Exome sequencing of refractory Diffuse Large B-Cell Lymphomas highlights candidate genes for targeted resequencing

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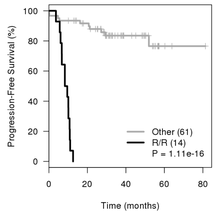
As the most common lymphoid malignancy, Diffuse Large B-Cell Lymphoma (DLBCL) has largely benefited from immunochemotherapy combinations developed in the past decade. However 30% to 40% of patients still do not respond to treatment, or relapse in a few months. The mechanisms involved in short term treatment failure are poorly understood, and biomarkers to predict refractoriness in newly diagnosed patients are still lacking.

Patients & Methods

14 Refractory / Relapsing DLBCLs (R/R)



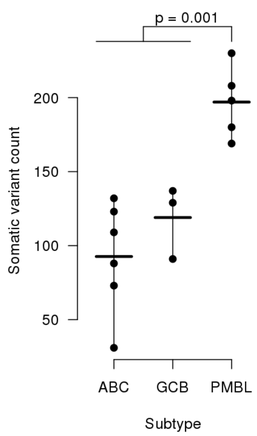
14 of 75 patients with de novo DLBCL were selected from a multicentric clinical trial led by the GELA, according to their clinical presentation (**relapse under treatment in less than a year**) and DNA sample availability. Molecular subtyping was performed using Affymetrix U133+2 transcriptomic arrays, revealing **6 ABC**, **3 GCB** and **5 PMBL** tumors.

Whole-Exome Sequencing (WES)

Normal and **tumoral** DNA pairs were sequenced on an **Illumina HiSeq 2000™** platform by a subcontractor (Integragen SA, Evry, France), producing an average of 92×10^6 76-bp long reads per sample in a "paired-end" fashion. A local analysis pipeline was set-up following the Broad Institute recommendations, including BWA-MEM, GATK BQSR, GATK near-indel realignment, and duplicate read filtering by Picard. An average sequencing depth of **79x [68-114]** was achieved across the coding exons defined in the Ensembl 72 database.

Single Nucleotide Variations (**SNVs**) and short insertion / deletions (indels) were called by VarScan 2, after estimating tumor cellularity. Copy Number Variations (**CNV**) and Copy-Neutral Loss of Heterozigosity (**CN-LOH**) were detected by ADTEx.

1 621 somatic SNVs and 277 somatic indels were called across the 14 samples, for an average of **135.6 variants per patient** [31-230]. The average mutation rate of 3.5 per Mb observed in these samples was consistent with previous reports, and significantly **higher in the PMBL subtype** (Mann-Whitney's p=0.001).

