Ultra-deep mutational landscape in chronic lymphocytic leukemia uncovers dynamics of resistance to targeted therapies

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SUPPLEMENTAL METHODS

DNA Duplex-seq library preparation

Given the small panel size (4843 bp), two rounds of hybridization capture were performed to increase efficiency.[1]

Duplex-seq analysis

Sequencing reads were analyzed using pipeline v2.1.2 available at https://github.com/Kennedy-Lab-UW/Duplex-Seq-Pipeline. First, raw reads were demultiplexed and grouped using the double stranded molecular tag included in the duplex adapters. Reads sharing the same tag were used to produce consensus Single-Strand Consensus Sequence (SSCS) reads. Then, SSCS reads with complementary tags were compared to produce a single, highly accurate Duplex Consensus Sequence (DCS) or "duplex read". Duplex reads were aligned to the human genome reference hg38 (GRCh38), end-trimmed (15 bp at 5', 5 bp at 3'), and overlap-trimmed. Samples with higher-than-average depth were subsampled using Samtools so that the mean depth per sample was similar across all samples from a particular patient. Variants were called using VarDict Java, and output VCF files were converted to MAF files using the Vcf2Maf script (https://github.com/mskcc/vcf2maf) with VEP version 104 and gene transcripts as indicated in Supplemental Table S3.[2] Masking was performed for *TP53* areas prone to sequencing artifacts.

For each sample, total number of sequenced raw reads, mean coding duplex depth, and total number of coding nucleotides were calculated (Supplemental Table S7). In Duplex-seq, the sensitivity of the assay is determined by the duplex depth, which is directly proportional to the

input amount of DNA assuming sufficient raw sequencing reads are allocated to build consensus sequences for most molecules.[3] Given the size of our library (4843 bp), for 500ng of DNA, we allocated ~15M raw reads per sample to achieve a target duplex depth of ~10,000x. Sequencing data matched expectations except of one sample (R001-D) that had less sequencing raw reads and proportionally lower depth (Supplementary Table S7). This sample was a replicate and was only used for validation analyses.

Mutational analysis

R scripts were used to process MAF files using R version 4.2.1 including the Tidyverse library.[4,5] Variants were discarded if they had depth < 50 reads or were in masked areas. Variants were annotated based on their classification in the MAF file as missense, nonsense, silent, indel, splice, UTR or intronic. Intronic mutations, mutations in UTRs, and mutations in intronic splice regions were considered non-coding. All other mutations were considered coding.

Variant allele frequencies (VAF) were calculated by dividing the number of mutant duplex reads (alternative counts) by the duplex depth at the mutated position. Variants that were present in all the samples from a given patient at VAF > 0.9 (homozygous) or VAF between 0.4 and 0.6 (heterozygous) and had a dbSNP identifier were considered single nucleotide polymorphisms (SNP)s (Supplemental Tables S5 and S6). Variants that had a dbSNP identifier, were present in all samples, and had a difference in VAF across samples (maximum minus minimum VAF) >0.2, were considered SNP-loss-of-heterozygosity (SNP-LOH). All variants that were not SNP or SNP-LOH and had depth > 1000 were considered mutations. For each sample, mutation

frequency (MF) was calculated as the number of mutant positions in coding regions divided by the total number of duplex nucleotides sequenced in coding regions.

Calculation of cancer cell fraction (CCF) for variants

To study clonal evolution of CLL under therapy, we converted the VAF of each mutation to its CCF (i.e., the fraction of cancer cells containing the mutation), which incorporates the tumor purity of the sample and the ploidy at the genomic location. In Duplex-seq, every duplex read corresponds to an original DNA molecule. While mutations in a single molecule are reliably detected, they are intrinsically subject to higher sampling error given the rarity of the event. Thus, we only focused on mutations detected in two or more molecules in a given sample to increase precision in CCF calculations. In patient R001, we analyzed clonal evolution using the four PB samples (A, B, C, and F) but not the two BMA samples (D, E) because they were technical replicates and collected only nine days after the third PB sample. In patient R002, sample R002-B was determined to have <1% tumor purity and therefore was excluded from clonal evolution analyses, resulting in a total of four samples available for this patient: one PB (E) and three BMA samples (A, C, and D).

Calculation of variant CCF of heterozygous mutations in a diploid scenario

Let *r* be the total number of duplex reads for mutation *i* occurring in a diploid region of the genome, with *v* being the number of variant duplex reads. Then $VAF_i = v/r$. The population of cells in the sample will be a mixture of normal cells and cancer cells. The tumor purity (or the percent disease), p , of a sample is the fraction of cancer cells in the sample. Thus, $r(1-p)$ reads are expected to correspond to normal cells, and rp reads are expected to come from cancer

cells. Furthermore, a somatic mutation in a diploid region is typically expected to be present in only one allele of a gene in question. In other words, the fraction of cancer cells containing mutation *i* is given by:

$$CCF_i = \frac{2v}{pr} = \frac{2VAF_i}{p}$$

An exception to this formula was *BAX* mutation p.E41Gfs*33 in patient R002, which reached a CCF of 100%. *BAX* is in an autosome (chr. 19). Thus, such high VAF indicates either the mutation occurred independently in both alleles or that the second allele was lost by LOH in most cells. Therefore, for this mutation we use the formula $\frac{VAF_i}{p}$.

Calculation of variant CCF of heterozygous mutations located on X chromosome for male patients

Of the genes included in the sequencing panel for this study, all are autosomal, except for *BTK* which is located on the X chromosome. Since there is only one allele of *BTK* for male patients, the CCF of *BTK* mutations is calculated as $\frac{VAF_i}{p}$. This formula is applied for the *BTK* mutations detected for the male patient R002.

Calculation of CCF for LOH

LOH is another type of genetic alteration relatively frequent in CLL. Our panel was not specifically designed to detect LOH, but several heterozygous SNPs were captured and indicated LOH for *TP53* in patient R001. As explained above, the availability of multiple samples allowed determination of LOH based on the comparison of the VAF of heterozygous SNPs across samples. As described in Methods, variants that had a dbSNP identifier, were present in all

samples, and had a difference in VAF across samples (maximum minus minimum VAF) > 0.2 were considered SNP-loss of heterozygosity (SNP-LOH). The VAF of these SNPs was used to infer the frequency of cells with SNP-LOH in the sample. To describe the methods in more detail, we consider the case of two SNPs on the two alleles of the same gene, SNP_a and SNP_b. There is a SNP-LOH in an unknown fraction of cells, *x*, in the sample, resulting in the loss of the allele containing SNP_a. Since SNP_a is only present in cells without SNP-LOH, it follows:

$$VAF_a = \frac{1-x}{2(1-x)+x} = \frac{1-x}{2-x}$$

The denominator takes into consideration that the total coverage at the locus is 2 in cells without SNP-LOH (frequency $= 1 - x$) and 1 in cells with SNP LOH (frequency $= x$), so total average coverage is $2(1-x) + x$. Note that $VAF_a < 0.5$ in this scenario, indicating that SNP_a is affected by LOH. Since SNP_b is present in all cells, we have:

$$VAF_b = \frac{1}{2-x}.$$

Similarly, $VAF_b > 0.5$, indicating that SNPb is not affected by LOH.

The ***fraction of cells*** with SNP-LOH in the sample, *x*, can be calculated from any of the two equations above. To produce a single value of x, we combine the equations for VAF_a and VAF_b, yielding

$$x = 1 - \frac{VAF_a}{VAF_b}.$$

To calculate the ***fraction of cancer cells*** with SNP-LOH (LOH CCF), we note that SNP-LOH should only be present in the cancer cell population. Thus, the fraction of cancer cells with SNP-LOH is x/p, where p is the tumor purity.

To validate our estimates of the ***fraction of cells*** with SNP-LOH, we cross referenced with clinical cytogenetic data from a combination of karyotype, FISH, and chromosomal genomic array testing (CGAT) on the same samples used for duplex sequencing (Supplemental Table S10). LOH was only observed for *TP53* in patient R001, with estimates very similar to CGAT data. For this patient we then calculated TP53 LOH CCF based on TP53 SNP-LOH (Supplemental Table S10) and tumor purity. *TP53* LOH/deletion was present at CCFs of 80-100% in all the samples (Supplemental Table S11).

Calculation of variant CCF in the presence of LOH

Here we calculate the CCF of a variant located in a gene affected by LOH. In this situation, one allele is mutated, and the other allele is lost. This situation only applied to the *TP53* mutations for patient R001.

We first need to determine *x*, the fraction of cells with LOH in the sample, using the methodology described in the previous section. Let *p* be the tumor purity of the sample. If mutation *j* is present in a fraction *f* of cancer cells with LOH and in a fraction *g* of cancer cells without LOH, then

$$VAF_j = \big(fx + g(p - x)\big)/(2 - x) \text{ and } CCF_j = \big(fx + g(p - x)\big)/p.$$

Combining the two equations, it follows that

$$CCF_j = \frac{VAF_j(2-x)}{p}.$$

Digital PCR

Digital polymerase chain reaction (dPCR) was performed using the kits, instrument, and software suite of the QIAcuity™ dPCR system. Custom dPCR LNA® Assays were used to screen for regions and variants of interest. Reaction setups and thermocycling conditions were modified from the QIAcuity™ Probe PCR Kit product insert's protocol; the volume of primer-probe mix was doubled, and the annealing/extension step of the thermocycling conditions was increased to 60 seconds. Wild-type and mutant targets were assayed in a single multiplex reaction, and reactions were performed on a 24-well nanoplate. The PCR template input was 50ng of DNA, which was measured to ensure each positive partition only contained a singlet positive reaction and no doublet positives. Experimental reactions were represented by six replicates. For analysis, the results for the six replicate wells were consolidated into a 6-well hyperwell to achieve screening by 144,000 partitions to augment sensitivity for measurable residual disease.

SUPPLEMENTAL TABLES

Supplemental Table S1. Patient R001 clinical results of peripheral blood and bone marrow.

Experimental sample #	Years post-diagnosis	Days post-pirtobrutinib	Tissue	Percent disease (%)	Hotspot mutation testing variant	Karyotype	FISH	CGAT
Not sequenced	0.0	-2281	BMA	73.0	NA	NA	NA	NA
Not sequenced	1.6	-1695	BMA	93.9	NA	46, XX, add(6)(p21.3), der(7)t(7;11)(q22q32), -10, del(11)(q21q23), add(17)(p 11.2), +mar1 [5] / 45, sl, dic(9;18) (p13;q21.3) [3] / 46, sl, add(19)(p13.3), -mar1, +mar2 [8] / 46, XX [4]	ish add(6)(MYB+), der(7)(ATM+), del(11)(ATM-), mar1(TP53+) [2] / add(6)(MYB+), der(7)(ATM+), del(11)(ATM-) mar2(TP53+) [1]. nuc ish (MYB,ATM,TP53) x2 [800], (CCND1,IGH) x2 [500], (D12Z3x2) [800], (D13S319-D13S25,163C9) x2 [800]	NA
Not sequenced	2.4	-1402	BMA	7.2	NA	NA	NA	NA
Not sequenced	2.9	-1232	BMA	69.5	NA	NA	NA	NA
R001-A	4.4	-687	PB	62.0	NA	46, XX, add(6)(p21.3), der(7)t(7;11)(q22;q32), -10, del(11)(q21q23), add(12)(p13), add(15(q22), add(17)(p11.2), del(20)(q11.2q13.3), -22, add(22)(q13), +1~2mar[cp14] / 46, XX [6]	nuc ish (MYBx2, ATMx2, TP53x1) [128/200]	CNAs: 9p21- (2.3 Mb), 10pterq21+ (58 Mb), 10p11- (6.4 Mb), and 17p- in ~60% of cells; Normal for 11q, 12, and 13q
Not sequenced	4.4	-660	BMA	78.1	NA	46, XX, add(6)(p21.3), der(7)t(7;11)(q22;q21), -10, del(11)(q21q23), add(12)(p13), add(14)(q32), add(17)(p11.2), del(20)(q11.2q13.3), +mar[cp18] / 46, XX [2]	NA	NA
Not sequenced	4.5	-636	BMA	33.1	NA	NA	NA	NA
Not sequenced	4.6	-617	PB	0.0	NA	NA	NA	NA
Not sequenced	4.6	-616	BMA	< 1.0	NA	46,XX[21]	NA	NA
Not sequenced	4.6	-591	PB	0.1	NA	NA	NA	NA
Not sequenced	4.7	-560	PB	15.3	NA	NA	NA	NA
R001-B	5.4	-294	PB	56.0	NA	46, X, t(X;18)(p22.1;q21.1), add(1)(q25), add(2)(q33), add(6)(p21.3), der(7)t(7;11)(q22;q21), -10,del(11)(q21q23), add(12)(p13), add(17)(p11.2), del(20)(q11.2q13.3), +mar[cp14] / 46, XX [6]	nuc ish (MYBx2, ATMx2, TP53x1) [153/200]	New aberrations: 1q-, 2q-, and 12q- in ~20% of cells; Persistent aberrations: 9p-, 10p+/-, 10q+, and -17p in ~60% of cells; Normal for 11q and 13q
R001-C	6.2	-15	PB	70.0†	NA	NA	NA	NA
R001-D/E*	6.2	-6	BMA	82.7	TP53 (p.H178D, NM_000546.5:c.532C>G, VAF 18%) BTK (p.C481S, NM_000061.3:c.1442G>C, VAF 10%) CARD11 (p.L341M, NM_032415.7:c.1021C>A, VAF 15%)	46, XX, add(1)(q25), add(2)(q33), add(6)(p21.3), der(7)t(7;11)(q22;q21), -10,del(11)(q21q23), add(17)(p11.2), +mar[11] / 46, XX [9]	nuc ish (MYBx2, ATMx2, TP53x1) [154/200]	Persistent CNAs: 1 q-, 2q-, 9p-, 10p+, 10p-, 10q+, 12q-, and -17p in ~20-80% of cells; (specifically -17p = 80%); Normal for 11q and 13q
R001-F	6.7	161	PB	95.0†	NA	NA	NA	NA

Abbreviations: Bone marrow aspirate (BMA); Peripheral blood (PB); Variant allele frequency (VAF); Fluorescence *in situ* hybridization (FISH); Chromosomal genomic array testing (CGAT); Copy number alterations (CNAs).

* Samples R001D and R001E are technical replicates from the same bone marrow aspiration procedure (independent specimen processing, DNA extraction and library preparation)

† No flow-cytometry or ClonoSeq data were available for these specimens. Tumor burden was inferred from CBC WBC counts on the samples' collection dates.

Supplemental Table S2. Patient R002 clinical results of peripheral blood and bone marrow.

