Author's response to reviews

Title: Phase I Study of TP300 in Patients with Advanced Solid Tumors with Pharmacokinetic, Pharmacogenetic and Pharmacodynamic Analyses

Authors:

D Alan Anthoney (Alan.Anthoney@leedsth.nhs.uk)
Jay Naik (Jay.Naik@leedsth.nhs.uk)
Iain R.J. MacPherson (Iain.MacPherson@glasgow.ac.uk)
Donna Crawford (Donna.Crawford2@ggc.scot.nhs.uk)
John M Hartley (john.hartley@ucl.ac.uk)
Janet A Hartley (janet.hartley@ucl.ac.uk)
Tomohisa Saito (saitotmh@chugai-pharm.co.jp)
Masaichi Abe (abemsi@chugai-pharm.co.jp)
Keith Jones (k.jones@chugai-pharm.co.uk)
Masanori Miwa (miwamsn@chugai-pharm.co.jp)
Christopher Twelves (c.j.twelves@leeds.ac.uk)
T RJE Evans (J.Evans@beatson.gla.ac.uk)

Version: 3 Date: 28 May 2012

Author's response to reviews: see over
Dear Sir/Madam

MS: 4122965266827777

**Phase I Study of TP300 in Patients with Advanced Solid Tumors with Pharmacokinetic, Pharmacogenetic and Pharmacodynamic Analyses**

D Alan Anthoney, Jay Naik, Iain R.J. MacPherson, Donna Crawford, John M Hartley, Janet A Hartley, Tomohisa Saito, Masaichi Abe, Keith Jones, Masanori Miwa, Christopher Twelves and T RJE Evans

Further to the email of 1st May 2012, we are pleased to provide our responses to the comments made by the two referees. A revised version of the manuscript has been uploaded to the website 28 May.
<table>
<thead>
<tr>
<th><strong>Reviewer: Mark McKeage</strong></th>
<th><strong>Author responses and actions.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction Paragraph 2 sentence 1: Please refer to Fig 1 that shows the chemical structures of the prodrug, and its topoisomerase inhibitor and active metabolite.</td>
<td>Agreed. We added the figure reference.</td>
</tr>
<tr>
<td>Introduction Paragraph 3 sentence 1: If this was the first-in-man trial of TP300 please say so here.</td>
<td>Added as requested and also to the abstract.</td>
</tr>
<tr>
<td>Results: Pharmacogenetic analysis: Sentence 1: Earlier in the paper mention was made about the role of AOX1 in TP300 metabolism, but no rationale for examining genetic variants of CYP26 and UGT1A1 is presented clearly here or elsewhere.</td>
<td>Not changed. The reasons are included in the methods, Pharmacogenomic analysis section. We do not think it necessary to repeat in the results section.</td>
</tr>
<tr>
<td>Results Pharmacodynamic analysis second sentence &quot;TM&quot; this abbreviation will not be familiar to many readers. Is it necessary or defined in the text?</td>
<td>TM stands for tail moment. Added into the results text. We already mentioned in Figure 6 &quot;tail moment&quot;.</td>
</tr>
<tr>
<td>Discussion paragraph 2 line 8: add dose units</td>
<td>Added. A phase IIa study has been completed. And we will report it in future. We did not want to make specific reference in this paper.</td>
</tr>
<tr>
<td>Discussion last paragraph: Are there plans for further clinical evaluation of TP300 Discretionary Revisions</td>
<td></td>
</tr>
<tr>
<td>References</td>
<td></td>
</tr>
<tr>
<td>Reference 9 is incomplete</td>
<td>Reference 9 is correct. We think you probably mean reference 8. We have added the information to reference 8.</td>
</tr>
</tbody>
</table>
### Reviewer: Laurent Rivory

**Major Compulsory**

1. The pharmacokinetics of the parent drug TP300 are surprisingly not provided in any form and the fact that both TP3011 and TP3076 will be present as a mixture of lactone and carboxylate forms appears to have been overlooked.

   As TP300 is a "prodrug" we concentrated on showing the active TP3076 and active metabolite TP3011 PK. We did of course measure TP300 PK too, but the conversion to TP3076 is very fast in physiological conditions, within minutes, and thus data is very limited to minimal points/no TP300 detected. In the paper, we have decided to add a figure showing TP300 PK, and also reference to it in text.

2. Page 5. The authors propose that a reduction in PK would reduce the likelihood of diarrhoea as a side effect of therapy (eg “be less inter-individual variation in activation and toxicity with TP300 than with irinotecan; specifically, it would be expected that severe diarrhea should not be an issue”). There is no basis for such a concept as the underlying cellular mechanisms and distribution are what determines the target organs, not variability.

   We agree to rephrase that part. Please see the modification.

3. Page 6. A proposed major finding is that "variability was small", but this is not quantitated or defined.

   Some text needs adding if possible to explain what we mean by variability eg to cf with SN38 variability or something.
There was linearity to 10 mg/m². The linearity has been determined with linear regression, analysis of variance, power model analysis and visual inspection. So we have added a statement to the "Method" section of the paper. Based on the results, TP3076 and TP3011 showed the linearity. More information is provided here, but we do not intend to include such detail in this paper.

