DISCOVERY OF BLOOD PHARMACODYNAMIC BIOMARKERS FOR ATR INHIBITORS

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Background The ataxia telangiectasia and rad3-related kinase (ATR) is a critical mediator of DNA damage response (DDR), which induce cell cycle arrest and facilitate DNA repair via downstream targets. Targeting ATR has become an attractive therapeutic strategy in cancer treatment and ATR inhibitors are currently being tested as anti-cancer agents in clinical trials, where pharmacodynamic (PD) biomarkers are crucial to help guide dose and scheduling and support mechanism of action studies. Tumor is the obvious tissue in which to measure PD effect but collecting tumor biopsies is often challenging for the patients. Phosphorylation of Chk1 or γH2AX has been reported to be PD markers of ATR inhibitors. However, these markers are difficult to measure directly using blood samples. ATG-018 is an oral, potent and selective small-molecule inhibitor of ATR. In this study, to identify clinically easy-to-use blood PD biomarkers of ATR inhibitor, we screened the gene expression changes in vitro using peripheral blood mononuclear cells (PBMCs) treated by different concentrations of ATG-018 and validated the marker in in vivo mouse models.

Methods We first assessed gene expression changes induced by ATG-018 on human PBMCs in vitro. Human PBMCs from three different donors were treated with different concentrations of ATG-018 (10, 100, 300, 1000 ng/ml), and NanoString technology was used for high-throughput gene expression profiling at transcriptional level. To further validate the identified potential biomarkers, wild type mice were treated with ATG-018 and Meso Scale Discovery was used to detect the protein expression of potential PD markers in plasma.

Results In in vitro test, a series of genes were found to change in a dose-dependent manner in response to ATG-018 in PBMCs (figure 1A). ATG-018 significantly inhibited the expression of chemokine genes, especially CCL2, CCL3/L1 and CCL4. Under the condition of 1000 ng/mL ATG-018 treatment, the log2 fold change of these three genes was more than -1.5 compared with the control group (figure 1B). In in vivo assay, the inhibition of CCL2, CCL3 and CCL4 protein expression could still be observed in plasma from Balb/c mice orally treated with ATG-018. Expression of CCL4 was significantly downregulated after administration of ATG-018 compared with the control group.

Conclusions In this study, we found the expression of chemokines, especially CCL2, CCL3/L1, and CCL4 are potential novel PD biomarkers of ATG-018, which could be detected in unmanipulated blood samples, and may guide dose and scheduling and support mechanism of action studies of both ATG-018 and other ATR inhibitors in clinic.

REFERENCE


Ethics Approval The protocol and any amendment(s) or procedures involving the care and use of animals in this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of CrownBio to execution with an AUP number or IACUC approval number for each animal study. During the study, the care and use of animals were conducted in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All studies were conducted following an approved IACUC protocol. AUP NO.: 2104–09–2108.