

Whole Genome Sequencing in Sequential Biopsies Reveals the Genetic Evolution of Follicular Lymphoma to Transformed Follicular Lymphoma

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Blood (2012) 120 (21) : 145.

<http://doi.org/10.1182/blood.V120.21.145.145>

Abstract

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Follicular lymphoma (FL) displays a heterogeneous clinical course with an initial good response followed by multiple relapses and the need for recurrent therapeutic interventions. Histological transformation (tFL) to a more aggressive phenotype remains a critical event in a subset of FL patients (20–30%) and is associated with poor clinical outcomes. Genetic profiling of serial FL biopsies and published cases of donor-derived FL in recipients of bone marrow transplantation suggest that the FL clones detected at diagnosis, relapse and transformation may arise by divergent evolution from a common progenitor B cell (CPC). An understanding of the clonal dynamics during disease progression together with the genetic events present at each time point will prove important for monitoring and effectively targeting these tumors.

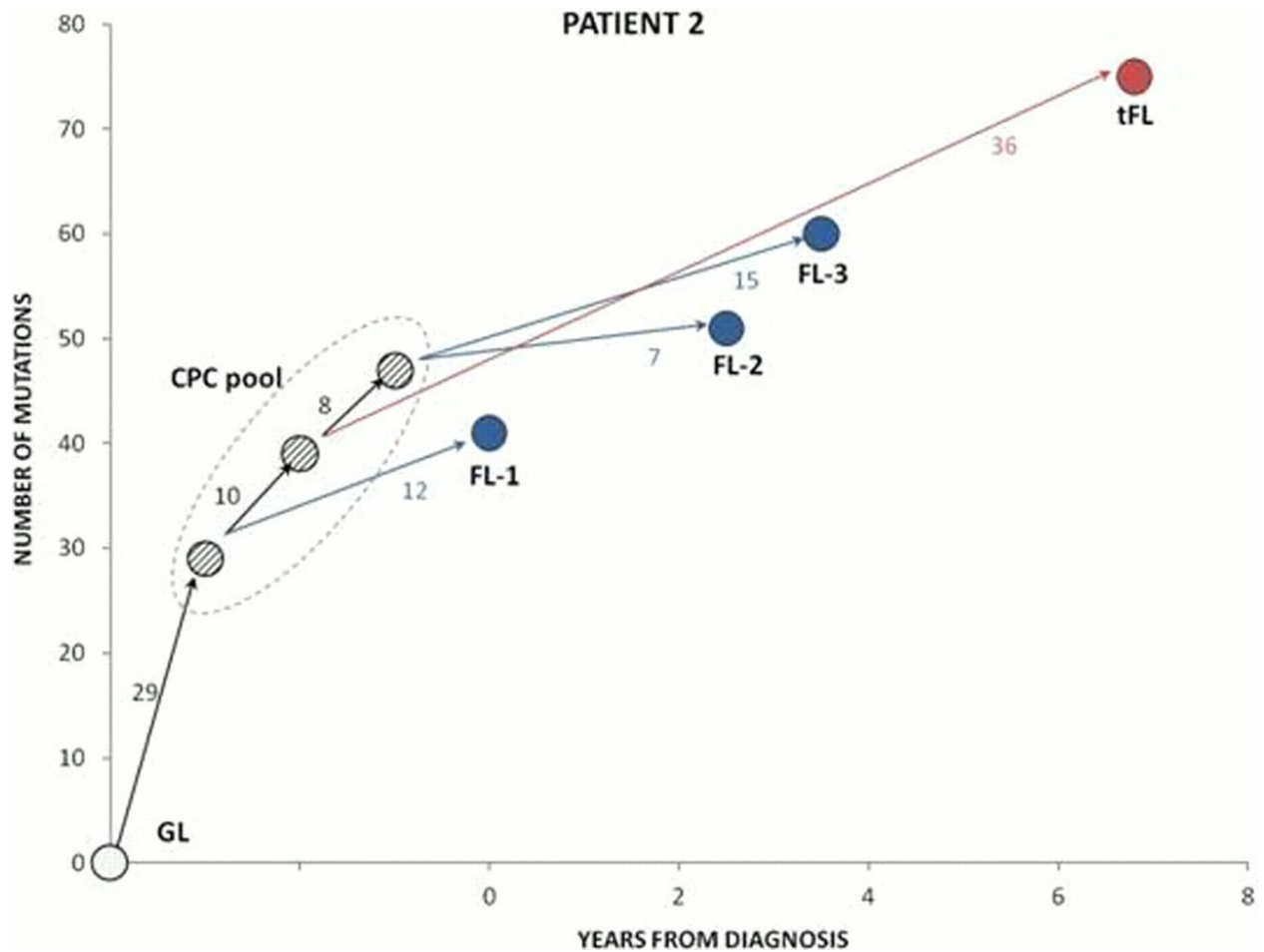
We performed whole genome sequencing (WGS) using 100bp paired-end reads on tumor DNA of lymph node (LN) biopsies from 6 patients who were selected on the basis of availability of a FL, subsequent tFL biopsy and paired germline sample (8 FL, 6 tFL, 6 germline). All 6 cases had received at least one line of therapy achieving a complete remission (CR) prior to undergoing histological transformation (number of therapies: 2–6; number of relapses: 1–6), with a mean time to transformation of 8.6 years (range: 1.6–15.6 yrs) from diagnosis. Tumor and germline genomes were sequenced at an average coverage of 37× of mapped individual reads.

