Biomarker Analysis of Zanubrutinib and Tislelizumab Combination Therapy in Patients with Relapsed/Refractory B-Cell Malignancies

Authors: Jiaoyan Lyu,[1] Xiaopeng Ma,[1] Ruiqi Huang,[2] Liyun Zhao,[2] Yiling Yu,[2] Oscar Puig,[3] Yang Liu,[2] and James Hilger[4]

Affiliations: [1]BeiGene (Beijing) Co., Ltd., Beijing, China; [2]BeiGene (Shanghai) Co., Ltd., Shanghai, China; [3]BeiGene USA, Inc., Ridgefield, NJ, USA; and [4]BeiGene USA, Inc., San Mateo, CA, USA

Background/Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma world-wide. Although frontline therapies have yielded positive outcomes in most patients with DLBCL, about one-third of patients are refractory to or relapse after standard therapy (Sehn et al. *J Clin Oncol* 2005;23(22):5027-33). The antitumor efficacy of zanubrutinib and tislelizumab combination therapy has been demonstrated in patients with B-cell malignancies (Tam et al. *Blood* 2019;134:1594). Herein, we performed a comprehensive analysis to examine biomarkers associated with response or resistance to zanubrutinib and tislelizumab combination therapy in patients with B-cell malignancies.

Methods: Biomarker analysis was performed in 24 patients with published response data determined by the investigator using Lugano 2014 criteria (Cheson et al. *J Clin Oncol* 2014;32(27):3059-68). Programmed death-ligand 1 (*PD-L1*) gene amplification was assessed in the baseline tumor of 11 patients with non-germinal center B-cell (GCB) subtype of DLBCL by fluorescence in situ hybridization (Empire Genomics). PD-L1 protein expression was examined in the baseline tumor (8 DLBCL) by immunohistochemistry (IHC) (Ventana PD-L1 [SP263] assay; Roche). DLBCL subtype and gene expression were examined in the baseline tumor (14 DLBCL) by HTG EdgeSeq DLBCL cell of origin assay (HTG Molecular Diagnostics). Gene mutation was examined in the baseline tumor (17 DLBCL) by DNA-seq (CGI NGS 220-Gene Panel assay; Cancer Genetic Inc.). PD-L1 and CD8 protein expression were examined in paired biopsy samples (1 DLBCL, 3 transformed follicular lymphoma [tFL], 1 follicular lymphoma [FL], 1 mantle cell lymphoma) before and 8 days after zanubrutinib treatment by IHC (Ventana SP263, CONFIRM CD8 SP57). Gene expression profiles were examined in paired biopsy samples (3 tFL, 1 FL, 1 chronic lymphocytic leukemia) by RNA-seq (RNA Access assay; Illumina).

Results: *PD-L1* gene alteration was observed in 2 of 11 patients with non-GCB DLBCL, including 1 with gene amplification and 1 with chromosome 9 polysomy. A higher overall response rate (ORR; 2/2 [100%] vs 3/9 [33.3%]) and complete response (CR) rate (2/2 [100%] vs 2/9 [22.2%]) was observed in patients with *PD-L1* gene alteration than in patients without. Two of 8 [25%] evaluable patients with DLBCL harbored PD-L1$^+$ tumor cells (defined using a PD-L1 protein expression cut-off ≥1%). A higher ORR (1/2 [50%] vs 2/6 [33%]) and CR rate (1/2 [50%] vs 1/6 [16.7%]) was observed in patients with PD-L1$^+$ tumor cells than in those without. High mRNA levels of *CD3D* ($P < 0.05$), *HLA-DRA* ($P < 0.07$), and *LAG3* ($P < 0.05$) were enriched in responders, suggesting an inflamed tumor microenvironment (TME). In contrast, non-responders harbored high mRNA levels of the NF-kB pathway-related gene *REL* ($P < 0.05$). High mRNA levels of *REL* were associated with an inferior clinical outcome to zanubrutinib and tislelizumab combination therapy ($P < 0.05$). Mutations in the tumor suppressor gene *TP53* (5/10 [50%] vs 0/7 [0%]) were enriched in non-responders. Non-responders harbored higher frequency of mutations in genes involved in immune evasion (3/10 [30%] vs 1/7 [14.3%]), epigenetic modifications (2/10 [20%] vs 1/7 [14.3%]), and cell survival (2/10 [20%] vs 0/7 [0%]) than responders, suggesting that non-responders may have complex resistance mechanisms. Results from paired tumor biopsies from 5 patients before and after zanubrutinib treatment revealed that zanubrutinib treatment downregulated B-cell and MYC target-related gene sets and upregulated the NK cell-related gene set *NK-2*. Zanubrutinib treatment seemed to have no effect on the frequency of PD-L1$^+$ cells and CD8$^+$ T cells (using a CD8 expression cutoff of ≥1%) in the TME, although the sample size was too small to draw a definitive conclusion.

Conclusion: Patients with *PD-L1* gene amplification, PD-L1^{+} tumor cells, and high mRNA levels of *CD3D, HLA-DRA,* and *LAG3* in baseline tumor tissue may be more responsive to zanubrutinib and tislelizumab combination therapy. A high mRNA level of *REL* or mutations in *TP53* may contribute to resistance of zanubrutinib and tislelizumab combination therapy. Due to the limited number of samples, results must be interpreted with caution.