Frequency of *IKZF3* L162R Mutation in Waldenström's Macroglobulinemia

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Introduction

- AIOLOS/IKZF3 is a lymphoid transcription factor important for B cell development.
- Recently, whole exome sequencing studies of CLL patients identified a hotspot mutation of *IKZF3* (L162R) as a putative driver of chronic lymphocytic leukemia (CLL).[1,2]
- This gain-of function mutation is localized within the DNA binding domain, altering DNA binding specificity and expression of IKZF3 target genes finally resulting in overexpression of B-cell receptor (BCR) pathway members.[3]
- Apart from CLL, *IKZF3* mutations are rarely described in Waldenström's Macroglobulinemia (MW).

Objective

- **Analysis of the distribution of the *IKZF3* mutations in B cell proliferations reported in the literature.**
- **Screening of the hotspot mutation *IKZF3* L162R in a large cohort of MW.**

Materials & Methods

- Retrospective cohort of 78 Waldenström's macroglobulinemia (WM) cases, all harboring the MYD88 L265P mutation.
- A droplet digital PCR (ddPCR) assay was designed to detect the *IKZF3* L162R mutation with a high sensitivity.
- Presence of the *IKZF3* L162R mutation was confirmed using NGS sequencing of a 13 genes-panel including exon 5 of IKZF3.

Results

Distribution of *IKZF3* mutations in lymphoproliferative B cell disorders

- By analyzing the frequencies of all *IKZF3* variants reported in the literature and through the cBioPortal and COSMIC databases, 43 *IKZF3* mutations were mentioned in hematological malignancies.
- *IKZF3* mutations are more frequent in CLL (45%) and DLBCL (35%) cases.
- The hotspot *IKZF3* L162R affecting the DNA binding domain is the most frequent mutation reported (35%).

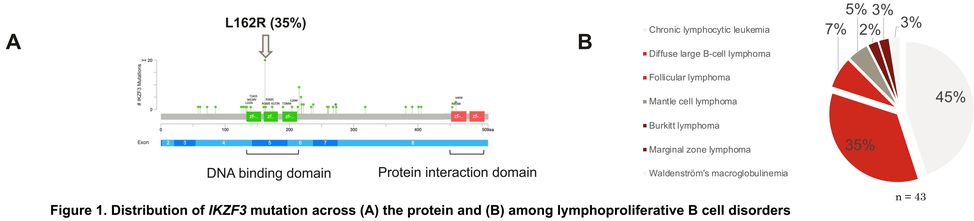


Figure 1. Distribution of *IKZF3* mutation across (A) the protein and (B) among lymphoproliferative B cell disorders

IKZF3 mutations are rarely reported in Waldenström's Macroglobulinemia ⇨ Lack of sensitivity of the sequencing techniques ? Low infiltration by tumoral cells of the samples ?

Screening of *IKZF3* L162R mutation in Waldenström Macroglobulinemia

- Among the 78 patients tested by ddPCR, the mutation was detected in 5 patients (5/78; 6%) with variant allelic frequency (VAF) comprised between 0.7% and 2.4%.
- Targeted NGS sequencing confirmed the presence of the *IKZF3*-L162R mutation in samples with VAF >1%.

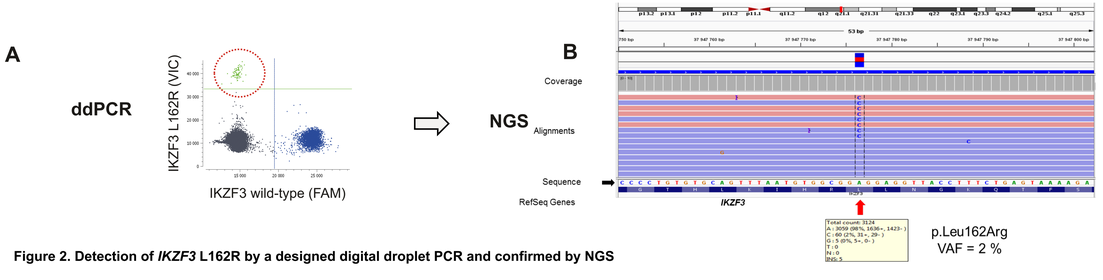


Figure 2. Detection of *IKZF3* L162R by a designed digital droplet PCR and confirmed by NGS

- *IKZF3* L162R mutation was found in the same range of VAF as MYD88 mutation in 2 cases and was subclonal in 3 cases.

Patients characteristics

Patient	IKZF3 (ddPCR)	MYD88	CXCR4	Disease	Age	Sex	Hb (g/dL)	Platelets (G/L)	IgM (g/L)	Anti-MAG antibodies	Traitement	Clinical complications
Patient 1	2.3 %	11%	neg	WM	65	F	15.3	217	2	Yes	No	Neuropathy
Patient 2	2.4 %	36%	neg	WM	73	F	4.7	140	60	Yes	BDR	Hyperviscosity/ Neuropathy
Patient 3	0.7 %	36%	neg	WM	61	M	12.6	207	27	Yes	No	Neuropathy
Patient 4	2%	1.5 %	neg	WM	65	F	12.4	229	10	No	R-Ibrutinib	Cryo/Neuropathy
Patient 5	0.7%	8%	neg	WM	88	M	11	373	28	No	BDR	Acrosyndrome/ Chronic inflammation

(BDR : bortezomib + dexamethasone + Rituximab)

- Almost all patients had a symptomatic disease requiering treatment (3/5).
- Neuropathy was the most frequent complication.
- None of the patients had a CXCR4 mutation.

Conclusion

- *IKZF3* L162R hotspot mutation is frequent in WM.
- This mutation is rarely reported in this disease, probably due to a lack of sensitivity of the sequencing technique used as WES and to a low infiltration of the samples by tumoral cells.
- The presence of this mutation in various lymphoproliferative B cell disorders suggest a common mechanism of lymphomagenesis.
- More samples including marginal zone lymphoma are currently sequenced to better appreciate the distribution of this mutation among B cell lymphomas

References

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2. Puente, X. S. et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. Nature 526, 519–524 (2015).
3. Lazarian, G. et al. A hotspot mutation in transcription factor IKZF3 drives B cell neoplasia via transcriptional dysregulation. Cancer Cell 39, 380-393.e8 (2021).