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# Molecular Profiling and Analysis of Genetic Aberrations Aimed at Identifying Potential Therapeutic Targets in Fibrolamellar Carcinoma of the Liver

**Imane El Dika, MD**[1,2], **Anita S. Bowman, MS**[1], **Michael F. Berger, MD**[1,2], **Marinela Capanu, PhD**[1], **Joanne F. Chou, MPH**[1], **Ryma Benayed, PhD**[1], **Ahmet Zehir, PhD**[1], **Jinru Shia, MD**[1,2], **Eileen M. O'Reilly, MD**[1,2], **David S. Klimstra, MD**[1,2], **David B. Solit, MD**[1,2], **Ghassan K. Abou-Alfa, MD, MBA**[1,2]

[1]Memorial Sloan Kettering Cancer Center, New York, New York

[2]Weill Cornell College of Medicine, New York, New York

## Abstract

**BACKGROUND:** Fibrolamellar carcinoma (FLC) is a rare primary liver cancer of young adults. A functional chimeric transcript resulting from the in-frame fusion of the DNAJ homolog, subfamily B, member 1 (*DNAJB1*), and the catalytic subunit of protein kinase A (*PRKACA*) genes on chromosome 19 is believed to be unique in FLC, with a possible role in pathogenesis, yet with no established therapeutic value. The objective of the current study was to understand the molecular landscape of FLC and to identify potential novel therapeutic targets.

**METHODS:** Archival fresh, formalin-fixed, paraffin-embedded samples from patients with FLC who prospectively consented to an institutional review board-approved protocol were analyzed using Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT), a next-generation sequencing assay encompassing up to 468 key cancer genes. Custom targeted RNA-Seq was performed in selected patients. Demographics, treatment, and outcome data were collected prospectively. Survival outcomes were estimated and correlated with mutation and/or copy number alterations.

**Corresponding Author**: Ghassan K. Abou-Alfa, MD, Memorial Sloan Kettering Cancer Center, 300 East 66th St, New York, NY 10065 (abou-alg@mskcc.org).

AUTHOR CONTRIBUTIONS

Conceptualization: **Michael F. Berger, Ryma Benayed, Ahmet Zehir, Jinru Shia, Eileen M. O'Reilly, David S. Klimstra, David B. Solit,** and **Ghassan K. Abou-Alfa.** Data curation: **Imane El Dika, Anita S. Bowman, Michael F. Berger, Marinela Capanu, Joanne F. Chou, Ryma Benayed, Ahmet Zehir, David B. Solit,** and **Ghassan K. Abou-Alfa.** Formal analysis: **Imane El Dika, Anita S. Bowman, Michael F. Berger, Marinela Capanu, Joanne F. Chou, Ryma Benayed, Ahmet Zehir, Jinru Shia, Eileen M. O'Reilly, David S. Klimstra, David B. Solit,** and **Ghassan K. Abou-Alfa.** Funding acquisition: **David B. Solit** and **Ghassan K. Abou-Alfa.** Investigation: **Imane El Dika, Anita S. Bowman, Michael F. Berger, Ryma Benayed, Ahmet Zehir, Jinru Shia, David B. Solit,** and **Ghassan K. Abou-Alfa.** Methodology: **Anita S. Bowman, Michael F. Berger, Ryma Benayed, Ahmet Zehir, David S. Klimstra, David B. Solit,** and **Ghassan K. Abou-Alfa.** Project administrator: **Anita S. Bowman, Michael F. Berger, Ahmet Zehir,** and **David B. Solit.** Supervision: **Michael F. Berger, Marinela Capanu, Ahmet Zehir, Jinru Shia, Eileen M. O'Reilly, David S. Klimstra, David B. Solit,** and **Ghassan K. Abou-Alfa.** Validation: **Michael F. Berger, Marinela Capanu, Joanne F. Chou, Ryma Benayed, Ahmet Zehir, Jinru Shia,** and **David B. Solit.** Visualization: **Imane El Dika, Anita S. Bowman, Michael F. Berger, Marinela Capanu, Joanne F. Chou, Ryma Benayed, Ahmet Zehir, Jinru Shia, Eileen M. O'Reilly, David S. Klimstra, David B. Solit,** and **Ghassan K. Abou-Alfa.** Writing–original draft: **Imane El Dika** and **Ghassan K. Abou-Alfa.** Writing–review and editing: **Imane El Dika, Anita S. Bowman, Michael F. Berger, Marinela Capanu, Joanne F. Chou, Ryma Benayed, Ahmet Zehir, Jinru Shia, Eileen M. O'Reilly, David S. Klimstra, David B. Solit,** and **Ghassan K. Abou-Alfa.**

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**RESULTS:** A total of 33 tumor samples from 31 patients with FLC were analyzed. The median age of the patients at the time of diagnosis was 18 years and approximately 53% were women. The *DNAJB1-PRKACA* fusion transcript was detected in 100% of patients. In 10 of 31 patients in which MSK-IMPACT did not detect the fusion, its presence was confirmed by targeted RNA-Seq. *TERT* promoter mutation was the second most common, and was detected in 7 patients. The median follow up was 30 months (range, 6-153 months). The 3-year overall survival rate was 84% (95% CI, 61%-93%).

**CONCLUSIONS:** The *DNAJB1-PRKACA* fusion transcript is nonspecific and nonsensitive to FLC. Its potential therapeutic value currently is under evaluation. Opportunities currently are under development for therapy that may be driven or related to the *DNAJB1-PRKACA* fusion transcript or any therapeutic target identified from next-generation sequencing in patients with FLC.

