Abstract: P1826

Title: PLCG2 Δ20-22 EXON-SKIPPED VARIANTS IN PRIMARY CLL

Abstract Type: e-Poster Presentation

Topic: Chronic lymphocytic leukemia and related disorders - Biology & translational research

Background:

Acquired BTK and PLCG2 mutations confer resistance to BTK inhibitors (BTKi). We reported novel splice-site mutation in PLCG2 acquired in tirabrutinib resistant REC-1 cells that led to exon skipping and activated enzyme (Qi J. et al., EHA, 2023). However, it is unknown if PLCG2 splice-site mutations and exon skipping would occur in samples from CLL patients treated with BTKi.

Aims:

To investigate if splice site mutations in the intronic regions and the deletion of exons coding for the regulatory domain of PLCG2 can occur in primary CLL.

Methods:

Targeted next generation sequencing (NGS) covering the splice sites of *PLCG2* was performed to identify mutations. High-resolution MetaPhor agarose gel electrophoresis was used to assess exon skipping in the cDNA. Sanger sequencing (Sanger-seq) was used to sequence the *PLCG2* amplicons from the cDNA. Quantitative reverse transcription PCR (qPCR) was used to quantify the mRNA expression of the *PLCG2* variants.

Results:

The novel *PLCG2* c.2236-1G>T splice site mutation acquired in tirabrutinib resistant REC-1 cells resulted deletion of exon 21 (Δ21) and Δ21-22, resulting in higher PLCG2 activity. However, review of NGS data from samples at baseline (BL) and at disease progression (PD) in the ibrutinib-based CLL2-GIVe (Huber H. et al., *Blood* 2023) and CLL12 (Langerbeins P. et al., *Blood* 2022) trials revealed no splice-sites mutations within the PLCG2 regulatory domain down to a variant allele frequency of 1.5%.

To verify if transcript variants with loss of auto-regulatory domain leading to PLCG2 activation can occur in CLL, we screened RNA samples from N=27 patients treated with ibrutinib at the Ulm University medical center and two truncated bands in addition to the larger (WT) band (Fig. 1A) in a subset of cases were observed. Sanger-seq confirmed that exons 22 and 20-22 (Δ22 and Δ20-22) were skipped in the RNA but no genomic deletions (Fig. 1B) or splice-site mutations were observed. Moreover, the occurrence of Δ22 and Δ20-22 PLCG2 was not related to *BTK/PLCG2* mutational status. While Δ21 (Qi J. et al.,*EHA*, 2023) and Δ20-22 (Ombrello MJ. et al., *NEJM*, 2012) exhibited higher PLCG2 enzyme activity, Δ22 resulted in premature stop codon. A correlation between the Δ22 and Δ20-22 *PLCG2* expression ($R2 = 0.71$, $p < 0.0001$) observed hints on a common mechanism behind the generation of these variants.

Of note, the Δ22, but not the Δ20-22 active PLCG2 variant was identified in PBMCs from healthy donors. Next, we performed qPCR to compare expressions of Δ20-22 and WT *PLCG2* between samples available at BL and PD from ibrutinib and venetoclax-treated CLL patients. In the ibrutinib-treated subgroup, Δ20-22 expression increased in N=6 of 20 cases (30%), remained stable in N=11 (55%), and decreased in N=3 cases (15%) at PD compared to BL (Fig. 1C, $P = 0.001$, Fisher's exact test, null hypothesis: no change). Conversely, in the venetoclax-based treatment, the variant expression decreased in N=12 of 20 cases (60%), was stable in N=4 (20%) and increased in N=4 cases (20%) at PD compared to BL (Fig. 1D, $P < 0.001$, Fisher's exact test, null hypothesis: no change). The ratio of cases with increasing *vs.* decreasing Δ20-22 expression was 2 in the ibrutinib-treated vs. 0.33 in the venetoclax-treated subgroup ($P = 0.087$, Fisher's exact test).

Summary/Conclusion:

The constitutively active *PLCG2* Δ20-22 variant is expressed in a subset of CLL samples but the mechanism behind its generation remains unclear. Though the mRNA expression of the variant varies at PD compared to BL based on treatment regimen, further studies are warranted to assess its clinical relevance.

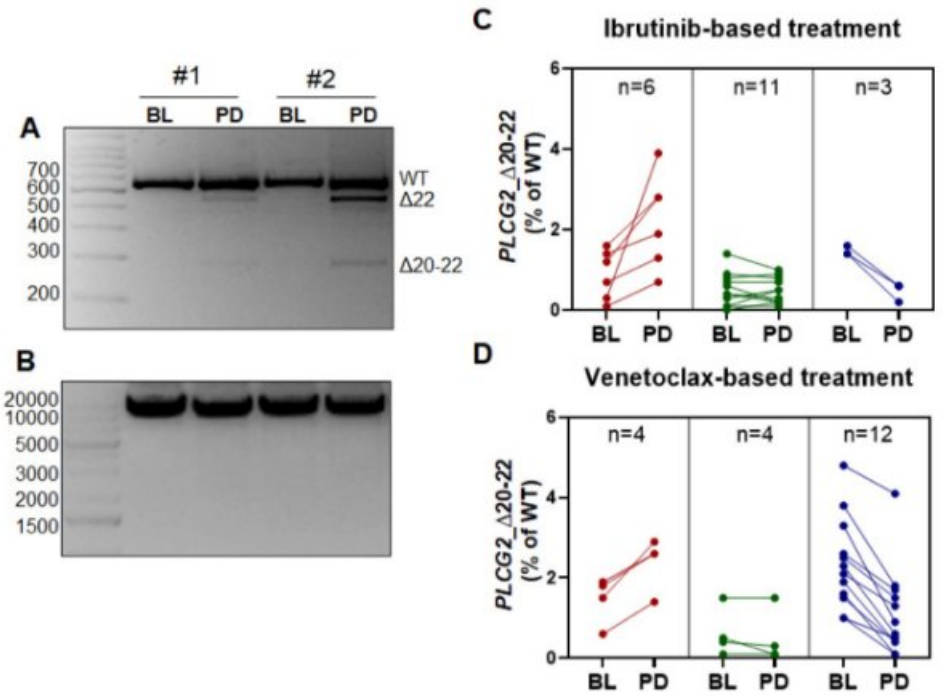

Figure 1. Gel electrophoresis of the PCR product from two representative patients using the template of cDNA (A) and DNA (B). The change in the expression of *PLCG2* Δ20-22 in ibrutinib (C) and venetoclax (D) treated CLL patients. Cases were assigned to three subgroups based on a change in expression exceeding 0.5% (of WT) between BL and PD. BL, baseline; PD, progressive disease; WT, wild type.

Keywords: Phospholipase C, Bruton's tyrosine kinase inhibitor (BTKi), B-CLL, Alternative splicing