targets (e.g. resulting in undesired activation or inhibition of other signaling pathways, or lack of activation or inhibition with respect to target isoforms), in the case of bi- or multispecific agents the right balance between the two or more binding specificities, suitable pharmacokinetic and –dynamic properties, an acceptable toxicological profile, and of course *in vivo* efficacy.

In view of the above, there is a need for novel therapeutic agents that allow an efficient treatment of several types of cancer diseases and tumors. It is thus an object of the invention to provide such pharmacologically active agents that can be used in the treatment of several cancer diseases, including NSCLC and TNBC.

In particular, it is an object of the invention to provide such pharmacologically active agents, compositions and/or methods of treatment that provide certain advantages compared to the agents, compositions and/or methods currently used and/or known in the art. These advantages include *in vivo* efficacy, improved therapeutic and pharmacological properties, less side effects, and other advantageous properties such as improved ease of preparation or reduced costs of goods, especially as compared to candidate drugs already known in the art.

BRIEF SUMMARY

According to a first aspect of the disclosure, the present disclosure provides polypeptides which specifically bind to LRP5 or LRP6, wherein such polypeptide of the disclosure comprises a first immunoglobulin single variable domain (a) selected from the group of immunoglobulin single variable domains (i) to (iii) defined by having the following CDR sequences:

(i):

CDR1: TYTVG (= SEQ ID NO:1)

CDR2: AIRRRGSSTYYADSVKG (= SEQ ID NO:2)

CDR3: DTRTVALLQYRYDY (= SEQ ID NO:3)

(ii):

CDR1: SYAMG (= SEQ ID NO:4)

CDR2: AIRRSGRRTYYADSVKG (= SEQ ID NO:5)

CDR3: ARRVRSSTRYNTGTWWWEY (= SEQ ID NO:6)

(iii):

CDR1: RYTMG (= SEQ ID NO:7)

CDR2: AIVRSGGSTYYADSVKG (= SEQ ID NO:8)

CDR3: DRRGRGENYILLYSSGRYEY (= SEQ ID NO:9),

and a second immunoglobulin single variable domain (b) selected from the group of immunoglobulin single variable domains (iv) and (v) defined by having the following CDR sequences:

(iv):

CDR1: SYAMG (= SEQ ID NO:10)

CDR2: AISWSGGSTYYADSVKG (= SEQ ID NO:11)

CDR3: SPIPYGSLLRRRNNYDY (= SEQ ID NO:12)

(v):

CDR1: SYAMG (= SEQ ID NO:13)

CDR2: AISWRSGSTYYADSVKG (= SEQ ID NO:14)

CDR3: DPRGYGVAYVSAYYEY (= SEQ ID NO:15).

The terms “first” and “second” with respect to such immunoglobulin single variable domains is solely intended to indicate that these domains are two different domains (as they will at least include different CDR sequences). Thus, these terms shall not be understood to refer to the exact order or sequence of the domains within such polypeptide chain.

The polypeptides of the disclosure optionally comprise a third immunoglobulin single variable domain, such as especially an albumin binding immunoglobulin single variable domain, such as the Alb11 domain, comprising the following CDRs:

CDR1(Alb11): SFGMS (= SEQ ID NO:16)

CDR2(Alb11): SISGSGSDTLYADSVKG (= SEQ ID NO:17)

CDR3(Alb11): GGSLSR (= SEQ ID NO:18).

According to a more specific example, the polypeptides of the disclosure include immunoglobulin single variable domains which are VHH domains, and preferably humanized VHH domains.

According to an even more specific example, the polypeptides of the disclosure include a first immunoglobulin single variable domain (a) selected from the group of immunoglobulin single variable domains (i) to (iii) having the following sequences:

(i):

AVQLVESGGGLVQPGGSLRLSCAASGRTFSTYTVGWFRQAPGKEREF
VAAIRRRGSSTYYADSVKGRFTISRDNSKNTVYLQMNSLRPEDTAVYY
CAADTRTVALLQYRYDYWGQGTLVTVSS
(= SEQ ID NO:19)

(ii):

AVQLVESGGGLVQPGGSLRLSCAASGGTFSSYAMGWFRQAPGKERE
FVAAIRRSGRRTYYADSVKGRFTISRDNSKNTVYLQMNSLRPEDTAVYY
CAAARRVRSSTRYNTGTWWWEYWGQGTLVTVSS
(= SEQ ID NO:20)

(iii):

