Reviewer's report

**Title:** A small molecular agent YL529 inhibits VEGF-D-induced lymphangiogenesis and metastasis in preclinical tumor models in addition to its known antitumor activities

**Version:** 6  **Date:** 25 November 2014

**Reviewer:** Steven Stacker

**Reviewer's report:**

The manuscript by Lu et al., describes the use of a small molecule inhibitor that targets the kinase domain of VEGFRs to explore its activity against the VEGFR-3 which is an important driver of lymphangiogenesis and tumor lymphangiogenesis.

**Major Compulsory Revisions**

The manuscript in its current form lacks certain key experiments which would allow the interpretation of the significance and specificity of the action of YL529 in this system.

While the preface to the analysis of the inhibitor YL529 is the modelling of the inhibitor and VEGFR-3 by homology modelling and molecular docking methods one could reasonably assume, given the previous history of other small molecule inhibitors to the VEGFR family, that some level of activity by the small molecule would be expected between two such highly homologous kinase domains.

The analysis relies on the activity of one of the VEGF growth factors VEGF-D. While this is fine from the perspective of testing the inhibitor and having a generic activator of the pathway, VEGF-C which is the other ligand for VEGFR-3 and indeed VEGFR-2 should be recognised, described in the text and it would have been preferable to look at whether VEGF-C stimulation could be inhibited.

Importantly YL529 also targets VEGFR-2 (which has been previously described) a receptor that can also be expressed on lymphatic endothelial cells, can be stimulated by VEGF-D and could be affected in biological or cellular assays where the YL529 inhibitor is being used. These caveats have not been addressed in the article. Can some of the activity in the various bioassays be attributed to VEGF-D stimulation of VEGFR-2? Can some of the inhibitory activity also be assigned to VEGFR-2? While p-VEGFR-3 alters how can we directly attribute the activity seen to VEGFR-3 rather than VEGFR-2?

VEGF-D is cleaved by proteolytic digestion (Stacker et al, JBC 1999, McColl J Exp Med 2003) and forms intermediate and mature forms of the growth factor that have variable affinity for VEGFR-2 and VEGFR-3. The SDS-PAGE/Western blot presented does not describe the form of VEGF-D presented in the gel. As VEGF-D can be cleaved into multiple forms (~52, ~30 and ~21 kD) which is these is seen in the gel. The 52 kD form is uncleaved and will have significantly
reduced activity on both VEGFR-2 and VEGFR-3. The fully mature form of 21 kD has the greatest affinity for the receptors and can bind and activate both VEGFr-2 and VEGFR-3. In these particular experiments knowing which form is generated is critical to the overall interpretation of the study and the interplay between VEGFr-3 and VEGFR-2.

The schematic diagram is an overly simplified version of the events and does not describe other participating receptors or pathways well. It is misleading to the non-expert and needs some clarity about other significant targets for YL529 in cells, particularly VEGF-D/VEGFR-2, VEGF-C/VEGFR-2 and VEGF-C/VEGFR-3.

Minor Essential Revisions:
Some English expression could be improved through editing
Results section, round down % reduction data eg. from 90.22% to 90%
Are (A) and (B) of Figure 3 transposed?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I am a shareholder in Circadian Technologies and Ark Therapeutics, both companies are involved in developing therapeutics for the treatment of angiogenesis and lymphangiogenesis in cancer and other diseases