### Keywords

catalytic subunit of protein kinase A (*PRKACA*); DNAJ homolog; subfamily B; member 1 (DNAJB1); fibrolamellar carcinoma (FLC); Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT); TERT

## INTRODUCTION

Fibrolamellar carcinoma (FLC) is an extremely rare form of liver cancer that is estimated to comprise <1% of all primary liver cancers and is distinct from conventional hepatocellular carcinoma.[1,2] FLC comprises up to 13.4% of all liver cancer patients occurring in patients aged <40 years.[1] Although FLC cells histologically have been characterized by well-differentiated neoplastic hepatocytes and thick fibrous bands in a noncirrhotic background,[3] to our knowledge the cell of origin in FLC has yet to be demonstrated. FLC occurs particularly in adolescents and young adults without evidence of underlying liver disease.[4–6] Survival is impacted mainly by lymph node and distant organ metastases.[7] FLC has a poor outcome due to its resistance to chemotherapy and limited therapeutic options. Although to our knowledge surgical resection remains the only curative approach, it also is performed in patients with recurrent and/or metastatic disease.[8–10] This is especially pertinent in the absence of alternative therapeutic approaches. FLC recently has gained more attention based on the discovery of an approximately 400-kilobase deletion in chromosome 19, which resulted in a fusion of the heat shock protein genes DNAJ homolog, subfamily B, member 1 (*DNAJB1*) and the catalytic subunit of protein kinase A (*PRKACA*).[11,12] It is interesting to note that the *DNAJB1-PRKACA* fusion transcript is not detected in adjacent normal tissue. Analysis of the RNA sequencing data from The Cancer Genome Atlas for >9100 tumors across approximately 30 cancer types suggested the *DNAJB1-PRKACA* fusion transcript to be specific to FLC.[13] However, recent data have demonstrated that the same fusion can occur in intraductal neoplasms of the pancreas and bile ducts,[14] and also is not present in all patients of FLC. Biallelic protein kinase CAMP-dependent type I regulatory subunit alpha (*PRKAR1A*) mutations with loss of *PRKAR1A* expression have been reported in FLC without *PRKACA* gene rearrangement[15] and another case of FLC characterized by *PRKACA* amplification without *PRKACA* rearrangement.[16] To further understand FLC and identifying therapeutic targets, recent efforts at Memorial Sloan

Kettering Cancer Center (MSKCC) using clustered regularly interspaced short palindromic repeats (CRISPR)–mediated mouse models demonstrated that the *DNAJB1-PRKACA* gene fusion actually drives tumorigenesis in mice, and that fusion to *DNAJB1* leads to the initiation of FLC more effectively than wild-type *PRKACA* overexpression.[15] The fact that *PRKACA* kinase domain appears to be conditional in tumor initiation suggests the potential value of developing kinase inhibitors of the fusion. Similarly, other investigators delivered CRISPR/Cas9 vectors designed to juxtapose exon 1 of *DNAJB1* with exon 2 of *PRKACA* to create the *DNAJB1-PRKACA* fusion, or control Cas9 vector, to livers of 8-week-old female mice. Twelve of 15 mice who were given the CRISPR/Cas9 vectors and none of the 11 control mice developed neoplasms in the liver parenchyma. The oncogenic lesions exhibited cytologic and histologic features of human FLC.[15] It is interesting to note that the generated mice tumors contained the *DNAJB1-PRKACA* gene fusion. Despite that, to our knowledge the predictive and targetable value of this fusion transcript has yet to be identified. The objective of the current study was to understand the molecular landscape of FLC past the *DNAJB1-PRKACA* fusion and to help to identify potential novel therapeutic targets.

## MATERIALS AND METHODS

Over a 3-year period between July 2014 and June 2017, patients with a confirmed histologic diagnosis of FLC were identified and included. Informed consent was obtained from all patients under ClinicalTrials.gov identifier NCT01775072 for tumor genomic profiling in patients evaluated for targeted cancer therapy. The study protocol was reviewed by the institutional review board at MSKCC. The current study was conducted in accordance with the principles of the Declaration of Helsinki.

Results from 31 patients with FLC were available at the time of analysis. Clinical data were collected including demographics (age, sex, and race), family and personal history of malignancy, treatment details, and survival outcomes including progression-free survival (PFS) and 3-year overall survival (OS).

### Sample Preparation

Histopathological review and microdissection of tissue from formalin-fixed, paraffin-embedded tumor samples were performed as needed to ensure adequate cellularity. Archival tissue (tissue obtained from prior surgical resection or biopsy) was used. Matched normal blood DNA from prospectively collected blood samples was analyzed in all patients to verify the somatic nature of the alterations detected. Germline mutation analysis also was performed if patients provided consent.

### Genetic Analysis

Tumors were profiled for genomic alterations using Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT), a US Food and Drug Administration–approved hybridization capture-based clinical sequencing assay.[16,17] Custom DNA probes were designed to capture all exons and selected introns (depending on the version of the test in use at the time the individual case was sequenced) of 341 (used for 8 patients), 410 (used for 12 patients), or 468 (used for 11 patients) oncogenes, tumor

suppressor genes, and members of pathways deemed potentially actionable by targeted therapies (see Supporting Tables 1–3).

Genomic DNA from tumor and patient-matched normal samples was analyzed as previously described.[16,18] Somatic copy number alterations were identified by comparing sequence coverage of targeted regions in the tumor sample relative to standard diploid normal. The resulting high-confidence single-nucleotide variant, indels, somatic copy number alterations, and structural variants as detected by MSK-IMPACT were used to produce a binary alteration matrix across all altered genes and samples. Genetic alterations were classified as actionable using a scale of 1 to 4, with level 1 to 2A alterations indicating standard therapeutic interventions and levels 2B to 4 suggesting investigational therapeutic alterations.[19] Classification was performed using the OncoKB[19] database, which integrates biologic, clinical, and therapeutic information curated from multiple resources, including recommendations derived from US Food and Drug Administration labeling, National Comprehensive Cancer Network guidelines, and the medical literature. The tumor mutational burden per sample was calculated as the total number of nonsynonymous mutations divided by the actual number of bases analyzed.[16]

## Targeted RNA Sequencing

Targeted RNA sequencing using Archer DX platform[18] was performed in patients that had undergone analysis using the previous MSK-IMPACT (version v3; 341 genes), which did not include the genes *DNAJB1* or *PRKACA*. Archer targeted RNA sequencing also was performed in patients in which the fusion was not detected through version 5 of MSK-IMPACT (468 genes) due to low tumor content. cDNA libraries were created using the Archer FusionPlex standard protocol (Illimunia Inc, San Diego, California), Archer MBC adapters (Illimunia Inc; catalog no. #SA0040-45), and our custom-designed gene-specific primer (GSP) pool kit. Fusion unidirectional GSPs were designed to target specific exons in 62 genes known to be involved in chromosomal rearrangements based on the current literature. GSPs, in combination with adapter-specific primers, enriched for known and novel fusion transcripts, were sequenced using an Illumina MiSeq sequencer (Illumina Inc). At the end of MiSeq sequencing, FASTQ files were generated automatically using the MiSeq reporter software (version 2.6.2.3; Illumina Inc) and analyzed using the Archer analysis software (version 5.0.4; Illumina Inc).[18]

