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603.LYMPHOID ONCOGENESIS: BASIC

Investigation of the Synergistic Combination of a Novel Celmod (CC-99282) and BET Inhibitors in Preclinical DLBCL

Chih-Chao Hsu, PhD[1], *Hsiling Chiu*[1], *Chad C. Bjorklund*[2], *Patrick R Hagner, PhD*[1], *Anita K. Gandhi, PhD*[1]

[1] Translational Development and Diagnostics, Bristol Myers Squibb, Summit, NJ
[2] Bristol Myers Squibb, Princeton, NJ

Background: Golcadomide (GOLCA; CC-99282) is a novel cereblon E3 ligase modulator (CELMoD), which binds to cereblon (CRBN), a substrate receptor of CRL4 CRBN E3 ubiquitin ligase, and induces proteasome-dependent degradation of Ikaros and Aiolos, transcription factors essential for B cell malignancies. GOLCA was shown to exhibit promising cell of origin independent activity in pre-clinical DLBCL models and is currently being investigated in early clinical trials (NCT03930953) for relapsed/refractory non-Hodgkin lymphomas (R/R NHL). Ikaros and Aiolos have previously been shown to have occupancy on the promoter of the proto-oncogene MYC, which is subsequently downregulated by CELMoDs in DLBCL models. Given MYC is a well-studied downstream target of BET inhibitors and a driver of lymphoma disease, we investigated the mechanistic synergy of anti-proliferative and pro-apoptotic effects of GOLCA in combination with BMS-986158, a potent BET inhibitor.
Results: Dose titration and analysis of the anti-proliferative effects of DLBCL cell lines (SU-DHL-4, WSU-DLCL2 and CARNAVAL) treated with either GOLCA or BMS-986158 suggests significant single agent activity of either agent. Bliss synergy score was determined and strong synergism of the two drugs was observed on day 5, with maximal bliss score (14.3-29.8) centered at low doses (1.25 and 2.5 nM) of BMS-986158 and sub-lethal doses of GOLCA (5-50 nM). Similar synergistic effects were found when combining GOLCA with JQ1, another tool compound BET inhibitor, confirming BET inhibition potentiates the anti-DLBCL effects of the CELMoD agent. Incucyte-based live cell image confirmed the enhanced anti-proliferative capacity of the combination compared to single agents. EdU (5-ethynyl-2′-deoxyuridine)-based cell cycle analysis revealed an increased proportion of G1-phase cells in combination treatment (67%) compared to GOLCA (38%), BMS-986158 (43%) and DMSO (35.4%) in the GOLCA resistant SU-DHL-4 cells.
To identify key pathways responsible for the observed synergistic combination effect, we performed transcriptomic analyses of SU-DHL-4 and WSU-DLCL2 cells treated with GOLCA/BMS-986158 for 72 hrs. Compared to either single agents, the combination treatment downregulated significantly more genes (~1000 and 500 genes in SU-DHL-4 and WSU-DLCL2, respectively), which were enriched with cell cycle pathways. Gene set enrichment analysis (GSEA) showed the top downregulated gene sets in combination treatment were E2F targets such as *CHEK1* and G2M checkpoint molecules including *AURKA* and *CDC45*. Given the critical role of *CDKN1A* (gene encodes p21) in cell cycle arrest, we examined its expression by real-time PCR. We found treatment of GOLCA induced *CDKN1A* mRNA expression (16.4 and 25.1-fold in SU-DHL-4 and WSU-DLCL2, respectively); remarkably, this induction was further enhanced by the combination with BMS-986158 (40.1 and 128.5-fold in SU-DHL-4 and WSU-DLCL2, respectively). Western blot confirmed the synergistic induction of p21 in combination compared to single agents, with a concomitant reduction of phosphorylated Rb, E2F target genes (Chk1, SKP2 and E2F1) and MYC.
Since the main targets of GOLCA and BMS-986158 are transcription factors (Ikaros and Aiolos) and transcriptional regulator (Brd4), respectively, we performed chromatin immunoprecipitation followed by sequencing (ChIP-seq) with antibodies targeting these three proteins along with various histone marks including H3K27ac and H3K4me3. Interestingly, the chromatin occupancies of Ikaros/Aiolos/Brd4 were highly overlapping in genes that are upregulated (*CDKN1A*) and downregulated (e.g. *CHEK1* and *MYC*) by the combination treatment. Further characterization of the interplay between Ikaros/Aiolos/Brd4 and their loci-specific interactomes may help elucidate the synergism at epigenetic levels.
Conclusions: BET inhibition potentiates anti-proliferative effects of GOLCA in DLBCL cells through synergistic induction of p21 and inhibition of E2F signaling. The synergetic effects of BMS-986158 and GOLCA suggest potential clinical benefits of combining lower doses of single agents to achieve better efficacy with reduced risk of adverse effects.

Disclosures Hsu: *BMS:* Current Employment, Current equity holder in publicly-traded company. **Chiu:** *BMS:* Current Employment, Current equity holder in publicly-traded company. **Bjorklund:** *Bristol Myers Squibb:* Current Employment, Current

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