Reviewer's report

Title: Targeting both IGF-1R and mTOR Synergistically Inhibits Growth of Renal Cell Carcinoma In Vitro

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Reviewer: Roman Nawroth

Reviewer's report:

In this article, the authors present antibody based methods to target the IGF-1 receptor or IFNalpha by using a multimerized antibody or antibody-ligand linkage based approach, a concept the authors presented already before successfully.

They used this system in combination with rapamycin derived mTOR inhibitors in renal cell carcinoma and were able to demonstrate synergistic effects on the activity of reductases in cells by using the MTS assay as a measure for cell growth.

Major essential revisions

1. An interesting finding is the different effect on cell growth by the antibodies used (mAB391 and hR1). The antibodies recognize two different epitopes on IGF1R and thus differ in mode of action. Given the better effect of multimerized antibodies on their target, one could conclude that interfering with the ligand-receptor interaction might be more efficient than targeting the receptor itself. I would either not include the mAB391 in this study or design the exact same multimeric AB structure with the mAB391 to directly compare effects based on the same mechanism. Otherwise the results dilute the statement about better effects conferred by multimerization of the AB.

2. Besides the demonstrated status of STAT, it is of crucial importance to show biochemical effects on the pathways targeted in order to examine specificity for the suggested target therapy. Thus, a characterization of the activation status for molecules such as Akt, S6K1, 4E-BP1, ERK, p53... is recommendable.

3. Since the in vitro effects on cell growth have been examined only with a very non-linear and indirect method, namely MTS, it would be very interesting to learn about effects on cell proliferation, apoptosis and cell survival. That could be easy to address using assays such as BrdU or 7AAD staining, Caspase activation and living cell counts.

4. The authors criticize the disappointing success of rapamycin derived compounds in clinical use. This statement is true and necessary to make and gives the rationale for the development of new drugs. However, in order to improve the situation it might be necessary not only to present different concepts for a better therapy design but one should also compare these concepts directly which has not been done in this study. Thus, a direct comparison in mode of action of the Hex-hR1 and the 1R-2b and on different cell lines with a different
genetic background is highly recommendable to learn about new and superior therapy concepts that might promise the best benefit for future treatment.

Discretionary revision:
Within the last three years, small molecules with a better inhibitory profile on mTOR have been developed and published. One should consider using those small molecules against mTOR in combination of rapamycin derivatives to examine thoroughly state of the art opportunities to interfere with these pathways.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests