WO2008/101985 describes immunoglobulin single variable domains from camelides (VHHs or "Nanobodies®, as defined herein) that bind to VEGF, and their use in the treatment of conditions and diseases characterized by excessive and/or pathological angiogenesis or neovascularization.

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

It has been a further object of the 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 disclosure~~first aspect~~, there are provided bispecific binding molecules, preferably bispecific immunoglobulins, preferably immunoglobulin single variable domains like VHHs and domain antibodies, which comprise at least one VEGF-binding component and at least one Ang2-binding component in a single molecule. Preferably, said bispecific binding molecules further comprise a serum albumin binding component.

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

According to an aspect, there is provided a bispecific binding molecule comprising a VEGF-binding immunoglobulin single variable domain, a serum albumin binding immunoglobulin single variable domain, and an Ang2-binding immunoglobulin single variable domain, wherein said VEGF-binding immunoglobulin single variable domain has the following CDR sequences:

CDR1: SYSMG

CDR2: AISKGGYKYDAVSLEG

CDR3: SRAYGSSRLRLADTYEY,

said serum albumin binding immunoglobulin single variable domain has the following CDR sequences:

CDR1: SFGMS (SEQ ID NO:255)

CDR2: SISGSGSDTLYADSVKG (SEQ ID NO:256)

CDR3: GGSLSR (SEQ ID NO:257)

said Ang2-binding immunoglobulin single variable domain has the following CDR sequences:

CDR1: DYAIG (SEQ ID NO:248)

CDR2: AIRSSGGSTYYADSVKG (SEQ ID NO:249)

CDR3: VPAGRLRYGEQWYPIYEYDA (SEQ ID NO:250)

According to another aspect, there is provided a bispecific binding molecule VEGFANGBII00022 (SEQ ID NO: 207).

According to preferred disclosure~~aspects of the invention~~, 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. Ang2 or VEGF, 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-Ang2 VHH and an (optionally humanized or sequence-optimized) anti-VEGF 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-Ang2 or anti-VEGF immunoglobulin single variable domains, such as domain antibodies.

In another aspect, there is provided~~the invention relates to~~ nucleic acids encoding the bispecific binding molecules of the disclosure~~invention as well as host cells containing same~~. In another aspect, there is provided a vector containing the nucleic acid molecule of the disclosure. In another aspect, there is provided host cells containing a vector of the disclosure.

The disclosure~~invention~~ 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.

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

The disclosureinvention 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 Ang2.

According to another aspect, there is provided a pharmaceutical composition containing at least one bispecific binding molecule as defined above as the active ingredient.

According to another aspect, there is provided the pharmaceutical composition as defined above for use in the treatment of cancer and cancerous diseases.

According to another aspect, there is provided the pharmaceutical composition as defined above for use in the treatment of eye diseases.

According to another aspect, there is provided a method of manufacturing a bispecific binding molecule as defined above comprising the steps of: culturing host cells as defined above under conditions that allow expression of said bispecific binding molecule; and recovering or isolating said bispecific binding molecule expressed by said host cells from the culture.

It has been found that the Ang2-binding component of the bispecific binding molecules according to the present disclosure~~invention~~ binds to and antagonizes 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 VEGF-binding component of the bi-specific binding molecules according to the present disclosure~~invention~~ binds to VEGF-A with an affinity of at least 1,000 times higher, preferebly at least 5,000 times higher, more preferably at least 10,000 times higher than to VEGF-B, VEGF-C, VEGF-D or PLGF. Due to the highly preferential binding to VEGF-A the signaling of VEGFR3, which modulates of lymph angiogenesis, is not interfered with.

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 (VEGF- 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 VEGF 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.

Affinities of for human serum albumin have been determined and are shown in Table 44. Briefly, human serum albumin (Sigma, St Louis, MO, USA) is immobilized on a CM5 chip via amine coupling. A multicycle kinetic approach is used: increasing concentrations of VHH (2-8-31-125-500 nM) are injected and allowed to associate for 2 min and to dissociate for 10 min at a flow rate of 100 µL/min. Between VHH injections, the surfaces are regenerated with a 10 sec pulse of 10 mM Glycine-HCl pH 1.5 and 60 sec stabilization period. Association/dissociation data are evaluated by fitting a 1:1 interaction model (Langmuir binding) or Heterogeneous Ligand model. The affinity constant K_D is calculated from resulting association and dissociation rate constants k_a and k_d (Table 44).