## Statistical Analysis

PFS was calculated from the date of diagnosis of FLC to the time of first disease progression or death, whichever occurred first. OS was calculated from the date of diagnosis to the date of death or last follow-up. OS and PFS were estimated using Kaplan-Meier methods[20]. For genes with a frequency of mutation of at least 5%, an association between patients with and without mutations and/or copy number alterations and survival outcomes was computed using the permuted log-rank test.[21] Among the subset of patients who underwent surgery (28 patients), PFS then was calculated from the date of surgery until the time of first disease progression or death and the log-rank test was used to compare PFS between patients who underwent an R0 versus R1 surgical resection. Patients who did not undergo surgery (3 patients) were excluded from this subgroup analysis. All statistical analyses were performed

using R statistical software (version 3.3.2; R Foundation for Statistical Computing, Vienna, Austria).[22] All *P* values were 2-sided and $P < .05$ was considered to indicate statistical significance.

## RESULTS

### Demographics

A total of 33 samples from 31 individual patients with histologically confirmed FLC were analyzed. Clinical characteristics are summarized in Table 1. All patients were white, with a slight female predominance (52%) noted. The majority of patients underwent upfront surgical resection (28 patients; 90%), 14 of whom underwent R0 resections.

### Landscape of Genomic Alterations in FLC

Genomic alterations were observed in all the examined samples. The *DNAJB1-PRKACA* fusion transcript was identified in all 31 patients (100%). In 12 patients, the *DNAJB1-PRKACA* fusion was not captured through MSK-IMPACT but rather through Archer targeted RNA sequencing: 8 samples tested negative when testing was performed using MSK-IMPACT version 3 (341 genes), which did not include the genes *DNAJB1* or *PRKACA,* and in 4 samples the fusion was not detected by MSK-IMPACT version 5 (468 genes) due to low tumor content. Genetic sequencing of brain metastasis samples from 3 patients demonstrated the *DNAJB-PRKACA* fusion transcript in all metastatic samples.

In 6 tumor samples, there was the suggestion of a different splice variant. In addition to the typical fusion that reads from the end of exon 1 of *DNAJB1* to the start of exon 2 of *PRKACA,* there were reads that started in the middle of exon 2 of *DNAJB1.* These findings were reported previously and suggested the presence of a predominant chimera and a second, minority chimera, incorporating the first exon and a part of the second exon of *DNAJB1.*[12] Samples with the minority chimera, as identified by MSK-IMPACT, all had a breakpoint within exon 2 of *DNAJB1.* We were unable to predict whether this was in-frame or not. Of these samples, only 2 had Archer targeted RNA sequencing results and 1 sample demonstrated evidence of the “minority chimera.” That single sample suggested retention of intron 2 following exon 2. Both chimeras continued with exons 2 to 10 of *PRKACA* (Fig. 1).

The second most frequently observed alterations were mutations in the *TERT* promoter and *TERT* amplifications (7 patients; 23%): 4 were promoter mutations, 2 were amplifications, and 1 was fusion. This was followed by mutations in *SF3B1, ZFHX3,* and *CTNNB1* (2 patients each), as illustrated in the oncoprint shown in Figure 2 and Table 2. Both patients harboring *CTNNB1* mutations were examined using immunohistochemistry, and neither was found to have abnormal nuclear β-catenin staining. Forty-three additional gene alterations were detected in 1 sample each (Table 2). No *mTOR* genetic alterations were identified. The tumor mutational burden score ranged from 0.9 to 5.3. Because to our knowledge there is no standard-of-care targeted therapy for patients with FLC, we looked for genetic alterations for which potentially active drugs were under development or drugs that have been used for other cancer types. To our knowledge, there currently is no specific agent to therapeutically target the *DNAJB1-PRKACA* fusion, and it remains unclear whether the *DNAJB1-*

*PRKACA* chimera harbors a potential target. The oncogenic and likely oncogenic mutations per OncoKB were *KRAS p.D119G, TERT* promoter mutations, *CTNNB1 p.K335I,* the truncating mutations *ARID1A p.Y1734*,* and *NCOR p.S1519* (Fig. 2) (Table 2). Several telomere-targeting strategies currently are in various stages of development.[23]

## Germline Mutations

Fourteen patients provided consent for germline sequencing. Of these, 3 patients (21%) had pathogenic germline mutations detected: MUTYH: c.1187G>A in 2 patients and NBN c.657_661delACAAA in one patient. In one of the patients with a *MUTYH* germline mutation, histology demonstrated characteristic foci of FLC features with certain regions in which such features are less well developed. Reportedly, the tumor cells were positive for CK7 in a diffuse pattern, and positive for HepPar-1. The patient with *NBN* germline mutation histology was found to be positive for HepPar-1, CK7, and CK8/18. Family history was negative for both patients with *MUTYH* germline mutations. The father of the patient with the *NBN* germline mutation had prostate cancer. Based on the transition and/or transversion rate ratio using the Single Nucleotide Polymorphism database (dbSNP), no increased transversions or double-strand breaks were noted.

## Clinical Outcomes and Genetic Correlation

The median follow-up among survivors was 30 months (range, 6-153 months). The most common sites of distant metastasis included the lungs (14 patients) and peritoneal cavity (10 patients). Three patients had bone metastases, 3 patients developed brain metastases, 1 patient had a urinary bladder lesion, and 1 patient had an intragastric metastasis.

Fifteen patients received cytotoxic therapy during their clinical course (5-fluorouracil, oxaliplatin, cisplatin, doxorubicin, temozolomide, and gemcitabine); the median number of therapies was 7, including local and systemic therapies. The 3-year OS rate was 83.7% (95% CI, 61%-93%) in the overall group (Fig. 3). The median PFS for the study population was 12 months (95% CI, 5-14 months) (Fig. 4). Four patients remained free of disease recurrence at the time of last follow-up. One may speculate a borderline association between PFS and surgical resection margin status (R0 vs R1). PFS from the time of surgery was 14 months (95% CI, 6-31 months) for patients who achieved an R0 surgical resection versus 5 months (95% CI, 0.98-12 months) for patients who underwent R1 surgical resections ($P = .10$) (Fig. 5). The median OS for the patients with R0 surgical resections was not reached, whereas the median OS for the R1 surgical resection group was 112 months ($P = .093$).