AVQLVESGGGLVQPGGSLRLSCAASGLTFSRYTMGWFRQAPGKEREF
VAAIVRSGGSTYYADSVKGRFTISRDNSKNTVYLQMNSLRPEDTAVYY
CAADRRGRGENYILLYSSGRYEYWGQGTLVTVSS
(= SEQ ID NO:21),

and a second immunoglobulin single variable domain (b) selected from the group consisting of immunoglobulin single variable domains (iv) and (v) having the following sequences:

(iv):

EVQLVESGGGLVQPGGSLRLSCAASGRTFSSYAMGWFRQAPGKERE
FVAAISWSGGSTYYADSVKGRFTISRDNSKNTVYLQMNSLRPEDTAVY
YCAASPIPYGSLLRRRNNYDYWGQGTLVTVSS

(= SEQ ID NO:22), and

(v):

EVQLVESGGGLVQPGGSLRLSCAASGGTFSSYAMGWFRQAPGKERE
FVAAISWRSGSTYYADSVKGRFTISRDNSKNTVYLQMNSLRPEDTAVY
YCAADPRGYGVAYVSAYYEYWGQGTLVTVSS

(= SEQ ID NO:23).

According to a specifically preferred example, the polypeptides of the disclosure additionally include a half-life extending moiety, wherein said half-life extending moiety is covalently linked to said polypeptide and is optionally selected from the group consisting of an albumin binding moiety, such as an albumin binding peptide or an albumin binding immunoglobulin domain, preferably an albumin binding immunoglobulin single variable domain, more preferably the Alb11 domain, a transferrin binding moiety, such as an anti-transferrin immunoglobulin domain, a polyethylene glycol molecule, human serum albumin, and a fragment of human serum albumin.

Specifically preferred are polypeptides which include, in addition to the two immunoglobulin single variable domains (a) and (b) as outlined above, an Alb11 domain having the following sequence:

EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEW
VSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTI
GGSLSRSSQGTLVTVSS

(= SEQ ID NO:24)

According to a further example, the disclosure specifically includes polypeptides comprising or consisting of any of the following three polypeptide chains:
F13500575, having the sequence SEQ ID NO:25,
F13500571, having the sequence SEQ ID NO:26, and
F13500720, having the sequence SEQ ID NO:27.

According to another aspect of the disclosure, there is provided a polypeptide selected from the group of polypeptides comprising or consisting of SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27.

The disclosure also relates to nucleic acid molecules, expression vectors, host cells, and methods of manufacturing used in the production of a polypeptide of the disclosure. Nucleic acid molecules encoding the polypeptides of the disclosure can be used, in an isolated form, for constructing respective expression vectors, which then may be transfected into host cells used for biopharmaceutical production of the polypeptides of the disclosure. Such method of manufacturing typically comprises the steps of culturing the host cell under conditions that allow expression of the polypeptide, recovering the polypeptide and purifying it according to methods known in the art.

According to another aspect of the disclosure, there is provided an expression vector comprising a nucleic acid encoding a polypeptide as defined herein.

According to another aspect of the disclosure, there is provided a host cell carrying an expression vector as defined herein.

According to another aspect of the disclosure, there is provided a method of manufacturing a polypeptide as defined herein, comprising the steps of culturing a host cell as defined herein under conditions that allow expression of a polypeptide as defined herein; and recovering and optionally purifying said polypeptide.

According to another aspect of the disclosure, there is provided a pharmaceutical composition comprising (i) as the active ingredient, the polypeptide as defined herein, and (ii) a pharmaceutically acceptable carrier, and optionally (iii) a diluent, excipient, adjuvant and/or stabilizer.

Further aspects, embodiments, examples, uses and methods involving the polypeptides of the disclosure will become clear from the following detailed description of the disclosure and from the appended claims.

The disclosure provides for novel molecules that allow a more efficient treatment of several cancer types, such as TNBC, CRC, and NSCLC, with less side effects. The polypeptides of the disclosure provide for a surprising therapeutic effect (i.e. efficacy) in the treatment of cancer patients, in that they may induce tumor regression resulting in pathological complete response (pCR). This, in turn, is expected to result in significant improvement of progression free survival and overall survival, especially in high unmet medical need indications such as e.g. in breast cancer. Thus, the polypeptides of the disclosure provide for novel therapeutic options in the treatment of several cancer types, esp. those showing a deregulated Wnt signaling pathway and beta-catenin accumulation.

