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

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 example 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 example 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 example, 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.

In one aspect, the disclosure refers to 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.

In one aspect, the disclosure refers to 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.

In one aspect, the disclosure refers to an isolated nucleic acid encoding a CDR polypeptide or ISVD polypeptide as disclosed herein.

In one aspect, the disclosure refers to a vector comprising the nucleic acid as disclosed herein.

In one aspect, the disclosure refers to a host cell expressing a CDR polypeptide or ISVD polypeptide as disclosed herein, or comprising a nucleic acid as disclosed herein, or comprising a vector as disclosed herein.

In one aspect, the disclosure refers to the use of the compound specifically inhibiting NBCe1/SLC4A4 as disclosed herein, isolated nucleic acid as disclosed herein, vector as disclosed herein, or host cell as disclosed herein in the manufacture of a medicament for treating or inhibiting a disease or disorder, or for 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.

In one aspect, the disclosure refers to a pharmaceutical composition comprising a compound specifically inhibiting NBCe1/SLC4A4 as disclosed herein, isolated nucleic acid as disclosed herein, vector as disclosed herein, or host cell as disclosed herein.

In one aspect, the disclosure refers to a method for producing a compound specifically inhibiting NBCe1/SLC4A4 as disclosed herein comprising: expressing a CDR polypeptide or ISVD polypeptide as disclosed herein in a host cell as disclosed herein, 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 **i**nhibition, 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,

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. Use of 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 in the manufacture of a medicament for treating or inhibiting a disease or disorder, or for 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.