The Landscape of Genetic Mutations in Patients with Chronic Lymphocytic Leukemia and Complex Karyotype

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Abstract

Context. Complex karyotype (CK) is associated with poor prognosis in patients with chronic lymphocytic leukemia (CLL). While mutations in TP53 have been associated with CK, overlap is not complete and the genetic landscape of patients with CLL and CK remains largely undescribed.

Design. We performed targeted next generation sequencing with a SureSelect custom panel of 295 genes (Agilent Technologies, Santa Clara, CA) on bone marrow aspirate collected between March 2003 and October 2015 from 71 patients with CLL and CK, and compared them to a control group of 90 patients without CK, age and sex-matched.

Results. In univariable analyses (UVA), CK was associated with Rai III-IV disease (61% vs 36%, p=0.002), beta-2 microglobulin (B2M) > 4 mg/L (67% vs 40%, p=0.001), del(17p) by FISH (33% vs 9%, p<0.001), and presence of any gene mutation (79% vs 59%, p=0.01). Among patients with CK, the most common mutations (>10% of patients) were TP53 (32%), SF3B1 (15.5%) and NOTCH1 (10%). When compared to the non-CK cohort, patients with CK showed a significantly higher prevalence of TP53 (32% vs 11%, p=0.001) and SETD2 mutations (6% vs 0%, p=0.04). On multivariable analysis (MVA), B2M >4 mg/L (odds ratio [OR] 2.5, 95% confidence interval [CI] 1.2-5.3; p=0.02) and del(17p) by FISH (OR 3.6, 95% CI 1.2-10.4, p=0.02) were independently associated with CK.

After a median follow-up of 17 months (range, 1-141 months), among the 71 patients with CLL and CK, 29 (41%) died, and median overall survival (OS) was 56 months (range, 1-141 months). Factors significantly associated with shorter OS in UVA were unmutated IGHV (32 months vs 132 months, p=0.02), mutated NOTCH1 (17 months vs 60 months, p=0.02) and mutated TP53 (32 months vs 62 months, p=0.05). In MVA, unmutated IGHV (hazard ratio [HR] 5.2, 95% CI 1.2-22.7; p=0.03) and mutated NOTCH1 (HR 5.8, 95% CI 1.6-20.4; p=0.007) maintained their association with shorter survival.

Conclusions. This is the first study specifically dissecting the genomic landscape of patients with CLL and CK. Further investigation of the role of mutated SETD2 and mutated NOTCH1 may improve the management and treatment of this very high risk population.

Background

- Complex karyotype (CK) is associated with poor prognosis in patients with chronic lymphocytic leukemia (CLL).
- While mutations in *TP53* have been associated with CK, overlap is not complete and the genetic landscape of patients with CLL and CK remains largely undescribed.

Methods

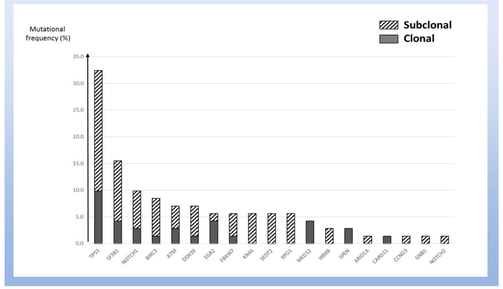
- CK was defined as the presence of 3 or more karyotype abnormalities by stimulated metaphase cytogenetic
- Targeted next generation sequencing of 295 genes was performed on bone marrow aspirate obtained from 71 patients with CLL and CK
- A control group of 90 patients without CK, matched by age, sex and number of previous therapies, was used for comparison

Baseline Characteristics (N=161)

	Number (percentage), median [range]		
Patients (N=161)	CK (N=71)	No CK (N=90)	p-value
Age ≥ 65	36 (51)	43 (48)	0.75
Age < 65	35 (49)	47 (52)	
Male	50 (70)	64 (71)	1
Female	21 (30)	26 (29)	
Rai stage III-IV	43 (61)	32 (36)	**0.002**
Rai stage 0-II	28 (39)	58 (64)	
B2M ≥4 mg/L	47 (67)	36 (40)	**0.001**
B2M <4 mg/L	23 (33)	54 (60)	
Unmutated *IGHV*	41 (75)	39 (67)	0.42
Mutated *IGHV*	14 (25)	19 (33)	
Del17p	23 (33)	8 (9)	**0.005**
Del11q	15 (22)	23 (26)	
+12	12 (17)	21 (24)	
Neg	5 (8)	12 (14)	
Del13q	14 (20)	24 (27)	
Del17p	23 (33)	8 (9)	**<0.001**
No del17p	46 (67)	80 (91)	
Previously treated*	37 (52)	35 (39)	0.11
Treatment-naive	34 (48)	55 (61)	
Mutation, present	56 (79)	53 (59)	**0.01**
Mutation, absent	15 (21)	37 (41)	
Mutations/pt ≥2	25 (35)	21 (23)	0.12
Mutations/pt <2	46 (65)	69 (77)	