According to another aspect of the disclosure, there is provided the polypeptide as defined herein for use as a medicament in a method for the treatment, prevention or alleviation of a disease, disorder or condition in a human being or an animal, preferably for use in a method (a) for the treatment of cancer, such as breast cancer, lung cancer, pancreatic cancer, colorectal cancer, sarcomas,

ovarian cancer or hepatocellular carcinoma, or (b) for the treatment of idiopathic pulmonary disease, or (c) for the treatment of a retinopathy caused by abnormal Wnt signaling.

According to another aspect, there is provided a polypeptide as defined herein for use in the treatment of cancer in combination with an immune checkpoint inhibitor selected from the group consisting of anti-PD1 antibodies, anti-PDL1 antibodies, anti-CTLA4 antibodies, anti-BTLA antibodies, anti-LAG3 antibodies, and anti-TIM3 antibodies, or in combination with a cancer vaccine.

Furthermore, the polypeptides of the disclosure are easy to manufacture, have a high stability and low antigenicity, and offer a variety of options regarding administration routes, in addition to injection and infusion.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic representation of biparatopic polypeptides antagonizing Wnt1 and Wnt3a signaling. They consist of three domains, with two domains binding to distinct epitopes of LRP5 and LRP6 (Wnt1 and Wnt3a blocker) and one domain for half-life extension (human serum albumin binder).

Figure 2 shows the lack of correlation between binding FACS and ELISA assays for a representative number of LRP6 binding VHHs derived from the llama immunization. The number “1” panel of VHHs is characterized by high affinity to cells expressing LRP6 on the plasma membrane, as detected by FACS binding assays (on the y axis MCF values are reported). The number “2” panel of VHHs is characterized by high affinity to recombinant human LRP6 ectodomain (rhLRP6-Fc), as detected by ELISA binding assays (on the x axis OD405 values are reported).

Figure 3 shows the binding of three half-life extended biparatopic LRP5/LRP6 cross-reactive VHH constructs to human LRP5 (Figure 3A) and human LRP6 (Figure 3B) overexpressing HEK293 cell lines when compared to the negative control consisting of a non-targeting binder (VHH construct that binds to a bacterial protein which is not expressed in HEK293 cells).

Figure 4 shows complete DKK1 competition of three half-life extended biparatopic LRP5/LRP6 cross-reactive VHH constructs for binding to both human LRP5 (Figure 4A) and human LRP6 (Figure 4B) overexpressing HEK293 cell lines, as detected by a FACS-based DKK1 competition assay.

Claims:

1. A polypeptide which specifically binds to LRP5 or LRP6, comprising

- a first immunoglobulin single variable domain selected from the group of immunoglobulin single variable domains (i) to (iii) defined by comprising the following CDR sequences:

(i):

CDR1: TYTVG (= SEQ ID NO:1)

CDR2: AIRRRGSSTYYADSVKG (= SEQ ID NO:2)

CDR3: DTRTVALLQYRYDY (= SEQ ID NO:3)

(ii):

CDR1: SYAMG (= SEQ ID NO:4)

CDR2: AIRRSGRRTYYADSVKG (= SEQ ID NO:5)

CDR3: ARRVRSSTRYNTGTWWWEY (= SEQ ID NO:6)

(iii):

CDR1: RYTMG (= SEQ ID NO:7)

CDR2: AIVRSGGSTYYADSVKG (= SEQ ID NO:8)

CDR3: DRRGRGENYILLYSSGRYEY (= SEQ ID NO:9),

and

- a second immunoglobulin single variable domain selected from the group of immunoglobulin single variable domains (iv) and (v) defined by comprising the following CDR sequences:

(iv):

CDR1: SYAMG (= SEQ ID NO:10)

CDR2: AISWSGGSTYYADSVKG (= SEQ ID NO:11)

CDR3: SPIPYGSLLRRRNNYDY (= SEQ ID NO:12)

(v):

CDR1: SYAMG (= SEQ ID NO:13)

CDR2: AISWRSGSTYYADSVKG (= SEQ ID NO:14)

CDR3: DPRGYGVAYVSAYYEY (= SEQ ID NO:15).

2. The polypeptide of claim 1 wherein

- said first immunoglobulin single variable domain comprises the following CDR sequences:

CDR1: TYTVG (= SEQ ID NO:1)

CDR2: AIRRRGSSTYYADSVKG (= SEQ ID NO:2)

CDR3: DTRTVALLQYRYDY (= SEQ ID NO:3)

and wherein

- said second immunoglobulin single variable domain comprises the following CDR sequences:

CDR1: SYAMG (= SEQ ID NO:10)

CDR2: AISWSGGSTYYADSVKG (= SEQ ID NO:11)

CDR3: SPIPYGSLLRRRNNYDY (= SEQ ID NO:12).