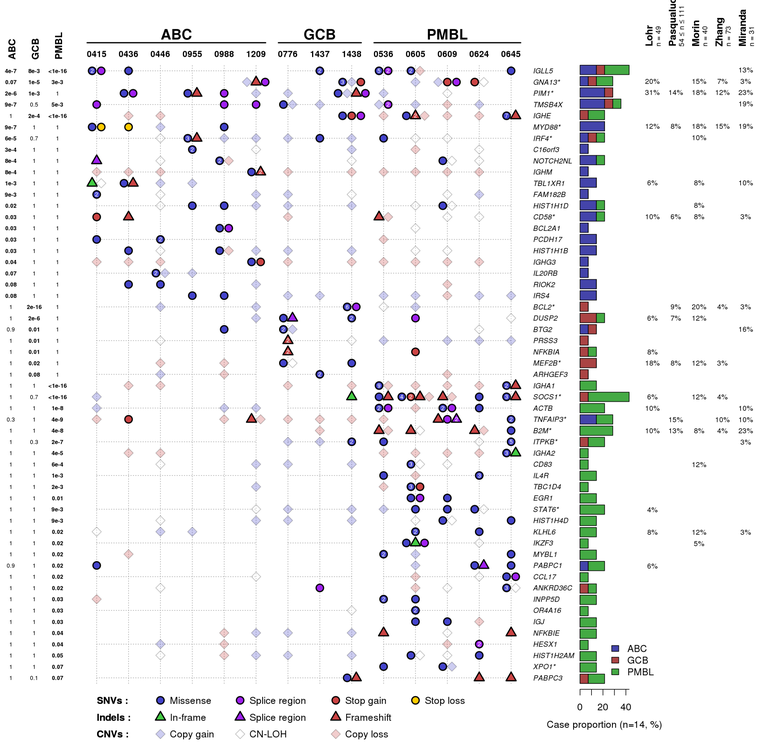


PGM™ validation

34 genes were resequenced in 12 tumoral samples using an orthogonal method (Life Technologies PGM™), allowing to estimate the true positive rate as [97;100] % and the false positive rate as [6;17] %.

Gene-level analysis

55 significantly mutated genes



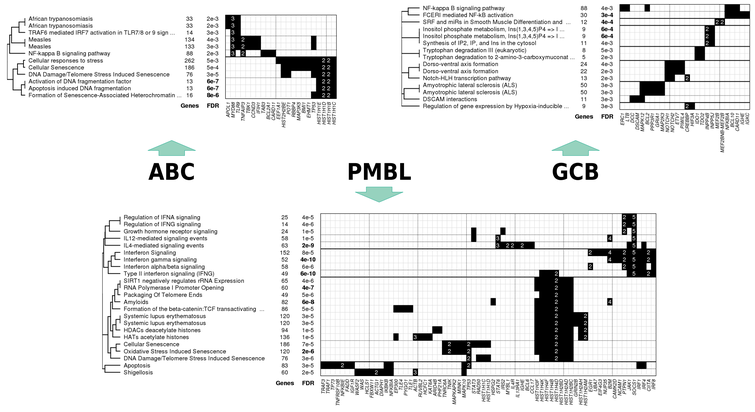
Genes significantly more mutated than expected according to the background mutation rate observed throughout the exomes were detected by MuSiC SMG (FDR < 1%). Several genes were confirmed as frequently mutated in DLBCLs, such as *MYD88* and *CD58* in ABC samples, *BCL2* and *MEF2B* in GCB samples, *SOCS1* and *STAT6* in PMBLs.

While the involvement of the **NFκB complex** is a well-established fact in ABC DLBCLs (via *MYD88* mutations notably), truncating events in *NFKBIA* and *NFKBIE* (NFκB inhibitors α and ε) in GCB and PMBL samples suggests this pathway as an interesting lead there too.

XPO1 was also revealed as a promising lead in PMBLs, with two E571K mutations that proved to be recurrent in a larger validation cohort (7/18 PMBLs, regardless of the R/R character). As a nucleus exportin with hundreds of identified cargos (P53, REL, BRCA1 ...), it represents a valuable target with experimental drugs in development (KPT-330).

Pathway-level analysis

118 significantly mutated pathways



2 632 pathways from the NCBI BioSystems database were screened for enrichment in mutations using Path-Scan (FDR < 1%). **Histone monomers** were found frequently mutated as previously reported, but pathway analysis proposed interesting relationships with mutations in *POT1* (telomere related senescence) or *TNIK* (oxidative stress related senescence). **Interferon and interleukins 4 / 12 signaling** pathways were also particularly disrupted in PMBLs, coupling to known *SOCS1*, *STAT6* and *PTPN1* mutations events in genes such as *B2M*, *CIITA*, *IL4R*, *IGHE* ... Frequent truncations of *B2M* observed here were confirmed in a larger validation cohort (6 truncating events out of 9/18 mutated PMBL patients).

Comparison with non-R/R cohorts

Simplified enrichment analyses based on the same principles and pathways were conducted in two published series of 39 DLBCL genomes (*Morin et al, Blood 2013*) and 73 exomes (*Zhang et al, PNAS 2013*) respectively. As our R/R cohort presented an **unexpectedly high proportion of PMBL** patients (a subtype known for its good prognosis), several PMBL-related pathways were found more enriched in our series (such as IL4 signaling pathways).

Non-PMBL pathways enriched exclusively in our R/R cohort included **tryptophan degradation** in ABC patients, which has been reported as an immune escape mechanism in several cancer types.

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