Reviewer's report

Title: Stat1 activation attenuates IL-6 induced Stat3 activity but does not alter apoptosis sensitivity in multiple myeloma

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Reviewer: Matthew Quinn

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The paper proposes an interesting question in the relationship between Stat1 and Stat3 and how the shifting of balance between these two opposing responses may affect the propensity of a cell to undergo apoptosis. The methods employed to investigate this question are appropriate and the approach is straightforward.

Major Compulsory Revisions

My concerns regarding the paper primarily relate to the completeness of the data and the perhaps over interpretation of their meaning. There seems to be a lack of consistency in which results were presented and with which experimental controls were included:

1) From the first figure, it seems that certain pieces were left out, and it is unclear why. In figure 1C, why were the blots related to the U-266-1970-wtStat1pcIneo treated cell line not presented? That seems to be a more appropriate comparison to the constitutive Stat1C expressing cell line that the vector alone stably transfected cells.

2) In figure 2A, the ratio of nuclear:cytoplasmic of the pStat1 doesn’t seem to change with treatment or even between vector alone and Stat1C cell lines. This may suggest the potential for constitutive gene activation in the absence of treatment, as is stated, but why don’t the Stat1C cells or treatment show increased nuclear localization?

3) Neither figure 2B nor figure S2 seem to support the claim that Stat1C transfected cell line have increased basal or IFN-# induced IRF-1 levels. There is no quantitation in Figure 2B to support a significant increase in IRF-1, especially considering the increase only seems to occur in the sample that also seems to include higher actin levels, and figure S2 seems to show very similar levels of IRF-1 relative to actin, considering the error bars included. There is also no indication on figure S2 of a significant difference between cell lines for any treatment condition.

There appears to be necessary controls lacking from a number of the experiments also, which makes it difficult to interpret the results:

4) In figure 4, there are no positive or negative controls to suggest that the appropriate cell lines are being presented, that the IFN-# treatment had any effect in this particular experiment, or that the levels of these proteins can change
in response to any stimulus or over the timescale that was tested. It is very hard to interpret the lack of effect on these protein levels when nothing changes at all.

5) Figure 7 also contains no positive controls for drug efficacy making it hard to determine whether small differences, such as those observed in figure 7B, are meaningful.

Minor Compulsory Revisions

6) In figure 6, the y axis is labeled as % Ann+/PI-, however the figure legend indicates that fold induction is displayed. Moreover the text indicates %s (such as 62 to 74%), but these percentages are not clearly marked on the graph. Significant difference comparisons are also not shown or mentioned. Together, these issues make it unclear exactly what is being shown in this figure.

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

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.