3. The polypeptide of claim 1 wherein

- said first immunoglobulin single variable domain comprises the following CDR sequences:

CDR1: SYAMG (= SEQ ID NO:4)

CDR2: AIRRSGRRTYYADSVKG (= SEQ ID NO:5)

CDR3: ARRVRSSTRYNTGTWWWEY (= SEQ ID NO:6)

and wherein

- said second immunoglobulin single variable domain comprises the following CDR sequences:

CDR1: SYAMG (= SEQ ID NO:13)

CDR2: AISWRSGSTYYADSVKG (= SEQ ID NO:14)

CDR3: DPRGYGVAYVSAYYEY (= SEQ ID NO:15).

4. The polypeptide of claim 1 wherein

- said first immunoglobulin single variable domain comprises the following CDR sequences:

CDR1: RYTMG (= SEQ ID NO:7)

CDR2: AIVRSGGSTYYADSVKG (= SEQ ID NO:8)

CDR3: DRRGRGENYILLYSSGRYEY (= SEQ ID NO:9)

and wherein

- said second immunoglobulin single variable domain comprises the following CDR sequences:

  CDR1: SYAMG (= SEQ ID NO:13)

  CDR2: AISWRSGSTYYADSVKG (= SEQ ID NO:14)

  CDR3: DPRGYGVAYVSAYYEY (= SEQ ID NO:15).

5. The polypeptide of any of claims 1 to 4, wherein said immunoglobulin single variable domains are VHH domains, and preferably humanized VHH domains.

6. The polypeptide of claim 1 or claim 2, wherein
   - said first immunoglobulin single variable domain has the amino acid sequence as shown in SEQ ID NO:19 and
   - said second immunoglobulin single variable domain has the amino acid sequence as shown in SEQ ID NO:22.

7. The polypeptide of claim 1 or claim 3, wherein
   - said first immunoglobulin single variable domain has the amino acid sequence as shown in SEQ ID NO:20 and
   - said second immunoglobulin single variable domain has the amino acid sequence as shown in SEQ ID NO:23.

8. The polypeptide of claim 1 or claim 4, wherein
   - said first immunoglobulin single variable domain has the amino acid sequence as shown in SEQ ID NO:21 and
   - said second immunoglobulin single variable domain has the amino acid sequence as shown in SEQ ID NO:23.

9. The polypeptide according to any of claims 1 to 8, wherein said first and said second immunoglobulin single variable domains are covalently linked by a linker peptide, wherein said linker peptide optionally comprises or consists of a third immunoglobulin single variable domain.

10. The polypeptide according to claim 9, wherein said third immunoglobulin single variable domain is an albumin binding immunoglobulin single variable domain, and preferably the Alb11 domain, defined by SEQ ID NO:24.

11. A polypeptide selected from the group of polypeptides comprising or consisting of SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27.

12. An expression vector comprising a nucleic acid encoding a polypeptide according to any of claims 1 to 11.

13. A host cell carrying an expression vector according to claim 12.

14. A method of manufacturing a polypeptide according to any of claims 1 to 11, comprising the steps of
    - culturing a host cell according to claim 13 under conditions that allow expression of a polypeptide according to any of claims 1 to 11; and
    - recovering and optionally purifying said polypeptide.

15. A pharmaceutical composition comprising (i) as the active ingredient, the polypeptide according to any of claims 1 to 11, and (ii) a pharmaceutically acceptable carrier, and optionally (iii) a diluent, excipient, adjuvant and/or stabilizer.

16. The polypeptide according to any of claims 1 to 11 for use as a medicament in a method for the treatment, prevention or alleviation of a disease, disorder or condition in a human being or an animal, preferably for use in a method (a) for the treatment of cancer, such as breast cancer, lung cancer, pancreatic cancer, colorectal cancer, sarcomas, ovarian cancer or hepatocellular carcinoma, or (b) for the treatment of idiopathic pulmonary disease, or (c) for the treatment of a retinopathy caused by abnormal Wnt signaling.

17. A polypeptide of any of claims 1 to 11 for use in the treatment of cancer in combination with an immune checkpoint inhibitor selected from the group consisting of anti-PD1 antibodies, anti-PDL1 antibodies, anti-CTLA4 antibodies, anti-BTLA antibodies, anti-LAG3 antibodies, and anti-TIM3 antibodies, or in combination with a cancer vaccine.