Reviewer's report

Title: EIF2A-dependent translational arrest protects leukemia cells from the energetic stress induced by NAMPT inhibition

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Reviewer: Versha Banerji

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1. Is the question posed by the authors well defined?

The authors aim to evaluate the role of AMPK in NAD depletion mediated cell death by FK866 and genetic loss of AMPK. The focus is the impact on the translation inhibition and differential regulation of mRNA.

They aim to characterize the “pre-toxic” events to define the molecular mechanisms favoring cell death or cell survival. This is due to the fact that protein synthesis inhibition represents an early response to FK866 induced energetic stress. They are trying to show that AMPK-EIF2A is the central hub for this process.

There is very little background to the role NAD and AMPK and translational modification provided. They conclude that LKB-1 low cells have better FK866 response, yet in the introduction they do not demonstrate why this link is important. They site one paper where autophagy is occurring in multiple myeloma. However they use T-ALL, breast and pancreatic cells. The rationale for selecting these lines is not described.

2. Are the methods appropriate and well described?

The methods are well described.

3. Are the data sound?

100nm is a very high dose of FK866. The levels used in patients and phase one trials is 13nM. The rationale for picking 100nM with 47% viable cells at 72 hours is not a good response.

If the goal was to take poorly responsive tumour types and make them more responsive that is not made clear in this paper. The data suggests a plateau effect after 2.5 nM.

The impact of the doses tested especially the 100nm dose is toxic to normal tissues. These effects were really emphasized at the higher doses. When you look at the % viable cells in Figure 1 A the 10nm dose is equivalent as the 100 so why was 100nM used? And there there is a plateau which if energy depletion is taking place should result in cell death.
It would be interesting to know if standard chemotherapeutics result in the same loss of translation at “pre toxic doses” to demonstrate that this is unique to FK866. It would be also important to see what happens with Rapamycin treatment as another control.

ACC is not defined in full form line 276
Line 278 should read as compared versus if compared to
Line 279. NA alone seems to increase pAMPK- how does its addition decreases the effect pf FK in Figure 3 B

Also figures do not indicate what the representative N for each experiment is.

Line 287 Figure 4: nampt is not expected to change with FK treatment. And should be stated. RNAi experiments using one shRNA are risky. At a minimum two shRNA against should be utilized to ensure that there are no off-target effects.

Figure 4d
The blot is over exposed and although the difference in pAMPK is obvious the smearing of the banks makes the EIF2A difficult to interpret. A second shRNAi hairpin should be tested to rule of off target effects.

Overall the rationale for skipping from cell line to cell line and disease background is poorly explained and needs to be clearly delineated and easy to follow.

4. Do the figures appear to be genuine, i.e. without evidence of manipulation?
Yes but the heading for the drug should include the name as some time pooled siRNA are indicated in concentration format.

5.

6. Does the manuscript adhere to the relevant standards for reporting and data deposition?
Yes

7. Are the discussion and conclusions well balanced and adequately supported by the data?
The discussion needs to reemphasize the thought process and demonstrate clarity.

8. Are limitations of the work clearly stated?
They do not highlight how these different backgrounds may be a limitation

9. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
Yes
10. Do the title and abstract accurately convey what has been found? 
yes
11. Is the writing acceptable? 
Please see my previous comments

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

**Quality of written English:** Acceptable

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

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