High deletion burden identified by whole genome sequencing is associated with enhanced risk in del17p CLL patients

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Introduction

- Deletion17p (del17p) that encompasses tumor protein p53 (TP53) is a well-established prognostic and predictive marker in patients with chronic lymphocytic leukemia (CLL).
- Presence of del17p has been associated with shorter time to treatment initiation, early relapse after therapy and inferior overall survival (OS)[1] However, there exists notable clinical heterogeneity within this group.[2,3]
- Previous reports have identified percentage of del17p nuclei by fluorescence in situ hybridization (FISH), having TP53 mutations on the second allele, copy number alterations, and complex karyotype (CK) as risk factors that add to that of del17p.[4,5]
- Here we profile a cohort of CLL patients that are wait and watch treatment-naive (n=14), treated (n=36), and relapsed/refractory(R/R) CLL (n=3) patients by whole genome sequencing (WGS) and RNA sequencing (RNAseq) to gain insights into genomic factors contributing to the high-risk nature of del17p across the spectrum of CLL as well as the underlying gene expression pathways and biology.

Methods

- 53 patients with del17p (identified by FISH testing in routine clinical practice from the Mayo Clinic CLL Database) with stored peripheral blood mononuclear cells (PBMCs) available pre-treatment (n= 50) and at relapse (n=3) were obtained.
- PBMCs were collected within 2 years from CLL diagnosis (n=44)
- RNA and DNA from matched peripheral blood (tumor) and DNA from CD19- (germline) samples pre-treatment (n=50) and relapse (n=3) was extracted using Qiagen RNeasy (RNA) and Purene (DNA) kits and subjected to RNAseq and WGS, respectively.
- WGS was processed using GATK best practices with MUTECT2 somatic variant caller. Samples were processed both as tumor-only pairs, and both tumor and normal samples were also processed in tumor only mode. Lenient filtering of the normal tumor-only sample was used to generate of set of secure germline variants, allowing for some tumor contamination. These germline variants were removed from the tumor sample.
- Gene deletion burden was defined as Enhanced High-Risk (EHR) for ≥514 deleted genes versus High-Risk (HR) for <514 deleted genes.
- RNA-seq was processed with STAR aligner and Salmon. Differential expression analysis was run with limma. GSEA score was calculated using the 'piano' R package with (geneSetStat='stouffer'), and (signifMethod='nullDist')
- FISH and CK by chromosomal banding analysis (CBA) were generated on these samples as part of clinical profiling.
- Baseline clinical characteristics, time to first treatment (TTT), and overall survival (OS) were obtained from the Mayo Clinic CLL Database (Table 1). TTT and OS were measured from sample collection date. The Mayo Clinic IRB approved this study.

Table 1. Clinical Characteristics of Cohort

Characteristic		N (%) or [range]
Median Age, years		69 [44-86]
Gender (Male)		31(60)
Unmutated *IGHV* (n=48)		29 (60)
Median time from CLL diagnosis to sample, years		0.1 [0.0 - 26.1]
Rai Stage(n=48)	0	15 (31)
	I or II	19 (40)
	III or IV	14 (29)
CLL FISH	Del11q	8 (15)
	Trisomy 12	8 (15)
	Del13q	30(58)
	6q	3(6)
	14	2(4)

Results

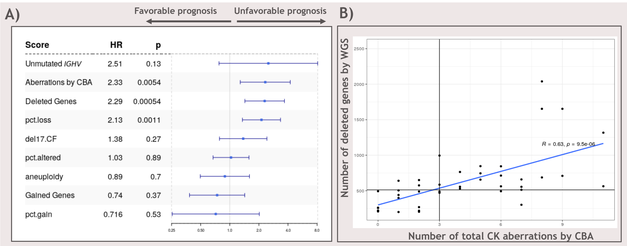

Figure 1. A) Forest plot of potential predictors of overall survival by high-risk factors. Associations reported as hazard ratios (HR); statistical testing by Cox PH test shown as p value. Aberrations by CBA, number of deleted genes, percent of genome lost, Del17p clonal fraction, percent of genome lost or gained, number of chromosome arms lost or gained and percent of genome gained were high-risk factors included **B) Correlation of Complex Karyotype by CBA with number of deleted genes defined by WGS.** Number of deleted genes (n=514) was identified as optimal cut-off to define increased risk using cutpointr package(corresponding to a CK≥3)

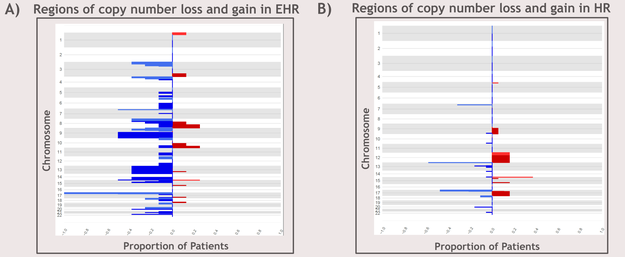

Figure 2. Recurrent copy number alterations in patients by deletion burden A) EHR (n=33) and B) HR (n=19) Gains (red) and loss(blue) across genome are indicated.

