RESEARCH LETTER

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TO THE EDITOR:

# Characteristics and outcomes of patients with CLL and *CDKN2A/B* deletion by fluorescence in situ hybridization

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The *CDKN2A* and *CDKN2B* (*CDKN2A/B*) locus on 9p21, a tumor suppressor hub, is the second most common genetically inactivated region after *TP53* in cancer.[1] *CDKN2A/B* deletion has been described in a wide variety of malignancies, including B-cell malignancies; acute lymphocytic leukemia and diffuse large B-cell lymphoma.[1-6] In chronic lymphocytic leukemia (CLL), *CDKN2A/B* loss has been described in a small subset of patients, but its significance is not well understood. It has been reported as the most common acquired abnormality found in ~19% to 30% of samples by single nucleotide polymorphism microarray or next-generation sequencing (NGS) at the time of transformation of CLL to an aggressive B-cell lymphoma (Richter transformation [RT]).[7-12] Previously, loss of *CDKN2A/B* was thought to only occur at RT but was later reported in 13 patients with CLL with high-risk disease, defined by either *TP53* aberration or refractory to purine analogs.[7,9,10] Homozygous loss of *CDKN2A/B* has been described in 3 patients who acquired resistance to venetoclax.[13] Most frequently, *CDKN2A/B* loss co-occurs with *TP53* deletion, with the concurrent loss of both tumor suppressors being a potential pathway for RT.[10,14] Because of the negative clinical impact of RT and the rarity of this genetic abnormality, we examined a large cohort of patients with CLL using fluorescence in situ hybridization (FISH) for *CDKN2A/B* deletion to identify the frequency of occurrence, population and genetic characteristics, and outcomes.

After Institutional Review Board approval, a retrospective study with chart review was conducted to identify patients with 1 or more samples submitted for CLL FISH panel analysis over a 3.5-year period. FISH and conventional chromosome analysis were performed on cells stimulated with pokeweed mitogen, phorbol myristate acetate, and CpG oligonucleotides in either peripheral blood or bone marrow aspirate or biopsy. FISH was performed using probes, according to the manufacturer's recommendations (supplemental Table 1). Patients were screened for abnormal *CDKN2A/B* results at diagnosis or on subsequent testing at a later stage of the disease. Abnormal *CDKN2A/B* results included homozygous loss, heterozygous loss, loss of 1 copy with loss of the chromosome 9 centromere, and relative loss of *CDKN2A/B* in a polyploid background (ie, 2 signals of *CDKN2A/B* with 4 signals of centromere 9). Karyotype complexity was counted as previously described.[15,16] Immunoglobulin heavy-chain variable region (IGHV) mutational status was determined using polymerase chain reaction. For patients who started therapy with a Bruton tyrosine kinase (BTK) inhibitor, mutational testing was performed using NGS or digital droplet polymerase chain reaction. A 50-gene hematologic sequencing panel was performed via ion torrent sequencing and annotated using the GenomOncology platform (supplemental Methods). A Cox regression model was used to evaluate overall survival (OS) after *CDKN2A/B* deletion. The Fine and Gray model was used to examine the correlation of variables regarding transformation, treating death without transformation as a competing risk.

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Data are available on request from the corresponding author, Cecelia Miller (cecelia.miller@osumc.edu).

The full-text version of this article contains a data supplement.

We identified 636 patients with CLL FISH panel analysis, of whom 43 (6.8%) had *CDKN2A/B* deletion. The cohort with *CDKN2A/B* deletion consisted of 28 (65.1%) males and 15 (34.9%) females, with a median age at diagnosis of 54.9 years (range, 41.9-77.7 years). Detection of *CDKN2A/B* loss occurred at a median of 7.8 years from diagnosis (range, diagnosis to 26.1 years). Five patients (11.6%) had *CDKN2A/B* loss within 1 year of diagnosis before receiving any therapy. For those who had been previously treated at the time of *CDKN2A/B* loss detection, the median number of lines of treatment was 5 (range, 1-13). Thirty-two (74.4%) patients had received prior therapy with a BTK inhibitor. BTK C481 mutation testing was performed for 27 (84.4%) patients, with alterations detected in 11 (34.4%). Of the patients with a known IGHV mutational status (n = 33), 30 (90.9%) had unmutated status.

Regarding *CDKN2A/B* deletion, 33 (76.7%), 7 (16.3%), and 3 (7.0%) patients had heterozygous, homozygous, or subclonal populations of heterozygous as well as homozygous loss, respectively. Complex (3-4 abnormalities), highly complex (5-9 abnormalities), and ultra complex (>10 abnormalities)[15] karyotypes were found in 6 (14.0%), 13 (30.2%), and 20 (46.5%) patients, respectively (Figure 1). A chromosomal abnormality involving the 9p21 locus was visible by conventional karyotyping in 72.1% of patients (supplemental Table 2). Additional FISH testing showed that 27 patients (62.8%) had *TP53* deletion; 25 (58.1%) had deletion of 13q14; 16 (37.2%) had gain, rearrangement, or amplification of *MYC*; 10 (23.3%) had gain of *REL*; 7 (16.3%) had *BCL6* gain or rearrangement; 7 (16.3%) had *ATM* deletion; 6 (14.0%) had *SEC63* deletion; and 6 (14.0%) had trisomy 12. Sequencing data were available for 15 (34.9%) patients; of note, 10 (66.7%) had mutations in *TP53*, with 4 (26.7%) also having mutations in *SF3B1*. *BRAF* mutations occurred in 2 patients, and 1 patient had mutations in *NOTCH1* and *XPO1*.