Experimental sample #	Years post-diagnosis	Days post-pirtobrutinib	Tissue	Percent disease (%)	Hotspot mutation testing variant	Karyotype	FISH	CGAT
Not sequenced	12.2	-797	BMA	9.8	NA	NA	NA	NA
Not sequenced	12.7	-615	BMA	2.7	NA	NA	NA	NA
Not sequenced	13.1	-489	BMA	91.0	NA	NA	NA	NA
Not sequenced	13.3	-428	PB	2.2	NA	46, XY, der(1)t(1;8)(q41;q13), der(7)t(3;7)(q26.1;p21) [9] / 46, XY [11]	Whole blood: nuc ish(MYB,ATM,TP53) x2 [500], (MYCx3) [29/200], (CDKN2Ax0,D9Z3x2) [12/500], (D13S319-D13S25,163C9)x2[500]. Enriched abnormal B-cells: nuc ish (D12Z3x2) [300]	CNAs: 1q41qter- (32 Mb), 3q26qter+ (36 Mb), 7pterp21- (12 Mb), 8q13qter+ (76 Mb), and 9p21-- (1.4 Mb, CDKN2A); Copy-neutral LOH (cnLOH) of 9pterp13 (35 Mb); Normal for 11q, 12, 13q, and 17p
R002-A	13.5	-331	BMA	12.9	NA	46, XY, der(1)t(1;8)(q41;q13), der(7)t(3;7)(q26.1;p21) [6] / 46, XY [14]	NA	NA
R002-B	13.6	-294	BMA	< 1.0	NA	46, XY [20]	NA	NA
Not sequenced	13.6	-288	BMA	75.3	NA	NA	NA	NA
R002-C	14.4	-6	BMA	95.0	MAP2K1 (p.K57N, NM_002755.3:c.171 G>C, VAF 48%)	46, XY, der(1)t(1;8)(q41;q13), der(7)t(3;7)(q26.1;p21) [16] / 46, XY [4]	nuc ish (MYBx2, ATMx3, TP53x2) [18/500]	CNAs: 1q41qter- (29 Mb), 3q26qter+ (35 Mb), 7pterp21- (12 Mb), 8q13qter+ (76 Mb), and 9p21-- (1.4 Mb, CDKN2A) in >80% of cells; Copy-neutral LOH (cnLOH) of 9pterp13 (36 Mb) in >80% of cells; Normal for 11q, 12, 13q, and
Not sequenced	14.6	70	PB	88.0	NA	NA	NA	NA
R002-D	14.7	82	BMA	91.0	TP53 (normal) MAP2K1 (p.K57N, NM_002755.3:c.171 G>C, VAF 43%) SF3B1 (p.E622V, NM_012433.3:c.1865A>T, VAF 37%) BTK (p.T474I, NM_000061.3:c.1421C>T, VAF 6%)	NA	nuc ish (MYBx2, ATMx3, TP53x2) [16/500], (D12Z3x2) [500], (D13S319-D13S25,163C9)x2 [500]	NA
R002-E	14.8	127	PB	95.0*	NA	NA	NA	NA

Abbreviations: Bone marrow aspirate (BMA); Peripheral blood (PB); Variant allele frequency (VAF); Fluorescence *in situ* hybridization (FISH); Chromosomal genomic array testing (CGAT) Copy number alterations (CNAs); Loss of heterozygosity (LOH).

* No flow-cytometry or ClonoSeq data was available for this specimen. Tumor burden was inferred from CBC WBC counts on the sample's collection date.

Supplemental Table S3. Duplex sequencing gene targets.

Gene	Gene NM	Transcript	Chromosome	Targeted area	Sequenced Exons	Source of Probes
BAX	NM_004324.4	ENST00000293288	19	hotspot codons	3,5	Integrated DNA Technologies
BCL2	NM_000633.3	ENST00000333681	18	hotspot codons	1	Integrated DNA Technologies
BTK	NM_000061.3	ENST00000308731	X	hotspot codons	10,14,15	Integrated DNA Technologies
PLCG2	NM_002661.5	ENST00000564138	16	hotspot codons	18,19,23,26	Integrated DNA Technologies
TP53	NM_000546.6	ENST00000269305	17	coding	2-11*	TwinStrand Biosciences Cat. Number 06-1004-XX

* Probes also covered alternative exons that were considered non-coding for analysis.

Supplemental Table S4. Custom probes for hotspots.

Gene	Probe ID	Coding Exon	Coordinates of Probe (hg38)	Probe Sequence
BCL2	693383_32637003_BCL2(596)_1a_12	1	chr18: 60985589-60985709	ACCCGGTCGCCAGGACCTCGCCGCTGCAGACCCCGGCTGCCCCCGGCGCCGCCGCGGGGCCTGCGCTCAGCCCGGTGCCACCTGTGGTCCACCTGACCCTCCGCCAGGCCGGCGACGACT
	693383_32637003_BCL2(596)_1a_13		chr18: 60985529-60985649	CTGCGCTCAGCCCGGTGCCACCTGTGGTCCACCTGACCCTCCGCCAGGCCGGCGACGACTTCTCCCGCCGCTACCGCCGCGACTTCGCCGAGATGTCCAGCCAGCTGCACCTGACGCCCT
BAX	693383_32636999_BAX(581)_3_2	3	chr19: 49458927-49459047	TCCATCCCCACTCTAGTTTCATCCAGGATCGAGCAGGGCGAATGGGGGGGGAGGCACCCGAGCTGGCCCTGGACCCGGTGCCTCAGGATGCGTCCACCAAGAAGCTGAGCGAGTGTCTCA
	693383_32636999_BAX(581)_3_3		chr19: 49458987-49459107	AGCTGGCCCTGGACCCGGTGCCTCAGGATGCGTCCACCAAGAAGCTGAGCGAGTGTCTCAAGCGCATCGGGGACGAACTGGACAGTAACATGGAGCTGCAGAGGTGTGGGCCCCTGAGGA
	693383_32637001_BAX(581)_6.1_4	5	chr19: 49464171-49464291	GTGAGACTCCTCAAGCCTCCTCACCCCCACCACCGCGCCCTCACCACCGCCCCTGCCCCACCGTCCCTGCCCCCCGCCACTCCTCTGGGACCCTGGGCCTTCTGGAGCAGGTCACAGTGG
	693383_32637001_BAX(581)_6.1_5		chr19: 49464231-49464351	CCGTCCCTGCCCCCCGCCACTCCTCTGGGACCCTGGGCCTTCTGGAGCAGGTCACAGTGGTGCCCTCTCCCCATCTTCAGATCATCAGATGTGGTCTATAATGCGTTTTCCTTACGTGTC
BTK	693383_32637018_BTK(695)_15a_2	15	chrX: 100611148-100611268	CCTTTCCTGTAGGAATCTTTCCCATGAGAAGCTGGTGCAGTTGTATGGCGTCTGCACCAAGCAGCGCCCCATCTTCATCATCACTGAGTACATGGCCAATGGCTGCCTCCTGAACTACCT
	693383_32637018_BTK(695)_15a_3		chrX: 100611088-100611208	GCAGCGCCCCATCTTCATCATCACTGAGTACATGGCCAATGGCTGCCTCCTGAACTACCTGAGGGAGATGCGCCACCGCTTCCAGACTCAGCAGCTGCTAGAGATGTGCAAGGATGTCTG
	693383_32637019_BTK(695)_16a_1	16	chrX: 100609650-100609770	TGGCTTCATTCTACTGGTCAGCAGAAGCTTTGTGCCTTTAACCTCTGTGCTGGGGACGGAGTCTCACTGGTCTCTGTTTGCACTACAGGCAGCTCGAAACTGTTTGGTAAACGATCAAGG
	693383_32637019_BTK(695)_16a_2		chrX: 100609590-100609710	GTCTCACTGGTCTCTGTTTGCACTACAGGCAGCTCGAAACTGTTTGGTAAACGATCAAGGAGTTGTTAAAGTATCTGATTTCGGCCTGTCCAGGTGAGTGTGGCTTTTTCATCTTTCCCT
	693383_32637014_BTK(695)_11a_2	11	chrX: 100613584-100613704	CTTCTTTTTCGTTGTTTCAGGGGAAAGAAGGAGGTTTCATTGTCAGAGACTCCAGCAAAGCTGGCAAATATACAGTGTCTGTGTTTGCTAAATCCACAGGGTGAGTGCTACTATTCCAAG
	693383_32637014_BTK(695)_11a_3		chrX: 100613524-100613644	CTGGCAAATATACAGTGTCTGTGTTTGCTAAATCCACAGGGTGAGTGCTACTATTCCAAGGCCCTGAGGACAAAGAACAGGGGTACCCTCCTAATAGCTCCTTGATGCTGTGCCCGTCCC
PLCG2	693383_32637040_PLCG2(5336)_19_2	19	chr16: 81946171-81946291	CTGGTCGTTTTCCCTGGCCCTGTGCCGCAGGTGGTACTATGACAGCCTGAGCCGCGGAGAGGCAGAGGACATGCTGATGAGGATTCCCCGGGACGGGGCCTTCCTGATCCGGAAGCGAGA
	693383_32637040_PLCG2(5336)_19_3		chr16: 81946231-81946351	GGCAGAGGACATGCTGATGAGGATTCCCCGGGACGGGGCCTTCCTGATCCGGAAGCGAGAGGGGAGCGACTCCTATGCCATCACCTTCAGGTGGGTGCGAGGGTGGGAGGCACATGCTCT
	693383_32637041_PLCG2(5336)_20.1_2	20	chr16: 81953054-81953174	TTGGCATGTCAACCCTGTGTTCTTCCTGCTCCAGGGCTAGGGGCAAGGTAAAGCATTGTCGCATCAACCGGGACGGCCGGCACTTTGTGCTGGGGACCTCCGCCTATTTTGAGAGTCTGG
	693383_32637041_PLCG2(5336)_20.1_3		chr16: 81953113-81953233	CGCATCAACCGGGACGGCCGGCACTTTGTGCTGGGGACCTCCGCCTATTTTGAGAGTCTGGTGGAGCTCGTCAGTTACTACGAGAAGCATTCACTCTACCGAAAGATGAGACTGCGCTAC
	693383_32637041_PLCG2(5336)_20.1_4		chr16: 81953172-81953292	GGTGGAGCTCGTCAGTTACTACGAGAAGCATTCACTCTACCGAAAGATGAGACTGCGCTACCCCGTGACCCCCGAGCTCCTGGAGCGCTACAATATGGTAGGTGGTGGACTCCCTTGTGA
	693383_32637045_PLCG2(5336)_24_1	24	chr16: 81962076-81962196	TCTGCTAAACGGTGTGCTTTGGAAACGGGTTTTCTTTTTATTATTCCCGTTACAACTAACGTGAGTTATGTCTTGTTTCTTCACAGATTATTGAAGACAATCCCTTAGGGTCTCTTTGCA
	693383_32637045_PLCG2(5336)_24_2		chr16: 81962136-81962256	GTGAGTTATGTCTTGTTTCTTCACAGATTATTGAAGACAATCCCTTAGGGTCTCTTTGCAGAGGAATATTGGACCTCAATACCTATAACGTCGGTACGTGCACACATCATCTTAGCCTGG
	693383_32637049_PLCG2(5336)_28_3	27	chr16: 81969818-81969938	CTGACAGCATCATCAGACAGAAGCCCGTCGACCTCCTGAAGTACAATCAAAAGGGCCTGACCCGCGTCTACCCAAAGGGACAAAGAGTTGACTCTTCAAACTACGACCCCTTCCGCCTCT
	693383_32637049_PLCG2(5336)_28_4		chr16: 81969878-81969998	CCCGCGTCTACCCAAAGGGACAAAGAGTTGACTCTTCAAACTACGACCCCTTCCGCCTCTGGCTGTGCGGTTCTCAGATGGTGGCACTCAATTTCCAGACGGCAGGTAAAGGCCGACTGA

Supplemental Table S5. Patient R001 duplex sequencing SNP data.

Chromosome	Gene	Position	HGVSc	HGVSp Short	Zygosity	LOH	R001-A VAF	R001-B VAF	R001-C VAF	R001-D VAF	R001-E VAF	R001-F VAF	R001-A duplex depth	R001-B duplex depth	R001-C duplex depth	R001-D duplex depth	R001-E duplex depth	R001-F duplex depth	R001-A duplex mutant molecules	R001-B duplex mutant molecules	R001-C duplex mutant molecules	R001-D duplex mutant molecules	R001-E duplex mutant molecules	R001-F duplex mutant molecules
chr16	PLCG2	81912361	c.1935-236C>T	NA	Heterozygous	no	0.5071	0.5005	0.4946	0.5027	0.5000	0.4875	1485	1882	1486	1098	1394	1887	753	942	735	552	697	920
chr16	PLCG2	81912818	c.2054+102C>T	NA	Heterozygous	no	0.4946	0.5041	0.5079	0.5060	0.5085	0.5099	7528	7437	7871	4844	7292	7403	3723	3749	3998	2451	3708	3775
chr16	PLCG2	81912866	c.2054+150G>T	NA	Heterozygous	no	0.5031	0.5134	0.5138	0.5052	0.5082	0.5069	4798	4866	4994	3078	4658	4841	2414	2498	2566	1555	2367	2454
chr16	PLCG2	81912881	c.2054+165G>A	NA	Heterozygous	no	0.5012	0.5085	0.5032	0.5023	0.5063	0.5042	4068	4240	4187	2580	3893	4159	2039	2156	2107	1296	1971	2097
chr16	PLCG2	81913109	c.2054+393T>C	NA	Heterozygous	no	0.4821	0.4831	0.4891	0.4972	0.4444	0.4801	195	296	184	181	117	377	94	143	90	90	52	181
chr16	PLCG2	81919162	c.2055-322C>G	NA	Heterozygous	no	0.4945	0.5154	0.4810	0.4938	0.5114	0.4851	457	586	447	563	352	1142	226	302	215	278	180	554
chr16	PLCG2	81919476	c.2055-8T>C	NA	Homozygous	NA	1.0000	1.0000	1.0000	1.0000	0.9999	1.0000	12705	12418	14084	10028	13263	15278	12705	12418	14084	10028	13262	15278
chr16	PLCG2	81919763	c.2235+99A>G	NA	Homozygous	NA	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	5651	6058	6218	4157	5820	6894	5651	6058	6218	4157	5820	6894
chr16	PLCG2	81936242	c.2916C>T	p.V972=	Heterozygous	no	0.4954	0.4858	0.4872	0.4856	0.4867	0.4919	12273	12045	13394	9028	13043	13958	6080	5851	6526	4384	6348	6866
chr16	PLCG2	81936583	c.3052+205C>G	NA	Homozygous	NA	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1716	2001	1709	1200	1607	2647	1716	2001	1709	1200	1607	2647
chr17	TP53	7670065	c.1101-375G>A	NA	Heterozygous	SNP-LOH*	0.3411	0.2966	0.3095	0.1489	0.1074	0.0815	214	263	168	188	121	466	73	78	52	28	13	38
chr17	TP53	7674797	c.672+62A>G	NA	Homozygous	NA	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	10438	10545	10873	6588	9298	8806	10438	10545	10873	6588	9298	8806
chr17	TP53	7675327	c.376-91G>A	NA	Homozygous	NA	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	6273	6241	6708	3982	5542	5575	6273	6241	6708	3982	5542	5575
chr17	TP53	7675519	c.376-283T>C	NA	Homozygous	NA	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1935	2354	2007	1212	1501	2065	1935	2354	2007	1212	1501	2065
chr17	TP53	7676154	c.215C>G	p.P72R	Homozygous	NA	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	11609	11461	12576	8478	10425	9927	11609	11461	12576	8478	10425	9927
chr17	TP53	7676278	c.97-6C>T	NA	Heterozygous	SNP-LOH*	0.6998	0.6943	0.7055	0.8191	0.8300	0.9120	10636	10400	11231	7993	9500	9263	7443	7221	7924	6547	7885	8448
chr19	BAX	48955313	c.35-235T>C	NA	Heterozygous	no	0.5243	0.5025	0.4384	0.5422	0.4762	0.4938	103	197	73	83	63	162	54	99	32	45	30	80
chr19	BAX	48955847	c.233+14A>G	NA	Heterozygous	no	0.5014	0.4904	0.4985	0.4909	0.4913	0.4992	11637	11512	12704	6851	11967	10932	5835	5646	6333	3363	5879	5457
chr19	BAX	48955955	c.233+122A>G	NA	Homozygous	NA	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	5001	5221	5483	3209	5156	5504	5001	5221	5483	3209	5156	5504
chrX	BTK	101354559	c.1631+71C>T	NA	Homozygous	NA	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	8105	8008	8306	6877	8858	10500	8105	8008	8306	6877	8858	10500
chrX	BTK	101356297	c.1350-29A>G	NA	Heterozygous	no	0.5157	0.5304	0.5071	0.5113	0.5206	0.4931	9096	9214	9378	6703	10062	10073	4691	4887	4756	3427	5238	4967

Abbreviations: Single nucleotide polymorphisms (SNP); Human Genome Variation Society nomenclature (HGVSc); Loss of heterozygosity (LOH); Variant allele frequency (VAF); Not applicable (NA).

