTSPYFPTTARKLIS DFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGGNPWWV CLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVI CSLMALPWYVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVF

SEQ ID NO:	ID	Sequence (amino acids labeled "X" in 1-letter code are defined by the description "Xaa" in 3-letter code)
		ILTGLSVFMAPILKFIPMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQ PDFIYLRHVPLRRVHLFTFLQVLCLALLWILKSTVAAIIFPVMILALVAVRK GMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKKKKKGSLDSDNDDSDCPYSE KVPSIKIPMDIMEQQPFLSDSKPSDRERSPTFLERHTSC
123	Mouse NBCe1-B (human EL2)	MEDEAVLDRGASFLKHVCDEEEVEGHHTIYIGVHVPKSYRRRRRHKRKAGHK EKKEKERISENYSDKSDVENADESSSSILKPLISPAAERIRFILGEEDDSPA PPQLFTELDELLAVDGQEMEWKETARWIKFEEKVEQGGERWSKPHVATLSLH SLFELRTCMEKGSIMLDREASSLPQLVEMIADHQIETGLLKPDLKDKVTYTL LRKHRHQTKKSNLRSLADIGKTVSSASRMFSNPDNGSPAMTHRNLTSSSLND ISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFIAFVRLQQAVMLG ALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKAKDRH DLIAGIDEFLDEVIVLPPGEWDPTIRIEPPKSLPSSDKRKNMYSGGENVQMN GDTPHDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQ ALSAILFIYLATVTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAG QPLTILSSTGPVLVFERLLFNFSKDNNFDYLEFRLWIGLWSAFMCLVLVATD ASFLVQYFTRFTEEGFSSLISFIFIYDAFKKMIKLADYYPINSDFKVGYNTH FSCACLPPDPVNLSVSNDTTLAPEDLPTISSTDMYHNVTFDWAYLSKKECVK YGGKLVGNNCDFVPDITLMSFILFLGTYTSSMAMKKFKTSRYFPTTARKLIS DFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGGNPWWV CLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVV CSFMALPWYVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVF ILTGLSVFMAPILKFIPMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQ PDFIYLRHVPLRRVHLFTFLQVLCLALLWILKSTVAAIIFPVMILALVAVRK GMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKKKKKGSLDSDNDDSDCPYSE KVPSIKIPMDIMEQQPFLSDNKPLDRERSSTFLERHTSC
124	Mouse NBCe1-B (human EL3)	MEDEAVLDRGASFLKHVCDEEEVEGHHTIYIGVHVPKSYRRRRRHKRKAGHK EKKEKERISENYSDKSDVENADESSSSILKPLISPAAERIRFILGEEDDSPA PPQLFTELDELLAVDGQEMEWKETARWIKFEEKVEQGGERWSKPHVATLSLH SLFELRTCMEKGSIMLDREASSLPQLVEMIADHQIETGLLKPDLKDKVTYTL LRKHRHQTKKSNLRSLADIGKTVSSASRMFSNPDNGSPAMTHRNLTSSSLND ISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFIAFVRLQQAVMLG ALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKAKDRH DLIAGIDEFLDEVIVLPPGEWDPTIRIEPPKSLPSSDKRKNMYSGGENVQMN GDTPHDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQ ALSAILFIYLATVTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAG QPLTILSSTGPVLVFERLLFNFSKDHNFDYLEFRLWIGLWSAFMCLVLVATD ASFLVQYFTRFTEEGFSSLISFIFIYDAFKKMIKLADYYPINSNFKVGYNTL FSCTCVPPDPANISISNDTTLAPEYLPTMSSTDMYHNTTFDWAFLSKKECSK YGGNLVGNNCNFVPDITLMSFILFLGTYTSSMAMKKFKTSRYFPTTARKLIS DFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGGNPWWV CLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVV CSFMALPWYVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVF ILTGLSVFMAPILKFIPMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQ PDFIYLRHVPLRRVHLFTFLQVLCLALLWILKSTVAAIIFPVMILALVAVRK GMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKKKKKGSLDSDNDDSDCPYSE KVPSIKIPMDIMEQQPFLSDNKPLDRERSSTFLERHTSC
125	Mouse NBCe1-B (human EL4)	MEDEAVLDRGASFLKHVCDEEEVEGHHTIYIGVHVPKSYRRRRRHKRKAGHK EKKEKERISENYSDKSDVENADESSSSILKPLISPAAERIRFILGEEDDSPA PPQLFTELDELLAVDGQEMEWKETARWIKFEEKVEQGGERWSKPHVATLSLH SLFELRTCMEKGSIMLDREASSLPQLVEMIADHQIETGLLKPDLKDKVTYTL LRKHRHQTKKSNLRSLADIGKTVSSASRMFSNPDNGSPAMTHRNLTSSSLND ISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFIAFVRLQQAVMLG

SEQ ID NO:	ID	Sequence (amino acids labeled "X" in 1-letter code are defined by the description "Xaa" in 3-letter code)
		ALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKAKDRH DLIAGIDEFLDEVIVLPPGEWDPTIRIEPPKSLPSSDKRKNMYSGGENVQMN GDTPHDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQ ALSAILFIYLATVTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAG QPLTILSSTGPVLVFERLLFNFSKDHNFDYLEFRLWIGLWSAFMCLVLVATD ASFLVQYFTRFTEEGFSSLISFIFIYDAFKKMIKLADYYPINSDFKVGYNTH FSCACLPPDPVNLSVSNDTTLAPEDLPTISSTDMYHNVTFDWAYLSKKECVK YGGKLVGNNCDFVPDITLMSFILFLGTYTSSMAMKKFKTSRYFPTTARKLIS DFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGENPWWV CLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVV CSFMALPWYVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVF ILTGLSVFMAPILKFIPMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQ PDFIYLRHVPLRRVHLFTFLQVLCLALLWILKSTVAAIIFPVMILALVAVRK GMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKKKKKGSLDSDNDDSDCPYSE KVPSIKIPMDIMEQQPFLSDNKPLDRERSSTFLERHTSC
126	Mouse NBCe1-B (hEL2+4)	MEDEAVLDRGASFLKHVCDEEEVEGHHTIYIGVHVPKSYRRRRRHKRKAGHK EKKEKERISENYSDKSDVENADESSSSILKPLISPAAERIRFILGEEDDSPA PPQLFTELDELLAVDGQEMEWKETARWIKFEEKVEQGGERWSKPHVATLSLH SLFELRTCMEKGSIMLDREASSLPQLVEMIADHQIETGLLKPDLKDKVTYTL LRKHRHQTKKSNLRSLADIGKTVSSASRMFSNPDNGSPAMTHRNLTSSSLND ISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFIAFVRLQQAVMLG ALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKAKDRH DLIAGIDEFLDEVIVLPPGEWDPTIRIEPPKSLPSSDKRKNMYSGGENVQMN GDTPHDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQ ALSAILFIYLATVTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAG QPLTILSSTGPVLVFERLLFNFSKDNNFDYLEFRLWIGLWSAFMCLVLVATD ASFLVQYFTRFTEEGFSSLISFIFIYDAFKKMIKLADYYPINSDFKVGYNTH FSCACLPPDPVNLSVSNDTTLAPEDLPTISSTDMYHNVTFDWAYLSKKECVK YGGKLVGNNCDFVPDITLMSFILFLGTYTSSMAMKKFKTSRYFPTTARKLIS DFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGENPWWV CLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVV CSFMALPWYVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVF ILTGLSVFMAPILKFIPMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQ PDFIYLRHVPLRRVHLFTFLQVLCLALLWILKSTVAAIIFPVMILALVAVRK GMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKKKKKGSLDSDNDDSDCPYSE KVPSIKIPMDIMEQQPFLSDNKPLDRERSSTFLERHTSC
127	Human NBCe1-B(D2-85)	MSPAAERIRFILGEEDDSPAPPQLFTELDELLAVDGQEMEWKETARWIKFEE KVEQGGERWSKPHVATLSLHSLFELRTCMEKGSIMLDREASSLPQLVEMIVD HQIETGLLKPELKDKVTYTLLRKHRHQTKKSNLRSLADIGKTVSSASRMFTN PDNGSPAMTHRNLTSSSLNDISDKPEKDQLKNKFMKKLPRDAEASNVLVGEV DFLDTPFIAFVRLQQAVMLGALTEVPVPTRFLFILLGPKGKAKSYHEIGRAI ATLMSDEVFHDIAYKAKDRHDLIAGIDEFLDEVIVLPPGEWDPAIRIEPPKS LPSSDKRKNMYSGGENVQMNGDTPHDGGHGGGGHGDCEELQRTGRFCGGLIK DIKRKAPFFASDFYDALNIQALSAILFIYLATVTNAITFGGLLGDATDNMQG VLESFLGTAVSGAIFCLFAGQPLTILSSTGPVLVFERLLFNFSKDNNFDYLE FRLWIGLWSAFLCLILVATDASFLVQYFTRFTEEGFSSLISFIFIYDAFKKM IKLADYYPINSNFKVGYNTLFSCTCVPPDPANISISNDTTLAPEYLPTMSST DMYHNTTFDWAFLSKKECSKYGGNLVGNNCNFVPDITLMSFILFLGTYTSSM ALKKFKTSPYFPTTARKLISDFAIILSILIFCVIDALVGVDTPKLIVPSEFK PTSPNRGWFVPPFGENPWWVCLAAAIPALLVTILIFMDQQITAVIVNRKEHK LKKGAGYHLDLFWVAILMVICSLMALPWYVAATVISIAHIDSLKMETETSAP GEQPKFLGVREQRVTGTLVFILTGLSVFMAPILKFIPMPVLYGVFLYMGVAS

