mutant RAS proteins, while impacting wild-type RAS to a much lesser degree, opening an avenue to the use of such molecules in situations where downregulation preferentially or specifically of a mutant RAS, particularly of the G12V mutant RAS, is desired, such as in diseases, including certain cancers, associated with or caused by a RAS mutation, such as in particular by the G12V RAS mutation.

Accordingly, ~~an aspect provides~~there is provided a non-naturally occurring molecule configured to form an intermolecular beta-sheet with the β-aggregation prone region (APR) of the amino acid sequence GFLCVFAIN (SEQ ID NO: 3) in human RAS protein. The capacity of such molecule to target said APR for the intermolecular β-sheet formation may manifest in particular as the molecule's ability to downregulate, decrease solubility of and/or induce aggregation or inclusion body formation of a RAS protein, such as in particular of the G12V mutant RAS protein, such as for example in an appropriate *in vitro*, cell culture or *in vivo* setup.

In one aspect, there is provided a non-naturally occurring molecule configured to form an intermolecular beta-sheet with the β-aggregation prone region (APR) of the amino acid sequence GFLCVFAIN (SEQ ID NO: 3) in human RAS protein.

In another aspect, there is provided a nucleic acid encoding the molecule as defined herein, wherein the molecule is a polypeptide.

Further, there is ~~aspects~~ provided any molecule as taught herein for use in medicine.

In another aspect, there is provided a molecule as defined herein or a nucleic acid as defined herein for use in medicine.

Further, ~~aspects~~ there is provided any molecule as taught herein for use in a method of treating a disease caused by or associated with a RAS mutation, such as in particular the G12V mutation in human RAS protein.

In another aspect, there is provided a molecule as defined herein or the nucleic acid as defined herein for use in treating a disease caused by or associated with a mutation in human RAS protein, preferably with a mutation at position 12 in human RAS protein, more preferably with G12V RAS mutation.

In another aspect, there is provided use of a molecule as defined herein or a nucleic acid as defined herein in the manufacture of a medicament for the treatment of a disease caused by or associated with a mutation in human RAS protein, preferably with a mutation at position 12 in human RAS protein, more preferably with G12V RAS mutation.

~~Related aspects~~There is provided a method for treating a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any molecule as taught herein.

In another aspect, there is provided a pharmaceutical composition comprising a molecule as defined herein; or a nucleic acid as defined herein.

Further, ~~aspects~~ there is provided a pharmaceutical composition comprising any molecule as taught herein.

It shall be appreciated that to the extent that the present molecules allow to target non-human mutant RAS molecules, (such as RAS molecules from other eukaryotes, particularly from yeast, fungi or animals, more particularly from animals, even more particularly warm-blooded animals, and still more particularly mammals, such as domesticated animals, farm animals, sport animals, or pets), for example owing to an acceptable degree of sequence identity between the targeted APR in human RAS and a corresponding APR in a non-human RAS, the molecules may also be used in non-human animals similarly as described herein for humans. Hence, medical interventions and pharmaceutical compositions as contemplated herein may also subsume veterinary treatments and compositions for veterinary use. By the same token, the present molecules may lend themselves for a variety of *in vitro*, *in cellulo* or *in vivo* applications (e.g., diagnostics, imaging, use in cell or non-human animal models, research tool use, etc.) not only in human cells or tissues, but also in non-human cells or tissues and in non-human animals.

Hence, also provided is an *in vitro* method for downregulating the amount or biological activity of a RAS in a cell (e.g., a human or non-human cell) expressing, e.g., endogenously or exogenously

expressing, the RAS, the method comprising contacting the cell with a RAS targeting pept-in as taught herein or with a nucleic acid molecule encoding the same (an alternative available for polypeptide peptins). Also provided is thus a method for downregulating the amount or biological activity of a RAS in a non-human organism expressing, e.g., endogenously or exogenously expressing, the RAS, the method comprising administering to the organism a RAS targeting pept-in as taught herein or a nucleic acid molecule encoding the same.