* SNP-LOH were SNPs present in all samples with difference in VAF across samples (maximum minus minimum VAF) > 0.2.

Supplemental Table S6. Patient R002 duplex sequencing SNP data.

Chromosome	Gene	Position	HGVSc	HGVSp Short	Zygosity	LOH	R002-A VAF	R002-B VAF	R002-C VAF	R002-D VAF	R002-E VAF	R002-A duplex depth	R002-B duplex depth	R002-C duplex depth	R002-D duplex depth	R002-E duplex depth	R002-A duplex mutant molecules	R002-B duplex mutant molecules	R002-C duplex mutant molecules	R002-D duplex mutant molecules	R002-E duplex mutant molecules
chr16	PLCG2	81912818	c.2054+102C>T	NA	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	6432	5829	6165	6038	5442	6432	5829	6165	6038	5442
chr16	PLCG2	81912866	c.2054+150G>T	NA	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	4108	3779	4014	3791	3353	4108	3779	4014	3791	3353
chr16	PLCG2	81919162	c.2055-322C>G	NA	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	749	702	655	625	191	749	702	655	625	191
chr16	PLCG2	81919219	c.2055-265T>C	NA	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	1222	1282	1137	1110	488	1222	1282	1137	1110	488
chr16	PLCG2	81919431	2055-50_2055-47c	NA	Heterozygous	no	0.4925	0.4859	0.4827	0.4920	0.4974	9664	9655	9409	9642	8385	4760	4691	4542	4744	4171
chr16	PLCG2	81919476	c.2055-8T>C	NA	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	12439	12144	11890	12126	11396	12439	12144	11890	12126	11396
chr16	PLCG2	81919763	c.2235+99A>G	NA	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	5453	5637	5264	5510	3749	5453	5637	5264	5510	3749
chr16	PLCG2	81928268	c.2515-290G>T	NA	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	1306	1337	1077	1238	248	1306	1337	1077	1238	248
chr16	PLCG2	81928466	c.2515-92A>C	NA	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	8418	8682	8358	8557	5809	8418	8682	8358	8557	5809
chr17	TP53	7674797	c.672+62A>G	NA	Homozygous	NA	0.9998	1.0	1.0	1.0	1.0	12033	11623	12568	12077	10325	12031	11623	12568	12077	10325
chr17	TP53	7675327	c.376-91G>A	NA	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	7923	7630	7927	7700	6646	7923	7630	7927	7700	6646
chr17	TP53	7675519	c.376-283T>C	NA	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	2510	2373	2368	2236	844	2510	2373	2368	2236	844
chr17	TP53	7676154	c.215C>G	p.P72R	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	13461	12771	13976	13424	14830	13461	12771	13976	13424	14830
chr18	BCL2	63318646	c.21A>G	p.T7=	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	1294	938	1029	745	1039	1294	938	1029	745	1039
chr19	BAX	48955513	c.35-35A>C	NA	Heterozygous	no	0.4864	0.5027	0.4846	0.5096	0.4809	1583	1647	1525	1513	863	770	828	739	771	415
chr19	BAX	48955847	c.233+14A>G	NA	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	10047	9811	9559	9889	9058	10047	9811	9559	9889	9057
chr19	BAX	48955955	c.233+122A>G	NA	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	4539	4417	4317	4265	3256	4539	4417	4317	4265	3256
chrX	BTK	101354559	c.1631+71C>T	NA	Homozygous	NA	1.0	1.0	1.0	1.0	1.0	5128	4992	5147	4975	3812	5128	4992	5147	4975	3812

Abbreviations: Single nucleotide polymorphisms (SNP); Human Genome Variation Society nomenclature (HGVSc); Loss of heterozygosity (LOH); Variant allele frequency (VAF); Not applicable (NA).

Supplemental Table S7. Duplex sequencing summary by sample.

Patient/ Sample Code	Sample type	Age at collection date	Days post-pirtobrutinib	Percent disease (%)	Raw reads (paired end)	Mean coding duplex depth	Coding nucleotides	Coding mutations	Coding MF	Included for clonal evolution analysis
R001-A	PB	65.4	-687	62.0	15,073,850	10227	32152379	27	8.40E-07	yes
R001-B	PB	66.5	-294	56.0	15,956,920	10185	32021158	25	7.80E-07	yes
R001-C	PB	67.3	-15	70.0	15,708,831	11170	35120001	41	1.20E-06	yes
R001-D*	BMA	67.3	-6	82.7	9,925,204	7812	24404287	32	1.30E-06	no
R001-E*	BMA	67.3	-6	82.7	15,019,519	10236	32152220	37	1.20E-06	no
R001-F	PB	67.8	161	95.0	19,197,071	10510	33042090	67	2.00E-06	yes
R002-A	BMA	72.2	-331	12.9	16,354,256	9192	28643615	31	1.10E-06	yes
R002-B	BMA	72.3	-294	< 1.0	18,129,500	9190	28314575	20	7.10E-07	no
R002-C	BMA	73.1	-6	95.0	16,470,890	9347	29042549	31	1.10E-06	yes
R002-D	BMA	73.3	82	91.0	17,769,765	9320	28548364	46	1.60E-06	yes
R002-E	PB	73.4	127	95.0	15,884,332	9605	29639794	65	2.20E-06	yes

Abbreviations: Peripheral blood (PB); Bone marrow aspirate (BMA); Mutation frequency (MF).
* Samples R001D and R001E are technical replicates of the same specimen.

Supplemental Table S8. List of coding mutations detected in samples from patient R001. The asterisks in the column "HGVSp Short" are standard Human Genome Variation Society nomenclature and do not represent footnotes. VAF > 0.01 are color coded in red/pink.

