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Chronic lymphocytic leukaemia (CLL)

Eurofins Biomnis offers two NGS panels for CLL:

- The **"LLCTR"** NGS panel for therapeutic purposes (*TP53* status and screening for BTKi, BCL2i and anti CD20 resistant mutations).
- The **"LLC"** NGS panel for diagnostic, prognostic and therapeutic purposes.

The "LLCTR" NGS panel consists of an analysis of 10 genes: *TP53*, *BTK*, *PLCG2*, *BCL2*, *CARD11*, *SF3B1*, *EP300*, *BAX*, *NOTCH1* and *MCL1*. As part of **the therapeutic decision**, it must be combined with 17p deletion (TP53 deletion) by the FISH technique and an IGHV mutation analysis.

TP53 status guides treatment decisions in symptomatic CLL (iwCLL criteria). The presence of a *TP53* mutation means that a BTKi (Bruton tyrosine kinase inhibitor) can be prescribed: ibrutinib or acalabrutinib. Secondary BTKi resistant mutations are classically reported in the *BTK* and *PLCG2* genes, and more exceptionally in *CARD11*, *SF3B1* or *EP300* genes.

In the absence of a *TP53* mutation, determination of *IGHV* mutation status makes it possible to prioritise treatment between a combination of BCL2 inhibitor (venetoclax) and anti-CD20 (obinutuzumab) or a BTKi. Venetoclax resistance mutations have been reported in the *BCL2*, *BAX*, *TP53* or *MCL1* GENES. Mutations in the *NOTCH1* gene have been reported to predict a poor response to 1st and 2nd generation anti-CD20 drugs.

To date, there is very little data in the literature on the notion of secondary resistant mutations to PI3Ki (idelalisib).

The "LLC" NGS panel consists of an analysis of 28 genes: *ARID1A/ATM/BAX/BCL2/BCOR/BIRC3/BRAF/BTK/CARD11/EGR2/EP300/FBXW7/HRAS/KRAS/MAP2K1/MCL1/MGA/MYD88/NFKBIE/NOTCH1/NRAS/PLCG2/POT1/RPS15/SAMHD1/SF3B1/TP53/XPO1.*

It has a triple **diagnostic, prognostic and theranostic** value and must be combined with a blood cytogenetic study and an analysis for IGHV mutations.

From a **theranostic** point of view, the CLL panel includes all the genes from the "LLCTR" panel presented above.

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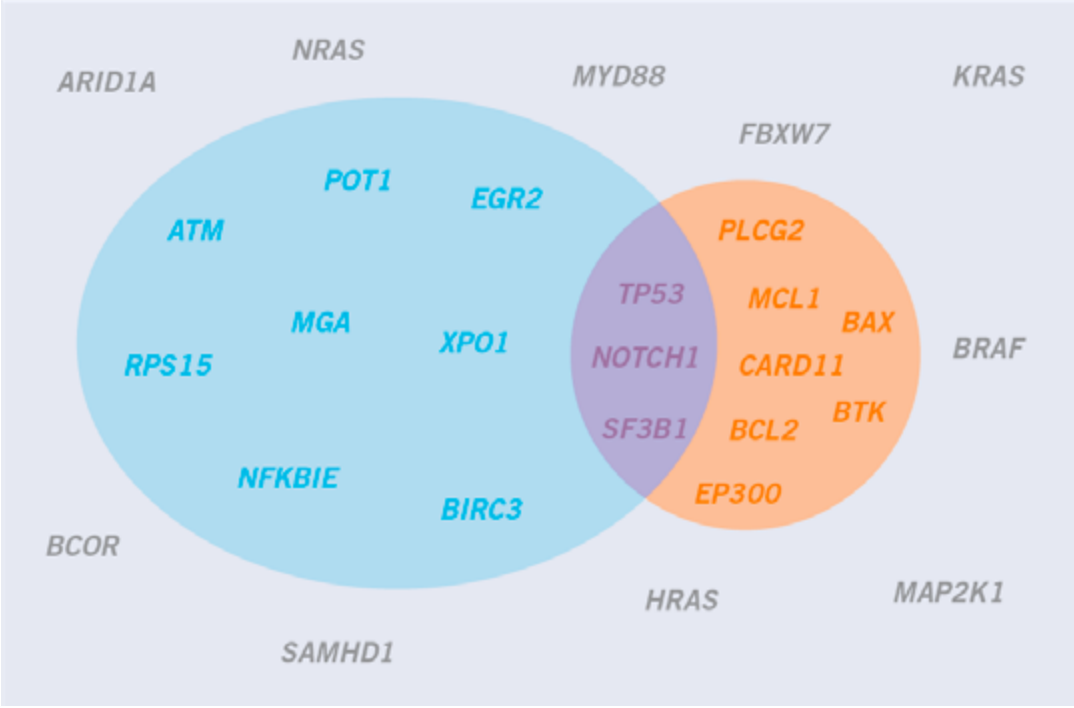
From a **prognostic** point of view, the presence of a *TP53* abnormality (del(17p) deletion or *TP53* mutation) is the predominant prognostic value in CLL, followed by IGHV status. The status of the two mutations are included in the CLL-IPI score. However, other mutations (*NOTCH1*, *SF3B1*, *ATM*, *BIRC3*, *XPO1*, *POT1*, *EGR2*, *RPS15*, *NFKBIE* or *MGA*) are also associated with a poor prognosis. The exhaustive molecular result obtained with this panel provides the clinician with an additional help for the prognostic evaluation of CLL, particularly in the context of Rai 0. The CLL-IPS-Early-Stage score already takes into account the mutation status of the *TP53*, *IGHV*, *ATM*, *MYD88*, *NOTCH1* and *SF3B1* genes. It should be noted that the presence of a *MYD88* mutation has no negative prognostic value in CLL.

