Supplemental Data

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Supplemental Figure A. Mutation landscape of rel/ref FL assessed by the Personalis ACE sequencing panel.

Supplemental Methods for Next-Generation Sequencing

Lymph node samples and sequencing

Samples were collected after informed consent from patients via biopsy prior to initiating ibrutinib therapy. Fresh needle core biopsies were snap frozen, processed to genomic DNA (AllPrep DNA/RNA kit, Qiagen), and submitted for custom-capture panel sequencing using the Accuracy and Content Enhanced (ACE) Panel, which targets >1,300 cancer genes.[1] Sequencing data generation was performed by Personalis (Menlo Park, CA) using the Illumina HiSeq system using standard manufacturer protocols (2 x 125 base pair reads). Variants were called using the Personalis commercial variant calling pipeline. All available lymph node samples (n=31) collected prior to ibrutinib therapy were included in this analysis. Non-malignant DNA was not available for comparison.

Variant annotation and filtering

BAM files with aligned reads (GRCh37) were provided to The McDonnell Genome Institute (MGI) as well as all identified variants. Using these inputs all SNVs and indels were annotated with Ensembl build 74, counts were generated for reference and variant reads, and variant allele frequencies (VAF) calculated using the Genome Modeling System.[2] Following gene annotation and read counting, the following filters were applied: Variants were excluded based on an upper (>5000X) and lower (<100X) coverage threshold, resulting in 685.80x mean depth for 168,971 variants across all 31 samples. Variants were excluded with less than 6 reads of support or tumor VAF less than 2.5%. Variant calls were excluded unless they were annotated as non-silent coding, splice site, or exonic non-coding RNA mutations. Sites were also excluded if they were annotated only to a novel or putative transcript. All variants were further filtered against an exome sequencing panel of normal blood and breast samples (n=905) from the TCGA breast

cancer sequencing project.[3] Sites were excluded if they were identified in 5 or more normal samples with >3 variant reads and >2.5% VAF in this panel of normal individuals as previously described.[4] Finally, common polymorphisms (adjusted allele frequency >0.001 in ExAC release 0.2)[5] were excluded, and sites were excluded in genes mutated < 3 times. Following these filtering steps, 1,426 sites remained. All sites were manually reviewed in the integrated genomics viewer (IGV)[6] to remove sequencing and variant caller artifacts, germline variants, and other low-quality variants (e.g., variants occurring in repeat regions or variants supported only by read pairs with short insert sizes). Following manual review, a total 764 variants (179 genes) were confirmed and used for analysis. To verify the presence or absence of known acquired ibrutinib resistance mutations previously identified in CLL, a targeted analysis was performed using bam-readcount (https://github.com/genome/bam-readcount) at *BTK* and *PLCG2.* Ibrutinib resistance variants were determined using several sources[7–10] and publicly available catalogues of genomic variants in cancer[11,12] Genomic coordinates were determined and verified using COSMIC [11] and TransVar[13]. The number of variant supporting reads for 19 variants presented in Supplemental Table C were counted and VAFs were determined for the 31 primary and 3 relapse FL samples (from PH1966, PH1990, PH2060). An inclusion threshold of 4 variant supporting reads and a VAF >1% was set. None of the samples had evidence for the 19 variants that met our inclusion criteria.

Statistical Analysis

Genes mutated in ≥4 individuals (see Supplemental Figure A and Supplemental Table A) were included in statistical analyses (n=65 genes, 462 variants). Time-to-event analyses were performed using a log-rank test to identify significant PFS (progression-free survival) and DOR (duration of response) differences between gene groups (mutated vs. wild-type). A chi-squared or Fisher's exact (for expected values =< 5) test was performed to examine the association

between mutation status and treatment response. The Benjamini-Hochberg method was used for multiple testing correction for all tests. Time-to-event analysis was performed using R (v3.2.3) with 'survival', 'multtest' and 'stat' packages and genomic visualizations created with 'GenVisR' package[14] and the ProteinPaint[15] web tool.