Chromosome	Gene	Start Position	HGVSc	HGVSp Short	BTKi resistance mutation*	BTKi resistance codon*	Number of samples with >1 mutant molecules	R001-A VAF	R001-B VAF	R001-C VAF	R001-D VAF†	R001-E VAF†	R001-F VAF	R001-A duplex depth	R001-B duplex depth	R001-C duplex depth	R001-D duplex depth	R001-E duplex depth	R001-F duplex depth	R001-A duplex mutant molecules	R001-B duplex mutant molecules	R001-C duplex mutant molecules	R001-D duplex mutant molecules	R001-E duplex mutant molecules	R001-F duplex mutant molecules
chr19	BAX	48955799	c.199G>A	p.G67R	NA	NA	4		0.0020	0.0008	0.0009	0.0005		12449	12179	13874	6673	12844	9834		24	11	6	7	
chr19	BAX	48955596	c.83_84del	p.Q28Rfs*45	NA	NA	0	0.0002						5258	5313	5730	3095	5093	4784	1					
chr19	BAX	48955713	c.121dup	p.E41Gfs*33	NA	NA	0	0.0001	0.0001				0.0001	12154	12099	13570	6509	12577	9526	1	1				1
chr19	BAX	48955780	c.180C>G	p.S60R	NA	NA	0			0.0001				12539	12231	14016	6225	13006	8842			1			
chr19	BAX	48955780	c.180C>T	p.S60=	NA	NA	0	0.0001						12539	12231	14016	6225	13006	8842	1					
chr19	BAX	48960836	c.396G>T	p.L132=	NA	NA	0						0.0001	5880	5830	6267	4159	5763	6871						1
chr19	BAX	48960857	c.417G>A	p.W139*	NA	NA	0	0.0001						6856	6880	7445	5214	6833	8338	1					
chr19	BAX	48960876	c.436G>T	p.E146*	NA	NA	0	0.0001						7167	7122	7750	6128	7190	9694	1					
chr19	BAX	48960896	c.456C>T	p.I152=	NA	NA	0			0.0001				7494	7519	8243	7252	7562	11252			1			
chr19	BAX	48960931	c.491C>T	p.P164L	NA	NA	0	0.0001						8606	8509	9502	9022	8829	13878	1					
chr19	BAX	48961037	c.597C>A	p.C195*	NA	NA	0			0.0001				11114	11148	12363	10043	11664	14767			1			
chr19	BAX	48961057	c.617C>G	p.S206*	NA	NA	0					0.0001		11107	11257	12279	9514	11606	14187					1	
chr18	BCL2	63318136	c.319_320dup	p.R107_R110dup	NA	NA	5	0.0039	0.0011	0.0010	0.0006	0.0010		9789	11016	11806	8181	10946	11456	38	12	12	5	11	
chr18	BCL2	63318329	c.338C>G	p.A113G	NA	NA	2	0.0011	0.0003				0.0001	9737	11047	11693	8058	10909	11261	11	3				1
chr18	BCL2	63318601	c.66G>A	p.K22=	NA	NA	2				0.0008		0.0008	3000	3325	3145	2529	2860	3677				2		3
chr18	BCL2	63318200	c.467T>A	p.V156D	NA	NA	1	0.0029						4095	4704	4885	3114	4538	4958	12					
chr18	BCL2	63318262	c.405G>A	p.E135=	NA	NA	1	0.0001		0.0001		0.0001	0.0002	7311	8272	8593	5647	8031	8310	1		1		1	2
chr18	BCL2	63318264	c.397_403del	p.V133Rfs*8	NA	NA	1	0.0003						7376	8366	8688	5695	8101	8374	2					
chr18	BCL2	63318329	c.338C>T	p.A113V	NA	NA	1	0.0002	0.0001					9737	11047	11693	8058	10909	11261	2	1				
chr18	BCL2	63318411	c.256C>T	p.L86F	NA	NA	1						0.0002	10073	11063	11741	8902	10787	12137						2
chr18	BCL2	63318531	c.136C>T	p.P46S	NA	NA	1						0.0003	6159	6798	6836	5284	6223	7666						2
chr18	BCL2	63318184	c.483C>T	p.S161=	NA	NA	0			0.0002				3445	3973	4117	2613	3775	4180			1			
chr18	BCL2	63318267	c.400G>A	p.V134M	NA	NA	0					0.0001		7529	8566	8928	5841	8295	8544					1	
chr18	BCL2	63318280	c.387C>T	p.R129=	NA	NA	0					0.0001		8290	9399	9844	6446	9195	9386					1	
chr18	BCL2	63318281	c.386G>A	p.R129H	NA	NA	0		0.0001					8272	9391	9834	6442	9184	9389		1				
chr18	BCL2	63318286	c.381G>A	p.R127=	NA	NA	0						0.0001	8530	9691	10162	6635	9486	9708						1
chr18	BCL2	63318288	c.379C>T	p.R127W	NA	NA	0		0.0001					8611	9753	10261	6724	9590	9840		1				
chr18	BCL2	63318312	c.355C>T	p.L119=	NA	NA	0						0.0001	9723	11020	11580	7834	10780	11041						1
chr18	BCL2	63318330	c.337G>C	p.A113P	NA	NA	0	0.0001						9782	11043	11731	8074	10937	11318	1					
chr18	BCL2	63318380	c.287C>T	p.T96I	NA	NA	0		0.0001					10003	11019	12016	8791	11080	11939		1				
chr18	BCL2	63318390	c.277G>A	p.V93I	NA	NA	0						0.0001	10029	10986	11843	8807	10893	12019						1
chr18	BCL2	63318393	c.274G>A	p.V92M	NA	NA	0			0.0001				10094	11028	11862	8815	10892	12081			1			
chr18	BCL2	63318399	c.268C>T	p.P90S	NA	NA	0			0.0001			0.0001	10139	11016	11840	8902	10914	12126			1			1
chr18	BCL2	63318406	c.261C>T	p.S87=	NA	NA	0						0.0001	10100	11014	11735	8893	10782	12084						1
chr18	BCL2	63318412	c.255C>A	p.A85=	NA	NA	0						0.0001	10043	11028	11706	8880	10742	12114						1
chr18	BCL2	63318434	c.233C>T	p.P78L	NA	NA	0			0.0001				9646	10589	11277	8435	10347	11760			1			
chr18	BCL2	63318438	c.229G>A	p.A77T	NA	NA	0					0.0001		9554	10497	11123	8351	10180	11653					1	
chr18	BCL2	63318441	c.226G>A	p.A76T	NA	NA	0						0.0001	9451	10324	10891	8296	10062	11551						1
chr18	BCL2	63318445	c.222C>T	p.T74=	NA	NA	0		0.0001				0.0001	9501	10370	10911	8283	10104	11626		1				1
chr18	BCL2	63318454	c.213G>C	p.P71=	NA	NA	0			0.0001				9263	10126	10513	8024	9685	11324			1			
chr18	BCL2	63318468	c.199G>A	p.A67T	NA	NA	0		0.0001				0.0001	8955	9905	10171	7782	9321	11087		1				
chr18	BCL2	63318471	c.178_196del	p.A60Sfs*30	NA	NA	0				0.0001			8836	9822	10013	7677	9154	10954				1		
chr18	BCL2	63318473	c.194C>T	p.P65L	NA	NA	0	0.0001						8815	9765	9931	7646	9098	10902	1					
chr18	BCL2	63318474	c.193C>T	p.P65S	NA	NA	0				0.0001			8790	9725	9889	7616	9051	10865				1		
chr18	BCL2	63318488	c.179C>T	p.A60V	NA	NA	0	0.0001	0.0001					8128	9055	9143	7028	8374	10148	1	1				
chr18	BCL2	63318492	c.175C>T	p.P59S	NA	NA	0				0.0001			7891	8870	8900	6843	8171	9911				1		
chr18	BCL2	63318510	c.157C>T	p.P53S	NA	NA	0	0.0001						7125	7921	8000	6103	7338	8877	1					
chr18	BCL2	63318511	c.156G>A	p.Q52=	NA	NA	0		0.0001				0.0001	7038	7833	7876	6046	7222	8751		1				1
chr18	BCL2	63318533	c.134C>T	p.A45V	NA	NA	0			0.0001				6006	6630	6668	5158	6061	7450			1			
chr18	BCL2	63318534	c.133G>A	p.A45T	NA	NA	0		0.0002					5977	6581	6624	5117	6010	7408		1				
chr18	BCL2	63318567	c.100G>A	p.E34N	NA	NA	0						0.0002	4634	5102	5065	3970	4653	5906						1
chr18	BCL2	63318572	c.95C>T	p.A32V	NA	NA	0		0.0002					4386	4802	4765	3748	4401	5294		1				
chr18	BCL2	63318586	c.81C>T	p.G27=	NA	NA	0			0.0003				3634	3973	3884	3099	3604	4416			1			
chr18	BCL2	63318600	c.67C>T	p.L23=	NA	NA	0		0.0003				0.0003	3021	3337	3151	2556	2872	3687		1				1
chr18	BCL2	63318633	c.34C>T	p.R12W	NA	NA	0			0.0005				1938	2440	1933	1672	1652	2530			1			
chr18	BCL2	63318634	c.33C>T	p.N11=	NA	NA	0				0.0006		0.0004	1928	2461	1917	1670	1641	2532				1		1
chr18	BCL2	63318640	c.27C>T	p.Y9=	NA	NA	0						0.0004	1749	2314	1725	1557	1447	2391						1
chr18	BCL2	63318663	c.4G>A	p.A2T	NA	NA	0				0.0007	0.0009		1443	2049	1389	1344	1165	2117				1	1	
chrX	BTK	101356176	c.1442G>C	p.C481S	YES	YES	4			0.0161	0.1061	0.1052	0.0064	14454	13976	15308	11565	15877	15332			247	1227	1671	98
chrX	BTK	101356177	c.1441T>A	p.C481S	YES	YES	4			0.0005	0.0034	0.0028	0.0005	14467	13988	15333	11585	15883	15371			8	39	45	8
chrX	BTK	101354678	c.1538T>G	p.L289W	YES	YES	3			0.0001	0.0006	0.0007	0.1677	13797	13316	14459	12013	15190	16022			1	7	11	2687
chrX	BTK	101354652	c.1609G>T	p.V537L	NO	YES	1						0.0001	13584	13233	14320	11922	14911	16376						2
chrX	BTK	101354639	c.1622G>T	p.G541V	NA	NA	0						0.0001	13507	13007	14105	11726	14722	16510						1
chrX	BTK	101354640	c.1621G>T	p.G541C	NA	NA	0					0.0001		13499	13021	14098	11714	14754	16457					1	
chrX	BTK	101354657	c.1604T>C	p.V535A	NA	NA	0				0.0001			13588	13186	14295	11927	14859	16138				1		
chrX	BTK	101356127	c.1491G>A	p.Q497=	NA	NA	0					0.0001		13433	12987	14149	9809	14835	12886					1	
chrX	BTK	101356128	c.1490A>T	p.Q497L	NA	NA	0			0.0001				13946	12887	14063	9778	14574	12819			1			
chrX	BTK	101356141	c.1477T>C	p.F493L	NA	NA	0	0.0001						13594	12977	14298	10321	14818	13564	1					
chrX	BTK	101356144	c.1476C>T	p.R492C	NA	NA	0					0.0001		13688	13077	14393	10487	14916	13705					1	
chrX	BTK	101356157	c.1461G>T	p.R487S	NA	NA	0					0.0001		13957	13402	14664	10948	15253	14338					1	
chrX	BTK	101356174	c.1444C>T	p.L482F	NA	NA	0				0.0001			14499	13963	15284	11513	15859	15280				1		
chrX	BTK	101356176	c.1442C>A	p.C481Y	YES	YES	0					0.0001		14454	13976	15308	11565	15877	15332					1	
chrX	BTK	101356215	c.1403G>A	p.R468H	NA	NA	0			0.0001				13968	13646	14774	11164	15298	15107			1			
chrX	BTK	101356284	c.1354C>T	p.L452F	NA	NA	0						0.0001	12008	12076	12657	9210	13340	13221						1
chrX	BTK	101358416	c.996T>G	p.R332=	NA	NA	0	0.0003						3983	4163	3992	3138	4207	4681	1					
chrX	BTK	101358433	c.979C>T	p.P327S	NA	NA	0					0.0002		4642	4819	4795	3750	5015	5526					1	
chrX	BTK	101358671	c.920G>T	p.R307I	NA	NA	0						0.0001	12980	12686	13396	11062	14300	14766						1
chrX	BTK	101358695	c.896G>T	p.G299V	NA	NA	0		0.0001					11492	11310	11806	9564	12667	13363		1				
chr16	PLCG2	81919549	c.2120C>T	p.S707F	NO	YES	4				0.0002	0.0007	0.0011	0.0006	13604	13212	15103	11062	14225	15577			3	8	15	10
chr16	PLCG2	81928578	c.2535A>C	p.L845F	NO	YES	4				0.0006	0.0048	0.0051	0.0418	11968	12129	13362	9622	12886	15105			8	46	66	631
chr16	PLCG2	81928580	c.2537G>T	p.G846V	NA	NA	4				0.0007	0.0022	0.0031	0.0013	11979	12122	13350	9624	12912	15100			9	21	40	20
chr16	PLCG2	81936300	c.2976G>T	p.V992F	NA	NA	4				0.0003	0.0007	0.0004	0.0028	13137	12779	14553	10216	13953	14806			4	7	5	41
chr16	PLCG2	81936303	c.2977G>A	p.D993N	NA	NA	4				0.0001	0.0003	0.0003	0.0029	13675	12744	14503	10182	13929	14712			2	3	4	43
chr16	PLCG2	81936303	c.2977G>C	p.D993H	NA	NA	4				0.0001	0.0002	0.0004	0.0008	13675	12744	14503	10182	13929	14712			2	2	6	12
chr16	PLCG2	81912655	c.1993C>T	p.R665W	NA	NA	3				0.0001	0.0008	0.0007	0.0030	13466	13346	14956	10681	13729	14762			1	9	9	44
chr16	PLCG2	81928578	c.2535A>T	p.L845F	NO	YES	3					0.0003	0.0003	0.0001	11968	12129	13362	9622	12886	15105				3	4	2
chr16	PLCG2	81928574	c.2531C>T	p.P844L	NA	NA	2				0.0001		0.0002	0.0006	11994	12192	13414	9656	12915	15190			1		2	9
chr16	PLCG2	81936304	c.2978A>T	p.D993V	NA	NA	2				0.0001		0.0001	0.0013	13000	12723	14448	10133	13862	14652			1		2	19
chr16	PLCG2	81928574	c.2531C>G	p.P844R	NA	NA	1						0.0002	11994	12192	13414	9656	12915	15190						3
chr16	PLCG2	81928580	c.2537G>A	p.G846E	NA	NA	1						0.0001	11979	12122	13350	9624	12912	15100						2
chr16	PLCG2	81936295	c.2969A>T	p.Q990L	NA	NA	1						0.0002	13106	12818	14521	10202	14006	14829						3
chr16	PLCG2	81912645	c.1983G>C	p.M661I	NA	NA	0						0.0001	13314	13219	14809	10534	13580	14602						1
chr16	PLCG2	81912656	c.1994G>A	p.R665Q	NA	NA	0						0.0001	13401	13340	14958	10679	13728	14768						1
chr16	PLCG2	81919518	c.2089C>T	p.R697W	NA	NA	0			0.0001				12654	12480	14158	10252	13317	14977			1			
chr16	PLCG2	81919549	c.2122_2124del	p.A708del	NA	NA	0					0.0001	0.0001	13604	13212	15103	11062	14225	15577					1	1
chr16	PLCG2	81919650	c.2221G>T	p.E741*	NA	NA	0					0.0001		12460	12282	13607	9457	13052	14416					1	
chr16	PLCG2	81919654	c.2225G>C	p.R742P	NA	NA	0						0.0001	12218	12060	13379	9285	12800	14243						1
chr16	PLCG2	81928572	c.2533T>A	p.N845K	NA	NA	0						0.0001	12071	12297	13529	9786	12996	13315						1
chr16	PLCG2	81928576	c.2533T>G	p.L845V	NO	YES	0				0.0001	0.0001	0.0001	11953	12121	13347	9628	12885	15113				1	1	1
chr16	PLCG2	81928579	c.2536G>A	p.G846R	NA	NA	0						0.0001	11988	12114	13369	9636	12901	15098						1
chr16	PLCG2	81928579	c.2536G>T	p.G846W	NA	NA	0						0.0001	11988	12114	13369	9636	12901	15098						1
chr16	PLCG2	81928586	c.2543T>G	p.L848R	NA	NA	0						0.0001	11943	12035	13250	9493	12835	14937						1
chr16	PLCG2	81928588	c.2545T>C	p.C849R	NA	NA	0			0.0001			0.0001	11804	11927	13131	9400	12674	14735			1			1
chr16	PLCG2	81928589	c.2546G>T	p.C849F	NA	NA	0					0.0001		11807	11916	13120	9386	12665	14732					1	
chr16	PLCG2	81936295	c.2969A>G	p.Q990R	NA	NA	0						0.0001	13106	12818	14521	10202	14006	14829						1
chr16	PLCG2	81936304	c.2978A>C	p.D993A	NA	NA	0						0.0001	13000	12723	14448	10133	13862	14652						1
chr16	PLCG2	81936308	c.2982T>G	p.S994=	NA	NA	0						0.0001	12926	12628	14372	10035	13820	14577						1
chr16	PLCG2	81936340	c.3014G>A	p.C1005Y	NA	NA	0						0.0001	12239	12047	13532	9320	13177	13976						1
chr16	PLCG2	81936373	c.3047C>A	p.T1016K	NA	NA	0				0.0001		0.0001	11442	11488	12348	8122	12149	13043				1		1
chr16	PLCG2	81936373	c.3047C>T	p.T1016M	NA	NA	0		0.0001					11442	11488	12348	8122	12149	13043		1				
chr17	TP53	7675094	c.518T>G	p.V173G	NA	NA	6	0.0206	0.0523	0.0227	0.0193	0.0215	0.0015	11034	10680	11875	7907	9969	8720	227	559	269	153	214	13
chr17	TP53	7674191	c.772G>A	p.E258K	NA	NA	5	0.0002	0.0008	0.0003	0.0003	0.0003		10920	10483	11610	7510	9578	9105	2	8	4	2	3	
chr17	TP53	7674263	c.700T>A	p.Y234N	NA	NA	5	0.0013	0.0035	0.0005	0.0006	0.0014	0.0001	8969	8916	9359	6167	7843	7718	12	31	5	4	11	1
chr17	TP53	7675093	c.532C>G	p.H178D	NA	NA	5		0.0773	0.1301	0.1701	0.1761	0.2785	11208	10759	11910	7995	9944	8833		832	1549	1390	1751	2460
chr17	TP53	7674262	c.701A>G	p.Y234C	NA	NA	2	0.0001	0.0001	0.0002		0.0003		8995	8998	9469	6227	7909	7792	1	1	2		2	
chr17	TP53	7675107	c.501_505del	p.Q167Hfs*12	NA	NA	1						0.0196	11269	10807	12159	8043	10216	8960						176
chr17	TP53	7669626	c.1165G>T	p.G389W	NA	NA	0		0.0001					8388	8360	8900	6547	7373	8851		1				
chr17	TP53	7670682	c.1027G>C	p.E343Q	NA	NA	0				0.0002			9591	9156	10102	6579	8933	8408				1		
chr17	TP53	7673728	c.892G>C	p.E298Q	NA	NA	0						0.0001	12050	11527	12860	8658	11176	9716						1
chr17	TP53	7673802	c.818G>A	p.R273H	NA	NA	0		0.0001	0.0001				11664	11324	12648	8237	10503	9397		1	1			
chr17	TP53	7673803	c.817C>T	p.R273C	NA	NA	0				0.0001			11680	11313	12649	8198	10525	9383				1		
chr17	TP53	7673816	c.804C>G	p.N268K	NA	NA	0			0.0001	0.0001			11404	11081	12416	8003	10312	9218			1	1		
chr17	TP53	7673820	c.800G>A	p.R267Q	NA	NA	0				0.0001			11386	11086	12441	7958	10298	9246				1		
chr17	TP53	7673838	c.783-1G>A	p.X261_splice	NA	NA	0				0.0001	0.0001		11151	10891	12311	7746	10040	9142				1	1	
chr17	TP53	7674194	c.769C>G	p.L257V	NA	NA	0	0.0001						10934	10500	11597	7543	9599	9079	1					
chr17	TP53	7674242	c.721T>A	p.S241T	NA	NA	0	0.0001						10009	9875	10563	6981	8859	8562	1					
chr17	TP53	7674274	c.689C>A	p.T230N	NA	NA	0			0.0001				8065	8049	8501	5594	7090	6968			1			
chr17	TP53	7674872	c.659A>G	p.Y220C	NA	NA	0	0.0001						10457	10380	11148	7048	9413	8923	1					
chr17	TP53	7674899	c.632C>T	p.T211I	NA	NA	0						0.0001	10039	9924	10856	7017	9049	8359						1
chr17	TP53	7674959	c.572C>G	p.P191R	NA	NA	0	0.0001						11204	10791	12138	7919	10046	9012	1					
chr17	TP53	7675088	c.524G>A	p.R175H	NA	NA	0	0.0001						11109	10730	11970	7945	9961	8836	1					
chr17	TP53	7675185	c.427G>T	p.V143L	NA	NA	0						0.0001	11298	11030	12284	7946	10130	9148						1
chr17	TP53	7675198	c.414C>T	p.A138=	NA	NA	0			0.0001				11131	10928	12162	7908	10039	9112			1			
chr17	TP53	7675224	c.388C>T	p.L130F	NA	NA	0						0.0001	10825	10710	11722	7536	9504	9040						1
chr17	TP53	7676007	c.362C>A	p.S121Y	NA	NA	0			0.0001				11251	11048	12267	8347	10287	9619			1			
chr17	TP53	7676073	c.296C>T	p.S99F	NA	NA	0						0.0001	11097	10981	11925	8314	10157	9426						1
chr17	TP53	7676096	c.273G>T	p.W91C	NA	NA	0	0.0001						11526	11395	12353	8491	10394	9701		1				
chr17	TP53	7676099	c.270C>G	p.S90=	NA	NA	0						0.0001	11600	11431	12399	8509	10466	9757						1
chr17	TP53	7676100	c.269C>T	p.S90F	NA	NA	0			0.0001				11712	11557	12520	8621	10582	9862			1			
chr17	TP53	7676177	c.192C>G	p.P64=	NA	NA	0						0.0001	11633	11396	12546	8461	10408	9871						1
chr17	TP53	7676396	c.82G>T	p.E28*	NA	NA	0						0.0001	10064	9645	11035	7437	8991	8915						1

Abbreviations: Human Genome Variation Society nomenclature (HGVSc); Variant allele frequency (VAF).

* BTKi resistance mutations and codons according to Kittai et al.[6]

† Samples R001-D and R001-E are technical replicates of the same specimen.

Supplemental Table S9. List of coding mutations detected in samples from patient R002. The asterisks in the column "HGVSp Short" are standard nomenclature for amino acid variants and do not represent footnotes. VAF > 0.01 are color coded in red/pink.

