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P615 BCL2 RESISTANCE MUTATIONS IN A REAL-WORLD COHORT OF PATIENTS WITH VENETOCLAX-TREATED CHRONIC LYMPHOCYTIC LEUKAEMIA

Topic: 05. Chronic lymphocytic leukemia and related disorders - Biology & Translational Research

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Background:

The Bcl-2 inhibitor venetoclax has transformed the therapeutic landscape of therapy refractory and treatment naïve chronic lymphocytic leukaemia (CLL). Despite the remarkable response rates in both patient groups, a subset of CLL patients receiving venetoclax treatment experience disease progression, frequently associated with pathogenic variants of the *BCL2* gene. Apart from the most common hotspot G101V and D103Y mutations, pathogenic variants affecting several different loci of the coding region of BCL2 have been described, however data and international recommendations on the sensitive detection and monitoring of these mutations are still absent to date.

Aims: Our aim was to develop a droplet digital PCR (ddPCR) based assay for the detection of *BCL2* G101V and D103Y mutations and to reveal further resistance mutations emerging at relapse by performing targeted ultra-deep next-generation sequencing (NGS).

Methods:

Peripheral blood samples from 71 patients treated with venetoclax-rituximab or first line venetoclax-obinutuzumab combination were collected from 11 Hungarian oncohematological centres. Genomic DNA was extracted from peripheral blood mononuclear cells and leukemic cell purity was assessed by flow cytometry. Fractional abundance of the most common hotspot *BCL2* mutations G101V and D103Y was assessed using the QX200 digital droplet PCR system. Following ddPCR analyses, targeted ultradeep NGS was performed on samples obtained at relapse using a custom-designed panel covering the whole coding regions of *BCL2*, *BTK*, *PLCG2* and *TP53* genes. Fractional abundance of *BCL2* mutations identified by NGS was assessed retrospectively in serial peripheral blood samples by ddPCR using custom-designed, allele-specific assays.

Results:

Lower quantitative limit of detection of the *BCL2* ddPCR assays was tested and could ubiquitously be established as 0.01%. With a median follow up of 14 months, *BCL2* G101V and D103Y mutations were detected in 8.5% (6/71) and 9.9% (7/71) of the patients, respectively, with 90% (9/10) of them experiencing relapse during the follow-up period. Three patients harbored both resistance mutations as detected by ddPCR. *BCL2* G101V and/or D103Y were observed in 43.5% (10/23) of the cases showing signs of disease progression. In four cases, emergence of the mutations

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predated the first clinical signs of relapse with a median 4 months. In samples obtained at relapse, targeted ultradeep NGS uncovered two additional *BCL2*variants (V156D and A113G), which were successfully backtracked and detected by ddPCR. Both V156D and A113G were detected in patients previously found to harbor G101V or D103Y, with no further *BCL2* resistance mutations identified in G101V and D103Y wild type cases.

Summary/Conclusion:

In a real-world cohort of CLL patients receiving venetoclax in combination therapy, comprehensive and sensitive screening of *BCL2* mutations can identify molecular mechanisms of venetoclax resistance in nearly half of the patients experiencing relapse. In patients harboring multiple *BCL2* mutations, convergent evolution of the CLL subclones may contribute to the driver mechanisms of resistance, justifying the comprehensive approach for the detection of these variants. In secondary venetoclax resistant cases displaying wild type *BCL2*, further molecular screening methods are required to reveal alternative genetic or non-genetic reasons for disease progression.