Dual targeting of angiogenesis pathways: combined blockade of VEGF and Ang2 signaling

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ABSTRACT

VEGF and Ang2 are important players in angiogenesis. VEGF is a well-known survival factor for endothelial cells, and several agents targeting VEGF or its receptor(s) are currently in clinical development. Ang2 is a negative regulator of tumor vessel plasticity, allowing vessels to respond to other angiogenic stimuli. However, little is known about the functional relationship between these angiogenic pathways, with Ang2 enhancing VEGF signaling, and VEGF regulating Ang2 expression on endothelial cells. Thus, combined inhibition of VEGF and Ang2 might well result in improved clinical efficacy compared to VEGF pathway blockade alone.

We have generated a humanized trispecific Nanobody® comprising two single variable domains blocking VEGF and Ang2, and an additional module for half-life extension in vivo. This molecule was tested in vivo for pathway inhibition and effect on endothelial cells. In addition, efficacy of the Nanobody® was tested in a series of patient-derived (PDX) xenograft models.

The VEGF/Ang2 Nanobody® was found to inhibit VEGF and Ang2 signaling and inhibit tumor growth in vivo. This new VEGF/Ang2 Nanobody® showed promising properties in vivo and in vitro, which strongly support the evaluation of this molecule in the clinic.

Nanobody® is an Alpharma trademark.

RESULTS

Inhibition of VEGF signaling

Figure 1: VEGF is a survival and growth factor for endothelial cells and induces proliferation of endothelial cells as well as sprouting and branching of new vessels. Ang2 promotes the detachment of pericytes from blood vessels and vessel destabilization. In addition, inhibition of Ang2-VEGF signaling was shown to suppress angiogenesis and tumor vessel formation.

Inhibition of Ang2 signaling

Figure 2: Nanobody® are antibody fragments consisting of a single variable domain, single-domain camelid antibodies are so specific as regular antibodies. Trispecific Nanobody® with variable domains blocking VEGF and Ang2 as well as an additional module for half-life extension in vivo were generated.

The VEGF/Ang2 Nanobody® is selective for human VEGF-A

Figure 3: The VEGF/Ang2 Nanobody targets VEGF-A, but not VEGF-B, VEGF-C or VEGF-D.

The VEGF/Ang2 Nanobody® impairs HUVEC survival

Figure 4: VEGF signals via the MAPK pathway. Phospho-ERK levels of VEGF-inhibited endothelial cells were measured to determine the presence of a blocking Nanobody® and its effect on proliferation. The VEGF/Ang2 Nanobody® efficiently blocked phosphorylation and induced cell death.

DISCUSSIONS

The authors I.A., A., F., P.S.C., N.R. and K.K. are employees of Boehringer Ingelheim. I.A. and J.B. were employees of Alpharma at the time of data generation.