Sixteen patients underwent subsequent surgery. Among this group, the median time from first treatment until subsequent surgery was approximately 8.98 months (range, 2.03-117.138 months). To examine the association between subsequent surgery and OS, subsequent surgery was treated as time-dependent in the Cox proportional hazards model. No significant association between subsequent surgery and OS was noted in this population (hazard ratio, 1.28; 95% CI, 0.29-5.52 [$P = .73$]).

No other clinical features were found to be associated with outcome. No association between OS and PFS and recurrently detected genetic alterations was noted (Table 3).

## DISCUSSION

Similar to previous efforts, the results of the current study helped to establish the presence of the chimeric *DNAJB1-PRKACA* transcript across examined FLC samples despite it not being universal and neither specific[14] nor sensitive.[15,16] The data from the current study are consistent with a previous report of transcriptome and whole-genome sequencing.[12]

In another report, whole-genome sequencing was performed on 10 paired tumor and adjacent normal liver tissues from an FLC cohort to identify recurrent oncogenic mutations[24]. There were relatively few somatic mutations, thereby placing FLC on the low end of the mutational spectrum. There were a few recurrent mutations, with the most frequently mutated gene being *MUC4* in 4 patients. Aside from the heterozygous deletion on chromosome 19 that encodes for the functional chimeric protein, there were no other recurrent structural variations that contributed to the tumor genotype. In the current study, we observed a different splicing pattern with breakpoints outside of the canonical region in 6 patients (19%), 2 of which were run on Archer RNA sequencing, which supported the presence of a minority chimera in 1 sample (Fig. 1). However, the small number of samples limited our ability to make conclusions.

In another report, a similar observation was made based on DNA sequencing. This finding then was validated at the RNA level using Sanger sequencing and at the protein level by Western blot analysis, with sodium dodecyl sulfate–polyacrylamide gel electrophoresis demonstrating the chimeric protein.[12]

The *DNAJB1-PRKACA* fusion previously was reported in a study that examined 26 samples of FLC. All neoplastic cells harbored the gene fusion, as demonstrated by fluorescence in situ hybridization analysis. The fusion was not detected in the background liver, suggesting that it is a rather early step in FLC carcinogenesis[25].

To our knowledge, the cell of origin in FLC is an area of controversy[26]; previous studies suggested a neuroendocrine signature. In the current study, we did not detect genes that are considered hallmarks of neuroendocrine differentiation. In one study evaluating genetic pathways in FLC tumors, among the genes that were found to be the most significantly overexpressed were prohormone convertase 1 (*PCSK1*), neurotensin, delta/notch-like *EGF,* and calcitonin, all of which were considered neuroendocrine genes[27]. Genetic studies have indicated that patients with FLC do not have genetic lesions that are common to other forms of liver cancer, such as loss-of-function mutations in *TP53* or *PTEN*[28]. Of the alterations observed in FLC, the most frequent are gains in 1q and 8q (which also frequently are noted in other hepatic tumors) and loss of 18q. In the MSKCC cohort in the current study, the second most common genetic alteration was *TERT* promoter mutations, amplifications, and fusions, which were detected in 22% of patients.

The chimeric transcript is predicted to code for a protein that includes the catalytic domain of protein kinase A. Upregulation of aurora kinase A (*AURKA*) may be responsible for subsequent tumor formation in FLC.[29] Targeting *AURKA* currently is underway in a recent study evaluating ENMD-2076 with selective activities against Aurora kinases[30] that recently was reported, disappointingly, as negative.[31]

Riehle et al examined mTOR pathway activation in 23 samples of FLC. Phosphorylated S6 ribosomal protein (*P-S6*), a downstream target of *mTORC1,* and fibroblast growth factor receptor 1 (*FGFR1*) demonstrated strong staining activity on the tissue microarray.[32] The results of the current study did not demonstrate expression of *mTOR* or *FGFR* pathway genes. Immunohistochemical staining for *P-S6* and *FGFR1* was not conducted. The *mTOR* inhibitor everolimus was evaluated alone and in combination with the aromatase inhibitor letrozole in 28 patients with FLC. Stable disease was reported in 6 patients (21%). The study was halted due to the low probability of extending PFS and the lack of efficacy.[33]

The *FGFR* pathway also was explored in another effort to understand the downstream targets of protein kinase A. Nineteen patients were evaluated: immunohistochemistry for *FGFR1* was negative in all examined patients.[34] There was modest *FGFR1* messenger RNA expression and weak or absent protein expression noted. Furthermore, the *FGFR2* rearrangement was not detected. To the best of our knowledge, this controversial and limited understanding of any potential role of the *FGFR* pathway in FLC has yet to be analyzed further, and would not exclude the exploration of *FGFR* inhibitors for the treatment of patients with FLC.

Whole-genome and RNA sequencing[11] revealed an FLC subset associated with the *EGF/ErbB2* gene signaling pathway, suggesting a potential therapeutic target. Neratinib, an irreversible *pan-HER* tyrosine kinase inhibitor, was evaluated as a single agent in patients with FLC in an open-label, multinational, phase 2 study (ClinicalTrials.gov identifier NCT01953926) that recently was discontinued because of a lack of efficacy.

This disappointing experience may suggest that future efforts should be directed toward targeting multiple pathways in FLC instead of using a single-target approach. Using immunohistochemistry to look for immune checkpoint targetable pathways in 32 tumor samples of FLC, *PD-L1* was found to be expressed in tumor cells in approximately 63% of patients, and significant expression of *B7-H3, PD-1,* and *IDO* was noted both in tumor cells and in the tumor microenvironment.[35] Checkpoint inhibitors have been used anecdotally with no significant results to our knowledge. Potential combination therapy as used for patients with hepatocellular carcinoma would be worth exploring.

The limitations of the current study included the small number of patients; nonetheless, its results have established the feasibility and potential clinical value for next-generation sequencing in patients with this rare disease. The use of multiple next-generation sequencing platforms (older versions of MSK-IMPACT) was a possible constraint to the study because some alterations may have been missed; however, the *DNAJB1-PRKACA* fusion was confirmed using Archer targeted RNA sequencing. Last, to the best of our knowledge, the etiology of FLC remains largely unknown, and the association between risk factors and a genetic signature could not be made; large-scale epidemiological studies are needed for this purpose.