These and further ~~aspects~~ examples ~~and preferred embodiments~~ of the ~~invention~~ disclosure are described in the following sections and in the appended claims. The subject-matter of the appended claims is hereby specifically incorporated in this specification.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 illustrates dose-response and IC_{50} determination of RAS-targeting molecules ('pept-ins') according to certain embodiments of the present invention and a negative control. Pept-ins were tested in a five-point dose-response using a one-in-two dilution series starting from 50µM as highest dose on adherently growing (2D) NCI-H441 cells. Viability was assessed after three days of exposure to the test compounds and normalized to vehicle conditions. Error bars represent the SD.

Fig. 2 illustrates IC_{50}s of RAS-targeting molecules ('pept-ins') according to certain embodiments of the present invention on suspension spheroid cultures. Waterfall plots showing the median IC_{50}s of RAS-targeting pept-ins on suspension spheroid cultures. Pept-ins were tested in a five-point dose-response using a one-in-two dilution series starting from 50µM as highest dose on spheroid suspension cultures on a set of cell lines with different KRAS mutations. Viability was assessed five days after of exposure to the test compounds. Error bars represent the SD on the median, if applicable.

Fig. 3 illustrates kinetic tinctorial aggregation assays on RAS-targeting molecules ('pept-ins') according to certain embodiments of the present invention. Aggregation behaviour of the RAS-targeting pept-ins was studied by performing kinetic tinctorial assays using the amyloid aggregate sensor dyes Thioflavin T (ThT; **lower panel**) and pentameric formyl thiophene acetic acid (p-FTAA; **upper panel**). All four biologically active pept-ins showed clear amyloid-aggregation kinetics with both dyes, while the inactive control showed no significant ThT signal and only a slight increase in p-FTAA signal over time.

Fig. 4 illustrates seeding of KRAS G12V by RAS-targeting molecules ('pept-ins') according to certain embodiments of the present invention. Seeding experiments of recombinant native KRAS G12V protein was performed with end-stage aggregates (**left panels**) or sonicated seeds (**right**

CLAIMS

1. A non-naturally occurring molecule configured to form an intermolecular beta-sheet with the β-aggregation prone region (APR) of the amino acid sequence GFLCVFAIN (SEQ ID NO: 3) in human RAS protein.

2. The molecule according to claim 1, wherein the RAS protein is KRAS, NRAS or HRAS protein, preferably KRAS protein.

3. The molecule according to claim 1 or 2, wherein the RAS protein is a mutant RAS protein, preferably a RAS protein mutated at position G12, G13 or Q61, more preferably at position G12.

4. The molecule according to claim 3, wherein the RAS protein is a G12V mutant RAS protein.

5. The molecule according to any one of claims 1 to 4, wherein the intermolecular beta-sheet involves at least 6 contiguous amino acids of the amino acid sequence GFLCVFAIN (SEQ ID NO: 3) in the human RAS protein.

6. The molecule according to claim 5, wherein the intermolecular beta-sheet involves the amino acid sequence LCVFAI (SEQ ID NO: 76) in the human RAS protein.

7. The molecule according to any one of claims 1 to 6, wherein the molecule is able to decrease the solubility or to induce the aggregation or inclusion body formation of the human RAS protein.

8. The molecule according to any one of claims 1 to 7, wherein the molecule comprises an amino acid stretch which participates in the intermolecular beta-sheet.

9. The molecule according to claim 8, wherein the amino acid stretch comprises at least 6 contiguous amino acids of the amino acid sequence GFLCVFAIN (SEQ ID NO: 3) or GFLSVFAIN (SEQ ID NO: 45).

10. The molecule according to claims 8 or 9, wherein the molecule comprises the amino acid stretch LSVFAI (SEQ ID NO: 6), FLSVFAI (SEQ ID NO: 46), GFLSVFAI (SEQ ID NO: 47), LSVFAIN (SEQ ID NO: 48), FLSVFAIN (SEQ ID NO: 49), or GFLSVFAIN (SEQ ID NO: 50).

11. The molecule according to any one of claims 8 to 10, wherein the molecule comprises the amino acid stretch LSVFAI (SEQ ID NO: 6).

12. The molecule according to any one of claims 8 to 11, wherein the amino acid stretch comprises one or more D-amino acids and/or analogues of one or more of its amino acids.

13. The molecule according to any one of claims 8 to 12, wherein the molecule comprises two or more, preferably two, said amino acid stretches, which are identical or different.

14. The molecule according to any one of claims 8 to 13, wherein the amino acid stretch or stretches are each independently flanked, on each end independently, by one or more amino acids that display low beta-sheet forming potential or a propensity to disrupt beta-sheets.

15. The molecule according to any one of claims 8 to 14, wherein the molecule comprises, consists essentially of or consists of the structure:

a) NGK1-P1-CGK1,

b) NGK1-P1-CGK1-Z1-NGK2-P2-CGK2,

c) NGK1-P1-CGK1-Z1-NGK2-P2-CGK2-Z2-NGK3-P3-CGK3, or

d) NGK1-P1-CGK1-Z1-NGK2-P2-CGK2-Z2-NGK3-P3-CGK3-Z3-NGK4-P4-CGK4,

wherein:

P1 to P4 each independently denote an amino acid stretch as defined in any one of claims 8 to 12,

NGK1 to NGK4 and CGK1 to CGK4 each independently denote 1 to 4 contiguous amino acids that display low beta-sheet forming potential or a propensity to disrupt beta-sheets, such as 1 to 4 contiguous amino acids selected from the group consisting of R, K, D, E, P, N, S, H, G, Q, and A, D-isomers and/or analogues thereof, and combinations thereof, preferably 1 to 4 contiguous amino acids selected from the group consisting of R, K, D, E, P, N, S, H, G, and Q, D-isomers and/or analogues thereof, and combinations thereof, more preferably 1 to 4 contiguous amino acids selected from the group consisting of R, K, D, E, and P, D-isomers and/or analogues thereof, and combinations thereof, and

Z1 to Z3 each independently denote a direct bond or preferably a linker~~.~~,

optionally wherein:

NGK1 to NGK4 and CGK1 to CGK4 is each independently 1 to 2 contiguous amino acids selected from the group consisting of R, K, A, and D, D-isomers and/or analogues thereof, and combinations thereof, preferably NGK1 to NGK4 and CGK1 to CGK4 is each independently 1 to 2 contiguous amino acids selected from the group consisting of R, K, and D, D-isomers and/or analogues thereof, and combinations thereof, such as wherein NGK1 to NGK4 and CGK1 to CGK4 is each independently K, R, D, A or KK, preferably each independently K, R, D or KK; and/or

each linker is independently selected from a stretch of between 1 and 10 units, preferably between 1 and 5 units, wherein a unit is each independently an amino acid or PEG, such as wherein each linker is independently GS, PP, AS, SA, GF, FF, or GSGS (SEQ ID NO: 51), or D-isomers

and/or analogues thereof, preferably each linker is independently GS, PP or GSGS (SEQ ID NO: 51), preferably GS, or D-isomers and/or analogues thereof.

~~16. The molecule according to claims 15, wherein:~~

~~NGK1 to NGK4 and CGK1 to CGK4 is each independently 1 to 2 contiguous amino acids selected from the group consisting of R, K, A, and D, D-isomers and/or analogues thereof, and combinations thereof, preferably NGK1 to NGK4 and CGK1 to CGK4 is each independently 1 to 2 contiguous amino acids selected from the group consisting of R, K, and D, D-isomers and/or analogues thereof, and combinations thereof, such as wherein NGK1 to NGK4 and CGK1 to CGK4 is each independently K, R, D, A or KK, preferably each independently K, R, D or KK; and/or~~

~~each linker is independently selected from a stretch of between 1 and 10 units, preferably between 1 and 5 units, wherein a unit is each independently an amino acid or PEG, such as wherein each linker is independently GS, PP, AS, SA, GF, FF, or GSGS (SEQ ID NO: 51), or D-isomers and/or analogues thereof, preferably each linker is independently GS, PP or GSGS (SEQ ID NO: 51), preferably GS, or D-isomers and/or analogues thereof.~~

~~17~~16. The molecule according to claim 15~~ or 16~~, wherein the molecules comprises, consists essentially of or consists of a peptide of the amino acid sequence KLSVFAIKGSKLSVFAIK (SEQ ID NO:7); optionally wherein the amino acid sequence comprises one or more D-amino acids and/or analogues of one or more of its amino acids, optionally wherein the N-terminal amino acid is acetylated and/or the C-terminal amino acid is amidated.

