tumor types (Noguera-Troise *et al.*, Nature. 2006 Dec 21; 444(7122):1032-7; Ridgway *et al.*, Nature. 2006 Dec 21;444(7122):1083-7).

Due to these results, Dll4 is being considered a promising target for cancer therapy, and several biological compounds that target Dll4 are in (pre-)clinical development have been described: REGN-421 (= SAR153192; Regeneron, Sanofi-Aventis; WO2008076379) and OPM-21M18 (OncoMed) (Hoey *et al.*, Cell Stem Cell. 2009 Aug 7; 5(2):168-77), both fully human Dll4 antibodies; YW152F (Genentech), a humanized Dll4 antibody (Ridgway *et al.*, Nature. 2006 Dec 21; 444(7122):1083-7); Dll4-Fc (Regeneron, Sanofi-Aventis), a recombinant fusion protein composed of the extracellular region of Dll4 and the Fc region of human IgG1 (Noguera-Troise *et al.*, Nature. 2006 Dec 21;444(7122)).

However, the state-of-the art monoclonal antibodies (MAbs) and fusion proteins have several shortcomings in view of their therapeutic application: To prevent their degradation, they must be stored at near freezing temperatures. Also, since they are quickly digested in the gut, they are not suited for oral administration. Another major restriction of MAbs for cancer therapy is poor transport, which results in low concentrations and a lack of targeting of all cells in a tumor.

It has been an object of the ~~present invention~~ embodiments disclosed herein to provide novel anti-angiogenic binding molecules for human therapy.

It has been a further object of the embodiments disclosed herein ~~invention~~ to provide methods for the prevention, treatment, alleviation and/or diagnosis of such diseases, disorders or conditions, involving the use and/or administration of such binding molecules and compositions comprising them. In particular, it is has been an object of the invention to provide such pharmacologically active binding molecules, compositions and/or methods that provide advantages compared to the agents, compositions and/or methods currently used and/or known in the art. These advantages include improved therapeutic and/or pharmacological properties and/or other advantageous properties, e.g. for manufacturing purposes, especially as compared to conventional antibodies as those described above, or fragments thereof.

BRIEF SUMMARY ~~OF THE INVENTION~~

According to ~~a first aspect~~the present disclosure, there are provided bispecific binding molecules, preferably bispecific immunoglobulins, preferably immunoglobulin single variable domains like VHHs and domain antibodies, which comprises at least one DLL4-binding component and at least one Ang2-binding component in a single molecule. These bispecific binding molecules may preferably comprise a further binding component, preferably a binding component binding to serum albumin. Hence, in a first aspect, there is provided a binding molecule comprising at least one Ang2-binding component, at least one Dll4-binding component, and at least one serum albumin binding component, wherein said Ang2-, Dll4- and serum albumin binding components are immunoglobulin single variable domains, each immunoglobulin single variable domain consisting of four framework regions and three complementarity determining regions (CDRs), and wherein said binding molecule is selected from the group consisting of binding molecules having (i) the CDR sequences as present in DLLANGBII00017 (SEQ ID NO: 476), (ii) the CDR sequences as present in DLLANGBII00018 (SEQ ID NO: 477), and (iii) the CDR sequences as present in DLLANGBII00019 (SEQ ID NO: 478).

More specifically, a bispecific binding molecule of the ~~invention~~ disclosure essentially comprises (i) at least one Dll4-binding component specifically binding to at least one epitope of Dll4 and (ii) at least one Ang2-binding component specifically binding to at least an epitope of Ang2, wherein the components are linked to each other in such a way that they simultaneously bind to Dll4 and Ang2 or that they bind to either Dll4 or Ang2 at a time.

According to ~~preferred aspects of the invention~~the disclosure, the two components comprise one or more immunoglobulin single variable domains that may be, independently of each other, VHHs or domain antibodies, and/or any other sort of immunoglobulin single variable domains, such as VL domains, as defined herein, provided that each of these immunoglobulin single variable domains will bind the antigen, i.e. Dll4 or Ang2, respectively.

According to a preferred embodiment, the immunoglobulin single variable domains are of the same type, in particular, all immunoglobulin single variable domains are VHHs or domain antibodies.