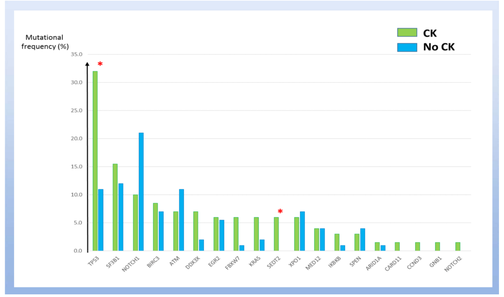
- B2M, beta-2-microglobulin; *IGHV*, immunoglobulin heavy chain variable gene; CK, complex karyotype
- (*): the median number of previous therapies did not significantly differ between CK and non-CK group (1 previous therapy, p=0.13)

Gene mutations clonality (N=71)



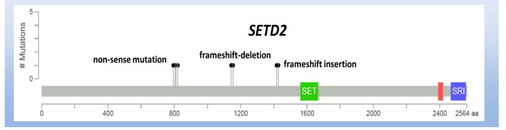
- Cancer cell fraction (CCF) < 85% = subclonal
- Only 1 (14%) of 7 patients with *NOTCH1* mutation had concomitant *TP53* mutation.
- None of the patients with *SETD2* mutation and CK had a concurrent *TP53* mutation
- Concurrent cytogenetic abnormalities in chromosome 17 by CKM were observed in 14 (61%) patients with *TP53* mutation, but no concurrent cytogenetic abnormalities in chromosome 3 or chromosome 9 were observed in patients with *SETD2* and *NOTCH1* mutation, respectively.
- SETD2 and TP53 mutations were equally divided between treatment-naïve and previously treated patients (2 cases each).

Gene mutations frequency (N=161)



- When adjusting by the Benjamini and Hochberg method, the only significant (false discovery rate [FDR] < 0.05) pairwise association was the one between CK and *TP53* mutation. No mutations mutually exclusive with CK were identified.

SETD2, *TP53* and *NOTCH1* mutations



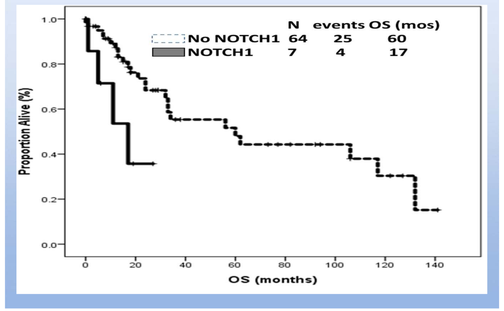
- *SETD2* mutations were exonic in 100% of cases (all involving exon 3 and resulting in truncated protein), median VAF was 24% (range, 11-33%), median CCF was 49% (range, 22-67)
- *TP53* mutations were exonic in 78% of cases (with no recurrent exonic involvement), median variant allele frequency (VAF) was 35% (range, 5-96%), median CCF was 70% (range, 10-100%)
- *NOTCH1* mutations were exonic in 100% of cases (all involving exon 34), median VAF was 36% (range, 15-50%), median CCF was 72% (range, 30-100%)
- **On MVA analysis, only B2M > 4 (OR 2.5, 95% CI 1.2-5.3; p=0.02) and del17p by FISH (OR 3.6, 95% CI 1.2-10.4, p=0.02) remained associated with CK.**

Overall Survival CK patients (N=71)

Patients (N=71)	OS (months)	p-value
Age ≥ 65	62	0.71
Age < 65	56	
Males	33	0.17
Females	117	
Rai III-IV	33	0.13
Rai 0-II	Not reached	
B2M ≥ 4 mg/L	34	0.14
B2M < 4 mg/L	Not reached	
IGHV unmutated	32	0.02
IGHV mutated	132	
Del17p	60	0.60
No del17p	Not reached	
Previously treated	34	0.13
Treatment-naive	Not reached	
Mutation, present	56	0.92
Mutation, absent	60	
Mutations/pt ≥ 2	33	0.12
Mutations/pt < 2	62	
Subsequent treatment	56	0.56
No subsequent treatment	Not reached	

- After a median follow-up of 17 months (range, 1-141 months), 29 (41%) died; median OS was 56 months (range, 1-141 months)

NOTCH1 and OS in CK pts (N=71)



- Genes significantly associated with shorter survival on UVA were *NOTCH1* (17 months vs 60 months, p=0.02) and mutated *TP53* (32 months vs 62 months, p=0.05).
- **On MVA, unmutated *IGHV* (HR 5.2, 95% CI 1.2-22.7; p=0.03) and mutated *NOTCH1* (HR 5.8, 95% CI 1.6-20.4; p=0.007) remained associated with shorter survival in this group of patients with CLL and CK.**
- At most recent follow-up, 4 (6%) patients developed biopsy-proven Richter syndrome (RS), one had mutated *NOTCH1*, none had mutated *TP53* prior to transformation.

Conclusions

- This is the first study specifically dissecting the genomic landscape of patients with CLL and CK.
- Further investigation of the role of mutated SETD2 and mutated NOTCH1 may improve the management and treatment of this very high risk population.

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