PFS	Gene	Mutated Individuals	Hazard Ratio	95% CI lower limit	95% CI upper limit	P-value	BH (q-value)
	CARD11	5	3.56	1.13	11.2	0.02	0.88
	IGLL5	8	0.322	0.108	0.961	0.033	0.88
DOR							
	KMT2D	11	0.076	0.007	0.858	0.007	0.096
	FOXO1	5	0.13	0.015	1.1	0.031	0.199

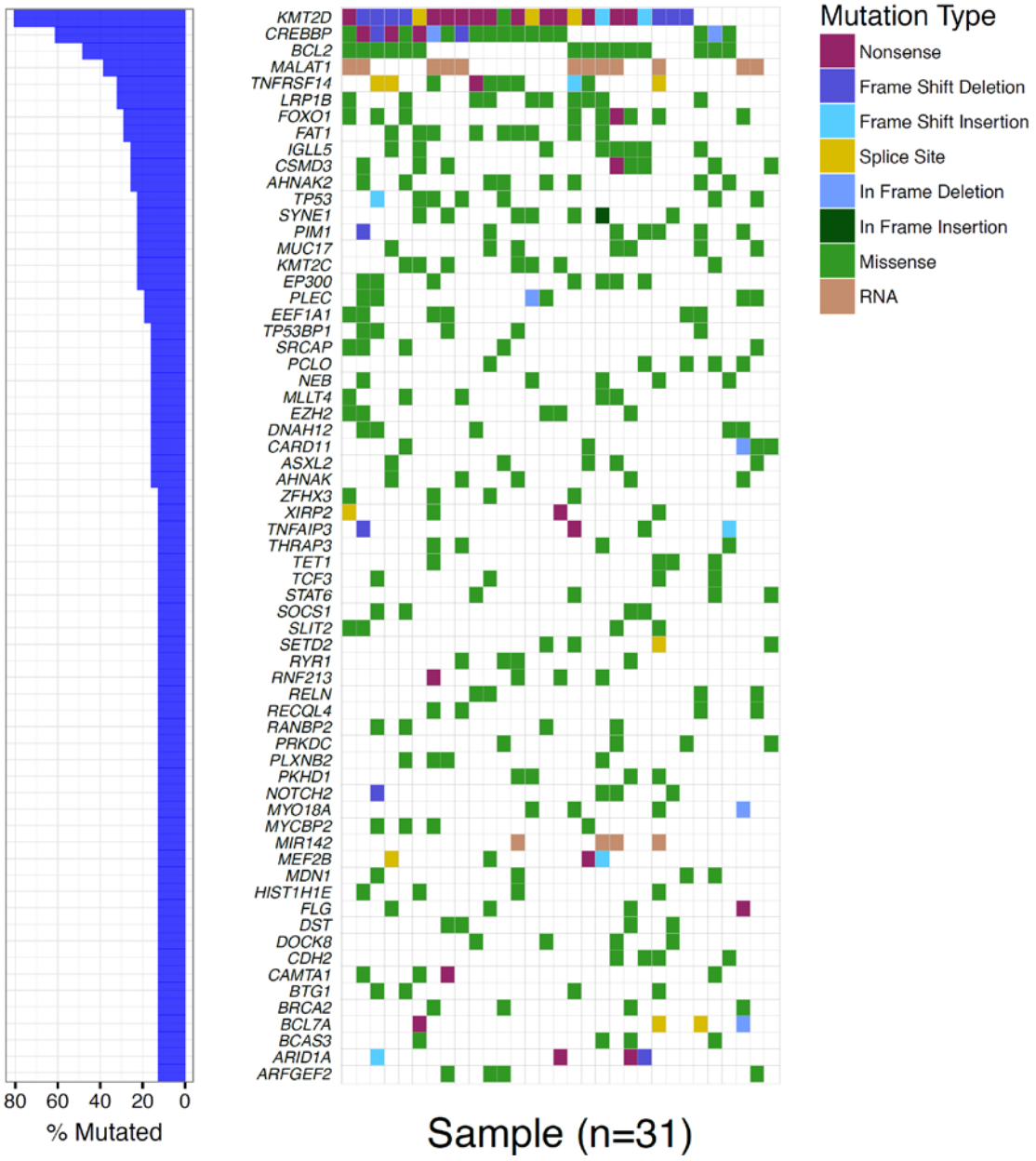
Supplemental Table A. Progression-free survival and duration of response correlations in genes mutated in >=4 individuals. Results are shown for genes with p <0.05. BH = Benjamini-Hochberg

