Abstract: S143

Title: NATURAL CLONAL EVOLUTION OF CHRONIC LYMPHOCYTIC LEUKEMIA WITH BIMODAL CD49D EXPRESSION REVEALS CD49D PLASTICITY

Abstract Type: Oral Presentation

Session Title: CLL biology & translational

Background:

CD49d, the alpha-chain of the VLA-4 integrin heterodimer, is one of the strongest negative prognosticators in chronic lymphocytic leukemia (CLL). CD49d expression is usually homogenously negative (homCD49d-) or positive (homCD49d+), although ~20% of CLL display a distinct bimodal pattern of CD49d expression (bimCD49d) with the co-presence of CD49d- and CD49d+ subpopulations, the latter tending to increase overtime, especially after therapy, given the higher propensity to proliferate and segregate in tissue sites.

Aims:

To investigate the interplay of natural clonal evolution, and dynamics of epigenetic regulation of CD49d expression in BimCD49d CLL.

Methods:

We collected 2-3 longitudinal samples at 3.3 years mean time interval (range 1.3-7.0) from 8 untreated bimCD49d CLL with overtime increase of the CD49d+ component, encompassing both logistic and exponential growth. WGS was carried out from sorted CLL cells (CD5+/CD19+) further split into CD49d+/CD49d- fractions in order to keep clonal dynamics of CD49d+/CD49d- subpopulations distinguishable. WGS was analysed according to Caravagna et al. (Nat Genet, 2020) and Lal et al. (PLoS Comput Biol, 2021). DNA methylation analysis of 119 CpG islands of the ITGA4/CD49d gene promoter was done by NGS (Nextera technology on bisulfite converted DNA). RNA-seq (6 bimCD49d, 9 homCD49d-, 9 homCD49d+) was performed with the Illumina Stranded mRNA prep library kit analyzed with R package DESeq2. ATAC-seq was done according to Illumina protocol in homCD49d- (n=3), homCD49d+ (n=7) and bimCD49d (n=3) CLL. Multiparametric flow cytometry was used to discriminate the CLL proliferative (CD5high/CXCR4dim, PF) and resting (CXCR4high/CD5dim, RF) fractions.

Results:

WGS analysis of the sorted CD49d+/CD49d- fractions of longitudinal samples revealed 4,815 median heterozygous SNVs (range: 3,177-15,547) and 61 driver mutations in 22 genes (Fig.A).

Two or more branching subclones with identical genetic origin were present in both the CD49d+ and CD49d- sorted components suggesting CD49d expression rewiring. Given the driving role of CD49d in CLL, such transitions occurred mainly toward the upregulation of CD49d expression in CD49d- cells, although the opposite switch was also observed. We hypothesized that the most parsimonious explanation for the presence of subclones across both components is a plastic pattern originating from a CD49d- cell rewiring CD49d expression to become CD49d+ (Fig.B).

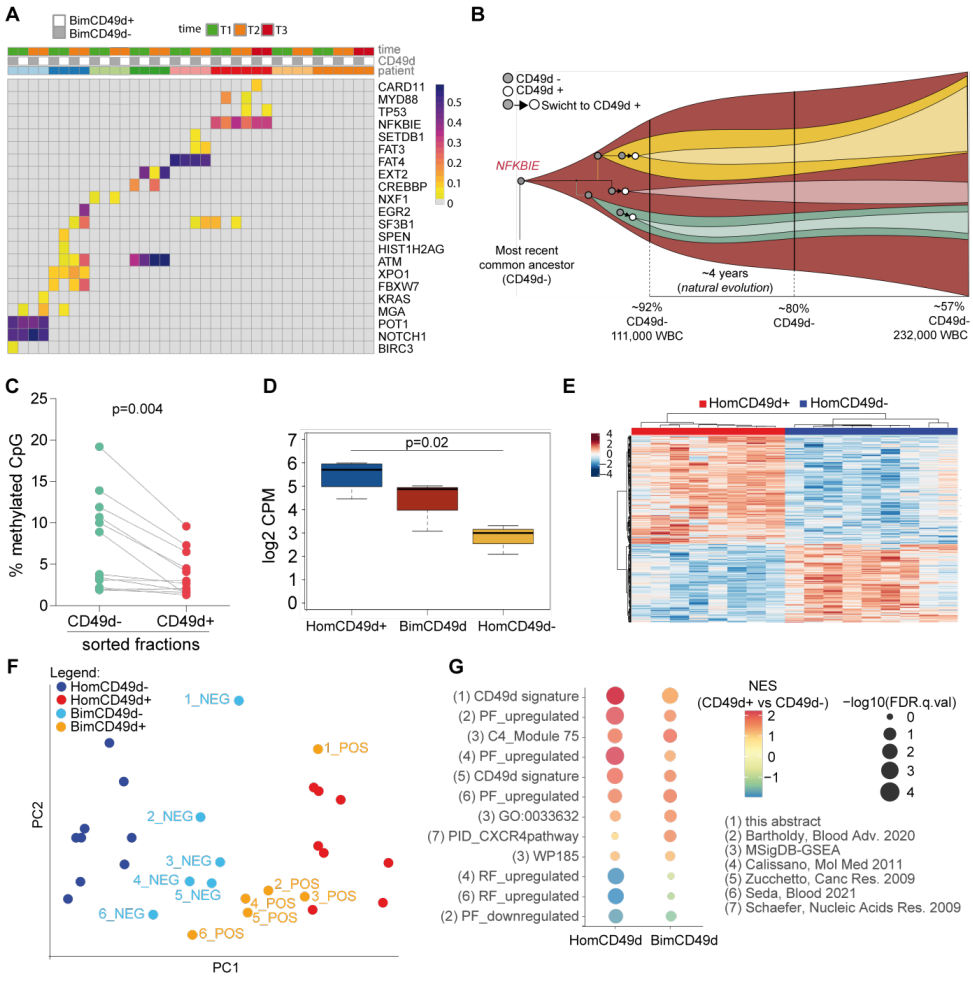
A higher degree of ITGA4 methylation was observed in CD49d- vs CD49d+ CLL fractions ($p=0.004$), indicating a methylation-driven epigenetic regulation of CD49d expression (Fig.C), as observed in other CLL settings (Zucchetto et al., Blood 2013). ATAC-seq of the ITGA4 region promoter showed higher chromatin accessibility in homCD49d+ compared to homCD49d- cases ($p=0.02$), bimCD49d cases showing intermediate levels (Fig.D); accessibility of the ITGA4 promoter correlated with the amount of ITGA4 mRNA ($p<0.0001$, $r=0.93$).

The CD49d- and CD49d+ fractions from bimCD49d cases expressed a transcription signature respectively similar to homCD49d- and homCD49d+ cases (Fig. EF). CD49d+ cells over-expressed the transcription signatures of CLL PF (Fig.G). Consistently, the CD49d+ fraction was significantly enriched in cells bearing the CXCR4dim/CD5bright

phenotype characterizing the PF, recently egressed from lymph nodes, distinct from the CXCR4high/CD5dim RF, marking quiescent cells long-lasting navigating the bloodstream (p=0.001).

Summary/Conclusion:

CD49d expression is plastic in CD49d bimodal CLL, its plasticity driven by a combination of genetic and epigenetic events.



Keywords: RNA-seq, Integrin, Chronic lymphocytic leukemia, Genomics