The correlations between PK parameters and dosage amount were investigated. It was shown that increases in Cmax, AUCt and AUCinf of TP3076 with increase in dosage amount. All dosage groups were evaluated by visual inspection, linear regression analysis, ANOVA, and power model. Linear regression analysis revealed that none of the intercept for Cmax, AUCt or AUCinf intercept showed any significant differences when compared (p values of the intercept for Cmax, AUCt and AUCinf are p=0.314, p=0.207, and p=0.21920, respectively). ANOVA revealed that dose normalized Cmax, AUCt and AUCinf showed significant difference (p values for Cmax, AUCt and AUCinf were p=0.006, p=0.0119, and p=0.017, respectively). Power model analysis revealed 95% confidence interval of the estimated slope for the estimated Cmax and AUCinf included by 1, but the 95% confidence interval for AUCt did not include 1 by only a narrow margin (the 95% confidence intervals for Cmax, AUCt and AUCinf are 0.922-1.251, 1.001-1.466, and 0.991-1.467, respectively).

In addition, the same analyses were performed using data of 1-10 mg/m² dosed groups. Linear regression analysis revealed that none of the intercept for the parameters showed any significant difference when compared (p values of the intercept for Cmax, AUCt and AUCinf are p=0.843, p=0.567, and p=0.568, respectively). According to ANOVA, none of the dose normalized parameters showed any significant difference (p values for Cmax, AUCt and AUCinf are p=0.329, p=0.290, and p=0.332, respectively). For the power model analysis, the 95% confidence interval of the estimated slope for all parameters included 1 (the 95% confidence intervals for Cmax, AUCt and AUCinf are 0.845-1.1527, 0.911-1.337, and 0.902-1.338, respectively).

In consideration of the above results, it was concluded that linearity was shown for Cmax and AUC of TP3076 at the levels of 1-10 m². The same analyses were performed for Cmax, AUCt and AUCinf of TP3011. Results showed that Cmax, AUCt and AUCinf of TP3011 increase with an
increase in dosage amount. All dosage groups were studied by linear regression analysis, ANOVA, and power model analysis. Linear regression analysis revealed that none of the intercept of the parameters exhibited any significant difference when compared (p values for Cmax, AUCt and AUCinf are p=0.437, p=0.413, and p=0.342, respectively). ANOVA findings revealed that none of the dose normalized parameters showed any significant differences (p values for Cmax, AUCt and AUCinf are p=0.397, p=0.6212, and p=0.344, respectively). For the power model analysis, the 95% confidence interval of the estimated slope for all parameters didn’t included 1 (the 95% confidence intervals for Cmax, AUCt and AUCinf are 1.075-1.687, 1.193-1.828, and 1.031-1.715, respectively).

5. Page 7 and elsewhere. The modelling of haematological toxicity with exposure is said to be “strong”, but no statistics (eg R2) are provided. We do not propose any changes. It isn’t a simple regression result, so R2 cannot be calculated. Instead, the accuracy of the model parameter can be shown. CV% of gamma and EC50 was 18.7% and 17.7%, respectively. Achieved convergence tolerance was 0.000007007.

6. Page 8. The Comet data are said to indicate a trend although there was no clear relationship/ “Although there was no clear relationship between TP300 dose and the extent of strand breaks, there was a trend towards higher doses being associated with greater DNA damage”. There is no evident trend from eye-balling the data and if not significant, then any “trend” is in any case meaningless. In our opinion, trends are not meaningless in the absence of statistics. Given the limited numbers of patients per cohort, suitable statistics was not possible or appropriate. However the vast majority of patients show a response which does make the data meaningful. Overall, we made a slight modification to the text to remove "trend".

7. Page 8 and elsewhere. It is claimed that TP3076 is rapidly converted, but no kinetic data are presented. For example, on page 9, it is claimed that “Pharmacokinetic data confirm that TP300 is rapidly converted in plasma to the active metabolite TP3076”. See the answer of inquiry 1.
8. Page 8. It is claimed that “Target interaction with the induction of DNA strand breaks was shown”, but the Comet data are inconclusive and there is no data shown of topoisomerase I inhibition.

As the comment above - the evidence is clear without statistics. We also state that measuring in cancer tissue may be a better measure, but in this study was limited chance to have tissue samples so we decided not to take them.

9. Page 9. Glucuronidated TP3076 was not detected, but it is not clear what attempts were made to detect it. Furthermore, the statement “reflecting UGT1A1 variant status had no influence on exposure to either TP3076 or TP3011” is somewhat of a non-sequitur as glucuronidation of TP3011 was not investigated.

Glucuronidated TP3076 was not detected in all patients with variable UGT1A1 in the study. The number of the patients in the study was limited but, it is possible to say that UGT1A1 variant status found in the study had no influence on exposure to either TP3076 or TP3011.