In the current study, we were unable to identify mutations with known clinical actionability or an association between clinical outcome and genetic signature. Beyond the chimera, the *TERT* pathway alternations appeared frequently in the current study cohort, therefore

justifying future therapeutic exploration. The results of the current study indicated *DNAJB1-PRKACA* as an ubiquitous fusion that can be detected by both targeted DNA and RNA sequencing in a clinical setting, despite its nonsecificity[14] and nonsensitivity.[15,16] Beyond the presence of *DNAJB1-PRKACA*, FLC tumorigenesis remains poorly understood, and ongoing efforts currently are underway to clarify oncogenesis and identify novel therapeutic targets.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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### CONFLICT OF INTEREST DISCLOSURES

Michael F. Berger has received personal fees from Roche and grants from Grail for work performed outside of the current study. Ahmet Zehir has received speaking honoraria from Illumina for work performed outside of the current study. Eileen M. O'Reilly has received grants from ActaBiologica, Agios, Astra Zeneca, Bayer, Beigene, Berry Genomics, BMS, Casi, Celgene, Exelixis, Genentech, Halozyme, Incyte, Mabvax, Puma, QED, Roche, Sillajen, Yiviva and has acted as a paid consultant for Agios, Astra Zeneca, Autem, Bayer, Beigene, Berry Genomics, Celgene, CytomX, Debio, Eisai, Eli Lilly, Flatiron, Genentech, Gilead, Incyte, Ipsen, LAM, Loxo, Merck, MINA, Polaris, QED, Redhill, Roche, Silenseed, Sillajen, Sobi, Therabionics, TWoxar, Vector, Yiviva. David B. Solit has acted as a paid member of the Scientific Advisory Boards for Pfizer, Loxo Oncology, Lilly Oncology, Illumina, and QED Therapeutics for work performed outside of the current study. Ghassan K. Abou-Alfa has received grants from ActaBiologica, Agios, Astra Zeneca, Bayer, Beigene, Berry Genomics, BMS, Casi, Celgene, Exelixis, Genentech, Halozyme, Incyte, Mabvax, Puma, QED, Roche, Sillajen, Yiviva and has acted as a paid consultant for Agios, Astra Zeneca, Autem, Bayer, Beigene, Berry Genomics, Celgene, CytomX, Debio, Eisai, Eli Lilly, Flatiron, Genentech, Gilead, Incyte, Ipsen, LAM, Loxo, Merck, MINA, Polaris, QED, Redhill, Roche, Silenseed, Sillajen, Sobi, Therabionics, Twoxar, Vector, Yiviva. In addition, Dr. Abou- Alfa has a patent "Articles and Methods for Preventing and Treating Dermatologic Adverse Events" identified by International Patent Application No. PCT/US2014/031545 filed on March 24, 2014 and priority application Serial No. 61/804,907 filed on March 25, 2013 issued. The other authors made no disclosures.

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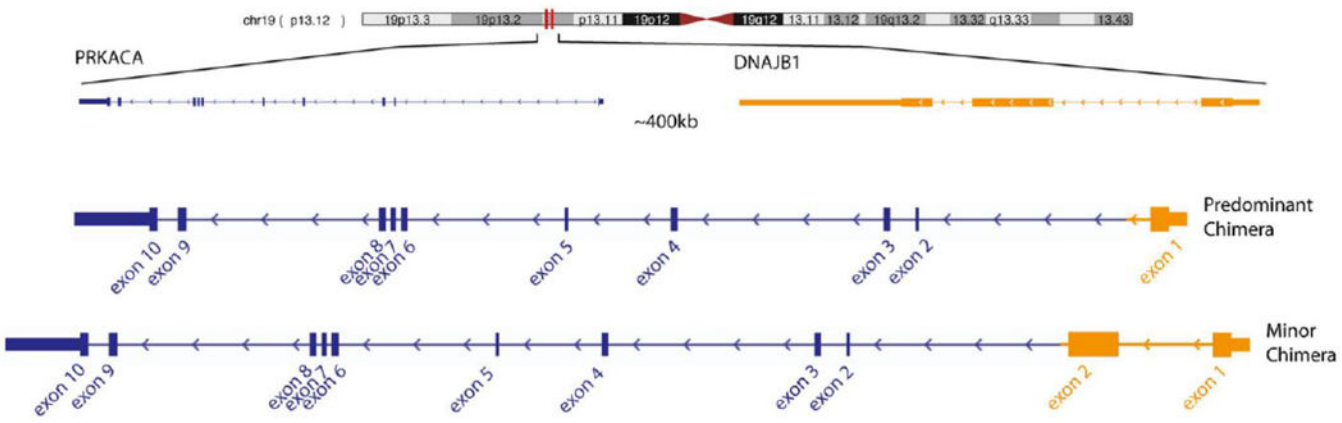


**FIGURE 1.**

Predominant chimera incorporating only the first exon of DNAJ homolog, subfamily B, member 1 (*DNAJB1*) and a second, minority chimera, incorporating both the first and a part of the second exon of *DNAJB1.* Both chimeras continue with exons 2 to 10 of catalytic subunit of protein kinase A (*PRKACA*).