In terms of outcomes, there were 30 deaths (69.8%) in the cohort. With a median follow-up period of 10.6 years among survivors, there was a median OS of 10.8 years from diagnosis (95% confidence interval [CI], 8.0-14.4). The median OS from the time of detection of *CDKN2A/B* deletion was 1.7 years (95% CI, 0.3-4.7), with a median follow-up period of 4.9 years. The treatments after *CDKN2A/B* deletion are provided in supplemental Table 3. Of the 43 patients in the cohort, 21 (49%) progressed to RT (n = 20) or prolymphocytic leukemia (n = 1). Two additional patients each underwent a bone marrow biopsy which noted concern for prolymphocytic leukemia transformation at the time of *CDKN2A/B* deletion; however, no follow-up data were available for confirmation. The median age at RT was 65.8 years (range, 56.0-83.9 years), and 1 patient was treatment naïve at the time of RT. The characteristics at the time of RT are shown in supplemental Figure 1. Of the 30 deaths, 17 occurred among those with RT and 13 among those without RT due to progressive disease (n = 8), infection (n = 2), complications of CLL therapy (n = 1), secondary cancer (n = 1), and respiratory failure (n = 1).

The time from *CDKN2A/B* deletion detection to RT ranged from 0 to 5.5 years, with all but 2 patients progressing to RT within 2 years. The cumulative incidence rates of RT from the initial CLL diagnosis were 4.7% (95% CI, 0.8-14.0) at 12 months and 9.3% (95% CI, 2.9-20.3) at 24 months. The cumulative incidence rates of RT after *CDKN2A/B* deletion were 23.5% (95% CI, 10.9-38.9) at 12 months and 29.4% (95% CI, 15.1-45.3) at 24 months. Although there were limited patients progressing to RT with the

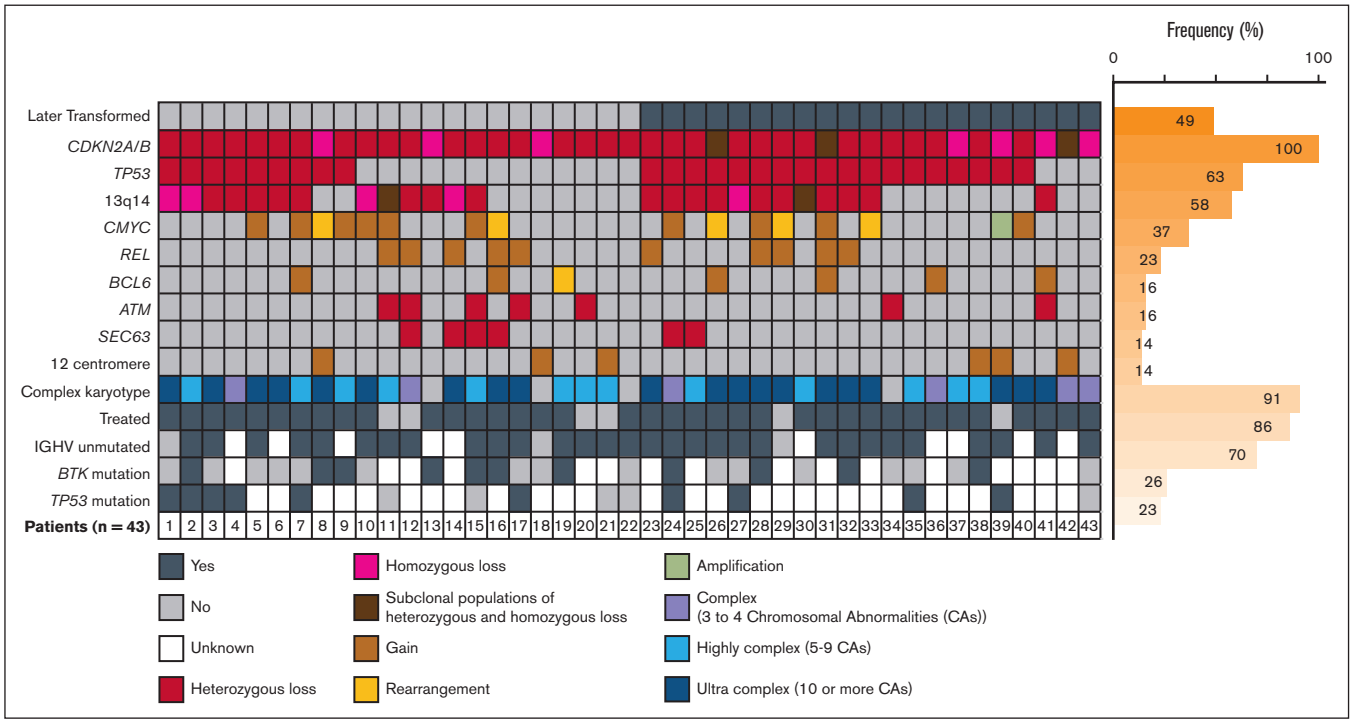


**Figure 1. Genetic profiles of patients with CLL with *CDKN2A/B* deletion.** Bone marrow or peripheral blood analysis at the time of the first *CDKN2A/B* deletion detection using FISH. Fine and Gray model for RT (n = 20) or prolymphocytic leukemia (n = 1). IGHV, immunoglobulin heavy-chain variable region.

