Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Clinical Response of Carcinomas Harboring the BRD4-NUT Oncoprotein to the Targeted Bromodomain Inhibitor OTX015/MK-8628

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1. Supplementary Methods

Fluorescence in situ hybridization (FISH)

BRD4 and *NUT* dual color split-apart FISH was performed as described[1] using *NUT* 3' telomeric probes, RP11-1H8 and RP11-64o3 (digoxigenin labeled, green), and BRD4 5' centromeric probes, RP11-207i16 and RP11-3055m5 (biotin labeled, red).

Immunohistochemistry

Immunohistochemistry for NUT, beta-catenin, and MYC was performed on 5-micron-thick, formalin-fixed, paraffin-embedded sections. Slides underwent heat-induced epitope retrieval in citrate buffer and were incubated with primary rabbit monoclonal anti-NUT (clone C52B1,1:50, Cell Signaling Technology, Danvers, MA), mouse monoclonal anti-beta-catenin (clone 14/beta-catenin, 1:1000, BD Transduction Laboratories, San Jose, CA), or rabbit monoclonal anti-MYC (clone Y69, ABCAM, Cambridge, MA), and visualized using Bond Polymer Refine Detection (Leica Microsystems, Buffalo Grove, IL).

OncoPanel

Targeted exome sequencing was performed on the following genes using a custom next-generation sequencing platform, termed 'OncoPanel', developed at Brigham and Women's Hospital Dana-Farber Cancer Institute according to the methods described[2,3]. Somatic DNA mutations, copy number variations, and gene rearrangements can be detected from formalin-fixed paraffin embedded sections of the tumor. DNA was isolated from tissue containing at least 20% tumor nuclei and analyzed by massively parallel sequencing using a solution-phase Agilent SureSelect hybrid capture kit and an Illumina HiSeq 2500 sequencer. Covered in the assay were exonic sequences of 300 genes and sequences of 113 introns across 35 genes.
For **patient tumor 1**, there were 16037717 unique, aligned, high-quality reads with a mean of 390 reads across all targeted exons and 99% of all exons having more than 30 reads. For **patient tumor 2**, pre-treatment, there were 11434742 unique, aligned, high-quality reads with a mean of 262 reads across all targeted exons and 99% of all exons having more than 30 reads. For **patient tumor 2**, treatment recurrence, there were 12593791 unique, aligned, high-quality with a mean of 303 reads across all targeted exons and 99% of all exons having more than 30 reads. For **patient tumor 4** (see discussion), there were 11366903 unique, aligned, high-quality reads with a mean of 288 reads across all targeted exons and 99% of all exons having more than 30 reads. No tumor tissue was available for patient tumor 3.

The 300 genes are: ABL1, AKT1, AKT2, AKT3, ALK, ALOX12B, APC, AR, ARAF, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, AURKA, AURKB, AXL, B2M, BAP1, BCL2, BCL2L1, BCL2L12, BCL6, BCOR, BCORL1, BLM, BMPR1A, BRAF, BRCA1, BRCA2, BRD4, BRIP1, BUB1B, CADM2, CARD11, CBL, CBLB, CCND1, CCND2, CCND3, CCNE1, CD274, CD58, CD79B, CDC73, CDH1, CDK1, CDK2, CDK4, CDK5, CDK6, CDK9, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CDKN2C, CEBPA, CHEK2, CIITA, CREBBP, CRKL, CRLF2, CRTC1, CRTC2, CSF1R, CSF3R, CTNNB1, CUX1, CYLD, DDB2, DDR2, DEPDC5, DICER1, DIS3, DMD, DNMT3A, EED,

