Reviewer's report

Title: In vitro prediction of stop-codon suppression by intravenous gentamycin in patients with cystic fibrosis; a pilot study.

Version: 1 Date: 13 November 2006

Reviewer: David M Bedwell

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General

In this study, the ability of gentamicin to suppress various premature stop mutations in the CFTR gene in both mammalian tissue culture as well as in vivo in CF patients was observed. Mouse embryo fibroblasts were transfected with a readthrough reporter that contained various naturally-occurring CFTR premature stop mutations (Y122X, W1282X, R1162X, and G542X) along with the surrounding CFTR sequence context. The cells were cultured in the absence and presence of 600µg/ml gentamicin and the level of readthrough was then determined. The Y122X mutation was found to produce the highest level of basal readthrough as well as the highest level of readthrough after gentamicin treatment. CF patients that carried the Y122X were administered gentamicin intravenously once daily at a concentration of 10mg/kg to achieve peak serum concentrations of 20-40 ug/ml for 15 days. In the majority of the Y122X patients that were treated with gentamicin, a restoration of CFTR protein was detected in nasal epithelial cells. In addition, CFTR-dependent chloride secretion was detected using NPD measurements and sweat chloride concentration levels decreased suggesting that readthrough of the Y122X mutation by gentamicin resulted in restoration of enough functional CFTR protein to alleviate the major physiological defects associated with CF.

This study provides important data concerning systemic administration of gentamicin to suppress premature stop mutations that cause CF. This clinical study is important since it represents one of the first studies to demonstrate that pharmacological suppression of CFTR premature stop mutations can restore adequate levels of functional CFTR protein to alleviate the CF disease phenotype. However, there are some issues that need to be addressed prior to its acceptance for publication, as indicated below.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. More extensive experimental details are needed for the in vitro readthrough experiments. There is also no explanation as to why a gentamicin concentration of 600µg/ml was used. Was this concentration found to be optimal for this cell line? Were other concentrations tried? It is also not clearly stated if during the 3-day course of the in vitro experiment gentamicin was maintained in the media throughout the 3 days and/or if the media was refreshed during the 3-day course. Also, how many times was this experiment performed and what was the experimental error for these experiments?

2. In Table 1, it is not clear why the sequences of complementary oligos for only the mutant were provided. It would have been clearer if the sense strand sequence of both the control and mutant constructs were provided in each case and the premature stop mutation were underlined. Finally, no statistics were provided for the readthrough data. These data should be expressed in terms of the mean and the standard deviation. Finally, there is a typo for the 600 µg/ml column heading (not 600 g/ml) in table 1.

3. The figure legends and figure numbers do not correspond. There is no figure that corresponds to figure legend 1. Figure legend 2 corresponds to figure 1 and figure legend 3 should be figure legend 2. The figure 1 legend should be removed and the remaining figures should be renumbered and the text should be changed accordingly.

4. In the sweat chloride figure and the NPD figures, representative data from normal controls should be included to give a better idea of the magnitude of the responses.

5. In the figure depicting immunostaining of CFTR, which antibody is used? Immunostaining from normal control cells is also needed for comparison in this figure.
6. No time scale is given for the nasal PD tracings. Since transient artifactual responses are sometimes observed, the time the recordings are maintained following isoproterenol addition during these experiments should be indicated.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Discretionary Revisions (which the author can choose to ignore)

**Which journal?:** Appropriate or potentially appropriate for BMC Medicine: an article of importance in its field

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

I am a consultant for PTC Therapeutics, which is currently carrying out phase 2 clinical trials to examine the use of PTC124 for the suppression of stop mutations in CF patients. I also hold a new-use patent for the use of aminoglycosides for the suppression of premature stop mutations that cause CF.