Reviewer’s report

Title: Target enzyme mutations are the molecular basis for resistance towards pharmacological inhibition of nicotinamide phosphoribosyltransferase.

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Reviewer: Fei-Fei Liu

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Olesen et al. have identified a CHS-828 analogue, TP201565, as an inhibitor of NAMPT, with greater potency than other NAMPT inhibitors, such as APO866. The authors have conducted a series of detailed studies demonstrating that acquired resistance towards NAMPT inhibitors may be primarily due to mutations in NAMPT. Overall, this is an interesting article, which contributes to the literature viz sensitivity to this class of inhibitors.

Major Compulsory Revisions

#1) Can the resistant phenotype of cell lines harboring mutations (Table 2B) be reversed by over-expression of WT NAMPT?

#2) Could over-expression of NAMPT in PC-3/TP201565 cells be due to increased gene copy number?

#3) Results, ‘Development of resistant cell lines’ section, last paragraph – The authors’ statement regarding up-regulation of NAMPT in specific cell lines is questionable (Figure 2). Due to the apparent unequal protein loading, the authors should quantify the western blot by densitometry, normalizing each sample to its corresponding GAPDH loading control.

#4) Is there any difference in doubling-time between the HEK293T cells with WT NAMPT versus mutant NAMPT containing H191R, D93del or Q388R/K342R (Figure 2C), similar to the in vivo xenograft data (Figure 4A)?

#5) Results, ‘APO866 binding and enzyme activity’ section – The statement “We found that the activity of NAMPTH191R was unaltered by the mutation when compared to wild type…” is incorrect since NAMPTH191R appears to have ~50% of WT activity (Figure 3A).

#6) What is the rationale for employing “two daily i.p. injections of 15-20 mg/kg APO866” as the xenograft treatment regimen?

#7) The images and descriptions of the H&E sections of xenograft tumours are qualitative and inconclusive (Figure 4B). It would be more valuable to conduct quantitative immunohistochemical analyses for differences in NAMPT expression, and markers for cell proliferation (e.g. Ki-67) and death (e.g. caspase-3, TUNEL) between the resistant and parental tumor xenografts.
#8) Why were two different assays used to measure NAMPT enzymatic activity (Figures 3 and 5)? For direct comparison purposes, it would be more ideal to use one assay (e.g. CMT) and measure the in vitro enzymatic activity of HEK293T cells with WT NAMPT and mutant NAMPT containing H191R, D93del or Q388R/K342R (as per Figure 5), instead of using purified recombinant NAMPT (Figure 3).

#9) Results, ‘PC-3/TP201565 and HEK293T/WT display’ section, last few lines – Explain the rationale behind selecting each of the additional inhibitor drugs to test for cross-resistance with the PC-3/TP201565 cell line.

#10) Results, ‘Docking studies confirm that APO866’ section, 6th line – For readers who are not familiar with docking studies, please indicate the significance of conducting docking analyses with and without water molecules.

#11) The authors state that CHS-828 and TP201565 are competitive inhibitors of NAMPT; however, data for only TP201565 and APO866 are shown (Figure 8B). Moreover, these data only show that TP201565 and APO866 can out-compete each other for binding to NAMPT. Does TP201565 compete directly with the nicotinamide substrate for NAMPT binding?

#12) Since NAD is synthesized through two metabolic pathways (de novo or salvage pathways), it would be interesting to see if the de novo pathway is upregulated (e.g. NAD synthetase activity) in resistant cells harboring NAMPT mutations and decreased enzymatic activity. And if this can compensate for the reduction in NAMPT-mediated NAD synthesis (i.e. measure total intracellular NAD levels). This might partially explain why resistant cancer cells with NAMPT mutations do not exhibit a growth disadvantage compared to WT cells.

Minor Essential Revisions
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#1) Background, first paragraph – Drug resistance “is”...
#2) Background, first paragraph – “multi-drug” (insert hyphen)
#3) Materials and Methods – spaces needed between numbers and units
#4) Results, first paragraph – “NAMPT inhibitor APO866:” (insert colon after APO866)
#5) Results, ‘Development of resistant cell lines’ section, second paragraph – Specify Tables “2A” and “2B”
#6) Results, ‘APO866 binding and enzyme activity’ section – “Figure 3A or B” label missing
#7) Results, ‘NAMPT mutations do not inhibit in vivo’ section, 4th line – Remove “(data not shown)” since tumor take rates between resistant and parental cell lines are shown
#8) Results, ‘PC-3/TP201565 and HEK293T/WT display’ section – “Figure 5” label missing

#9) Results, ‘Docking studies confirm that APO866’ section, 9th line – Capitalize “Fig 6Aa”

#10) Results, ‘Docking studies confirm that APO866’ section, second paragraph – “overall” has no hyphen

Discretionary Revisions
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#1) Perhaps the authors can comment on how they discovered the novel potent analogue of CHS-828, TP201565. How does TP201565 compare to CHS-828 (or even siRNAs against NAMPT) in terms of NAMPT inhibition? Is there a therapeutic window between cancer versus normal cells?

#2) Have the NAMPT mutations listed in Table 1 been identified as SNPs in cancer patients resistant to NAMPT inhibitors?

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

Quality of written English: Needs some language corrections before being published

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