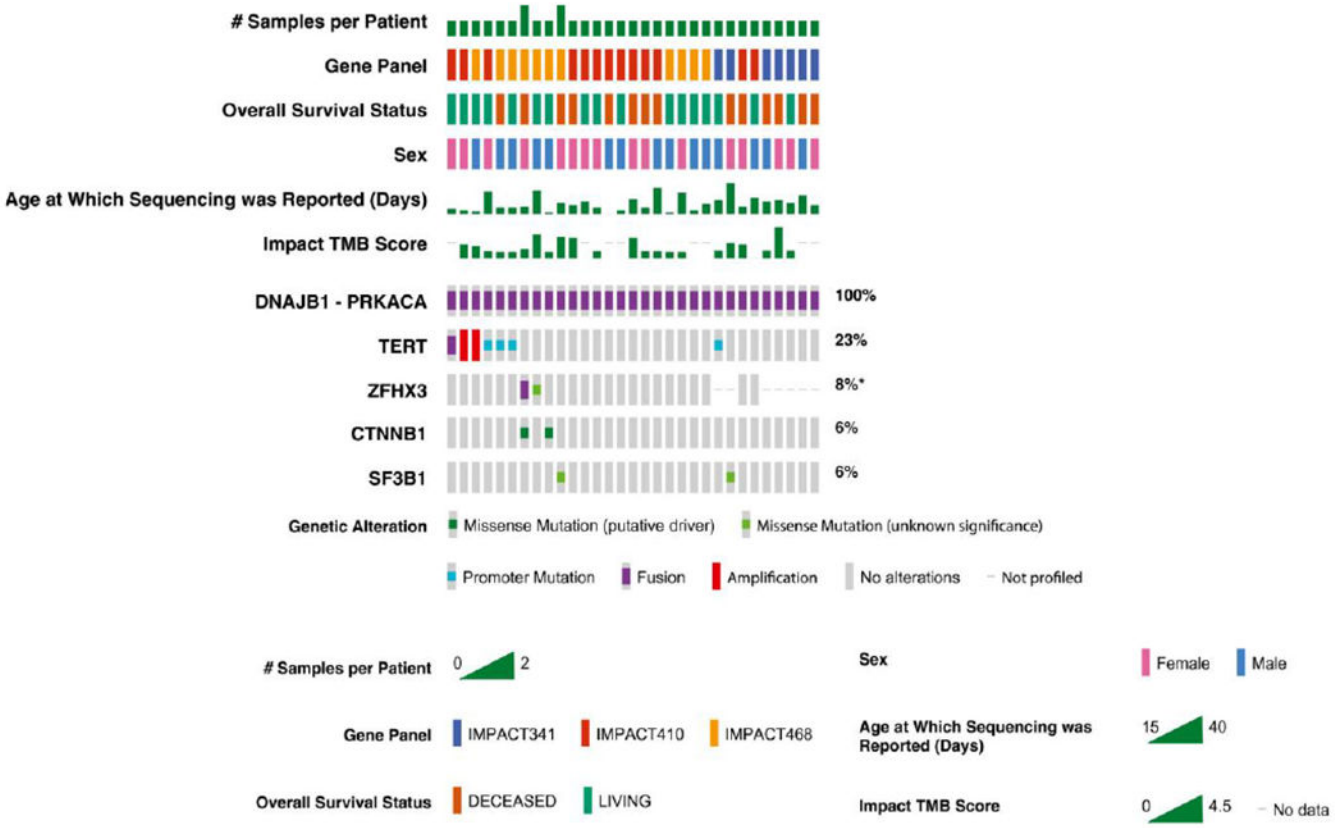


**FIGURE 2.**

Oncoprint per sample evaluated. *DNAJB1* indicates DNAJ homolog, subfamily B, member 1; IMPACT, Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets; *PRKACA*, catalytic subunit of protein kinase A; TMB, tumor mutational burden.