According to a particularly preferred embodiment, all immunoglobulin single variable domains are VHHs, preferably humanized (or "sequence-optimized", as defined herein) VHHs. Accordingly, the disclosure ~~invention~~ relates to bispecific binding molecules comprising an (optionally humanized or sequence-optimized) anti-Dll4 VHH and an (optionally humanized or sequence-optimized) anti-Ang2 VHH.

However, it will be clear to the skilled person that the teaching herein may be applied analogously to bispecific binding molecules including other anti-Dll4 or anti-Ang2 immunoglobulin single variable domains, such as domain antibodies.

In another aspect, the disclosure ~~invention~~ relates to a nucleic acid~~s~~ molecule encoding ~~the bispecific~~ a binding molecule~~s of the invention~~ as defined herein. In another aspect, the disclosure relates to a vector containing a nucleic acid molecule as defined herein. In another aspect, the disclosure relates to a ~~as well as~~ host cell~~s~~ containing a nucleic acid molecule as defined herein or a vector as defined herein~~same~~.

The ~~invention~~ disclosure further relates to a product or composition containing or comprising at least one bispecific binding molecule of the disclosure ~~invention~~ and optionally one or more further components of such compositions. In another aspect, the disclosure relates to a pharmaceutical composition containing at least one binding molecule as defined herein as the active ingredient.

The disclosure ~~invention~~ further relates to methods for preparing or generating the bispecific binding molecules, nucleic acids, host cells, products and compositions described herein.

The disclosure ~~invention~~ further relates to applications and uses of the bispecific binding molecules, nucleic acids, host cells, products and compositions described herein, as well as to methods for the prevention and/or treatment for diseases and disorders that can be modulated by inhibition of Dll4. In another aspect, the disclosure relates to the pharmaceutical composition as defined herein for the treatment of a disease that is associated with Dll4-mediated and/or Ang2-mediated effects on angiogenesis.

It has been found that the Ang2-binding component of the bispecific binding molecules according to the present disclosure ~~invention~~ binds to Ang2 with a potency at least 5,000 times higher, preferably 10,000 times higher than to Ang1 or Ang4. This will largely avoid blocking activation of Ang1-mediated signalling, which would counter the intended anti-angiogenetic effect.

It has further been found that the DLL4-binding component of the bi-specific binding molecules according to the present disclosure ~~invention~~ binds to DLL4-A with an affinity of at least 1,000 times higher than to Dll1, Jagged1 and preferably also against Jagged2. Due to this selectivity unwanted side reactions can be avoided.

In a preferred embodiment the bispecific binding molecules of the present disclosure

~~invention~~ are provided as linked VHH domains. Such molecules are significantly smaller than conventional antibodies and have thus the potential for penetrating into a tumor deeper than such conventional antibodies. This benefit is further accentuated by the specific sequences disclosed herein after being free of glycosylation sites.

Further, due to the bispecific nature (Dll4- and Ang2-binding components in one molecule) the tumor penetration of both functionalities will be necessarily equal, which will ensure that the beneficial effects of the combined antagonism of Dll4 and Ang2 will be provided within the whole depth of penetration of the tumor. This is an advantage over the combination of individual antagonists against these targets, since

the depth of penetration of individual antagonists will always vary to some degree. Another advantage of a preferred bispecific binding molecules of the present disclosure ~~invention~~ is their increased serum half-like due to a serum albumin binding component such as a serum albumin binding molecule as described herein.

These and other aspects, embodiments, advantages and applications of the disclosure ~~invention~~ will become clear from the further description hereinbelow.

DEFINITIONS

Unless indicated or defined otherwise, all terms used have their usual meaning in the art, which will be clear to the skilled person. Reference is for example made to the standard handbooks, such as Sambrook *et al*, "Molecular Cloning: A Laboratory Manual" (2nd Ed.), Vols. 1-3, Cold Spring Harbor Laboratory Press (1989); Lewin, "Genes IV", Oxford University Press, New York, (1990), and Roitt *et al.*, "Immunology" (2nd Ed.), Gower Medical Publishing, London, New York (1989), as well as to the general background art cited herein; Furthermore, unless indicated otherwise, all methods, steps, techniques and manipulations that are not specifically described in detail can be performed and have been performed in a manner known *per se*, as will be clear to the skilled person. Reference is for example again made to the standard handbooks, to the general background art referred to above and to the further references cited therein.