	Best Response to Treatment					
Gene	CR	PR	PD	SD	p-value	q-value
SOCS1						
Wild type	1	9	2	15	0.001	0.054
Mutant	3	0	1	0		
CARD11						
Wild type	4	9	0	13	0.002	0.054
Mutant	0	0	3	2		
LRP1B						
Wild type	4	2	2	13	0.003	0.059
Mutant	0	7	1	2		
ARID1A						
Wild type	1	9	3	14	0.005	0.08
Mutant	3	0	0	1		
RANBP2						
Wild type	2	8	2	15	0.021	0.272
Mutant	2	1	1	0		
FOXO1						
Wild type	1	7	1	13	0.042	0.45
Mutant	3	2	2	2		

Supplemental Table B. Best response correlations for genes mutated in >=4 individuals. Results are shown for genes with $p < 0.05$.

Gene Name	Transcript	AA Mutation	cDNA Mutation	gDNA Mutation	Strand	Chromosome
BTK	ENST00000308731	p.C481F	c.1442G>T	g.100611164C>A	-	X
BTK	ENST00000308731	p.C481R	c.1441T>C	g.100611165A>G	-	X
BTK	ENST00000308731	p.C481S	c.1441T>A	g.100611165A>T	-	X
BTK	ENST00000308731	p.C481S	c.1442G>C	g.100611164C>G	-	X
BTK	ENST00000308731	p.C481S	c.1442_1443GC>CT	g.100611163_100611164delinsAG	-	X
BTK	ENST00000308731	p.C481Y	c.1442G>A	g.100611164C>T	-	X
BTK	ENST00000308731	p.T316A	c.946A>G	g.100613633T>C	-	X
BTK	ENST00000308731	p.C481A	c.1441_1442delTGinsGC	g.100611164_100611165delCAinsGC	-	X
BTK	ENST00000308731	p.T474I	c.1421C>T	g.100611185G>A	-	X
BTK	ENST00000308731	p.T474S	c.1421C>G	g.100611185G>C	-	X
BTK	ENST00000308731	p.L528W	c.1583T>G	g.100609666A>C	-	X
PLCG2	ENST00000359376	p.R665W	c.1993C>T	g.81946260C>T	+	chr16
PLCG2	ENST00000359376	p.S707P	c.2119T>C	g.81953153T>C	+	chr16
PLCG2	ENST00000359376	p.S707F	c.2120C>T	g.81953154C>T	+	chr16
PLCG2	ENST00000359376	p.S707Y	c.2120C>A	g.81953154C>A	+	chr16
PLCG2	ENST00000359376	p.L845F	c.2535A>T	g.81962183A>T	+	chr16
PLCG2	ENST00000359376	p.D1140G	c.3419A>G	g.81973602A>G	+	chr16
PLCG2	ENST00000359376	p.D334H	c.1000G>C	g.81927327G>C	+	chr16
PLCG2	ENST00000359376	p.R742P	c.2225G>C	g.81953259G>C	+	chr16

Supplemental Table C. Known acquired Ibrutinib resistance mutations identified in CLL. Evidence for these variants was assessed in primary and relapse samples using bam-readcount. All genomic positions are in reference to GRCh37/hg19.



Supplemental Figure A. Mutation landscape of rel/ref FL assessed by the Personalis ACE sequencing panel. Genes mutated in >=4 individuals and the types of mutations observed are shown in each row. Columns represent an individual. Columns are ordered by mutation presence in the most frequent to least frequently mutated gene. The histogram on the left describes the frequency of mutations in the gene in the corresponding row of the mutation landscape plot. For patients with multiple mutations in one gene, one mutation type is shown using the priority order indicated by the 'Mutation Type' legend where the mutation type at the top is given highest priority. Mutation landscape (waterfall) plot was created using the R 'GenVisR' package.[14]

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