**Table 1. Fine and Gray model for RT**

| | Univariable models | | Multivariable model | |
|---|---|---|---|---|
| **21 events** | **Hazard ratio (95% CI)** | ***P* value** | **Hazard ratio (95% CI)** | ***P* value** |
| Age at diagnosis, 1 y increase | 1.06 (1.02-1.11) | 0.0076 | – | – |
| Male vs female | 0.88 (0.37-2.07) | 0.77 | – | – |
| Rai stage, 1 unit increase | 0.70 (0.38-1.32) | 0.27 | – | – |
| IGHV unmutated vs mutated | 1.60 (0.14-18.08) | 0.70 | – | – |
| B2M at first visit, 1 unit increase | 0.97 (0.77-1.23) | 0.82 | – | – |
| BTK mutation vs no | 1.13 (0.37-3.50) | 0.83 | – | – |
| **Complexity vs 0** | | | – | – |
| 3-4 | 4.06 (0.35-46.71) | 0.26 | | |
| 5-9 | 1.48 (0.14-15.67) | 0.75 | | |
| 10-14 | 1.29 (0.12-14.54) | 0.84 | | |
| >15 | 6.92 (0.57-83.72) | 0.13 | | |
| *REL* gain vs no | 1.44 (0.53-3.97) | 0.48 | – | – |
| *BCL6* abnormality vs no | 1.49 (0.45-5.00) | 0.52 | – | – |
| *SEC63* deletion vs no | 0.81 (0.27-2.39) | 0.70 | – | – |
| *CMYC* abnormality vs no | 1.07 (0.46-2.51) | 0.88 | – | – |
| ***CDKN2A/B* vs het** | | | | |
| Hom | 1.55 (0.49-4.92) | 0.46 | 2.22 (0.65-7.61) | 0.20 |
| Both | 7.22 (3.03-17.24) | <.0001 | 6.08 (2.13-17.39) | 0.0008 |
| *ATM* deletion vs no | 0.75 (0.14-3.95) | 0.74 | – | – |
| *TP53* deletion vs no | 4.25 (1.22-14.86) | 0.02 | 4.53 (1.33-15.43) | 0.02 |
| Trisomy 12 vs no | 1.24 (0.32-4.82) | 0.76 | – | – |
| D13S319 deletion vs no | 0.95 (0.40-2.23) | 0.90 | – | – |
| Lines of treatment, 1 increase | 0.92 (0.80-1.06) | 0.26 | – | – |

Fine and Gray model for RT (n = 20) and prolymphocytic leukemia (n = 1).
B2M, beta-2-microglobulin; both, heterozygous and homozygous populations; het, heterozygous; hom, homozygous; IGHV, immunoglobulin heavy-chain variable region.

detection of *CDKN2A/B* deletion, the multivariable model found that loss of *TP53* ($P = .02$; hazard ratio, 4.53 [95% CI, 1.33-15.43]) and homozygous and heterozygous loss of *CDKN2A/B* ($P = .0008$; hazard ratio, 6.08 [95% CI, 2.13-17.39]) were independent significant variables associated with RT (Table 1). The frequencies of *MYC*, *REL*, and *BCL6* abnormalities and karyotype complexity were similar between patients with and without RT.

To our knowledge, this study examined the largest cohort of patients with CLL and *CDKN2A/B* deletion and demonstrated poor outcomes after detection. This retrospective study has various limitations. Serial samples were unavailable for many patients (supplemental Table 4); thus, the timing of acquisition of deletion remains unclear. We were unable to assess whether CLL and subsequent RT were clonally related, and NGS data were available for a limited subset of patients with *CDKN2A/B* not part of the panel. Loss of *CDKN2A/B* was a rare event in patients with CLL. Among those with the abnormality, 18.6% died due to progressive CLL and 48.8% progressed with RT. Here, we show that FISH analysis is a clinically practical option that can be incorporated into routine FISH testing for CLL, particularly for high-risk patients with *TP53* abnormalities. Our findings indicate that future prospective analysis of *CDKN2A/B* deletion as a prognostic variable is warranted.

**Contribution:** S.M.T. compiled data from chart review; S.M.T. and C.R.M. wrote the manuscript; Y.H. conducted the statistical analysis; S.M.T., Y.H., A.S.K., S.A.B., M.G., K.A.R., W.Z., D.J., J.C.B., M.R.A., N.A.H., J.A.W., and C.R.M. provided clinical data, reviewed the manuscript, and approved the final version; and J.A.W. and C.R.M. supervised the study.

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