magnetic resonance imaging method, but not by the standard method of glomerular filtration rate (clearance rate of the endogenous creatinine). Like low dose (11 mg/kg) of adefovir, low dose of tenofovir only also caused minor effects on the renal structure while higher doses of tenofovir caused severely gastrointestinal toxicity within 2 weeks. No renal toxicity was seen for either entecavir or telbivudine, highlighting a high degree of safety compared with others. This preclinical study provides key insight before a clinical study on the direct head to head comparison of these 4 drugs is available. View Abstract—Lu Cai

Letter to the Editor

Role of Mitochondrial Toxicity in BMS-986094-Induced Toxicity

We read with interest the paper by Baumgart et al titled "Effect of BMS-986094, a Guanosine Nucleotide Analog, on Mitochondrial DNA Synthesis and Function" (Baumgart *et al.*, 2016). Whereas non-clinical studies in mice and monkeys were able to identify the heart and kidney as target organs (Robertson et al., 2014), correlating with clinical adverse events, the authors conclude that the toxicity observed was not due to mitochondrial toxicity. This conclusion was based on the absence of selective depletion of mitochondrial DNA (mtDNA) and transcripts observed in vitro, and the lack of changes in mtDNA content, ATP and ex vivo mitochondrial respiration during toxicology studies in monkeys. Similarly, a distinct prodrug of the same pharmacologically active nucleotide analog, 2′-C-methyl guanosine triphosphate (2'CMeG-TP), IDX14184, was also reported to have heart and kidney toxicities in rodents and monkeys (Luo *et al.*, 2015). In this study, swollen mitochondria were detected in the kidney but not the heart in monkeys, using transmission electron microscopy.

We have also studied the mechanism of toxicity of nucleotide analogs including BMS-986094 *in vitro* (Arnold *et al.*, 2012; Feng *et al.*, 2016). These studies demonstrated efficient incorporation of 2′-CMeG-TP by mitochondrial RNA polymerase (POLRMT), correlating with selective reduction of the protein expression of a mitochondrial gene [cytochrome c-oxidase subunit 1 (COX1)], and, ultimately, a functional reduction in mitochondrial respiration at clinically relevant concentrations of BMS-986094. Of note, a primary effect on mitochondrial function was observed both in a cultured cell-line and in freshly isolated rat cardiomyocytes. The effect on COX1 protein expression was potentiated by ribavirin, an inhibitor of endogenous guanosine triphosphate formation, consistent with competitive inhibition of a host polymerase by BMS-986094.

Whereas the results, reported in Baumgart et al. and our work, are seemingly at odds, we feel that this reflects the technical difficulties in accurately assessing mitochondrial toxicity. In particular, we question whether toxicity of a POLRMT inhibitor would necessarily be observed in the endpoints assessed by Baumgart et al. For example, mtDNA was monitored, but DNA depletion would not be expected for an inhibitor of mitochondrial transcription. Furthermore, ATP is not a specific measure of mitochondrial respiration, as it can also be formed by glycolysis. Consistent with a shift towards glycolysis, increased lactic acid was reported during in vitro incubations with BMS-986094 previously (Furman *et al.*, 2011). In fact, the observation by Baumgart et al. of cardiomyopathy resulting in reduced left ventricular ejection fraction, in and of itself, may be suggestive of an effect on mitochondrial function. This cardiac finding has been associated with a number of mitochondrial toxicants (eg, 2′,3′-dideoxycytidine, 2′-azidothymidine, and chloramphenicol) and mutations in genes involved in mitochondrial biogenesis (Domanski *et al.*, 1995; Haack *et al.*, 2013; Skuta *et al.*, 1999; Suarez and Ow, 1992).

It is important to understand the mechanistic basis for toxicity so that future clinical candidates can be appropriately assessed. In the absence of an alternative mechanism, we do not feel that the role of mitochondrial toxicity, and the inhibition of POLRMT in particular, can be definitely excluded for BMS-986094.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

REFERENCES

Available as a Supplementary data.

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