Chromosome	Gene	Start Position	HGVSc	HGVSp Short	BTKi resistance mutation*	BTKi resistance codon*	Number of samples with >1 mutant molecules	R002-A VAF	R002-B VAF	R002-C VAF	R002-D VAF	R002-E VAF	R002-A duplex depth	R002-B duplex depth	R002-C duplex depth	R002-D duplex depth	R002-E duplex depth	R002-A duplex mutant molecules	R002-B duplex mutant molecules	R002-C duplex mutant molecules	R002-D duplex mutant molecules	R002-E duplex mutant molecules
chr19	BAX	48955709	c.109C>T	p.R37*	NA	NA	4		0.0003	0.0010	0.0003	0.0003	9706	9822	8385	8739	7802		3	8	3	2
chr19	BAX	48955713	c.121dup	p.E41Gfs*33	NA	NA	4	0.0118		0.8753	0.8253	0.9947	9814	9858	8460	8817	7859	116		7474	7349	7909
chr19	BAX	48955713	c.120_121dup	p.E41Gfs*20	NA	NA	3			0.0020	0.0025	0.0024	9814	9858	8460	8817	7859			17	22	19
chr19	BAX	48955714	c.121del	p.E41Rfs*19	NA	NA	3	0.0002		0.0006	0.0002	0.0001	9750	9822	8456	8802	7873	2		5	2	1
chr19	BAX	48955780	c.180C>T	p.S60=	NA	NA	2	0.0002	0.0010	0.0001		0.0001	9571	9983	9418	9581	9571	2	10	1		1
chr19	BAX	48955579	c.66_67del	p.A24Pfs*49	NA	NA	0	0.0003		0.0003			3168	3211	3122	3204	2378	1		1		
chr19	BAX	48955583	c.70G>T	p.A24S	NA	NA	0					0.0004	3466	3485	3392	3463	2637					1
chr19	BAX	48955596	c.86+1dup	NA	NA	NA	0					0.0003	4172	4191	3991	4110	3257					1
chr19	BAX	48955597	c.84G>T	p.Q28H	NA	NA	0					0.0003	4234	4259	4062	4172	3314					1
chr19	BAX	48955775	c.175C>G	p.L59V	NA	NA	0		0.0001				9942	9981	9388	9557	9515		1			
chr19	BAX	48955785	c.185_192del	p.G62Sfs*9	NA	NA	0	0.0001					9838	9879	9378	9534	9498	1				
chr19	BAX	48955799	c.199G>A	p.G67R	NA	NA	0	0.0001					10001	9978	9711	9839	9776	1				
chr19	BAX	48960824	c.385del	p.V129Cfs*4	NA	NA	0	0.0002					4569	4249	4431	4242	4121	1				
chr19	BAX	48960873	c.433C>T	p.R145W	NA	NA	0	0.0002					5894	5465	5655	5594	5895	1				
chr19	BAX	48960915	c.475G>A	p.V159M	NA	NA	0	0.0001					6671	6151	6344	6430	7007	1				
chr19	BAX	48960968	c.528T>A	p.P176=	NA	NA	0		0.0001		0.0001		7510	6972	7213	7181	7973		1		1	
chr19	BAX	48960982	c.542T>A	p.L181Q	NA	NA	0		0.0001				8217	7707	7964	7858	8587		1			
chr19	BAX	48960983	c.549dup	p.A184Rfs*29	NA	NA	0			0.0001			8171	7679	7912	7822	8553			1		
chr19	BAX	48961076	c.636T>A	p.N212K	NA	NA	0					0.0001	8290	8072	8139	8247	8059					1
chr19	BAX	48961080	c.640T>C	p.F214L	NA	NA	0				0.0001		8041	7846	7926	8040	7782				1	
chr18	BCL2	63318259	c.408G>T	p.E136D	NA	NA	0				0.0002		5171	5298	5132	4867	5555				1	
chr18	BCL2	63318338	c.309C>T	p.D103=	NA	NA	0					0.0001	6947	7064	6785	6112	8963					1
chr18	BCL2	63318411	c.256C>T	p.L86F	NA	NA	0					0.0001	7213	7095	6759	6203	9250					1
chr18	BCL2	63318426	c.241G>A	p.A81T	NA	NA	0					0.0001	7138	7075	6748	6284	9115					1
chr18	BCL2	63318438	c.229G>T	p.A77S	NA	NA	0					0.0001	6897	6785	6517	6150	8682					1
chr18	BCL2	63318560	c.107G>T	p.G36V	NA	NA	0					0.0002	4190	3526	3471	2765	4472					1
chr18	BCL2	63318600	c.67C>T	p.L23=	NA	NA	0				0.0007		2438	1965	1968	1500	2355				1	
chrX	BTK	101356168	c.1450A>G	p.N484D	NA	NA	3			0.0087	0.0030	0.0006	6436	6573	6904	6776	6542			60	20	4
chrX	BTK	101356177	c.1441T>C	p.C481R	YES	YES	3			0.0023	0.0092	0.0031	6386	6554	6865	6764	6543			16	62	20
chrX	BTK	101356197	c.1421C>T	p.T474I	NO	YES	3			0.0003	0.0388	0.0203	6376	6532	6816	6796	6606			2	262	134
chrX	BTK	101358689	c.902A>G	p.E301G	NA	NA	3			0.0003	0.0005	0.0003	6009	5941	6157	6142	5915			2	3	2
chrX	BTK	101356157	c.1421C>A	p.T474N	NO	YES	2				0.0010	0.0003	6376	6532	6816	6796	6606				7	2
chrX	BTK	101356242	c.1376A>C	p.Q459P	NA	NA	2				0.0005	0.0003	5849	6057	6362	6438	5859				3	2
chrX	BTK	101358645	c.946A>G	p.T316A	YES	YES	2				0.0018	0.0022	6430	6265	6622	6505	6772				12	15
chrX	BTK	101354679	c.1582T>G	p.L528V	NO	YES	1				0.0003		7122	6905	7415	6959	6851				2	
chrX	BTK	101356176	c.1442G>C	p.C481S	YES	YES	1			0.0003	0.0001	0.0002	6363	6557	6854	6753	6516			2	1	1
chrX	BTK	101356239	c.1379T>C	p.L460S	NA	NA	1				0.0005		5954	6140	6446	6550	5940				3	
chrX	BTK	101356250	c.1368G>T	p.K456N	NA	NA	1					0.0005	5927	6066	6389	6495	5801					3
chrX	BTK	101354640	c.1621G>A	p.G541S	NA	NA	0				0.0001		7525	7305	7698	7370	6661				1	
chrX	BTK	101354645	c.1616A>G	p.D539G	NA	NA	0					0.0001	7516	7277	7720	7340	6726					1
chrX	BTK	101354678	c.1583T>C	p.L528S	NO	YES	0					0.0001	7136	6920	7420	6982	6846					1
chrX	BTK	101356123	c.1495C>T	p.L499=	NA	NA	0		0.0002				5837	6219	6480	6387	5800		1			
chrX	BTK	101356209	c.1405T>A	p.L470N	NA	NA	0				0.0002		6185	6335	6602	6610	6315				1	
chrX	BTK	101356210	c.1408A>G	p.I470V	NA	NA	0				0.0002		6186	6335	6600	6589	6297				1	
chrX	BTK	101356215	c.1403G>A	p.R468H	NA	NA	0		0.0002				6216	6359	6618	6608	6297			1		
chrX	BTK	101356242	c.1376A>G	p.Q459R	NA	NA	0					0.0002	5849	6057	6362	6438	5859					1
chr16	PLCG2	81912605	c.1943A>G	p.Y648C	NA	NA	2			0.0001	0.0006	0.0009	8437	9002	9037	8931	10575			1	5	9
chr16	PLCG2	81928576	c.2531T>G	p.L845V	NO	YES	2				0.0002	0.0004	12176	12287	11573	12212	9726				2	4
chr16	PLCG2	81919578	c.2149G>A	p.V717I	NA	NA	1		0.0009				12214	11711	11452	11914	12506		10			
chr16	PLCG2	81919626	c.2197T>G	p.Y733D	NA	NA	1			0.0003			11851	11754	11414	11744	11204			3		
chr16	PLCG2	81912628	c.1966G>C	p.A656P	NA	NA	0			0.0001			9039	9353	9482	9363	11287			1		
chr16	PLCG2	81912650	c.1988T>A	p.I663N	NA	NA	0					0.0001	9650	9775	9935	9832	11988					1
chr16	PLCG2	81912655	c.1993C>T	p.R665W	NA	NA	0			0.0001	0.0001		9678	9809	9589	9911	12080			1	1	
chr16	PLCG2	81919503	c.2074C>G	p.H692D	NA	NA	0					0.0001	12200	11692	11536	11769	11747					1
chr16	PLCG2	81919521	c.2092G>T	p.D698Y	NA	NA	0					0.0001	12156	12287	11573	12212	9726					1
chr16	PLCG2	81919548	c.2119T>C	p.S707P	NO	YES	0				0.0001		12749	12098	11921	12360	12867				1	
chr16	PLCG2	81919549	c.2120C>A	p.S707Y	YES	YES	0					0.0001	12714	12065	11904	12339	12883					1
chr16	PLCG2	81919549	c.2120C>T	p.S707F	NO	YES	0				0.0001		12714	12065	11904	12339	12883				1	
chr16	PLCG2	81919551	c.2122G>A	p.A708T	NA	NA	0			0.0001	0.0001	0.0001	12631	12000	11814	12278	12873			1	1	1
chr16	PLCG2	81919554	c.2125T>G	p.Y709D	NA	NA	0					0.0001	12414	11824	11591	12142	12760					1
chr16	PLCG2	81919575	c.2146C>A	p.L716I	NA	NA	0					0.0001	12197	11702	11457	11943	12488					1
chr16	PLCG2	81919645	c.2216T>C	p.L739P	NA	NA	0				0.0001	0.0001	11826	11890	11513	11788	10707				1	1
chr16	PLCG2	81928570	c.2527A>G	p.N843D	NA	NA	0					0.0001	12433	12352	11694	12266	9853					1
chr16	PLCG2	81928572	c.2529T>G	p.N843K	NA	NA	0					0.0001	12487	12399	11705	12329	9848					1
chr16	PLCG2	81928574	c.2531C>A	p.P844H	NA	NA	0				0.0001		12428	12329	11594	12236	9794				1	
chr16	PLCG2	81928577	c.2534T>C	p.L845S	NO	YES	0					0.0001	12393	12335	11593	12231	9760					1
chr16	PLCG2	81928586	c.2543T>G	p.L848R	NA	NA	0					0.0001	12448	12366	11643	12235	9653					1
chr16	PLCG2	81928588	c.2545T>C	p.C849R	NA	NA	0				0.0001		12260	12171	11474	12096	9509				1	
chr16	PLCG2	81928615	c.2572T>C	p.Y858H	NA	NA	0					0.0001	11297	11248	10753	11440	8728					1
chr16	PLCG2	81936192	c.2866C>T	p.R956C	NA	NA	0			0.0001			8819	9027	8925	9403	6804			1		
chr16	PLCG2	81936204	c.2878G>T	p.E960*	NA	NA	0					0.0001	9745	9949	9895	10410	7702					1
chr16	PLCG2	81936220	c.2894G>A	p.S965N	NA	NA	0	0.0001					10597	10820	10765	11257	8615	1				
chr16	PLCG2	81936300	c.2974G>A	p.V992I	NA	NA	0				0.0001		12089	12284	12193	12495	11063				1	
chr16	PLCG2	81936302	c.2976T>C	p.Y992=	NA	NA	0					0.0001	12056	12192	12166	12448	11030					1
chr16	PLCG2	81936303	c.2977G>A	p.D993N	NA	NA	0				0.0001	0.0001	11992	12163	12139	12421	10988				1	1
chr16	PLCG2	81936304	c.2978A>C	p.D993A	NA	NA	0				0.0001		11939	12125	12079	12356	10968				1	
chr16	PLCG2	81936317	c.2991C>T	p.Y997=	NA	NA	0	0.0001					11531	11888	11692	12020	10465	1				
chr16	PLCG2	81936356	c.3030G>C	p.V1010=	NA	NA	0				0.0001		10858	11508	11183	11594	9257				1	
chr17	TP53	7674200	c.763A>T	p.I255F	NA	NA	4	0.0027	0.0002	0.0012	0.0002		12475	12037	12835	12544	12208	34	2	16	3	
chr17	TP53	7674217	c.746G>C	p.R249T	NA	NA	3	0.0003			0.0003	0.0002	11802	11436	12360	11894	11660	4			3	2
chr17	TP53	7674900	c.631A>G	p.T211A	NA	NA	3	0.0032	0.0027	0.0001	0.0009		10813	10886	11803	11402	12025	35	29	1	10	
chr17	TP53	7675205	c.407A>T	p.Q136L	NA	NA	3			0.0002	0.0003	0.0001	11921	11724	12558	12047	13827			3	4	2
chr17	TP53	7675238	c.376-2A>T	p.X126_splice	NA	NA	3			0.0004	0.0003	0.0002	11984	11615	12283	11765	12618			5	3	2
chr17	TP53	7673838	c.783-1G>A	p.X261_splice	NA	NA	1	0.0002					12278	12325	12960	12621	13154	2				
chr17	TP53	7674189	c.774A>C	p.E258D	NA	NA	1			0.0001		0.0003	12545	12074	12919	12598	12236			1		4
chr17	TP53	7674220	c.748G>A	p.R248Q	NA	NA	1	0.0003					11727	11378	12297	11831	11548	3				
chr17	TP53	7675184	c.428T>C	p.V143A	NA	NA	1	0.0005			0.0001	0.0001	12343	12028	12843	12392	14362	6			1	1
chr17	TP53	7675218	c.394A>G	p.K132E	NA	NA	1	0.0002					12168	11859	12613	12133	13577	3				
chr17	TP53	7676552	c.43A>G	p.S15G	NA	NA	1					0.0002	9678	9729	10558	9878	10012					2
chr17	TP53	7669637	c.1154T>G	p.F385C	NA	NA	0	0.0001					10373	10500	10851	10701	8891	1				
chr17	TP53	7669692	c.1101-2A>G	p.X367_splice	NA	NA	0			0.0001			11569	11763	12024	11995	9568			1		
chr17	TP53	7670620	c.1089G>T	p.R363S	NA	NA	0					0.0001	12015	12035	12336	12539	10551					1
chr17	TP53	7670652	c.1057G>A	p.A353T	NA	NA	0					0.0001	12001	12108	12590	12519	10871					1
chr17	TP53	7670672	c.1037A>G	p.E346G	NA	NA	0					0.0001	11462	11709	12150	12098	10417					1
chr17	TP53	7670706	c.1003C>T	p.R335C	NA	NA	0			0.0001			10397	11020	11215	11293	9210			1		
chr17	TP53	7673581	c.947C>A	p.P316H	NA	NA	0					0.0001	14858	14400	14883	14673	16512					1
chr17	TP53	7673595	c.933C>A	p.N311K	NA	NA	0					0.0001	14525	14092	14582	14426	16291					1
chr17	TP53	7673728	c.892G>T	p.E298*	NA	NA	0					0.0001	14356	13556	14541	13976	15792					1
chr17	TP53	7673755	c.865C>A	p.L289I	NA	NA	0					0.0001	13319	12942	13813	13421	14996					1
chr17	TP53	7673765	c.855G>T	p.E285D	NA	NA	0			0.0001			13010	12623	13496	13051	14499			1		
chr17	TP53	7673782	c.838A>G	p.R280G	NA	NA	0	0.0001					12809	12516	13355	12986	14268	1				
chr17	TP53	7673784	c.836G>A	p.G279E	NA	NA	0		0.0001				12943	12652	13491	13098	14409		1			
chr17	TP53	7673790	c.830G>T	p.C277F	NA	NA	0	0.0001					12886	12685	13508	13200	14453	1				
chr17	TP53	7673803	c.817C>T	p.R273C	NA	NA	0			0.0001			12924	12846	13588	13321	14310			1		
chr17	TP53	7673837	c.783T>C	p.S261=	NA	NA	0					0.0001	12303	12356	12996	12633	13198					1
chr17	TP53	7674180	c.782+1G>A	p.X261_splice	NA	NA	0					0.0001	12718	12139	13074	12775	12275					1
chr17	TP53	7674182	c.781A>T	p.S261C	NA	NA	0					0.0001	12666	12119	13040	12750	12241					1
chr17	TP53	7674199	c.764T>C	p.I255T	NA	NA	0		0.0001				12565	12119	12940	12665	12270		1			
chr17	TP53	7674221	c.742C>T	p.R248W	NA	NA	0		0.0001				11771	11476	12399	11965	11587		1			
chr17	TP53	7674223	c.740A>C	p.N247T	NA	NA	0				0.0001		11633	11326	12223	11807	11501				1	
chr17	TP53	7674233	c.730G>A	p.G244S	NA	NA	0	0.0001	0.0001				11656	11351	12296	11752	11330	1	1			
chr17	TP53	7674246	c.717C>A	p.N239K	NA	NA	0	0.0001					11139	11051	11860	11424	10751	1				
chr17	TP53	7674250	c.713G>A	p.C238Y	NA	NA	0		0.0001				11159	11116	11931	11437	10736		1			
chr17	TP53	7674251	c.712T>C	p.C238R	NA	NA	0			0.0001			11011	10981	11791	11354	10597			1		
chr17	TP53	7674275	c.688A>G	p.T230A	NA	NA	0				0.0001		9065	8987	9658	9248	8618				1	
chr17	TP53	7674281	c.682G>A	p.D228N	NA	NA	0		0.0001				8525	8468	9130	8710	8068		1			
chr17	TP53	7674868	c.663G>T	p.R221D	NA	NA	0				0.0001		11454	11544	12347	11884	11588				1	
chr17	TP53	7674885	c.646G>A	p.V216M	NA	NA	0				0.0001		11092	11074	12011	11573	11738				1	
chr17	TP53	7674927	c.604C>T	p.R202C	NA	NA	0	0.0001	0.0001				11263	11335	12203	11827	13140	1	1			
chr17	TP53	7674937	c.585_594delinsT	p.R196_E198del	NA	NA	0	0.0001					11327	11510	12294	11946	13354	1				
chr17	TP53	7674950	c.581T>A	p.L194H	NA	NA	0	0.0001					11604	11573	12510	12095	13840	1				
chr17	TP53	7674956	c.575A>G	p.Q192R	NA	NA	0				0.0001		11802	11823	12735	12333	14214				1	
chr17	TP53	7674964	c.567C>A	p.A189=	NA	NA	0					0.0001	12166	12093	13012	12560	14600					1
chr17	TP53	7675063	c.549A>T	p.S183=	NA	NA	0					0.0001	11622	11711	12612	12080	14808					1
chr17	TP53	7675064	c.548C>G	p.S183*	NA	NA	0	0.0001					11586	11680	12570	12048	14793	1				
chr17	TP53	7675077	c.535C>T	p.H179Y	NA	NA	0	0.0001					11680	11680	12558	12155	14792	1				
chr17	TP53	7675086	c.526T>C	p.C176R	NA	NA	0					0.0001	11576	11577	12338	12067	14766					1
chr17	TP53	7675089	c.523C>T	p.R175C	NA	NA	0			0.0001			11496	11490	12296	12036	14667			1		
chr17	TP53	7675098	c.514G>T	p.V172F	NA	NA	0	0.0001					11393	11374	12170	11943	14554	1				
chr17	TP53	7675108	c.504C>A	p.H168Q	NA	NA	0					0.0001	11356	11445	12236	11969	14656					1
chr17	TP53	7675126	c.486C>T	p.I162=	NA	NA	0				0.0001		10793	10929	11684	11549	14044				1	
chr17	TP53	7675145	c.467G>A	p.R156H	NA	NA	0			0.0001			10823	10760	11585	11371	13374			1		
chr17	TP53	7675204	c.408A>G	p.Q136=	NA	NA	0		0.0001				12036	11827	12676	12174	13995		1			
chr17	TP53	7675208	c.404G>A	p.C135Y	NA	NA	0		0.0001				12032	11823	12599	12068	13781		1			
chr17	TP53	7675993	c.375+1G>T	p.X125_splice	NA	NA	0	0.0001					12781	12830	13783	13314	13542	1				
chr17	TP53	7676018	c.351G>C	p.G117=	NA	NA	0		0.0001				12429	12513	13360	13091	13572		1			
chr17	TP53	7676077	c.292C>A	p.P98T	NA	NA	0					0.0001	12168	12068	13162	12711	13679					1
chr17	TP53	7676097	c.272G>C	p.W91S	NA	NA	0	0.0001					13010	12706	13796	13354	14376	1				
chr17	TP53	7676170	c.199C>T	p.P67S	NA	NA	0		0.0001				13453	12802	13881	13431	15097		1			

