Reviewer's report

Title: Functional activation of endogenous p53 by combined, but not individual, p19Arf gene transfer and nutlin-3 drug treatment reduced viability of B16 cells

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Reviewer: Charles Giardina

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General comments
This manuscript describes a series of experiments aimed at determining the extent to which different p53 activators function independently and in combination to regulate the proliferation of cancer cells. Two cancer cell lines are compared in these studies; one is sensitive to p53 activation and growth regulation by p19Arf and the anticancer agents Nutlin-3 and doxorubicin (C6 cells). P53 in the other cell line appears to be more resistant to activation (B16 cells). Experiments show that p53 activation in the resistant B16 cell line requires two independent inducers. In particular the Nutlin-3, p19Arf combination appears to be particularly effective.

The findings reported here have potential implications for the use of viral vectors designed to control carcinogenesis through activation and/or accentuation of the p53 pathway (if such an approach ever becomes clinically feasible). These experiments also provide insight into the interplay between the endogenous p53 activator p19Arf and pharmacological agents. Although the experiments shown are convincing and carefully interpreted, some additional information would serve to make the findings more complete. Some suggestions are outlined below.

Specific comments
1. Some of the figures were difficult to interpret due to the use of numeric identifiers. The authors should consider replacing the number codes with direct labels that indicate the cell line and the viral vector employed.

2. The legend for Figure 1 needs to include information on what the different regions of the virus are and what their purpose is.

3. The data in Figure 4 is difficult to reconcile with Figure 2. The p19Arf infection appears to be sufficient to activate p21 expression, whereas it did not stabilize p53 in Figure 2. This could be due to transient p53 activation, or perhaps a p53-independent activation of p21. Additional discussion of these data is required.

4. It would also seem logical to include additional time points examining p53 stabilization after the various treatments. This information would provide important insight into the mechanism by which the different treatments interact.

5. It is somewhat surprising that p19Arf and Nutlin-3 provide an effective combination since theoretically they should be accomplishing the same thing:
Mdm2 neutralization. The authors raise the possibility that p19Arf is inhibiting Mdm4 while Nutlin-3 neutralizes Mdm2. Data on Mdm4 and Mdm2 expression in the cell lines would therefore be of interest.

**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.