Associations between "cytogenetic abnormalities" and "molecular abnormalities" have also been reported: del(11q) deletion and *ATM*, *BIRC3*, *SF3B1* or *XPO1* mutations, trisomy 12 and *NOTCH1*, *BIRC3* or *FBXW7* mutations, del(13q) deletion and *MYD88* or *POT1* mutation. In addition, the association of these mutations could also have a more pronounced unfavourable prognostic value. Mutational co-occurrences have been reported (e.g., *TP53-NOTCH1-XPO1*).

All the genes in the NGS "CLL" panel belong to different signalling pathways involved in CLL and thus contribute to the diagnostic value. The most frequently mutated genes are *NOTCH1* (10~15%), *SF3B1* (~10%), *TP53* (5~8%), *ATM* (~5%), *BIRC3*, *EGR2*, *FBXW7*, *MYD88*, *NFKBIE*, *POT1* and *XPO1* (3~5%).

Diagnostic, prognostic and theranostic distribution (with resistance mutations) of mutated genes in CLL



Legend:

- *Mutations reported in CLL*
- *Mutations associated with a poor prognosis*
- *Mutations guiding therapeutic choice and treatment resistance mutations*

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Targeted genes

"LLCTR" NGS Panel

Gene	Transcript	Exon rank
BAX	NM_138761	Full coding region
BCL2	NM_000633	Full coding region
BTK	NM_000061	Full coding region
CARD11	NM_032415	Full coding region
EP300	NM_001429	Full coding region
MCL1	NM_021960	Full coding region
NOTCH1	NM_017617	Full coding region
PLCG2	NM_002661	Full coding region
SF3B1	NM_012433	Full coding region
TP53	NM_000546	Full coding region

Test code: LLCTR

"CLL" NGS Panel

Gene	Transcript	Exon rank
ARID1A	NM_006015	Full coding region
ATM	NM_000051	Full coding region
BAX	NM_138761	Full coding region
BCL2	NM_000633	Full coding region
BCOR	NM_017745	Full coding region
BIRC3	NM_001165	Full coding region
BRAF	NM_004333	Full coding region
BTK	NM_000061	Full coding region
CARD11	NM_032415	Full coding region
EGR2	NM_000399	Full coding region
EP300	NM_001429	Full coding region
FBXW7	NM_033632	Full coding region
HRAS	NM_176795	Full coding region
KRAS	NM_033360	Full coding region
MAP2K1	NM_002755	Full coding region
MCL1	NM_021960	Full coding region
MGA	NM_001164273	Full coding region
MYD88	NM_002468	Full coding region
NFKBIE	NM_004556	Full coding region
NOTCH1	NM_017617	Full coding region
NRAS	NM_002524	Full coding region
PLCG2	NM_002661	Full coding region
POT1	NM_015450	Full coding region
RPS15	NM_001018	Full coding region
SAMHD1	NM_015474	Full coding region
SF3B1	NM_012433	Full coding region
TP53	NM_000546	Full coding region
XPO1	NM_003400	Full coding region

Test code: LLC

Pre-analytical requirements: EDTA peripheral blood or bone marrow sample

Turnaround time: 10 days (Results may require an extended turnaround time of oneweek, depending on the confirmation tests required by Sanger sequencing)

Contact

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References

Different prognostic impact of recurrent gene mutations in chronic lymphocytic leukemia depending on IGHV gene somatic hypermutation status: a study by ERIC in HARMONY. Mansouri L et al. Leukemia. 2023 Feb;37(2):339-347. PMID: 36566271

The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Alaggio R et al. Leukemia. 2022 Jul;36(7):1720-1748. PMID: 35732829

The International Consensus Classification of Mature Lymphoid Neoplasms: a report from the Clinical Advisory Committee. Campo E et al. Blood. 2022 Sep 15;140(11):1229-1253. PMID: 35653592

Mechanisms of venetoclax resistance and solutions. Liu J et al. Front Oncol. 2022 Oct, 12:1005659. PMID: 36313732

Multiple Mechanisms of NOTCH1 Activation in Chronic Lymphocytic Leukemia: NOTCH1 Mutations and Beyond. Pozzo F et al. Cancers (Basel). 2022 Jun 17;14(12):2997. PMID: 35740661

The resistance mechanisms and treatment strategies of BTK inhibitors in B-cell lymphoma.
Wang H et al. Hematol Oncol. 2021 Dec;39(5):605-615. PMID: 34651869

International prognostic score for asymptomatic early-stage chronic lymphocytic leukemia. Condoluci A et al. Blood 2020 May 21;135(21):1859-1869. PMID: 32267500

Ibrutinib Resistance Mechanisms and Treatment Strategies for B-Cell lymphomas. George B et al. Cancers (Basel). 2020 May 22;12(5):1328. PMID: 32455989

The Need for a Consensus Next-generation Sequencing Panel for Mature Lymphoid Malignancies. Sujobert P et al. Hemasphere. 2018 Dec 27;3(1):e169. PMID: 31723808

iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Hallek M et al. Blood. 2018 Jun 21;131(25):2745-2760. PMID: 29540348

ERIC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia-update on methodological approaches and results interpretation. Malcikova J et al. Leukemia. 2018 May;32(5):1070-1080. PMID: 29467486

An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. International CLL-IPI working group. Lancet Oncol. 2016 Jun;17(6):779-790. PMID: 27185642

Recommandations FILO :
https://www.filo-leucemie.org/actualites/traitements/llc-lesrecommandations-du-filo-2023/

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