Our analysis identified mutations in the coding and non-coding (5' and 3' UTR, miRNA, lincRNA and CpG islands) regions of the FL and t-FL genome. Variants were validated by Sanger sequencing. Mutations were detected in the coding region of 587 genes. Each tumor had an average of 72 somatic mutations (range: 41–143) with higher frequencies in the tFL genomes (mean: FL 64.8; tFL 83.6), indicating ongoing genetic evolution with disease progression. In addition to identifying somatic mutations in previously described genes involved in histone methylation (*MLL2*, *EZH2*) and histone acetylation (*CREBBP*, *MEF2B*), we also report mutations in other chromatin regulators including linker and core histones (*HIST1H1 B-E*, *HIST1H2BG*) that facilitate folding of chromatin into higher order structure and *ZMYM3*, a regulator of gene expression at the H3K4me3 mark. Interestingly, all biopsies showed co-occurrence of mutations in *MLL2* with at least one additional chromatin regulator, and frequent acquisition of novel epimutations at transformation. Moreover, we observe recurrent mutations in genes involved in the NF-κB pathway, B cell receptor signaling, B cell development, DNA repair and apoptosis regulation. In all cases, we show an overlap of somatic variants (based on comparisons between non-synonymous, synonymous and UTR variants) between the paired FL/tFL biopsies, although this clonal relationship varied across the patients (range: 7.0–86.9%; mean 52.4%). The overall pattern of FL evolution to transformation was consistent with the existence of a shared common origin, the CPC (Figure 1). We did not identify any compelling single mutational event that was restricted to the tFL 'branch' that could immediately be linked with transformation. However, the 'truncal' CPC mutations present in a patient's biopsies highlighted key biological pathways that are commonly deregulated in both FL and tFL.

Although the existence of such complex patterns of heterogeneity and progression has cast doubt on the possibility of developing personalized therapies capable of targeting multiple variants simultaneously in solid tumors, the detection of recurring events common to FL, tFL and the CPC clone in our study, suggests that a stratified medicine approach targeting these specific pathways in FL warrants consideration.

Figure 1: Clonal divergence of follicular lymphoma in patient 2 illustrating the presence of shared 'truncal' and unique 'branching' somatic mutations at various tumour time points. The numbers above each branch

indicate the number of non-synonymous mutations. CPC, common progenitor cell; GL, germline; FL-1, 1st relapse; tFL, transformed FL



Disclosures:

Gribben: *Celgene*: Honoraria; *Roche*: Honoraria; *Merck*: Honoraria; *Mundipharma*: Honoraria; *Pharmacyclics*: Honoraria.

Author notes

* Asterisk with author names denotes non-ASH members.

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