SEQ ID NO:	ID	Sequence (amino acids labeled "X" in 1-letter code are defined by the description "Xaa" in 3-letter code)
		LNGVQFMDRLKLLLMPLKHQPDFIYLRHVPLRRVHLFTFLQVLCLALLWILK STVAAIIFPVMILALVAVRKGMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKK KKKKGSLDSDNDDSDCPYSEKVPSIKIPMDIMEQQPFLSDSKPSDRERSPTF LERHTSC
131	SA26h5	EVQLVESGGGLVQPGGSLRLSCAASGRNIKEYVMGWFRQAPGKEREFVAAIS WSAGNIYYADSVKGRFTISRDNSKNTVYLQMNSLRPEDTAVYYCAAGRYSAW YVAAYEYDYWGQGTLVTVSS
132	sortase motif	LPXTG wherein Xaa at position 3 can by any amino acid
133	sortase motif	LPETG
134	asparaginyl ligase motif	NXL wherein Xaa at position 2 can be Gly, Ser, Ala, Gln
164	human IGHV3/JH germline consensus sequences	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVSVIS SDGSSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARWGQGTL VTVSS

EXAMPLE 19. Sequence optimization of VHHs P01400014 and VHH P01400040

The amino acid sequences of the SLC4A4-blocking VHHs P01400014 and P01400040 were modified to improve them in terms of humanization towards human IGHV3 and JH germline consensus sequences (defined by SEQ ID NO: 164, see Table 15), as well as in terms of chemical and biophysical stability, while minimizing the impact on target binding and inhibition. To this end, different mutant variants were generated for each VHH, leaving the VHH hallmark residues unchanged (see Figure 21, and evaluating the effect of changes of VHH amino acids into human IGHV3 and JH germline consensus sequences amino acids. The VHH variants were then compared for their capacity to compete for binding on human and cynomolgus monkey SLC4A4 (and mouse SLC4A4 in case of P01400040) versus their respective parental sequences by means of competition flow cytometry. Similar potency comparisons were performed in the functional acid shock recovery assay. MeltI) temperatures of the different variants were determined. The variants (at 1 mg/ml in PBS) were furthermore subjected to temperature stress (1 week at 40 °C) followed by analytical size exclusion chromatography to assess their oligomerization propensity. In addition, the VHHs (at 1 mg/ml in PBS) were subjected to long term temperature stress (4 weeks at 40 °C) and forced oxidative stress (10 mM H_2O_2 for 3 hours @ 37 °C), followed by detailed peptide mapping mass spectrometry to asses amino acid stability, more in particular evaluation of the post-translational modifications deamidation (Asn/Gln), isomerization (Asp), oxidation (Met/Trp), N-terminal cyclization (pyroGlu) and C-terminal truncations. Amino acid residues with poor chemical stability (>5% modification after relevant stress) were replaced by suitable alternatives.