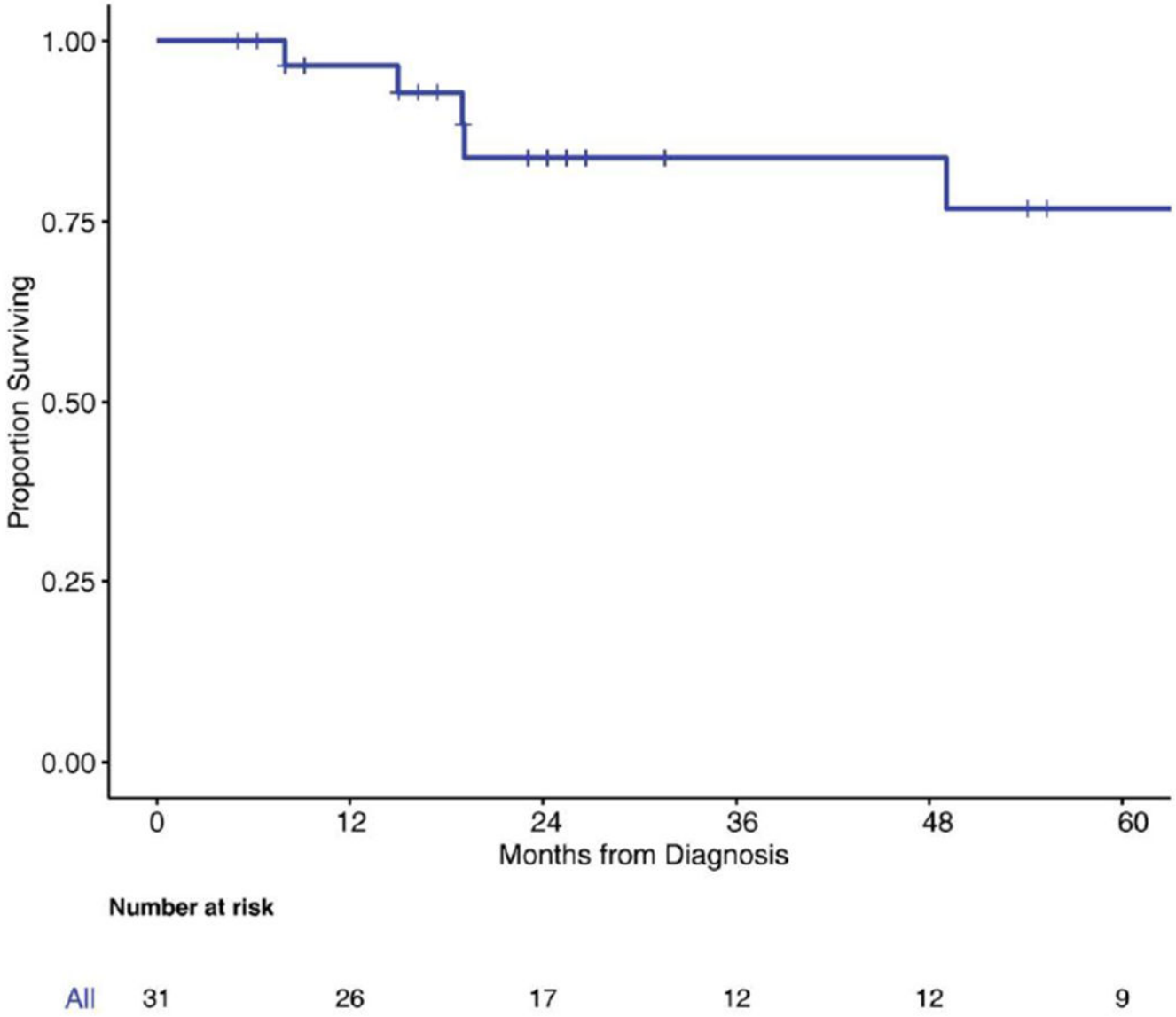


**FIGURE 3.**
Kaplan-Meier curve for overall survival.

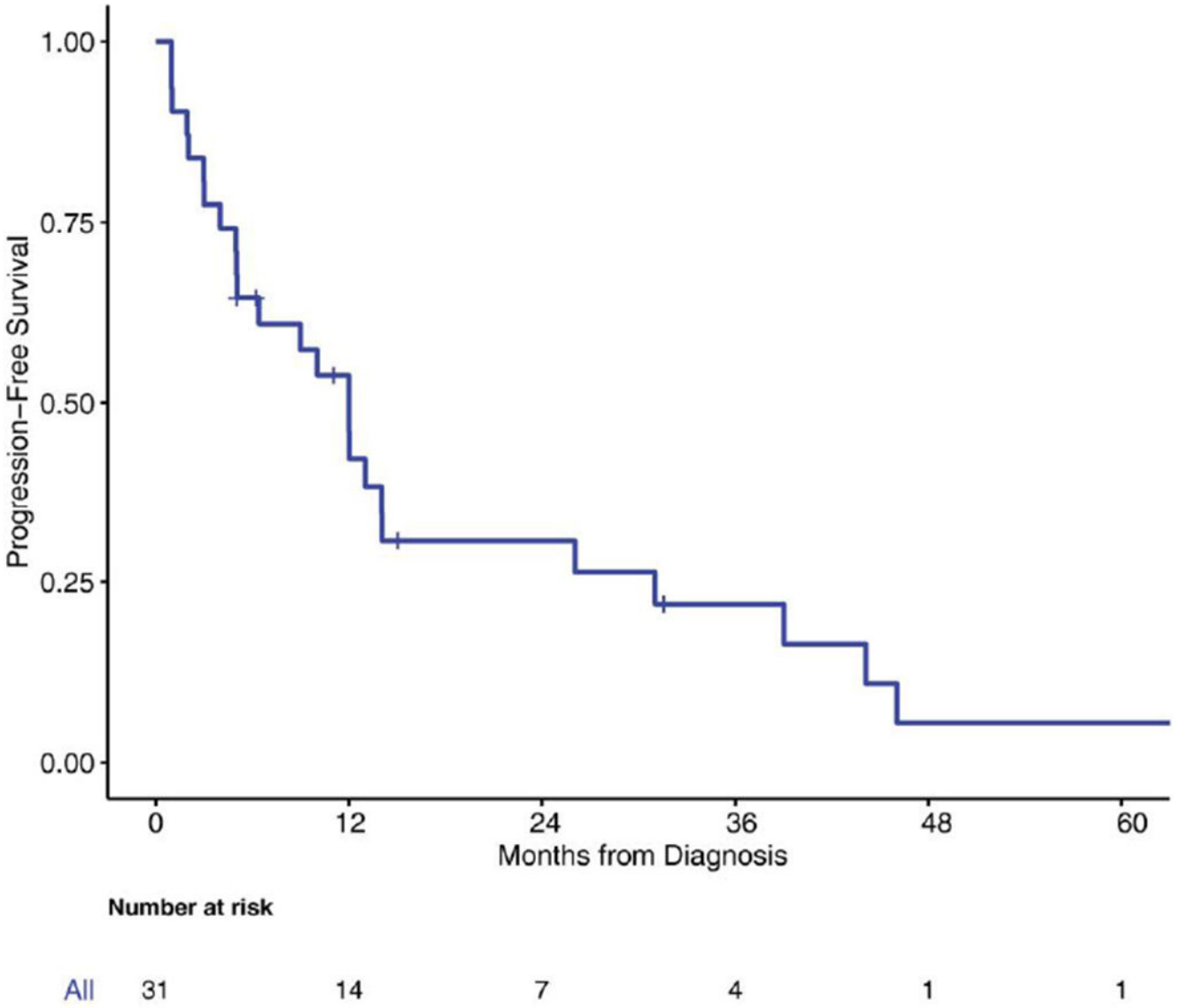


**FIGURE 4.**
Kaplan-Meier curve for progression-free survival.

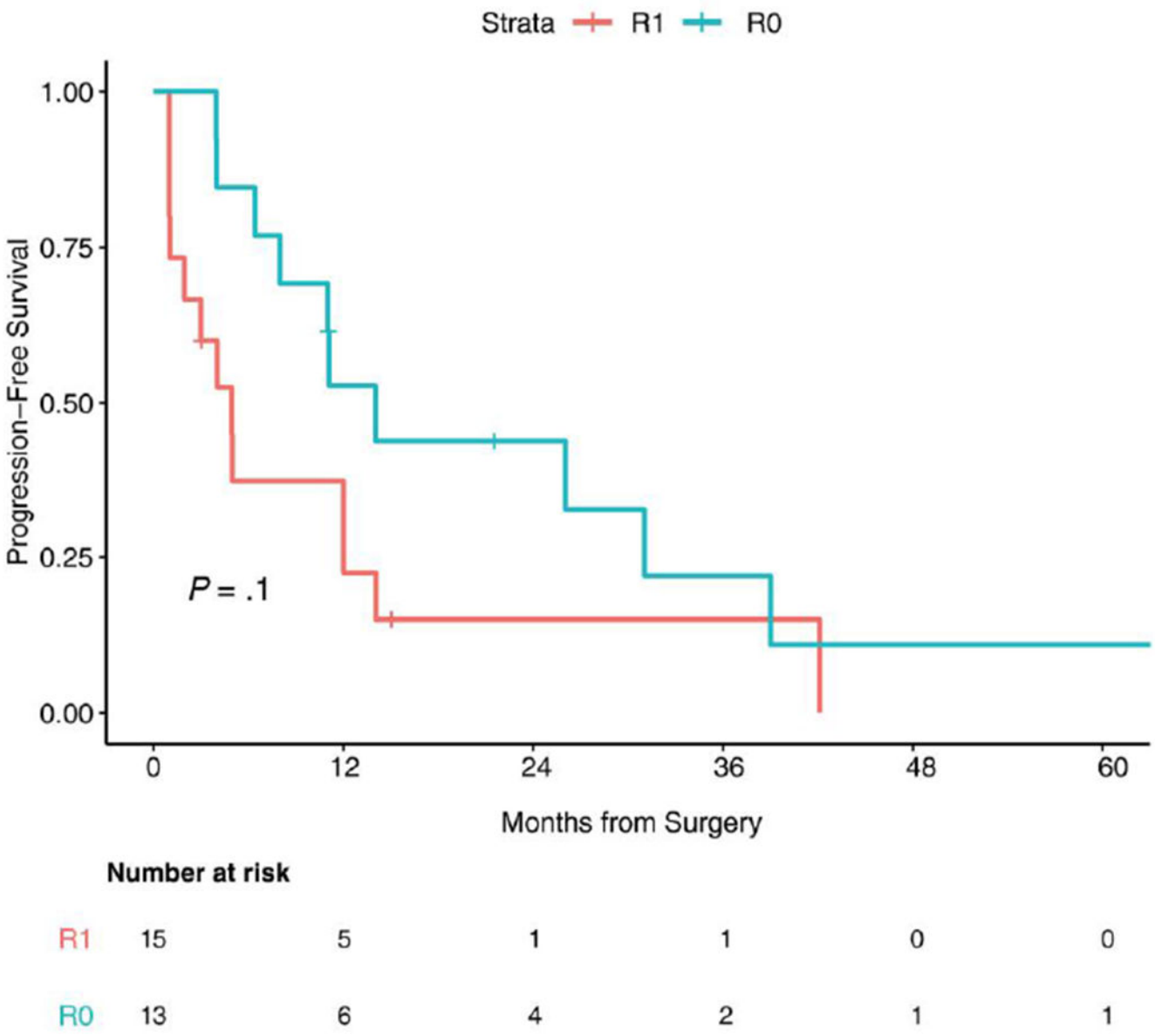


**FIGURE 5.**
Kaplan-Meier curve for progression-free survival based on surgical resection margin status (R0 vs R1).

**Table 1.**

Patient Characteristics (N = 31)

| Variable | Value |
|---|---|
| Age (range), y | 18 (17-24.5) |
| Sex, no.(%) | |
| Female | 16 (51.6) |
| Male | 15 (48.4) |
| Race, no. (96) | |
| White | 31 (100.0) |
| Stage at of disease at the time of diagrams, no. (%) (AJCC 8th edition) | |
| I | 1 (3.2) |
| II | 9 (29.0) |
| II/III | 1 (3.2) |
| III | 7 (22.6) |
| IV | 13 (41.9) |
| OCP use, no. (%) | |
| No | 25 (80.6) |
| PCOS | 1 (3.2) |
| Unknown | 2 (6.5) |
| Yes | 3 (9.7) |
| Family history of cancer | 12 (38.7%) |

Abbreviations: OCP, oral contraceptive pill; PCOS, polycystic ovary syndrome.

**Table 2.**

Oncogenic Mutations

| Gene | cDNA | Amino Acid |
|---|---|---|
| APC | c.1585C>T | p.L529F |
| ARID1A | c.5202T>G | p.Y1734* |
| ATR | c.4430C>G | p.P1477R |
| BCOR | c.2360C>T | p.P787L |
| BRD4 | c.886A>C | p.T296P |
| CHEK1 | c.1235T>C | p.V412A |
| CHEK2 | c.680G>A | p.G227E |
| CIC | c.89G>A | p.R30H |
| CTNNB1 | c.1004A>T | p.K335I |
| CTNNB1 | c.122C>T | p.T41I |
| DIS3 | c.2417A>G | p.D806G |
| EIF4A2 | c.881A>G | p.H294R |
