Reviewer's report

Title: Striking reduction of amyloid plaque burden in an Alzheimer's mouse model after chronic administration of carmustine

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Reviewer: Mary Jo LaDu

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Overall
In this manuscript, based on evidence that the proposed relationship between cell cycle re-entry and Alzheimer’s disease, the authors screened all FDA approved oncology drugs to determine the effect on lowering A# levels. The authors demonstrated that BCNU reduces A# levels in vitro and in vivo, with data indicating these effects are independent on BACE or gamma-secretase inhibition. These data are novel and highlight the potential of BCNU as an Alzheimer’s disease agent. However, as presented, data are lacking on the exact mechanism(s) by which BCNU decreases A# levels in vitro and in vivo. Further experiments are required to determine the metabolic properties of BCNU, the mechanism by which BCNU may lower A# in vivo, and the consequence of this drug on neuronal and behavioral phenotypes in vivo.

Major
• The potential mechanism(s) by which BCNU decreases A# levels in vivo are unexplored. It is important to address these issues before BCNU could be considered for AD therapy:
  o PK data indicate that it is an active metabolite of BCNU that is most likely response for the A# lowering effects of BCNU. It is would be important to identify the active drug metabolite, as this may also lead to a better understanding of the mechanism of action of the drug both in vitro and in vivo.
  o It is unclear whether any of the proposed mechanisms of actions identified in vitro are mediating the decreased amyloid levels in vivo:
    • Does BCNU effect A# production pathway or clearance rates in vivo after short-term treatment? This presumably would result in less-plaques formed? Also, it is important that APP and APP-fragments are measured in vivo to confirm the in vitro findings.
    • Did BCNU over the short or long term increase the clearance of plaques by microglia? Indeed, other anti-cancer drugs that have been tested in vivo act partially by this mechanism.
    • The conclusion that ‘BCNU reduces A# generation and plaque burden at non-toxic concentrations through the TGF# pathway’ is an over statement of the data. There are no in vivo data presented that BCNU increases TGF# levels.
    • Neuronal markers of viability and perhaps behavioral read-outs would also
strengthen the case that BCNU is not neurotoxic and may prevent A# induced neurotoxicity.

• In the introduction and discussion a strong case is introduced for cell-cycle re-entry as a significant factor in AD pathogenesis. However, there are no attempts to determine whether BCNU has indeed affected the cell cycle.

• A comment on why MTT toxicity was determined on a different cell type (N2A) to the cells used for demonstrating drug activity (CHO) is required.

• Fig1G, 4B, 6B and 7B. The X-axis is not plotted on a linear scale, which is the standard method of representing drug effect and pharmacokinetic data which can often enable estimations of drug activity for example EC50 calculations. Indeed comparing the activity of BCNU to BACE or y-secretase inhibitors would also enable an appreciation of target validation.

• The introduction could also be updated in light of recent data:
  o Bapineuzumab could be added to the list of failed amyloid beta therapies.
  o Referring to opponents of anti-amyloid therapies as ‘naysayers’ and the description that follows is too simplistic classification for this complex field. For example, one can argue against amyloid therapies (since amyloid refers to a structure not a specific protein), but still agree that lowering soluble and oligomeric A#42 levels is a viable target for AD.
  o There are now multiple BACE inhibitors that demonstrate effects at lowering CNS A# levels in vivo.

**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