VHH P01400131 was selected as reference variant of VHH P01400014 and comprises, relative to the amino acid sequence of VHH P01400014/SEQ ID NO:15, the Ala to Ser (A to S) substitution at position 71 – thus resulting in the amino acid sequence SEQ ID NO:139 for VHH P01400131, with the recited amino acid positions being relative to SEQ ID NO:15 (see Table 14).

VHH P01400119 was selected as reference variant of VHH P01400040 and comprises, relative to the amino acid sequence of VHH P01400040/SEQ ID NO:20, the Ala to Pro (A to P) substitution at position 14, the Asn to Ser (N to S) substitution at position 85, the Lys to Arg (K to R) substitution at position 87, and the Ser to Pro (S to P) substitution at position 88 – thus resulting in the amino acid sequence SEQ ID NO:138 for VHH P01400119, with the recited amino acid positions being relative to SEQ ID NO:20 (see Table 14).

HIS6-tagged sequence optimization variants of VHH P01400014 were compared to reference variant VHH P01400131. The only major chemical liability observed was cyclization of the free N-terminal Glu residue which is easily solved by a substitution to Asp (E1D) as exemplified in variants VHH P01400225 and VHH P01400226 (Table 16). The Asn deamidation motif in the CDR3 of VHH P01400131 (N111.1, IMGT numbering; N104 in SEQ ID NO:139) is not sensitive to deamidation after 4 weeks at 40 °C. However, both N111.1G and N111.1S (N104G and N104S in SEQ ID NO:139, respectively) substitutions are acceptable with minor potency losses on human and cynomolgus SLC4A4 as exemplified in variants VHH P01400225 and VHH P01400226 (Table 16). None of the variants showed any oligomerization propensity as assessed by the absence of pre-peaks in analytical size exclusion chromatography. The S82N and S85N mutations (IMGT numbering; S74N and S77N in SEQ ID NO:139, respectively) were not retained in the final variants. VHH P01400134 was selected as the final variant (with A79S and T87V mutations (IMGT numbering; T71S and T79V, respectively, relative to SEQ ID NO:139), with comparable binding and potency to VHH P01400131 on human and cyno SLC4A4. The amino acid sequence of VHH P01400134 with either E or D at the N-terminal position (E/D variation at position 1) is defined by SEQ ID NO:140. A derivative of VHH P01400134 including the E/D variation at position 1 and/or either N104G or N104S is defined by SEQ ID NO:141. An alternative derivative of VHH P01400134 including the E/D variation at position 1, the S/N variation at position 74 and/or the S/N variation at position 77 is defined by SEQ ID NO:142. A further alternative derivative of VHH P01400134 including the E/D variation at position 1, the S/N variation at position 74 and/or the S/N variation at position 77 and/or the N/G/S variation at position 104 is defined by SEQ ID NO:143. The E1D mutation should preferably be included in therapeutic formats where VHH P01400134 is in the N-terminal position.

The N/G/S variation at position 104 is located in the CDR3 region and leads to an alternative sequence definition of this CDR as being defined by SEQ ID NO:149 (amino acid position 6 therein chosen from N, G or S) – the same CDR3 region (native sequence defined by SEQ ID NO:54) was hereinabove described as harboring another possible amino acid substitution (amino acid position 5 therein chosen from T or R), thus combined leading to a further alternative sequence definition, i.e. as defined by SEQ ID NO:150.

