PrePrint Journal Club Review: Small-molecule targeting of MUSASHI RNA-binding activity in acute myeloid leukemia

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Title: Small-molecule targeting of MUSASHI RNA-binding activity in acute myeloid leukemia.

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Dear Authors,

Thank you for posting your manuscript titled *Small-molecule targeting of MUSASHI RNA-binding activity in acute myeloid leukemia* as a preprint on bioRxiv! We reviewed this work at our journal club at the Structural Genomics Consortium, University of Toronto. Compiled comments from the attendants are below. To structure the feedback, we used the quick worksheet guidelines published on PREreview.

We hope this feedback will be useful to improve the manuscript.

Kind regards,

PreReview Journal Club Members, Structural Genomics Consortium, University of Toronto

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What is the main question the study attempts to answer?

- Can chemical inhibition of Musashi2's RNA-binding function be used to selectively target myeloid leukemia cells?
- How does Ro 08-2750 inhibit MSI RNA-binding activity?

What is (are) the hypothesis?

Small molecule antagonism of the RNA binding domain of MSI2 has therapeutic potential in the treatment of acute myeloid leukemia. Ro-08-2750 binds at an RNA-interacting site and competitively inhibits RNA binding of MSI2.

What techniques/analyses do the researchers adopt to test their hypothesis(es)?

The authors use biophysical assays, computational structural biology, cell biology techniques, and an animal model. These methods are all common to preclinical drug development and are appropriate to address the outlined hypothesis and in most cases adequately address the question being asked.

Why is this study relevant?

Understanding of RNA biology and its importance in human health has expanded greatly in recent years. While proteins with RNA binding domains have been implicated in disease, they are typically thought of as "undruggable" as they often lack defined pockets. MSI2 overexpression is common in AML patients with poor clinical prognosis. This study highlights that (1) RRM domains can be targeted by a small molecule antagonist and (2) that this approach may have therapeutic value in the treatment of AML.

Write here any general comments you might have about the research approach.

Structural studies

- The structure-activity-relationship was clearly described considering a lack of a co-crystal structure.
- Given that residues F66, F97 and R100 of MSI2 are also conserved in MSI1, it would have been interesting to see how Ro 08-2750 compares between both proteins.

Biophysical Assays

- It would be interesting to see measurements of compound binding to MSI2, for example ITC or thermal shift assays.

Cell Biology

- The assays used appeared to be well thought out and provided good evidence for target engagement and therapeutic potential in cellular models of AML. However, given the genetic diversity of AML, it would be informative to include a sentence to describe why MOLM13 and K562 cells were selected for these studies.

Write here any specific comment you might have about experimental approaches and methods used in the study.

- In the discussion the authors state that Ro 08-2750 is the first "selective MSI inhibitor". MSI2 was compared to SYNCRIP for selectivity; however, given the extensive repertoire of RNA binding domains found in the human genome, we feel that a more extensive characterization is warranted to make a definitive statement. This could be accomplished by either utilizing a biotinylated compound for pulldowns and MS-id or by measuring binding to a panel of RRMs.
- It would be interesting to see KD/KO studies alongside compound treatment to evaluate to what degree this compound may phenocopy genetic perturbation of MSI2 in AML. In addition, it would also be cool to see how the mutants deficient in Ro binding are functional in cells, which may hint at potential mechanisms of resistance.
- For the mouse data presented it would be informative to the reader to include both survival and tumour volume data. Additionally, have the authors done any experiments to test the suitability of this compound for in vivo use (ex. What is the PK of Ro?).
- Additional biophysical assay to measure direct binding of the compound to MSI2's RRM domain would be informative, ex. ITC or thermal shift assay.

Structural studies

- We found that the molecular dynamics analysis greatly complemented the structural data and enabled a thorough description of the potential binding mechanism for the Ro compounds. It would be useful to note whether co-crystallization of MSI2 and Ro was attempted, though the mutagenesis experiments provided evidence of the importance of interactions with F66, R100, and F97. It is unclear why YANK

was selected to perform alchemical analysis given that the program has not yet been extensively validated and carries a "Use at your own risk!" warning. It would have been reassuring to see these calculations carried out with a more validated software.

Write here any specific comment/note about figures in the paper (this could be related to the way data are displayed and your ability to understand the results just by looking at the figures).

- Extended figure 7 appears to be mislabeled in text and extended figure 8 is missing.
- For figure 3d, we felt it would be easier to interpret if annexin V + cells were normalized to the DMSO control.
- For the RNA-IP experiments, we would suggest that this data be presented as % input, akin to a ChIP experiment. This would account for any changes in gene expression that may confound the interpretation of this result as well as allow the authors to probe RNAs that should not change in response to compound (ie. negative control)

Write here any additional comment you might have (this includes minor concerns such as typos and structure of the manuscript).

- On line 216: what does "proximal" mean here?
- Classically, the term inhibitor would be reserved for a compound disrupting an enzymatic activity. Here**,** antagonist may be a more appropriate term.
- Any supplementary tables listed in the text should be included for readers/reviewers.