XIX CONGRESSO NAZIONALE SIES2026
Firenze | 4-6 marzo 2026 Palazzo degli Affari

XIX Congress of the Italian Society of Experimental Hematology ABSTRACT N. CO13

Chronic lymphocytic leukemia and Chronic Lymphoproliferative Disorders

DISSECTION OF BIALLELIC *NOTCH1* DISRUPTION IN CHRONIC LYMPHOCYTIC LEUKEMIA REVEALS CHROMOSOME 9 COPY NEUTRAL-LOSS OF HETEROZYGOSITY

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Introduction: *NOTCH1* is frequently mutated in CLL, with an incidence of 12–25%, and shorter TTFT and OS compared to wild-type cases, with subclonal mutations also exerting a residual prognostic impact. However, the prognostic significance of *NOTCH1* disruption with biallelic involvement (VAF > 50%) has not yet been specifically evaluated.

Methods: A total of 4222 peripheral blood samples from 3564 CLL patients referred to the Clinical and Experimental Onco-Hematology Unit, CRO Aviano, were screened for *NOTCH1* mutations by NGS (exon 34 and part of 3′UTR). Copy number analysis of *NOTCH1* was performed using TaqMan probes for exon 34 (Hs00124234_cn) and exon 31 (Hs00088335_cn) by ddPCR/qPCR, or FISH on fixed nuclei. Whole-exome sequencing (WES) was conducted on sorted CD19+ (tumor) and CD3+ (germline) fractions from frozen samples. The PureCN R package was used to estimate copy number from exome sequencing (WES) data.

Results: *NOTCH1* mutations were identified in 753 samples (17%), including 46 (6%) with VAF > 50% (high-burden). Copy number analysis was performed to assess biallelic involvement and to exclude Chr9 deletions. ddPCR demonstrated that most high-burden samples were diploid, with only 3/46 (6.5%) showing deletions. FISH confirmed these findings, revealing 19/20 (95%) diploid samples without Chr9 deletions and 1 sample with a 56% loss. However, NGS of *NOTCH1* exon 34 revealed a peculiar polymorphic unbalancing of D2185D rs2229974 SNP (e.g. VAF 0.1-0.4 or 0.6-0.9) in heterozygous samples. Most of such samples with *NOTCH1* mutations above 50% were unbalanced (13/20). To investigate the hypothesis of tumor-restricted allelic imbalance, separate sequencing on FACS-sorted CD19+ and CD3+ fractions from 7 high-burden unbalanced samples (plus 1 deleted control) showed that both *NOTCH1* mutation and D2185D unbalancing were confined to CD19+ cells. Complete allelic loss of 9q34 was observed in 3 samples and partial loss in the other 3 samples. However confirmed a diploid (2n) status in both tumor and germline fractions (1n in the deleted control). WES revealed strong allelic imbalance across multiple loci on Chr9 compared to germinal controls, suggesting chromosomal deletions, whereas PureCN predicted diploid status in 5 samples and confirmed deletion only in the control. FISH on 9q22 confirmed diploid status in these samples. WES also detected alterations on other chromosomes (Chr8q gain/loss, del11q, del13q, del22), all confirmed by FISH with specific probes. Overall, NGS data suggest presence of a D2185D imbalance, and thus likely Chr9 alterations, in 150 (3.5%) samples, independently of *NOTCH1* mutations.

Conclusion: This study describes, for the first time in CLL, a non-rare copy-neutral loss of heterozygosity (cn-LOH) mechanism on chromosome 9, where high-burden *NOTCH1* disruption coexists with a normal (2n) copy number. Ongoing analyses aim to clarify the clinical implications of cn-LOH in both *NOTCH1*-mutated and wild-type CLL cases.