Table 16. Summary of selected sequence optimization variants of VHH P01400014

	Mutations relative to VHH P01400131 (IMGT numbering)				Binding competition		Functional inhibition
Variant VHH ID	E1	T87	N111.1	Intrinsic fluorescence Tm (°C)	pIC50 on human NBCe1-B	pIC50 on cyno NBCe1-B	pIC50 on human NBCe1-B
P01400131				85	8.0	7.9	7.6
P01400134		V		88	7.9	7.6	7.4
P01400225	D	V	G	87	7.9	7.1	7.4
P01400226	D	V	S	87	8.0	7.1	7.4

HIS6-tagged sequence optimization variants of VHH P01400040 were compared to reference variant VHH P01400119. The only major chemical liability observed was cyclization of the free N-terminal Glu residue which is easily solved by a substitution to Asp (E1D) as exemplified in variants VHHs P01400161, 01400214 and 01400247 (Table 17). The Asp isomerization motif in the CDR3 of VHH P01400119 (D111.3, IMGT numbering; D106 in SEQ ID NO:138) is only very weakly (±5%) sensitive to deamidation after 4 weeks at 40 °C. As such this does not constitute a major developability liability. But in case this would not be acceptable, the D111.3G (D106G in SEQ ID NO:138) substitution as exemplified in variant VHH P01400161 is well tolerated in terms of potency and species cross-reactivity (Table 17). None of the variants showed any oligomerization propensity as assessed by the absence of pre-peaks in analytical size exclusion chromatography. The N69D, A84K and T85N mutations (IMGT numbering; N62D, A76K and T77N, respectively, in SEQ ID NO:138) were not retained in the final variants. As related cluster members VHHs P01400041 and P01400046 displayed comparable activity (see Example 8), we decided to explore some of their sequence features in some sequence optimization variants. A series of chimeric VHH P01400119 variants were conceived as exemplified in variants VHH P01400161 and VHH P01400247 (Table 17). VHH P01400161 was selected as the final variant (with T24A, L68A, P82S, L83Y, A84Q, D111.3G and R120Q mutations (IMGT numbering; T23A, L61A, P74S, L75Y, A76Q, D106G and R119Q, respectively, relative to SEQ ID NO:138), with improved binding and potency on human and mouse SLC4A4 and comparable binding on cynomolgus SLC4A4 to P01400119. The E1D mutation should not be included in therapeutic formats where P01400161 is not in the N-terminal position.

The amino acid sequence of VHH P01400161 with either E or D at the N-terminal position (E/D variation at position 1) is defined by SEQ ID NO:144. A derivative of VHH P01400161 including the E/D variation at position 1 and/or the D/G variation at position 106 is defined by SEQ ID NO:145.

An alternative derivative of VHH P01400161 including the E/D variation at position 1 and/or the N/D variation at position 62 and/or the T/N variation at position 77 is defined by SEQ ID NO:146.

A further alternative derivative of VHH P01400134 including the E/D variation at position 1 and/or the N/D variation at position 62 and/or the T/N variation at position 77 and/or the D/G variation at position 106 is defined by SEQ ID NO:147.

The D/G variation at position 106 is located in the CDR3 region and leads to an alternative sequence definition of this CDR as being defined by SEQ ID NO:148 (amino acid position 8 therein chosen from D or G).