EGFR, EP300, EPHA3, EPHA5, EPHA7, ERBB2, ERBB3, ERBB4, ERCC2, ERCC3, ERCC4, ERCC5, ESR1, ETV1, ETV4, ETV5, ETV6, EWSR1, EXT1, EXT2, EZH2, FAM46C, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FAS, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FH, FKBP9, FLCN, FLT1, FLT3, FLT4, FUS, GATA3, GATA4, GATA6, GLI1, GLI2, GLI3, GNA11, GNAQ, GNAS, GNB2L1, GPC3, GSTM5, H3F3A, HNF1A, HRAS, ID3, IDH1, IDH2, IGF1R, IKZF1, IKZF3, INSIG1, JAK2, JAK3, KCNIP1, KDM5C, KDM6A, KDM6B, KDR, KEAP1, KIT, KRAS, LINC00894, LMO1, LMO2, LMO3, MAP2K1, MAP2K4, MAP3K1, MAPK1, MCL1, MDM2, MDM4, MECOM, MEF2B, MEN1, MET, MITF, MLH1, MLL, MLL2, MPL, MSH2, MSH6, MTOR, MUTYH, MYB, MYBL1, MYC, MYCL1, MYCN, MYD88, NBN, NEGR1, NF1, NF2, NFE2L2, NFKBIA, NFKBIZ, NKX2-1, NOTCH1, NOTCH2, NPM1, NPRL2, NPRL3, NRAS, NTRK1, NTRK2, NTRK3, PALB2, PARK2, PAX5, PBRM1, PDCD1LG2, PDGFRA, PDGFRB, PHF6, PHOX2B, PIK3C2B, PIK3CA, PIK3R1, PIM1, PMS1, PMS2, PNRC1, PRAME, PRDM1, PRF1, PRKAR1A, PRKCI, PRKCZ, PRKDC, PRPF40B, PRPF8, PSMD13, PTCH1, PTEN, PTK2, PTPN11, PTPRD, QKI, RAD21, RAF1, RARA, RB1, RBL2, RECQL4, REL, RET, RFWD2, RHEB, RHPN2, ROS1, RPL26, RUNX1, SBDS, SDHA, SDHAF2, SDHB, SDHC, SDHD, SETBP1, SETD2, SF1, SF3B1, SH2B3, SLITRK6, SMAD2, SMAD4, SMARCA4, SMARCB1, SMC1A, SMC3, SMO, SOCS1, SOX2, SOX9, SQSTM1, SRC, SRSF2, STAG1, STAG2, STAT3, STAT6, STK11, SUFU, SUZ12, SYK, TCF3, TCF7L1, TCF7L2, TERC, TERT, TET2, TLR4, TNFAIP3, TP53, TSC1, TSC2, U2AF1, VHL, WRN, WT1, XPA, XPC, XPO1, ZNF217, ZNF708, ZRSR2.

Intronic regions are tiled on specific introns of ABL1, AKT3, ALK, BCL2, BCL6, BRAF, CIITA, EGFR, ERG, ETV1, EWSR1 , FGFR1, FGFR2, FGFR3, FUS, IGH@, IGL@, JAK2, MLL, MYC, NPM1, NTRK1, PAX5, PDGFRA, PDGFRB, PPARG, RAF1, RARA, RET, ROS1, SS18, TRA@, TRB@, TRG@, TMPRSS2.

Table S1. Genomic alterations identified by OncoPanel

Single-nucleotide variants

Patient no.	Gene	exon	DNA	Protein	Variant type	Variant allele frequency	Predicted effect	Genotype
1	*CSF1R*	9	*c.1407G>C*	*p.Q469H*	substitution	42% of 201 reads	unknown	heterozygous
1	*CUX1*	24	*c.4316C>G*	*p.P1439R*	substitution	58% of 85 reads	unknown	heterozygous
1	*ERCC3*	10	*c.1558C>G*	*p.R520G*	substitution	47% of 470 reads	unknown	heterozygous
1	*GATA3*	2	*c.85C>T*	*p.P29S*	substitution	49% of 233 reads	unknown	heterozygous
1	*NOTCH1*	26	*c.4793G>A*	*p.R1598H*	substitution	53% of 249 reads	oncogenic	heterozygous
1	*PIK3CA*	15	*c.2217G>T*	*p.M739I*	substitution	48% of 379 reads	unknown	heterozygous
2 pre-treatment	*EPHA7*	*5*	*c.1083C>G*	*p.N361K*	substitution	30% of 549 reads	unknown	heterozygous
2 pre-treatment	*FLT4*	*23*	*c.3209G>C*	*p.R1070P*	substitution	49% of 152 reads	unknown	heterozygous
2 during treatment	*EPHA7*	*5*	*c.1083C>G*	*p.N361K*	substitution	39% of 558 reads	unknown	heterozygous
2 during treatment	*FLT4*	*23*	*c.3209G>C*	*p.R1070P*	substitution	37% of 120 reads	unknown	heterozygous
4	*MECOM*	*7*	*c.1823T>C*	*p.V608A*	substitution	53% of 315 reads	unknown	heterozygous
4	*ZNF708*	*4*	*c.1229A>G*	*p.H410R*	substitution	47% of 296 reads	unknown	heterozygous

Copy number alterations

Patient no.	Chromosome	Gene	Aberration
1	19p13.12	*BRD4*	Single copy deletion
2 pre-treatment	N/A	N/A	none
2 during treatment	N/A	N/A	none
4	N/A	N/A	none