~~18~~17. The molecule according to any one of claims 1 to ~~17~~16, which comprises a detectable label, a moiety that allows for isolation of the molecule, a moiety increasing the stability or half-life of the molecule, a moiety increasing the solubility of the molecule, a moiety increasing the cellular uptake of the molecule, and/or a moiety effecting targeting of the molecule to cells.

18. A nucleic acid encoding the molecule according to any one of claims 1 to 17, wherein the molecule is a polypeptide.

19. The molecule according to any one of claims 1 to ~~18~~ 17 or the nucleic acid according to claim 18 for use in medicine~~; or~~

~~a nucleic acid encoding the molecule according to any one of claims 1 to 18, wherein the molecule is a polypeptide, for use in medicine~~.

20. The molecule according to any one of claims 1 to ~~18~~ 17 or the nucleic acid according to claim 18 for use in ~~a method of~~ treating a disease caused by or associated with a mutation in human RAS protein, preferably with a mutation at position 12 in human RAS protein, more preferably with G12V RAS mutation~~; or~~

~~a nucleic acid encoding the molecule according to any one of claims 1 to 18, wherein the molecule is a polypeptide, for use in a method of treating a disease caused by or associated with a mutation in human RAS protein, preferably with a mutation at position 12 in human RAS protein, more preferably with G12V RAS mutation.~~

21. The molecule or nucleic acid for use according to claim 20, wherein the disease is a neoplastic disease, particularly cancer~~.~~, optionally wherein the disease is pancreatic ductal adenocarcinoma, colorectal adenocarcinoma, multiple myeloma, lung adenocarcinoma, skin cutaneous melanoma, uterine corpus endometrioid carcinoma, uterine carcinosarcoma, thyroid carcinoma, acute myeloid leukaemia, bladder urothelial carcinoma, gastric adenocarcinoma, cervical adenocarcinoma, head and neck squamous cell carcinoma, non-small cell lung cancer (NSCLC), or colorectal cancer.

~~22. The molecule or nucleic acid for use according to claim 20 or 21, wherein the disease is pancreatic ductal adenocarcinoma, colorectal adenocarcinoma, multiple myeloma, lung adenocarcinoma, skin cutaneous melanoma, uterine corpus endometrioid carcinoma, uterine carcinosarcoma, thyroid carcinoma, acute myeloid leukaemia, bladder urothelial carcinoma, gastric adenocarcinoma, cervical adenocarcinoma, head and neck squamous cell carcinoma, non-small cell lung cancer (NSCLC), or colorectal cancer.~~

22. Use of the molecule according to any one of claims 1 to 17 or the nucleic acid according to claim 18 in the manufacture of a medicament for the treatment of a disease caused by or associated with a mutation in human RAS protein, preferably with a mutation at position 12 in human RAS protein, more preferably with G12V RAS mutation.

23. The use according to claim 22, wherein the disease is a neoplastic disease, particularly cancer, optionally wherein the disease is pancreatic ductal adenocarcinoma, colorectal adenocarcinoma, multiple myeloma, lung adenocarcinoma, skin cutaneous melanoma, uterine corpus endometrioid carcinoma, uterine carcinosarcoma, thyroid carcinoma, acute myeloid leukaemia, bladder urothelial carcinoma, gastric adenocarcinoma, cervical adenocarcinoma, head and neck squamous cell carcinoma, non-small cell lung cancer (NSCLC), or colorectal cancer.

~~23~~24. A pharmaceutical composition comprising

the molecule according to any one of claims 1 to ~~18~~17; or

~~a~~ the nucleic acid ~~encoding the molecule~~ according to ~~any one of~~ claim~~s 1 to~~ 18~~, wherein the molecule is a polypeptide~~.