| EPHA5 | c.2382G>C | p.M794I |
| ERBB3 | c.621_622delinsCC | p.K207_T208delinsNP |
| FAT1 | c.12791C>T | p.S4264L |
| GRIN2A | c.3211C>A | p.H1071N |
| HGF | c.1504C>G | p.R502G |
| INPP4B | c.7delA | p.I3fs |
| KMT2C | c.8539G>A | p.D2847N |
| KRAS | c.356A>G | p.D119G |
| MED12 | c.6118G>T | p.A2040S |
| MPL | c.1586G>A | p.W529* |
| NCOR1 | c.4556delC | p.S1519* |
| NKX3-1 | c.225G>C | p.Q75H |
| NOTCH1 | c.3340C>T | p.R1114C |
| NOTCH3 | c.6743C>T | p.S2248F |
| NSD1 | c.6531G>A | p.M2177I |
| KTRK2 | c.1784A>G | p.D595G |
| PAK7 | c.996T>A | p.D332E |
| RASA1 | c.143C>A | p.P48H |
| ROS1 | c.377G>A | p.G126E |
| SF3B1 | c.1447G>T | p.D483Y |
| SF3B1 | c.674G>T | p.G225V |
| SYK | c.1850C>A | p.P617H |
| TERT | g.1295228C>T | |
| TET1 | c.5973T>A | p.D1991E |
| WT1 | c.811C>A | p.H271N |
| YAP1 | c.370C>G | p.R124G |
| ZFHX3 | c.3195G>C | p.E1065D |

Abbreviation: cDNA, complementary DNA.

*Means Substitution - Nonsense.

**Table 3.**

Study of the Association Between Recurrently Mutated Genes and Survival Outcomes

| Characteristics | No. at Risk | No. Dead | 36-Month OS (95% CI) | *P* |
|---|---|---|---|---|
| TERT | | | | .105 |
| 0 | 24 | 8 | 0.79 (0.63-1) | |
| 1 | 7 | 0 | 1 (1-1) | |
| CTNNB1 | | | | .604 |
| 0 | 28 | 8 | 0.83 (0.68-1) | |
| 1 | 2 | 0 | NA | |
| ZFHX3 | | | | .668 |
| 0 | 28 | 8 | 0.83 (0.69-1) | |
| 1 | 2 | 0 | NA | |

Abbrevations: NA, not applicable; OS, overall survival.