Rearrangement analysis

Patient no.	Genes	Chr	DNA	Break-point 1	Break-point 2	Variant type	Predicted effect
1	*BRD4, NUT*	15, 19	t(15;19)(q14;p13.1) (hg19 chr15:g.34637920; chr19:g15366077)	intron 1 of *NUT*	intron10 of *BRD4*	Translo-cation	*BRD4-NUT* fusion
2 pre-treatment	*BRD4, NUT*	15, 19	t(15;19)(q14;p13.1) (hg19 chr15:g.34639242; chr19:g15350745)	intron 1 of *NUT*	exon 15 of *BRD4*	Translo-cation	*BRD4-NUT* fusion
2 during treatment	*BRD4, NUT*	15, 19	t(15;19)(q14;p13.1) (hg19 chr15:g.34639242; chr19:g15350745)	intron 1 of *NUT*	exon 15 of *BRD4*	Translo-cation	*BRD4-NUT* fusion
4	none	N/A	N/A			N/A	N/A

Table S2. BRD4-NUT fusion sequences identified by OncoPanel

Breakpoint patient 1

Fusion Contig

CGGCAGGTGTGACGCACTCGACCTCGCGATACAGTGGAGGACAAACGCCTTCTTTTCCTTTGGAGTTCCACACGAA
CCCGGGGGACCCGTACCCCAAGTTTCTCCGTTTGTAGTTAACACAACGGTTTGACATCAAACTTCCCTTAAGGACCA
AAAAAGTATGAATGATCTTGGG

Intron 10, BRD4, 19: 15366078-15366175

CGGCAGGTGTGACGCACTCGACCTCGCGATACAGTGGAGGACAAACGCCTTCTTTTCCTTTGGAGTTCCACACGAA
CCCGGGGGACCCGTACCCCAAG

Intron 1, *NUT*, 15: 34637921-34637997

TTTCTCCGTTTGTAGTTAACACAACGGTTTGACATCAAACTTCCCTTAAGGACCAAAAAAGTATGAATGATCTTGGG

Breakpoint patient 2, pre-treatment

GAAGAGGATCCAGTGGAGGCGCTTCGGGGGAGGGGCGAATACTATGTAAGGGGGGTCTACAGTGTCAAGGTCT
CGGACTGGGTGGTCAGAGGTGGGGTTCACTAAAACTAGAATGAAGAGCAACTCTTACTGACGTAATTTCTTCAAC
GAAGAAAAAGTTGTTCTGTGTGTCTGGGGATAGACGGTTCTTCTCTGTTCGGGTTCGGTAGTGACGGA

Exon 15, *BRD4*, 19:15350746-15350843

GAAGAGGATCCAGTGGAGGCGCTTCGGGGGAGGGGCGAATACTATGTAAGGGGGGTCTACAGTGTCAAGGTCT
CGGACTGGGTGGTCAGAGGTGGGGT

Intron 1, *NUT*, 15: 34639120-34639242

TCACTAAAACTAGAATGAAGAGCAACTCTTACTGACGTAATTTCTTCAACGAAGAAAAAGTTGTTCTGTGTGTCTG
GGGATAGACGGTTCTTCTCTGTTCGGGTTCGGTAGTGACGGA

Breakpoint patient 2, during treatment

GAAGAGGATCCAGTGGAGGCGCTTCGGGGGAGGGGCGAATACTATGTAAGGGGGGTCTACAGTGT
CAAGGTCTCGGACTGGGTGGTCAGAGGTGGGGTTCACTAAAACTAGAATGAAGAGCAACTCTTACT
GACGTAATTTCTTCAACGAAGAAAAAGTTGTTCTGTGTGTCTGG

Exon 15, *BRD4*, 19: 15350746-15350843

GAAGAGGATCCAGTGGAGGCGCTTCGGGGGAGGGGCGAATACTATGTAAGGGGGGTCTACAGTGT
CAAGGTCTCGGACTGGGTGGTCAGAGGTGGGGT

Intron 1, *NUT*, 15: 34639161 -34639242

TCACTAAAACTAGAATGAAGAGCAACTCTTACTGACGTAATTTCTTCAACGAAGAAAAAGTTGTTCTGT
GTGTCTGG

3. **Supplementary References**

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3. Abo RP, Ducar M, Garcia EP, et al. BreaKmer: detection of structural variation in targeted massively parallel sequencing data using kmers. Nucleic acids research 2015;43:e19.