Reviewer’s report

**Title:** Effects of small interfering RNA targeting thymidylate synthase on survival of ACC3 cells from salivary adenoid cystic carcinoma

**Version:** 4  **Date:** 20 August 2008

**Reviewer:** Nobuhiko Oridate

**Reviewer’s report:**

Discretionary Revisions

I do not think that the authors responded well to my concerns on this study.

Specific comments:

1) I have not yet found the authors’ explanation for the presence of apoptosis without increase in sub-G1 population.

(My previous comment 2: The authors suggested the induction of apoptosis by the TS siRNA introduction in figure 5 C and D. However, cell cycle analyses (figure 3) showed no obvious increase in sub-G1 population. The authors need to discuss this discrepancy.)

2) I am still confused about the mechanism by which up-regulation of p53 and p21 following TS inhibition induces apoptosis without G1 arrest.

(My previous comment 3: It would be better to explain more in detail the association of S-phase accumulation and increased apoptosis induced by TS inhibition. Was induction of apoptosis resulted from up-regulation of p21 or activation of S-phase checkpoint instead of G1 checkpoint? Is there some other mechanism(s) to explain for these results? The authors need to discuss this point.

**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