Abbreviations: Human Genome Variation Society nomenclature (HGVSc); Variant allele frequency (VAF).
* BTKi resistance mutations and codons according to Kittai et al.[6]

Supplemental Table S10. Comparison of copy number alterations identified by chromosomal genomic array testing (CGAT) and duplex sequencing.

	BTK		*BAX*		*PLCG2*		*BCL2*		*TP53*	
Samples	**CGAT (%)**	**Duplex SNP-LOH (%)**	**CGAT (%)**	**Duplex SNP-LOH (%)**	**CGAT (%)**	**Duplex SNP-LOH (%)**	**CGAT (%)**	**Duplex SNP-LOH (%)**	**CGAT (%)**	**Duplex SNP-LOH (%)**
R001-A	0	no LOH	0	no LOH	0	no LOH	0	NA	del 17p, 60.0	51.3
R001-B	0	no LOH	0	no LOH	0	no LOH	0	NA	del 17p, 60.0	57.3
R001-C	NP	no LOH	NP	no LOH	NP	no LOH	NP	NA	NP	56.1
R001-D	0	no LOH	0	no LOH	0	no LOH	0	NA	del 17p, 80.0	81.8
R001-E	0	no LOH	0	no LOH	0	no LOH	0	NA	del 17p, 80.0	87.1
R001-F	NP	no LOH	NP	no LOH	NP	no LOH	NP	NA	NP	91.1
R002-A	NP	NA	NP	no LOH	NP	no LOH	NP	NA	NP	NA
R002-B	NP	NA	NP	no LOH	NP	no LOH	NP	NA	NP	NA
R002-C	0	NA	0	no LOH	0	no LOH	0	NA	0%	NA
R002-D	NP	NA	NP	no LOH	NP	no LOH	NP	NA	NP	NA
R002-E	NP	NA	NP	no LOH	NP	no LOH	NP	NA	NP	NA

Abbreviations: Chromosomal Genomic Array Testing (CGAT); Single nucleotide polymorphisms (SNP); Loss of heterozygosity (LOH); Cancer cell fraction (CCF); Not available because no heterozygous SNPs were sequenced (NA); Not performed (NP).

Supplemental Table S11. Patient R001 cancer cell fraction (CCF) for variants with two or more mutant molecules in at least one sample.

Patient	Sample	Gene	Chromosome	Position	Reference allele	Alternate allele	Mutation type	HGVSp short	HGVSc	Duplex depth	Duplex mutant reads	VAF	Variant CCF	Days post-pirtobrutinib	TP53 LOH CCF	Sample type	Percent disease (%) (Tumor purity)
R001	B	BAX	chr19	48955799	G	A	Missense	p.G67R	c.199G>A	12179	24	0.0020	0.0070	-294	NA	PB	56.0
R001	C	BAX	chr19	48955799	G	A	Missense	p.G67R	c.199G>A	13874	11	0.0008	0.0023	-15	NA	PB	70.0
R001	A	BCL2	chr18	63318200	A	T	Missense	p.V156D	c.467T>A	4095	12	0.0029	0.0095	-687	NA	PB	62.0
R001	A	BCL2	chr18	63318262	C	T	Silent	p.E135=	c.405G>A	7311	1	0.0001	0.0004	-687	NA	PB	62.0
R001	C	BCL2	chr18	63318262	C	T	Silent	p.E135=	c.405G>A	8593	1	0.0001	0.0003	-15	NA	PB	70.0
R001	F	BCL2	chr18	63318262	C	T	Silent	p.E135=	c.405G>A	8310	2	0.0002	0.0005	161	NA	PB	95.0
R001	A	BCL2	chr18	63318264	CCACCAC	-	Indel	p.V133Rfs*8	c.397_403del	7376	2	0.0003	0.0009	-687	NA	PB	62.0
R001	A	BCL2	chr18	63318329	G	C	Missense	p.A113G	c.338C>G	9737	11	0.0011	0.0036	-687	NA	PB	62.0
R001	A	BCL2	chr18	63318329	G	A	Missense	p.A113V	c.338C>T	9737	2	0.0002	0.0007	-687	NA	PB	62.0
R001	B	BCL2	chr18	63318329	G	C	Missense	p.A113G	c.338C>G	11047	3	0.0003	0.0010	-294	NA	PB	56.0
R001	B	BCL2	chr18	63318329	G	A	Missense	p.A113V	c.338C>T	11047	1	0.0001	0.0003	-294	NA	PB	56.0
R001	F	BCL2	chr18	63318329	G	C	Missense	p.A113G	c.338C>G	11261	1	0.0001	0.0002	161	NA	PB	95.0
R001	A	BCL2	chr18	63318336	-	GGCGGTAG	Indel	p.R107_R110dup	c.319_330dup	9789	38	0.0039	0.0125	-687	NA	PB	62.0
R001	B	BCL2	chr18	63318336	-	GGCGGTAG	Indel	p.R107_R110dup	c.319_330dup	11016	12	0.0011	0.0039	-294	NA	PB	56.0
R001	C	BCL2	chr18	63318336	-	GGCGGTAG	Indel	p.R107_R110dup	c.319_330dup	11806	12	0.0010	0.0029	-15	NA	PB	70.0
R001	F	BCL2	chr18	63318411	G	A	Missense	p.L86F	c.256C>T	12137	2	0.0002	0.0003	161	NA	PB	95.0
R001	F	BCL2	chr18	63318531	G	A	Missense	p.P46S	c.136C>T	7666	2	0.0003	0.0005	161	NA	PB	95.0
R001	F	BCL2	chr18	63318601	C	T	Silent	p.K22=	c.66G>A	3677	3	0.0008	0.0017	161	NA	PB	95.0
R001	F	BTK	chrX	101354652	C	A	Missense	p.V537L	c.1609G>T	16376	2	0.0001	0.0003	161	NA	PB	95.0
R001	C	BTK	chrX	101354678	A	C	Missense	p.L528W	c.1583T>G	14459	1	0.0001	0.0002	-15	NA	PB	70.0
R001	F	BTK	chrX	101354678	A	C	Missense	p.L528W	c.1583T>G	16022	2687	0.1677	0.3531	161	NA	PB	95.0
R001	C	BTK	chrX	101356176	C	G	Missense	p.C481S	c.1442G>C	15308	247	0.0161	0.0461	-15	NA	PB	70.0
R001	F	BTK	chrX	101356176	C	G	Missense	p.C481S	c.1442G>C	15332	98	0.0064	0.0135	161	NA	PB	95.0
R001	C	BTK	chrX	101356177	A	T	Missense	p.C481S	c.1441T>A	15333	8	0.0005	0.0015	-15	NA	PB	70.0
R001	F	BTK	chrX	101356177	A	T	Missense	p.C481S	c.1441T>A	15371	8	0.0005	0.0011	161	NA	PB	95.0
R001	C	PLCG2	chr16	81912655	C	T	Missense	p.R665W	c.1993C>T	14956	1	0.0001	0.0002	-15	NA	PB	70.0
R001	F	PLCG2	chr16	81912655	C	T	Missense	p.R665W	c.1993C>T	14762	44	0.0030	0.0063	161	NA	PB	95.0
R001	C	PLCG2	chr16	81919549	C	T	Missense	p.S707F	c.2120C>T	15103	3	0.0002	0.0006	-15	NA	PB	70.0
R001	F	PLCG2	chr16	81919549	C	T	Missense	p.S707F	c.2120C>T	15577	10	0.0006	0.0014	161	NA	PB	95.0
R001	C	PLCG2	chr16	81928574	C	T	Missense	p.P844L	c.2531C>T	13414	1	0.0001	0.0002	-15	NA	PB	70.0
R001	F	PLCG2	chr16	81928574	C	T	Missense	p.P844L	c.2531C>T	15190	9	0.0006	0.0012	161	NA	PB	95.0
R001	F	PLCG2	chr16	81928574	C	G	Missense	p.P844R	c.2531C>G	15190	3	0.0002	0.0004	161	NA	PB	95.0
R001	C	PLCG2	chr16	81928578	A	C	Missense	p.L845F	c.2535A>C	13362	8	0.0006	0.0017	-15	NA	PB	70.0
R001	F	PLCG2	chr16	81928578	A	C	Missense	p.L845F	c.2535A>C	15105	631	0.0418	0.0879	161	NA	PB	95.0
R001	F	PLCG2	chr16	81928578	A	T	Missense	p.L845F	c.2535A>T	15105	2	0.0001	0.0003	161	NA	PB	95.0
R001	C	PLCG2	chr16	81928580	G	T	Missense	p.G846V	c.2537G>T	13350	9	0.0007	0.0019	-15	NA	PB	70.0
R001	F	PLCG2	chr16	81928580	G	T	Missense	p.G846V	c.2537G>T	15100	20	0.0013	0.0028	161	NA	PB	95.0
R001	F	PLCG2	chr16	81928580	G	A	Missense	p.G846E	c.2537G>A	15100	2	0.0001	0.0003	161	NA	PB	95.0
R001	F	PLCG2	chr16	81936295	A	T	Missense	p.Q990L	c.2969A>T	14829	3	0.0002	0.0004	161	NA	PB	95.0
R001	C	PLCG2	chr16	81936300	G	T	Missense	p.V992F	c.2974G>T	14553	4	0.0003	0.0008	-15	NA	PB	70.0
R001	F	PLCG2	chr16	81936300	G	T	Missense	p.V992F	c.2974G>T	14806	41	0.0028	0.0058	161	NA	PB	95.0
R001	C	PLCG2	chr16	81936303	G	A	Missense	p.D993N	c.2977G>A	14503	2	0.0001	0.0004	-15	NA	PB	70.0
R001	C	PLCG2	chr16	81936303	G	C	Missense	p.D993H	c.2977G>C	14503	2	0.0001	0.0004	-15	NA	PB	70.0
R001	F	PLCG2	chr16	81936303	G	A	Missense	p.D993N	c.2977G>A	14712	43	0.0029	0.0062	161	NA	PB	95.0
R001	F	PLCG2	chr16	81936303	G	C	Missense	p.D993H	c.2977G>C	14712	12	0.0008	0.0017	161	NA	PB	95.0
R001	C	PLCG2	chr16	81936304	A	T	Missense	p.D993V	c.2978A>T	14448	1	0.0001	0.0002	-15	NA	PB	70.0
R001	F	PLCG2	chr16	81936304	A	T	Missense	p.D993V	c.2978A>T	14652	19	0.0013	0.0027	161	NA	PB	95.0
R001	A	TP53	chr17	7674191	C	T	Missense	p.E258K	c.772G>A	10920	2	0.0002	0.0004	-687	0.8267	PB	62.0
R001	B	TP53	chr17	7674191	C	T	Missense	p.E258K	c.772G>A	10483	8	0.0008	0.0019	-294	1.0000	PB	56.0
R001	C	TP53	chr17	7674191	C	T	Missense	p.E258K	c.772G>A	11610	4	0.0003	0.0007	-15	0.8019	PB	70.0
R001	A	TP53	chr17	7674262	T	C	Missense	p.Y234C	c.701A>G	8995	1	0.0001	0.0003	-687	0.8267	PB	62.0
R001	B	TP53	chr17	7674262	T	C	Missense	p.Y234C	c.701A>G	8998	1	0.0001	0.0003	-294	1.0000	PB	56.0
R001	C	TP53	chr17	7674262	T	C	Missense	p.Y234C	c.701A>G	9469	2	0.0002	0.0004	-15	0.8019	PB	70.0
R001	A	TP53	chr17	7674263	A	T	Missense	p.Y234N	c.700T>A	8909	12	0.0013	0.0032	-687	0.8267	PB	62.0
R001	B	TP53	chr17	7674263	A	T	Missense	p.Y234N	c.700T>A	8916	31	0.0035	0.0089	-294	1.0000	PB	56.0
R001	C	TP53	chr17	7674263	A	T	Missense	p.Y234N	c.700T>A	9359	5	0.0005	0.0011	-15	0.8019	PB	70.0
R001	F	TP53	chr17	7674263	A	T	Missense	p.Y234N	c.700T>A	7718	1	0.0001	0.0001	161	0.9585	PB	95.0
R001	B	TP53	chr17	7675080	G	C	Missense	p.H178D	c.532C>G	10759	832	0.0773	0.1971	-294	1.0000	PB	56.0
R001	C	TP53	chr17	7675080	G	C	Missense	p.H178D	c.532C>G	11910	1549	0.1301	0.2673	-15	0.8019	PB	70.0
R001	F	TP53	chr17	7675080	G	C	Missense	p.H178D	c.532C>G	8833	2460	0.2785	0.3194	161	0.9585	PB	95.0
R001	A	TP53	chr17	7675094	A	C	Missense	p.V173G	c.518T>G	11034	227	0.0206	0.0494	-687	0.8267	PB	62.0
R001	B	TP53	chr17	7675094	A	C	Missense	p.V173G	c.518T>G	10680	559	0.0523	0.1334	-294	1.0000	PB	56.0
R001	C	TP53	chr17	7675094	A	C	Missense	p.V173G	c.518T>G	11875	269	0.0227	0.0466	-15	0.8019	PB	70.0
R001	F	TP53	chr17	7675094	A	C	Missense	p.V173G	c.518T>G	8720	13	0.0015	0.0017	161	0.9585	PB	95.0
R001	F	TP53	chr17	7675107	TGTGC	-	Indel	p.Q167Hfs*12	c.501_505del	8960	176	0.0196	0.0225	161	0.9585	PB	95.0

Abbreviations: Human Genome Variation Society nomenclature (HGVSc); Variant allele frequency (VAF); Cancer cell fraction (CCF); Loss of heterozygosity (LOH); Peripheral blood (PB).

Supplemental Table S12. Patient R002 cancer cell fraction for variants with two or more mutant molecules in at least one sample.

