Peer Review File

Article information: https://dx.doi.org/10.21037/atm-21-1103

Reviewer Comments

Comment: The authors do not have a full understanding of either this ADC or the character of the antibody used, which internalizes. Sacituzumab govitecan (SG) is not a conventional ADC, since it does not conform to the design features dictated by the ADC paradigm.

Reply: We agree with the reviewer that SG is not a conventional ADC; indeed, that is one major message of the commentary.

Comment: It cannot be characterized as a prodrug, since preclinical data were published showing specificity, and clinical inference to indicate better outcome with higher Trop-2 expression. Efficacy in low Trop-2 tumors may be due to their sensitivity to SN-38 because of impaired HRR (defective DNA repair).

Reply: SG is indeed a prodrug, which is defined as: "a biologically inactive compound which can be converted in the body to produce a drug". No one, including the authors of papers on SG denies (see Ref. 2) that most of the SN-38 released from SG is by simple chemical hydrolysis (i.e. by definition, a prodrug). And, we do not state that SG is only a prodrug; we propose that it acts as either or both an ADC and a prodrug, depending on circumstance/system. We agree that there is clinical inference that that SG may be more active in high Trop2 tumors in TNBC, but this is not the case for mSCLC [*Gray et al., 2017; SCLC treated with Trodelvy, Clin Cancer Res*) found ORR~17% in mSCLC and state: "no significant difference in PFS or OS was found with regard to (Trop2) IHC score."]. We have reworded para 1 of the MS to make these facts perfectly clear to readers.

Comment: With regard to internalization, Shih et al., probably 20 years ago, showed that the murine anti-Trop-2 mAb of SG internalized very well, yet there was also ample mAb retained on the cell surface.

Reply: We do recognize and refer to the Shih paper published 26 years ago (ref 4). In the last para of p. 2 of the commentary, we state: "In early efforts to establish Trop2 targeting, tumor uptake of the carrier mAb ^{131}I-RS7 was only ~7- to 16% of the initial dose/gm in a Trop2 TNBC xenograft – only ~2-fold higher than a control ^{131}I-mAb by comparison, Trastuzumab shows an

uptake of ~40% of the initial dose/gm in a HER2-positive tumor[5].” *Note that targeted-mediated internalization of* ^{131}I-RS7 *translates to only 3.5 to 8% of the initial dose/gm*, which is more reminiscent of passive internalization by EPR than it is of active targeted antibodies. So, the internalization of SG is certainly not very efficient in mouse xenografts.

Comment: Studies with I-131 are not as convincing as when using a residualizing radiolabel, such as In-111, to show internalization and retention, because I-131 is released from the tumor due to intracellular processing. A residualizing radiolabel, such as In-111, needs to be used to compare specific vs. non-specific accretion.

Reply: We agree Shih et al. (ref 4) should have used a residualizing radiolabel such as In-111, but they did not—certainly, we can't be responsible for Shih et al. doing the correct isotope or predicting what the outcome might have been if they did.

Comment: There have been a number of non-targeted SN-38 formulations examined clinically (PEG-SN-38; PEG-Irinotecan; SN-38 nanoparticle; liposomal SN-38), yet none has shown clinical efficacy comparable to SG.

Reply: Some (e.g. Nektar and Merrimack Pharma) would argue that the Nektar-102 (PEG-Irinotecan) or Merrimack Oncovyde (liposomal irinotecan approved for Panc CA) did pretty well. But that seems irrelevant for the present purpose since they all have different PK than SG and each other, and were never studied in the same patient populations. Indeed, adequately comparing SG to a SN-38 prodrug is exactly what we propose in the 2nd to last sentence of the paper: “comparing the efficacy of a long-acting non-targeted SN-38 prodrug to sacituzumab-govitecan at doses that provide equal exposure may resolve to what extent sacituzumab-govitecan acts as a SN-38 prodrug versus a targeted ADC.”

Comment: The fact that high and medium Trop-2 expression show better clinical benefit compared to tumors with low Trop-2 already suggests that SG acts like an ADC.

Reply: As before, it is NOT a fact in SCLC that high and medium Trop-2 expression show better clinical benefit compared to tumors with low Trop-2 (see *Gray et al., 2017; SCLC treated with Trodelvy, Clin Cancer Res*); i.e. they state there is no difference.

Comment: If it were just a prodrug, it would not be much different than Enzon's PEG-SN-38.

Reply: We disagree. As before, the drugs have completely different PK parameters, and were never studied in the same patient populations, so no direct comparisons are possible. However, Sapra et al. (Clin Cancer Res 2008;14(6) March 15, 2008) reports remarkable tumor accumulation and anti-tumor efficacy of a single dose of Enz2208 in mouse xenografts, much

more than multiple SG doses in mouse xenografts. Note the striking effect of a single injection of Enz2208 on a TNBC xenograft, and its tumor accumulation/retention; SG doesn't come close to this in animal tumors.

However, mice are not men, so the relevance of such models are rightly criticized (although the reviewer uses such models to claim superiority of SG). Also, the reviewers statement seems to imply that all prodrugs are equal. Indeed, the prodrugs he cites - PEG-SN-38; PEG-Irinotecan; SN-38 nanoparticle; liposomal SN-38/CPT-11 – all have greatly differing PK and antitumor activities.