Table 17. Summary of selected sequence optimization variants of VHH P01400040

	Mutations relative to VHH P01400119 (IMGT numbering)									Binding competition			Functional inhibition
Variant VHH ID	E1	T24	L68	P82	L83	A84	D111.3	R120	Intrinsic fluorescence Tm (°C)	pIC50 on human NBCe1-B	pIC50 on cyno NBCe1-B	pIC50 on mouse NBCe1-B	pIC50 on human NBCe1-B
P01400119									81	7.8	7.1	8.0	7.5
P01400161	D	A	A	S	Y	Q	G	Q	81	8.1	7.0	8.5	7.6
P01400214	D	A	A	N	A			Q	85	7.7	6.5	7.9	7.4
P01400247	D	A	A	S	Y	Q		Q	82	7.7	6.6	8.2	7.5

Next, a number of exemplary multivalent constructs comprising sequence optimized VHHs and a half-life extending VHH were generated. Optimized building blocks VHH P01400134 and VHH P01400161 and VHH SA26h5 (serum albumin binding VHH for half-life extension) were separated by long 35GS linkers. The multivalent constructs were compared to their monovalent counterparts VHH P01400134 and VHH P01400161 in binding competition flow cytometry and the acid shock recovery functional assay on both human and cynomolgus NBCe1-B. Results are summarized in Table 18. Overall, these multivalent constructs have potency/efficacy profiles mirroring the profiles of the monovalent VHH building blocks. One notable remark concerns the addition of SA26h5 to the C-terminus of VHH P01400161 (VHH P01400255 in Table 10) resulting in substantial loss in potency/efficacy compared to the monovalent control, this loss was mitigated by adding a second P01400161 building block (VHHs P01400250 and P01400253 in Table 10) improves potency/efficacy, therewith further corroborating the conclusion of Example 13.

Table 18. Competition for binding to and functional inhibition of human or cynomolgous ("cyno") NBCe1-B by multivalent constructs containing variants VHH P014000134 ("134") and/or VHH P014000161 ("161") and the serum albumin binding VHH SA26h5. ND: not determined

				Competition binding vs. VHH P01400040		Competition binding vs. VHH P01400014		Functional inhibition	
VHH ID	VHH1	VHH2	VHH3	Human NBCe1-B efficacy (%)	Cyno NBCe1-B efficacy (%)	Human NBCe1-B efficacy (%)	Cyno NBCe1-B efficacy (%)	Human NBCe1-B % inhibition	Cyno NBCe1-B % inhibition
P01400250	161	161	SA26h5	113	131	ND	67	84	94
P01400253	161	SA26h5	161	105	93	ND	58	28	ND
P01400255	161	SA26h5		100	36	ND	43	30	18
P01400161	161			100	100	ND	42	101	ND
P01400251	134	161	SA26h5	102	109	124	143	114	ND
P01400254	134	SA26h5	161	94	133	103	150	158	162
P01400249	134	134	SA26h5	50	163	124	150	144	150
P01400252	134	SA26h5	134	71	166	115	151	160	185
P01400134	134			21	128	100	100	121	46

CLAIMS

1. A compound specifically inhibiting Electrogenic Sodium Bicarbonate Cotransporter 1 (NBCe1)/solute carrier family member 4A4 (SLC4A4), wherein the compound is binding to and inhibiting the activity of human NBCe1-B, is binding to human NBCe1-B and human NBCe1-B(Δ2-85) expressed by a wild-type cell, and is not binding to amino acids 629-644 of human NBCe1-B.

2. A compound specifically inhibiting NBCe1/SLC4A4, wherein the compound is selected from NBCe1/SLC4A4-inhibiting immunoglobulin single variable domain antibody (ISVD) polypeptide, compounds comprising at least one CDR polypeptide of a NBCe1/SLC4A4-inhibiting ISVD polypeptide, and compounds comprising a NBCe1/SLC4A4-inhibiting ISVD polypeptide, wherein the ISVD polypeptide is chosen from the ISVD polypeptides defined by SEQ ID NOs:14-22, 135, and 138-147.

3. The compound specifically inhibiting NBCe1/SLC4A4 according to claim 2, wherein the at least one CDR polypeptide chosen from the CDR1 polypeptides defined by SEQ ID nOs: 23-31, from the CDR2 polypeptides defined by SEQ ID nOs: 32-44, and/or from the CDR3 polypeptides defined by SEQ ID NOs: 45-55, 136-137, and 148-150.