Patient	Sample	Gene	Chromosome	Position	Reference allele	Alternate allele	Mutation type	HGVSp short	HGVSc	Duplex depth	Duplex mutant reads	VAF	Variant CCF	Days post-pirtobrutinib	Sample type	Percent disease (%) (Tumor purity)
R002	C	BAX	chr19	48955709	C	T	Nonsense	p.R37*	c.109C>T	8385	8	0.0010	0.0020	-6	BMA	95.0
R002	D	BAX	chr19	48955709	C	T	Nonsense	p.R37*	c.109C>T	8739	3	0.0003	0.0008	82	BMA	91.0
R002	E	BAX	chr19	48955709	C	T	Nonsense	p.R37*	c.109C>T	7802	2	0.0003	0.0005	127	PB	95.0
R002	A	BAX	chr19	48955713	-	G	Indel	p.E41Gfs*33	c.121dup	9814	116	0.0118	0.0916	-331	BMA	12.9
R002	C	BAX	chr19	48955713	-	G	Indel	p.E41Gfs*33	c.121dup	8539	7474	0.8753	0.9213	-6	BMA	95.0
R002	C	BAX	chr19	48955713	-	GG	Indel	p.E41Gfs*20	c.120_121dup	8539	17	0.0020	0.0042	-6	BMA	95.0
R002	D	BAX	chr19	48955713	-	G	Indel	p.E41Gfs*33	c.121dup	8905	7349	0.8253	0.9069	82	BMA	91.0
R002	D	BAX	chr19	48955713	-	GG	Indel	p.E41Gfs*20	c.120_121dup	8905	22	0.0025	0.0054	82	BMA	91.0
R002	E	BAX	chr19	48955713	-	G	Indel	p.E41Gfs*33	c.121dup	7951	7909	0.9947	1.0000	127	PB	95.0
R002	E	BAX	chr19	48955713	-	GG	Indel	p.E41Gfs*20	c.120_121dup	7951	19	0.0024	0.0050	127	PB	95.0
R002	A	BAX	chr19	48955714	G	-	Indel	p.E41Rfs*19	c.121del	9750	2	0.0002	0.0032	-331	BMA	12.9
R002	C	BAX	chr19	48955714	G	-	Indel	p.E41Rfs*19	c.121del	8456	5	0.0006	0.0012	-6	BMA	95.0
R002	D	BAX	chr19	48955714	G	-	Indel	p.E41Rfs*19	c.121del	8802	2	0.0002	0.0005	82	BMA	91.0
R002	E	BAX	chr19	48955714	G	-	Indel	p.E41Rfs*19	c.121del	7873	1	0.0001	0.0003	127	PB	95.0
R002	A	BAX	chr19	48955780	C	T	Silent	p.S60=	c.180C>T	9971	2	0.0002	0.0031	-331	BMA	12.9
R002	C	BAX	chr19	48955780	C	T	Silent	p.S60=	c.180C>T	9418	1	0.0001	0.0002	-6	BMA	95.0
R002	E	BAX	chr19	48955780	C	T	Silent	p.S60=	c.180C>T	9571	1	0.0001	0.0002	127	PB	95.0
R002	D	BTK	chrX	101354679	A	C	Missense	p.L528V	c.1582T>G	6959	2	0.0003	0.0003	82	BMA	91.0
R002	C	BTK	chrX	101356168	T	C	Missense	p.N484D	c.1450A>G	6904	60	0.0087	0.0091	-6	BMA	95.0
R002	D	BTK	chrX	101356168	T	C	Missense	p.N484D	c.1450A>G	6776	20	0.0030	0.0032	82	BMA	91.0
R002	E	BTK	chrX	101356168	T	C	Missense	p.N484D	c.1450A>G	6542	4	0.0006	0.0006	127	PB	95.0
R002	C	BTK	chrX	101356176	C	G	Missense	p.C481S	c.1442G>C	6854	2	0.0003	0.0003	-6	BMA	95.0
R002	D	BTK	chrX	101356176	C	G	Missense	p.C481S	c.1442G>C	6753	1	0.0001	0.0002	82	BMA	91.0
R002	E	BTK	chrX	101356176	C	G	Missense	p.C481S	c.1442G>C	6516	1	0.0002	0.0002	127	PB	95.0
R002	C	BTK	chrX	101356177	A	G	Missense	p.C481R	c.1441T>C	6865	16	0.0023	0.0025	-6	BMA	95.0
R002	D	BTK	chrX	101356177	A	G	Missense	p.C481R	c.1441T>C	6764	62	0.0092	0.0101	82	BMA	91.0
R002	E	BTK	chrX	101356177	A	G	Missense	p.C481R	c.1441T>C	6543	20	0.0031	0.0032	127	PB	95.0
R002	C	BTK	chrX	101356197	G	A	Missense	p.T474I	c.1421C>T	6816	2	0.0003	0.0003	-6	BMA	95.0
R002	D	BTK	chrX	101356197	G	A	Missense	p.T474I	c.1421C>T	6796	262	0.0386	0.0424	82	BMA	91.0
R002	D	BTK	chrX	101356197	G	T	Missense	p.T474N	c.1421C>A	6796	7	0.0010	0.0011	82	BMA	91.0
R002	E	BTK	chrX	101356197	G	A	Missense	p.T474I	c.1421C>T	6606	134	0.0203	0.0214	127	PB	95.0
R002	E	BTK	chrX	101356197	G	T	Missense	p.T474N	c.1421C>A	6606	2	0.0003	0.0003	127	PB	95.0
R002	D	BTK	chrX	101356239	A	G	Missense	p.L460S	c.1379T>C	6550	3	0.0005	0.0005	82	BMA	91.0
R002	D	BTK	chrX	101356242	T	G	Missense	p.Q459P	c.1376A>C	6438	3	0.0005	0.0005	82	BMA	91.0
R002	E	BTK	chrX	101356242	T	G	Missense	p.Q459P	c.1376A>C	5859	2	0.0003	0.0004	127	PB	95.0
R002	E	BTK	chrX	101356250	C	A	Missense	p.K456N	c.1368G>T	5801	3	0.0005	0.0005	127	PB	95.0
R002	D	BTK	chrX	101358645	T	C	Missense	p.T316A	c.946A>G	6505	12	0.0018	0.0020	82	BMA	91.0
R002	E	BTK	chrX	101358645	T	C	Missense	p.T316A	c.946A>G	6772	15	0.0022	0.0023	127	PB	95.0
R002	C	BTK	chrX	101358689	T	C	Missense	p.E301G	c.902A>G	6157	2	0.0003	0.0003	-6	BMA	95.0
R002	D	BTK	chrX	101358689	T	C	Missense	p.E301G	c.902A>G	6142	3	0.0005	0.0005	82	BMA	91.0
R002	E	BTK	chrX	101358689	T	C	Missense	p.E301G	c.902A>G	5915	2	0.0003	0.0004	127	PB	95.0
R002	C	PLCG2	chr16	81912605	A	G	Missense	p.Y648C	c.1943A>G	9037	1	0.0001	0.0002	-6	BMA	95.0
R002	D	PLCG2	chr16	81912605	A	G	Missense	p.Y648C	c.1943A>G	8931	5	0.0006	0.0012	82	BMA	91.0
R002	E	PLCG2	chr16	81912605	A	G	Missense	p.Y648C	c.1943A>G	10575	9	0.0009	0.0018	127	PB	95.0
R002	C	PLCG2	chr16	81919626	T	G	Missense	p.Y733D	c.2197T>G	11414	3	0.0003	0.0006	-6	BMA	95.0
R002	D	PLCG2	chr16	81928576	T	G	Missense	p.L845V	c.2533T>G	12212	2	0.0002	0.0004	82	BMA	91.0
R002	E	PLCG2	chr16	81928576	T	G	Missense	p.L845V	c.2533T>G	9726	4	0.0004	0.0009	127	PB	95.0
R002	A	TP53	chr17	7673838	C	T	Splice	p.X261_splice	c.783-1G>A	12278	2	0.0002	0.0025	-331	BMA	12.9
R002	C	TP53	chr17	7674189	T	G	Missense	p.E258D	c.774A>C	12919	1	0.0001	0.0002	-6	BMA	95.0
R002	E	TP53	chr17	7674189	T	G	Missense	p.E258D	c.774A>C	12236	4	0.0003	0.0007	127	PB	95.0
R002	A	TP53	chr17	7674200	T	A	Missense	p.I255F	c.763A>T	12475	34	0.0027	0.0423	-331	BMA	12.9
R002	C	TP53	chr17	7674200	T	A	Missense	p.I255F	c.763A>T	12835	16	0.0012	0.0026	-6	BMA	95.0
R002	D	TP53	chr17	7674200	T	A	Missense	p.I255F	c.763A>T	12544	3	0.0002	0.0005	82	BMA	91.0
R002	A	TP53	chr17	7674217	C	G	Missense	p.R249T	c.746G>C	11802	4	0.0003	0.0053	-331	BMA	12.9
R002	D	TP53	chr17	7674217	C	G	Missense	p.R249T	c.746G>C	11894	3	0.0003	0.0006	82	BMA	91.0
R002	E	TP53	chr17	7674217	C	G	Missense	p.R249T	c.746G>C	11660	2	0.0002	0.0004	127	PB	95.0
R002	A	TP53	chr17	7674220	C	T	Missense	p.R248Q	c.743G>A	11727	3	0.0003	0.0040	-331	BMA	12.9
R002	A	TP53	chr17	7674900	T	C	Missense	p.T211A	c.631A>G	10813	35	0.0032	0.0502	-331	BMA	12.9
R002	C	TP53	chr17	7674900	T	C	Missense	p.T211A	c.631A>G	11803	1	0.0001	0.0002	-6	BMA	95.0
R002	D	TP53	chr17	7674900	T	C	Missense	p.T211A	c.631A>G	11402	10	0.0009	0.0019	82	BMA	91.0
R002	A	TP53	chr17	7675184	A	G	Missense	p.V143A	c.428T>C	12343	6	0.0005	0.0075	-331	BMA	12.9
R002	D	TP53	chr17	7675184	A	G	Missense	p.V143A	c.428T>C	12392	1	0.0001	0.0002	82	BMA	91.0
R002	E	TP53	chr17	7675184	A	G	Missense	p.V143A	c.428T>C	14362	1	0.0001	0.0001	127	PB	95.0
R002	C	TP53	chr17	7675205	T	A	Missense	p.Q136L	c.407A>T	12558	3	0.0002	0.0005	-6	BMA	95.0
R002	D	TP53	chr17	7675205	T	A	Missense	p.Q136L	c.407A>T	12047	4	0.0003	0.0007	82	BMA	91.0
R002	E	TP53	chr17	7675205	T	A	Missense	p.Q136L	c.407A>T	13827	2	0.0001	0.0003	127	PB	95.0
R002	A	TP53	chr17	7675218	T	C	Missense	p.K132E	c.394A>G	12168	3	0.0002	0.0038	-331	BMA	12.9
R002	C	TP53	chr17	7675238	T	A	Splice	p.X126_splice	c.376-2A>T	12283	5	0.0004	0.0009	-6	BMA	95.0
R002	D	TP53	chr17	7675238	T	A	Splice	p.X126_splice	c.376-2A>T	11765	3	0.0003	0.0006	82	BMA	91.0
R002	E	TP53	chr17	7675238	T	A	Splice	p.X126_splice	c.376-2A>T	12618	2	0.0002	0.0003	127	PB	95.0
R002	E	TP53	chr17	7676552	T	C	Missense	p.S15G	c.43A>G	10012	2	0.0002	0.0004	127	PB	95.0

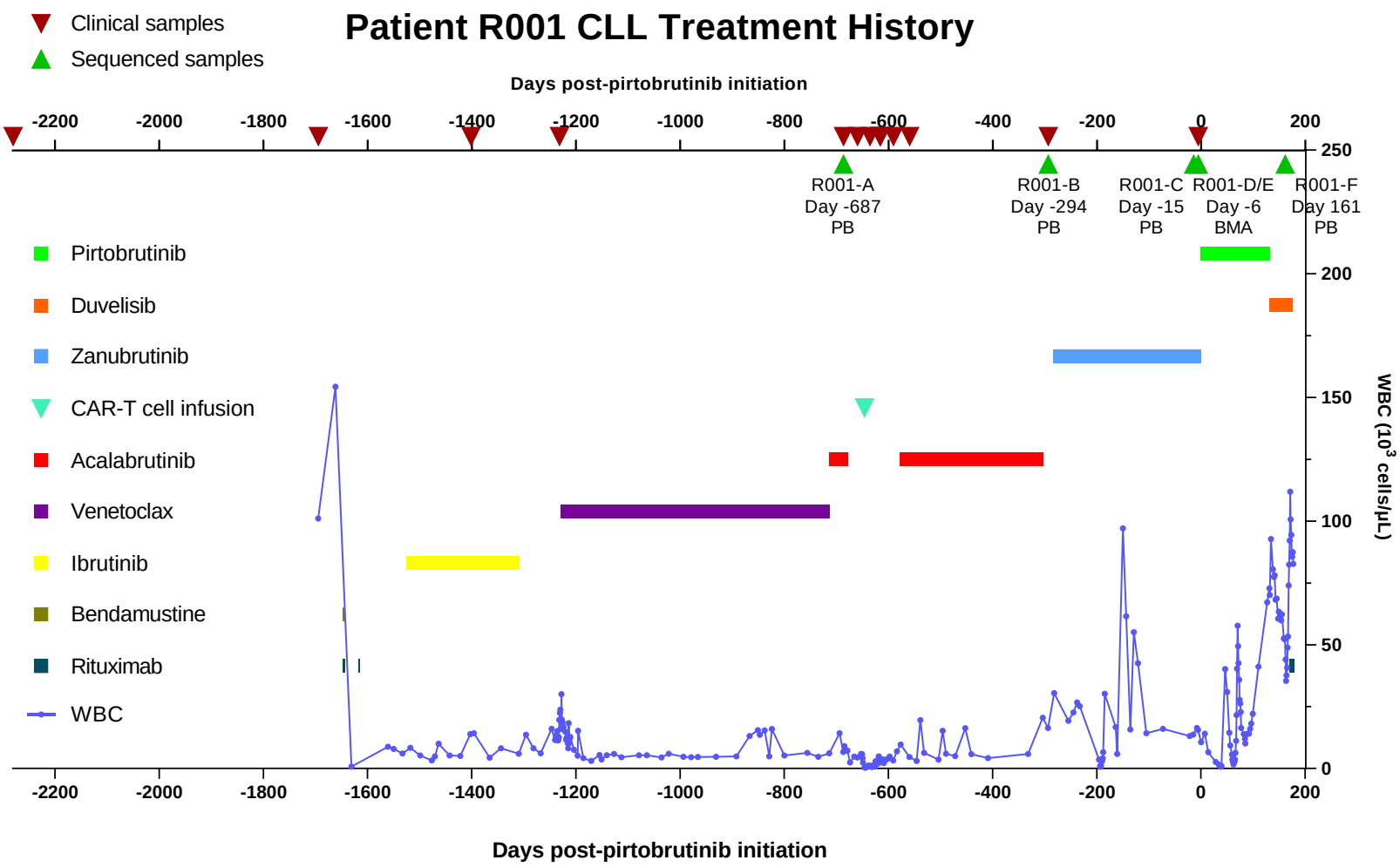
Abbreviations: Human Genome Variation Society nomenclature (HGVSc); Variant allele frequency (VAF); Cancer cell fraction (CCF); Bone marrow aspirate (BMA); Peripheral blood (PB).

Supplemental Table S13. Comparison of NGS and duplex sequencing results.