The term "bispecific binding molecule" refers to a molecule comprising at least one Ang2-binding molecule (or "Ang2-binding component") and at least one Dll4-binding molecule (or "Dll4-binding component"). A bispecific binding molecule may contain more than one Ang2-binding molecule and/or more than one Dll4-binding molecule, i.e. in the case that the bispecific binding molecule contains a biparatopic (as defined below) Ang2-binding molecule and/or a biparatopic Dll4-binding molecule, in the part of the molecule that binds to Ang2 or to Dll4, i.e. in its "Ang2-binding component" (or anti-Ang2 component) or "Dll4-binding component" (or anti-Dll4 component), respectively. The word "bispecific" in this context is however not to be construed as to exclude further binding components with binding specificity to molecules other than Dll4 and Ang2 from the bispecific binding molecule. Non-limiting examples of such further binding components are binding components binding to serum albumin.

Claims

1. A ~~bispecific~~ binding molecule comprising
- at least one Ang2-binding component,
- at least one Dll4-binding component, and
- at least one serum albumin binding component,

wherein said Ang2-, Dll4- and serum albumin binding components are immunoglobulin single variable domains, each immunoglobulin single variable domain consisting of four framework regions and three complementarity determining regions (CDRs), and

wherein said ~~bispecific~~ binding molecule is selected from the group consisting of ~~bispecific~~ binding molecules having
(i) the CDR sequences as present in DLLANGBII00017 (SEQ ID NO: 476),
(ii) the CDR sequences as present in DLLANGBII00018 (SEQ ID NO: 477), and
(iii) the CDR sequences as present in DLLANGBII00019 (SEQ ID NO: 478).

2. The ~~bispecific~~ binding molecule of claim 1, wherein said immunoglobulin single variable domains are VHHs.

3. The ~~bispecific~~ binding molecule of claim 2, selected from the group consisting of ~~bispecific~~ binding molecules (i) to (iii),
the molecules (i) comprising
- the DLLBII00018 VHH domain as the Dll4-binding component,
- the Alb11 domain (SEQ ID NO: 519) as the serum albumin binding component,
and
- two ANGBII000921 VHH domains (SEQ ID NO: 485) as the Ang2-binding domains,
in this order;
the molecules (ii) comprising
- one Ang2-binding domain, having a CDR1 having the sequence DYAIG (= SEQ ID NOs: 495, 498, 513, 516), a CDR2 having the sequence AIRSSGGSTYYADSVKG (= SEQ ID NOs: 514, 517), and a CDR3 having the sequence VPAGRLRYGEQWYPIYEYDA (= SEQ ID NO: 515),
- the DLLBII00018 domain as the Dll4-binding component, and
- the Alb11 domain (SEQ ID NO: 519) as the serum albumin binding component,
in this order;

and

the molecules (iii) comprising

- two Ang2-binding domains, each having a CDR1 having the sequence DYAIG (= SEQ ID NOs: 495, 498, 513, 516), a CDR2 having the sequence AIRSSGGSTYYADSVKG (= SEQ ID NOs: 514, 517), and a CDR3 having the sequence VPAGRLRFGEQWYPLYEYDA (= SEQ ID NO: 518),
- the DLL00018 domain as the Dll4-binding component, and
- the Alb11 domain (SEQ ID NO: 519) as the serum albumin binding component, in this order.

4. The ~~bispecific~~ binding molecule of claim 3, selected from the group consisting of
(i) DLLANGBII00017 (SEQ ID NO: 476),
(ii) DLLANGBII00018 (SEQ ID NO: 477), and
(iii) DLLANGBII00019 (SEQ ID NO: 478).

5. A nucleic acid molecule encoding a ~~bispecific~~ binding molecule of any one of claims 1 to 4.

6. A vector containing a nucleic acid molecule of claim 5.

7. A host cell containing a nucleic acid molecule of claim 5 or a vector of claim 6.

8. A pharmaceutical composition containing at least one ~~bispecific~~ binding molecule of any one of claims 1 to 4 as the active ingredient.

9. The pharmaceutical composition of claim 8 for the treatment of a disease that is associated with Dll4-mediated and/or Ang2-mediated effects on angiogenesis.

10. The pharmaceutical composition of claim 8 or 9 for the treatment of cancer and cancerous diseases.

11. The pharmaceutical composition of claim 8 or 9 for the treatment of eye diseases.