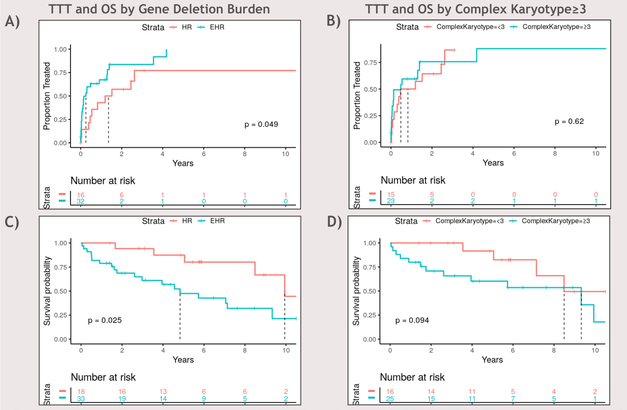

Figure 3. Time to Treatment, TTT (A and B) and Overall Survival, OS (C and D). Patients were stratified as Enhanced High Risk and High Risk Del17p CLL patients by number of deleted genes ≥ 514 (A and C) and by Complex Karyotype (CK≥3)

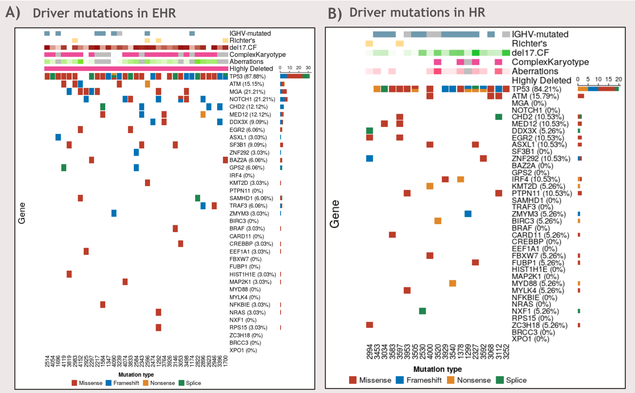

Figure 4. Recurrently mutated driver genes profile in A) EHR Del17p CLL (n= 33) and B) HR Del17p CLL (n=19) stratified by deletion burden. Mutation types are color-coded as indicated. Additional clinical data on IGHV mutation status, Richter transformation, clonal cell fraction of del17p by FISH, Complex Karyotype (CK≥3), total aberrations by CBA and deletion group are indicated on top

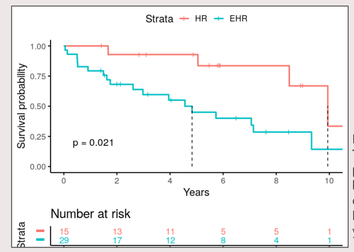

Figure 5. Overall Survival curve in bi-allelic TP53 aberration (Del17p and P53 mutation) patients (n= 44/52; 84.6%) by gene deletion burden. High deletion burden is associated with decreased survival compared to low deletion burden (Median OS in EHR=4.8 years vs HR=9.9 years).

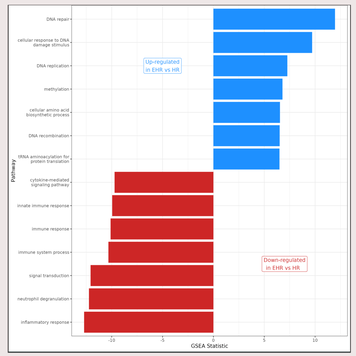

Figure 6. Pathway analysis in EHR Del17p vs HR Del17p. Increased gene deletion burden is associated with upregulation of DNA repair and cellular response to DNA damage stimulus pathways. Immune response and Inflammatory response pathways are downregulated in EHR sub-group in comparison to HR sub-group

Results (Continued)

- Total number of deletions by WGS and number of chromosomal aberrations by CBA as a continuous variable were significantly associated with unfavorable OS in the entire cohort (n=53) in a univariate analysis (Fig.1)
- In patients with CK data (n=41), deletion burden was highly correlated with chromosomal aberrations by CBA.
- Del17p patients with increased gene deletion burden showed enrichment across the genome with deletions in 3p, 4p, 8p, 9p, 9q and gains in 2p, 8q, 15q and 17q chromosomal lesions. (Fig.2)
- TP53 (86%), ATM (15%), NOTCH1 (13%), MGA (13%), CHD2 and MED12 (11%) are top mutations in the full cohort occurring at a prevalence of >10%. Total number of driver genes mutated was not significantly different between EHR and HR subgroups, but EHR subset was significantly enriched with mutations in NOTCH1 (21%), and MGA (21%) as compared to no mutations in the HR group in these latter genes (Fig.4)
- Within the biallelic TP53 aberration patients, increased deletion burden is associated with decreased survival in unadjusted analyses. (Fig 5)
- Pathway analysis shows enrichment of DNA repair and downregulation of immune/ inflammatory response pathways in EHR group (Fig. 6)

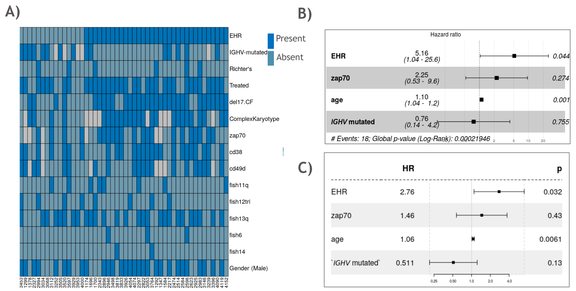

Figure 7.A) Heatmap of clinical and genetic characteristics across EHR and HR group B) Multivariable Cox model of OS by gene deletion burden adjusted for other clinical characteristics. C) Corresponding Univariate Cox model statistics

Conclusions

- CK is a known prognostic marker which includes both chromosomal losses and gains.
- Using genome wide sequencing, we identify increasing genomic deletions as a feature of del17p patients with shorter time to treatment and decreased survival.
- EHR subgroup remained significant in OS after adjusting for other known prognostic variables.
- Gene deletion burden cut-off identified here was specific to our research method and requires further validation in independent cohorts.

References

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Presented at the International Workshop on Chronic Lymphocytic Leukemia Meeting 2023; October 6-9, 2023; Boston, MA, USA

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