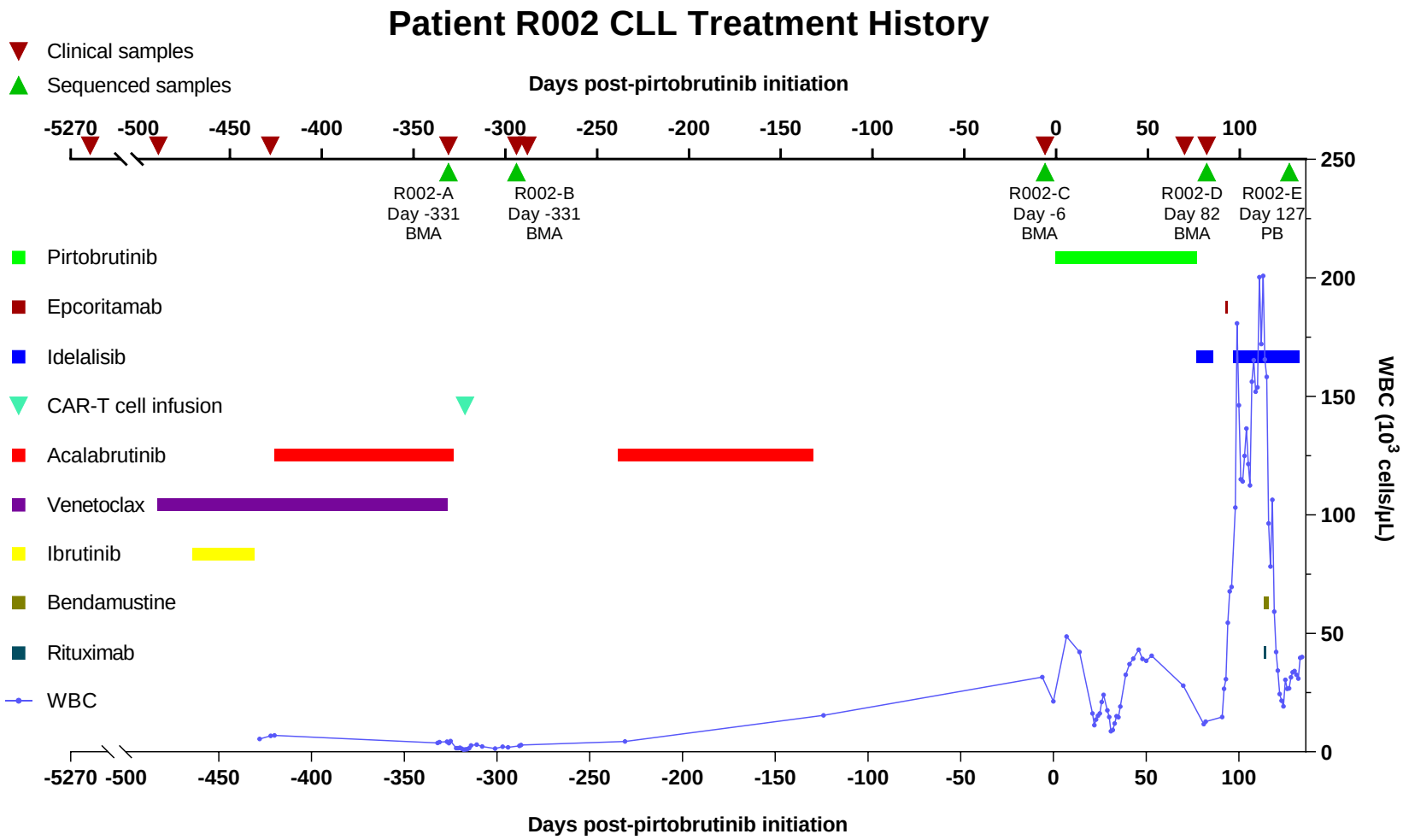
Patient/ sample code	Gene	Variant	NGS VAF	Duplex VAF
R001-A	BTK	p.C481S, c.1442G>C	NP	0
R001-B	BTK	p.C481S, c.1442G>C	NP	0
R001-C	BTK	p.C481S, c.1442G>C	NP	0.0161
R001-D	BTK	p.C481S, c.1442G>C	0.1	0.1061
R001-E	BTK	p.C481S, c.1442G>C	0.1	0.1052
R001-F	BTK	p.C481S, c.1442G>C	NP	0.0064
R001-A	TP53	p.H178D, c.532C>G	NP	0
R001-B	TP53	p.H178D, c.532C>G	NP	0.0773
R001-C	TP53	p.H178D, c.532C>G	NP	0.1301
R001-D	TP53	p.H178D, c.532C>G	0.18	0.1701
R001-E	TP53	p.H178D, c.532C>G	0.18	0.1761
R001-F	TP53	p.H178D, c.532C>G	NP	0.2785
R002-A	BTK	p.T474I, c.1421C>T	NP	0
R002-B	BTK	p.T474I, c.1421C>T	NP	0
R002-C	BTK	p.T474I, c.1421C>T	0	0.0003
R002-D	BTK	p.T474I, c.1421C>T	0.06	0.0386
R002-E	BTK	p.T474I, c.1421C>T	NP	0.0203

Abbreviations: Next-generation sequencing (NGS); Variant allele frequency (VAF); Clinical testing not performed (NP).

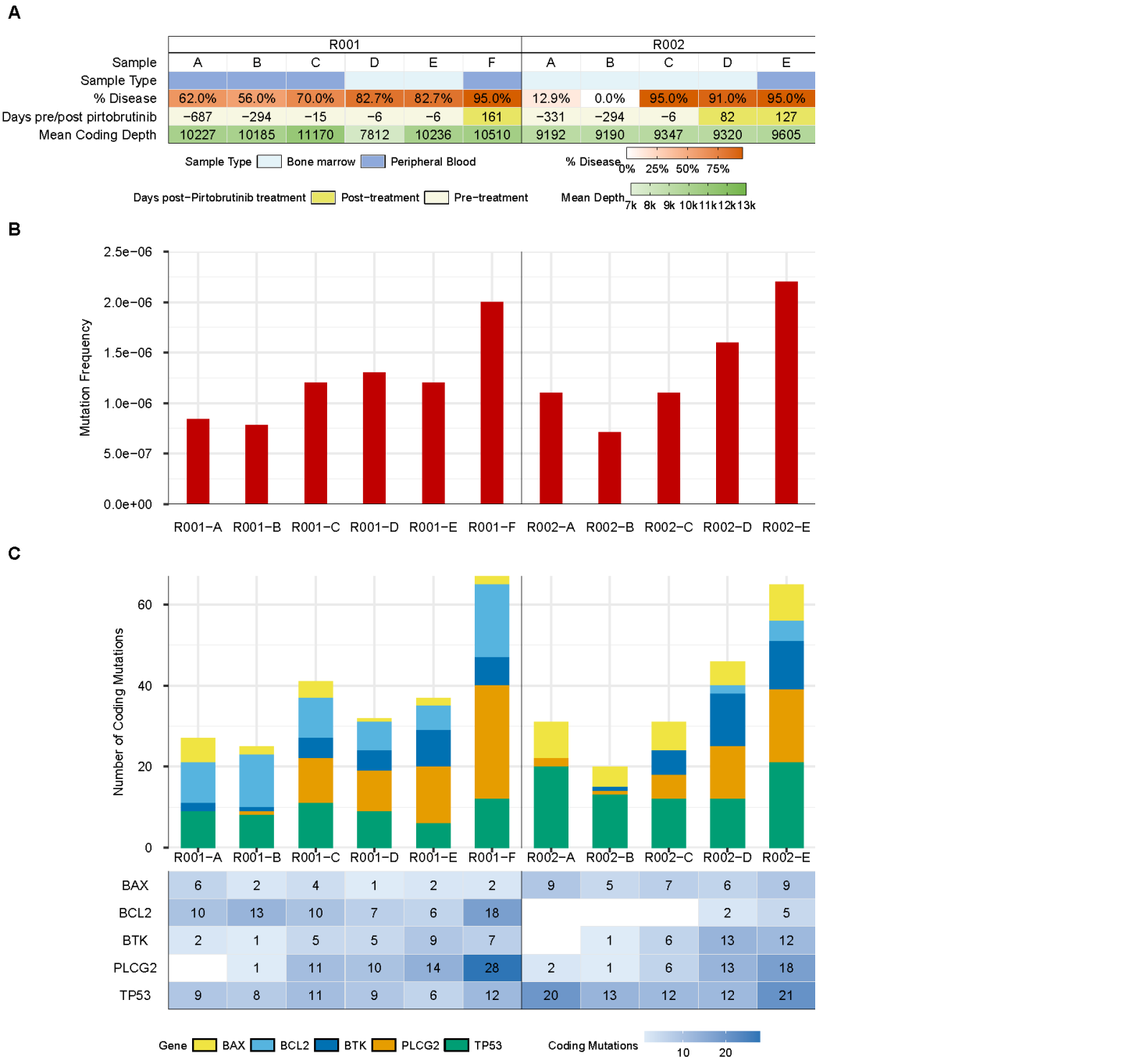
SUPPLEMENTAL FIGURES



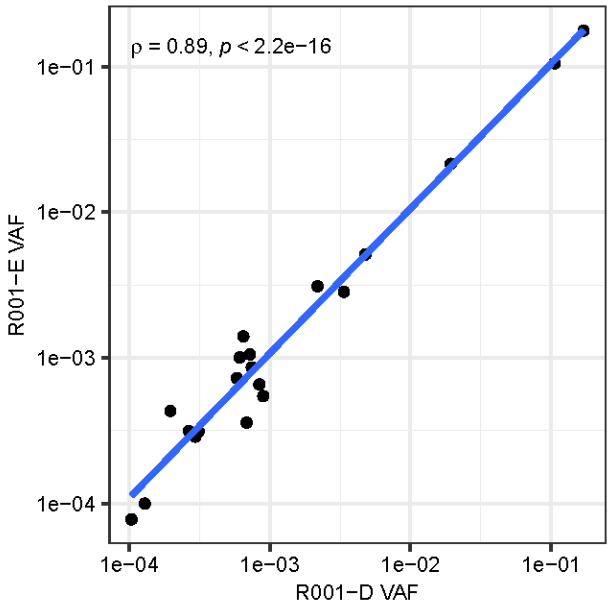
Supplemental Figure S1. Timeline of CLL treatments, PB and BM studies, and white blood cell (WBC) counts during patient R001's CLL history. The WBC line graph may be used to monitor disease severity. Time in the x-axis is centered on the first day of pirtobrutinib treatment. The length of each treatment bar is proportional to treatment duration, and the turquoise "CAR-T cell infusion" triangle is centered on the day of infusion. On the upper-x-axis, green triangles indicate the sequenced samples of the study, and maroon inverted triangles indicate clinical samples.



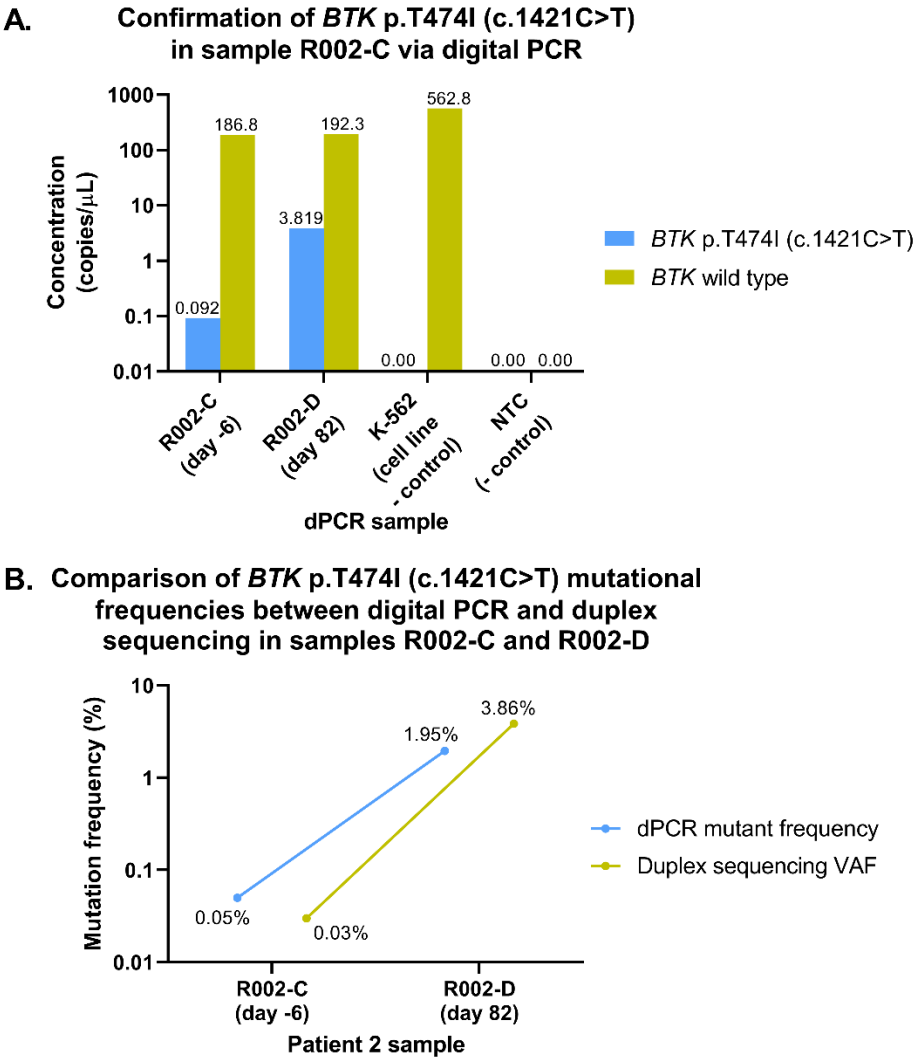
Supplemental Figure S2. Timeline of CLL treatments, PB and BM studies, and white blood cell (WBC) counts during patient R002's CLL history. The WBC line graph may be used to monitor disease severity. Time in the x-axis is centered on the first day of pirtobrutinib treatment. The length of each treatment bar is proportional to treatment duration, and the turquoise "CAR-T cell infusion" triangle is centered on the day of infusion. On the upper-x-axis, green triangles indicate the sequenced samples of the study, and maroon inverted triangles indicate clinical samples.



Supplemental Figure S3. Characterization of mutations identified pre- and post- treatment. A) Description of samples collected over time for both patients R001 (A, B, C, D, E, F) and R002 (A, B, C, D, E). Sample types include bone marrow and peripheral blood. Values for percent (%) disease, days pre-/post-pirtobrutinib treatment, and mean coding depth for duplex sequencing are shown for each sample. B) Mutation frequency for coding and non-coding mutations for each sample collected over time. C) Number of coding mutations found in genes associated with drug resistance in CLL for each sample. Genes associated with CLL resistance and covered by duplex sequencing probes include *BAX*, *Bcl2*, *BTK*, *PLCG2*, and *TP53*. Each sample is represented by a single column. Mutated genes are color-coded and represented as a fraction within the column with the number of mutation counts indicated underneath with a blue-colored gradient scale.



Supplemental Figure S4. Reproducibility of duplex sequencing data. Two DNA samples extracted from the same bone marrow sample from patient R001 (R001-D and R001-E) were independently processed for DNA extraction and duplex sequencing. Variant allele frequencies (VAF) were calculated by dividing the number of mutant duplex reads (alternative counts) by the duplex sequencing depth at the mutated position. The correlation plot includes all mutations identified in both samples (n=20). Spearman's rank correlation coefficient and p-value demonstrate high reproducibility of measurements.



Supplemental Table S5. Orthogonal confirmation of *BTK* p.T474I (c.1421C>T) in sample R002-C using digital PCR (dPCR). Figure S5A illustrates the detection via dPCR of *BTK* p.T474I (c.1421C>T) variant at 0.092 copies per 40µL reaction, controlled by assaying sample R002-D, which carried our target variant at a greater concentration, and K-562 cell line DNA lacking the target variant. Figure S5B compares the mutational frequencies for *BTK* p.T474I (c.1421C>T) in samples R002-C and R002-D as reported by dPCR versus duplex sequencing, demonstrating concordant mutational frequencies between the two techniques.

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