Peer Review File

Reviewer Comments

Authors present a manuscript that reviews molecular predictive determinants for sensitivity to aurora B kinase inhibitors using mainly preclinical models. They discuss molecular biology of these genes, its mechanisms of actions, gene interactions and tumour cell biology. They identify molecular markers including p53, Rb. myc amplification, which in the presence of AURKB inhibitors would result in synthetic lethality in small cell lung carcinoma and they discuss their mechanisms of interaction.

Comment 1: This is an interesting overview of the literature that points to new direction in the challenging area of treatment of SCLC involving predictive molecular targets in line with the principles of precision oncology.

Reply 1: Thank you for your comment.

Comment 2: Ultimately the aim of this review is to point to new strategies for choosing patients that would benefit from AURKB inhibitors. However, authors give a very cursory mention of current clinical trials utilizing this strategy. Considering that previously attempts using aurk inhibitors have failed, it would be of interest to know the current state of these new clinical trials and why these attempts may succeed where others failed.

Reply 2: Thank you for this comment. In response we have modified the concluding remarks section to point out that past trials failed due to toxicity issues and poor clinical responses. We discuss that AURKB inhibitor trials going forward may succeed by inclusion of immune checkpoint inhibitors to improve responses, new drug formulations that can reduce toxicity, and by the development of predictive biomarkers.
Changes in text: The concluding remarks section is rewritten as follows, with altered writing in red.

Past clinical trials with AURKB inhibitors failed or were halted due to toxicity issues and poor clinical response in patients. At least three factors are likely to improve AURKB trials going forward. First is the inclusion of immune checkpoint inhibitors, which have already shown promise for improving outcomes in SCLC patients (1). At the time of this writing, there is at least one active clinical trial combining AURKB inhibitor with an immune checkpoint inhibitor in extensive stage SCLC (NCT04745689). A second factor that may improve AURKB inhibitor trial success is improved drug formulations. The best example here is the AURKB inhibitor

AZD2811. Nanoparticle formulations of AZD2811 have been shown to provide a slow but extended drug release that reduces toxicity while maintaining anti-tumor activity (23). A third factor that may improve AURKB inhibitor trial success is the development of predictive biomarkers of response. This is an on-going challenge. Past trials examined expressions of AURKA and B and also different proliferation and mitotic markers for their correlation with response and their potential as predictive markers (24). However, no consistent correlations were made and there are currently no established markers to differentiate responsive and non-responsive tumors. Recent studies suggested that the mutation/deletion status of *RB* and *P53*, and/or the amplification status of *MYC*, can be used to identify responsive tumors. The findings from Byers and colleagues suggest this is not necessarily the case. Specifically, their findings indicate BCL2 high vs low expression can also identify responsive vs non-responsive SCLC tumors. It is likely that combined analysis of *RB*/*P53* status, *MYC* amplification, and BCL2 high vs low expression status in tumors would best identify patients most likely to benefit from AURKB inhibitor treatment. Combining these analyses with gene expression patterns may also help stratify SCLC tumors into responsive and non-responsive groups even further (25). Related to toxicity, combination treatments that sensitize tumors to AURKB inhibition will allow lower treatment doses of AURKB inhibitor to be given and could therefore reduce toxicity. Results from the Byers lab study suggest the BCL2 inhibitor venetoclax can sensitize SCLC cells and tumors to the AURKB inhibitor AZD2811, which is in current clinical trials. Notably, venetoclax is already FDA approved for AML, suggesting the potential for rapid clinical translation in SCLC for venetoclax in combination with AURKB inhibitors like AZD2811.