10. Page 9. It is claimed that “TP300 has advantages over other agents in this class in terms of tolerability and the predictability of its principle toxicity, myelosuppression”, but this has not been formally assessed. Although the comparison with CPT-11 is interesting, I would have expected Topotecan to be the more appropriate drug for comparing profiles.

The major concept for TP300 was based on CPT11 which is much more widely used. Also target indications for Topotecan does not include Gastric or GE. junction cancer. That’s why CPT11 (irinotecan) was compared to TP300.

11. Page 11. Exclusion criteria included “prior cytotoxic chemotherapy” which is not consistent with the fact that several patients had previously been treated with CPT-11.

The exclusion criteria applied a 4 week period before the TP300 study, with no chemo/cytotoxics. Entry was open to CPT11 treated patients - just not to those who had shown life-threatening drug allergy or hypersensitivity. The text in the paper still seems to be ok so we do not propose changes.
12. Page 11. I am concerned that the details regarding the infusion medium are not provided. TP300 is said to spontaneously convert to TP3076 at pH>5. Most infusion solutions are not buffered but some are, so the pH of the drug infusion solution will depend on the formulation of TP300 and the dose of TP300 (ie if TP300 formulation is acidic, then increased dose in a fixed infusion volume will cause a reduction in pH). Furthermore, and in the absence of more details on TP300, the motivation for using TP300 is not evident if it is required to be maintained at pH<5, as this is a limitation also with the conventional 20(S)OH camptothecins.

The pH was less than 2 for re-constituted TP300 for infusion. There was no additional buffering. There were no issues with administration. Some additional text is added to paper. The intention with longer term development had been to reduce dose time below 60 minutes if possible - but this was in the end not explored. However, the actual formulation would always have to be low pH, as suggested. We had no problems using this clinically.

13. Page 12. Where was blood drawn from? What was the extraction method? What was the analytical method? Were samples acidified so as to measure total TP3011 and TP3076 or were only the lactone forms assayed? The methodology is insufficiently described.

Agreed. We have added more text. Yes venous blood/plasma was acidified before being stored frozen. Method details are added too.

14. Page 12. Some of the data points in Figure 2 are below the limit of quantification.

When the value was below the limit of quantification, the value was set as 0 and summary statistics was calculated. That's why some point has mean value even below the limit of quantification. No change.
Minor

15. Page 5. I think that UGT1A1 can also form acyl and N-glucuronides, so “lacks the phenolic-OH group required for glucuronidation” is not strictly correct.

Agreed. We will modify text. As TP3076 has no phenolic-OH group in its structure in which SN-38 has, TP300 cannot receive the glucuroniate conjugate by UGT1A1 easily as compared with SN-38. (In fact that no glucuroniate conjugate body of TP300 were detected in the P1 study.)

16. Page 6 and elsewhere. TP300 PK should be shown in Figure 2.

Agreed. Now corrected.

17. Page 8. It is not just the side-chain of CPT-11 that accounts for its acetylcholinesterase inhibition, so “the side chain present in CPT-11 that has acetylcholine esterase activity being absent” should be rephrased.

Agree. We have modified the sentence to remove reference to the side chain.

18. Page 9. “as acute cholinergic reactions are associated with the piperidine moiety of irinotecan, not found in TP300” should be referenced.

Agree. We have modified the sentence and added reference.

19. Page 9. “Hepatic aldehyde oxidase converts TP3076 to a further metabolite TP3011” should be referenced.

Added Reference in "Background" section page 4.
20. Page 12. What were the two animal species tested?

We used Rat as rodent species and Dog as non-rodent. We did not think it necessary to give that level of detail, as the tox studies supported Phase start dose only and this paper is about Phase I data not preclinical. They were GLP compliant and included with the CTA/IMPD in UK. We do not plan to change the paper.

21. Page 13. Was the urinary ratio adjusted for the molecular weight difference between the parent drug and the metabolites?

Yes, it was but we do not think it is necessary to state that in the paper.

22. Page 13. The PCR method needs to be referenced or details provided.

Details are included in the text.

Other

23. Page 4. “TP300 has activity in the nanomolar level across a range of tumour types” could be rephrased.

Agreed. Now corrected.

24. Page 4. Need to accord plural in “TP3011 and TP3076 are equipotent as Topo-1 inhibitors, with an IC50 in the”

Agreed. Now corrected.

25. Page 6. Punctuation needs to be fixed in “lasting between 1.5-5 months; Five”.

Agreed. Now corrected.

26. Page 11: Punctuation needs to be fixed in “Standard Phase I trial exclusion criteria included, exposure.”

Agreed. Now corrected.


Agreed. Now corrected.

28. References: #7 – United misspelt. #8, #17 – page numbers missing.

Agreed. Now corrected.
We trust that these comments and the changes made to the manuscript will be acceptable to the referees and the editor. Please do not hesitate to contact myself or other authors if any further details/changes are needed.

Best wishes
Yours sincerely

Mr Keith Jones
Director of Non-clinical R&D, Chugai Pharma Europe Ltd.