4. The compound specifically inhibiting NBCe1/SLC4A4 according to claim 2 or 3 wherein said compound is inhibiting human NBCe-1B, is inhibiting NBCe1-B(Δ2-85), and is not inhibiting human NBC1e-A.

5. The compound specifically inhibiting NBCe1/SLC4A4 according to any one of claims 2 to 4 wherein said compound is binding to extracellular loop 3 of human NBCe1-B exposed by a cell, is binding to human NBCe1-B and human NBCe1-B(Δ2-85) expressed by a wild-type cell, and is not binding to amino acids 629-644 of human NBCe1-B.

6. The compound specifically inhibiting NBCe1/SLC4A4 according to claim 4 or 5, wherein the compound is comprising an ISVD polypeptide chosen from the ISVD polypeptides defined by SEQ ID NOs:19-21, 138, 144-147, or wherein said compound is comprising at least one CDR polypeptide chosen from the CDR1 polypeptide defined by SEQ ID NO: 23, from the CDR2 polypeptides defined by SEQ ID NOs: 32-38, and/or from the CDR3 polypeptides defined by SEQ ID NOs: 45-50, 148.

7. The compound specifically inhibiting NBCe1/SLC4A4 according to claim 2 or 3 wherein said compound is inhibiting human NBCe-1B, is inhibiting NBCe1-B(Δ2-85), and is inhibiting human NBC1e-A.

8. The compound specifically inhibiting NBCe1/SLC4A4 according to any one of claims 2, 3 and 7 wherein said compound is binding to amino acid glutamate 775 in extracellular loop 4 (amino acid position 775 of human NBCe-1B) of human NBCe1-B exposed by a cell.

9. The compound specifically inhibiting NBCe1/SLC4A4 according to any one of claims 2, 3, 7 and 8 wherein said compound is binding to extracellular loop 2 and extracellular loop 4 of human NBCe1 exposed by a cell.

10. The compound specifically inhibiting NBCe1/SLC4A4 according to any one of claims 7 to 9, wherein the compound is comprising an ISVD polypeptide chosen from the ISVD polypeptides defined by SEQ ID NOs:14-18, 135, and 139-143, or wherein said compound is comprising at least one CDR polypeptide chosen from the CDR1 polypeptide defined by SEQ ID NOs: 24-31, from the CDR2 polypeptides defined by SEQ ID NOs: 39-44, and/or from the CDR3 polypeptides defined by SEQ ID NOs: 51-55, 136-137, and 149-150.

11. An isolated nucleic acid encoding a CDR polypeptide or ISVD polypeptide according to any of claims 2 to 10.

12. A vector comprising the nucleic acid according to claim 11.

13. A host cell expressing a CDR polypeptide or ISVD polypeptide according to any of claims 2 to 10, or comprising a nucleic acid according to claim 11, or comprising a vector according to claim 12.

14. The compound specifically inhibiting NBCe1/SLC4A4 according to any of claims 1 to 10, isolated nucleic acid according to claim 11, vector according to claim 12, or host cell according to claim 13 for use as a medicament.

15. The compound specifically inhibiting NBCe1/SLC4A4 according to any of claims 1 to 10, isolated nucleic acid according to claim 11, vector according to claim 12, or host cell according to claim 13 for use in treating or inhibiting a disease or disorder, or for use in inhibiting progression or relapse of a disease or disorder, wherein the disease or disorder is cancer, diabetes, ischemic heart disease, cardiac ischemia, or reperfusion injury.

16. A pharmaceutical composition comprising a compound specifically inhibiting NBCe1/SLC4A4 according to any of claims 1 to 10, isolated nucleic acid according to claim 11, vector according to claim 12, or host cell according to claim 13.

17. A method for producing a compound specifically inhibiting NBCe1/SLC4A4 according to any of claims 2 to 10 comprising:
    - expressing a CDR polypeptide or ISVD polypeptide according to any of claims 2 to 10 in a host cell according to claim 13, or synthetic manufacture of the polypeptide;
    - purifying the expressed or manufactured CDR polypeptide or ISVD polypeptide.