JNCCN

National Comprehensive Cancer Network®

JOURNAL OF THE NATIONAL COMPREHENSIVE CANCER NETWORK

Supplemental online content for:

KRAS G12V Mutation in Acquired Resistance to Combined BRAF and MEK Inhibition in Papillary Thyroid Cancer

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J Natl Compr Canc Netw 2019;17(5):409–413

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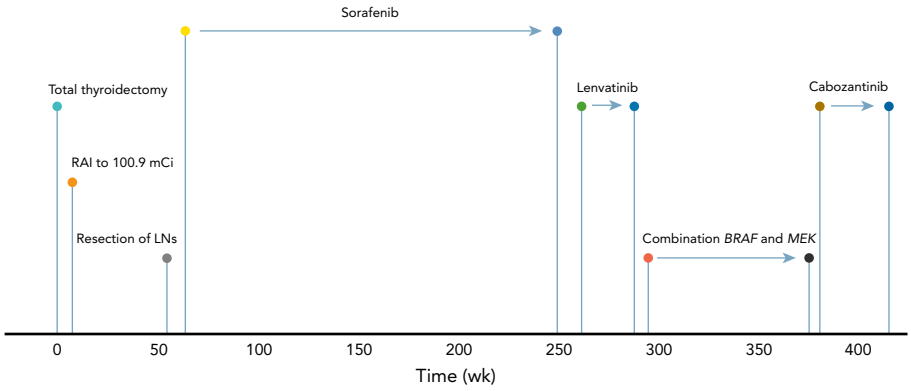

eFigure 1. Treatment timeline from initial surgery.
Abbreviations: LNs, lymph nodes; RAI, radioactive iodine.

eTable 1. Candidate Mutations Detected at Time of Progression

Gene	Mutation	Allelic Frequency	Consequence	Condel Score
KRAS	G12V	0.322	Missense	Damaging
UGGT1	E1391Q	0.134	Missense	Damaging
RASGEF1C	A164P	0.317	Missense	Neutral
ZNF35	S291*	0.448	Stop-gain	N/A
LEUTX	S104*	0.115	Stop-gain	N/A
PDHA1	W421*	0.522	Stop-gain	N/A

Mutations detected at time of progression not present in archival specimen. Mutations detected at progression were cross-referenced with Condel, SIFT, PolyPhen, and PROVEAN and were considered a candidate if the mutation had deleterious or damaging scores on at least 3 of the 4 platforms (or stop codon).
Abbreviation: N/A, not applicable.

eTable 2. All Mutations Detected on Progression Tissue Sample

Gene	Amino Acid Change	Position	Progression Allelic Frequency	Archival Allelic Frequency	Consequence	Condel Score
CDKN2C	A/P	144	0.903	0	Missense_variant	Neutral, 0.516
PPCS	S/L	113	0.842	0	Missense_variant	Neutral, 0.513
KLHL21	F/L	144	0.778	0	Missense_variant	Neutral, 0.519
TBXA2R	V/M	153	0.595	0	Missense_variant	Damaging, 0.534
USP29	S/Y	534	0.566	0	Missense_variant	Neutral, 0.33
P4HTM	Y/F	179	0.565	0	Missense_variant	Damaging, 0.547
XPO1	L/V	23	0.552	0	Missense_variant	Neutral, 0.504
PDHA1	W/*	421	0.522	0	Stop-gain	
WNK4	K/N	387	0.492	0	Missense_variant	Neutral, 0.41
KPNA3	E/G	8	0.475	0	Missense_variant	Neutral, 0.454
KPNA4	E/G	9	0.475	0	Missense_variant	Neutral, 0.454
ZNF35	S/*	291	0.448	0	Stop-gain	
HELZ	R/*	597	0.440	0.01	Stop-gain	
OR10H2	V/A	124	0.385	0	Missense_variant	Neutral, 0.276
KRAS	G/V	12	0.322	0	Missense_variant	Damaging, 0.699
RASGEF1C	A/P	164	0.317	0	Missense_variant	Neutral, 0.415
FOXRED2	F/C	345	0.250	0	Missense_variant	Neutral, 0.426
IGFN1	E/K	1718	0.240	0	Missense_variant	Neutral, 0.464
ADCY10	E/D	847	0.239	0	Missense_variant	Neutral, 0.445
KIF14	S/A	1298	0.233	0.01	Missense_variant	Damaging, 0.533
TRAF3IP3	E/K	456	0.211	0	Missense_variant	Damaging, 0.538
RELN	M/V	1	0.194	0	Start_lost	Neutral, 0.312
CDC40	E/Q	86	0.160	0	Missense_variant	Damaging, 0.593
IKBKAP	Q/E	1127	0.147	0	Missense_variant	Neutral, 0.416
UGGT1	E/Q	1391	0.134	0	Missense_variant	Damaging, 0.560
PRKG1	D/N	117	0.120	0	Missense_variant	Damaging, 0.599
LEUTX	S/*	104	0.115	0	Stop-gain	
EPS8L2	Q/H	274	0.108	0	Missense_variant	Neutral, 0.481

eTable 3. Mutations Detected on Archival Tissue Specimen

Gene	Amino Acid Change	Position	Archival Allelic Frequency	Progression Allelic Frequency	Consequence	Condel Score
POTEC	A/T	119	0.333	0.179	Missense_variant	Neutral, 0.417
IFIT1B	D/N	455	0.129	0.547	Missense_variant	Neutral, 0.416
ESF1	E/Q	750	0.289	0.419	Missense_variant	Neutral, 0.391
RBM26	F/L	311	0.227	0.543	Missense_variant	Neutral, 0.46
BRAF	V/E	600	0.250	0.467	Missense_variant	Damaging, 0.536
SLC25A24	R/W	6	0.465	0.750	Missense_variant	Neutral, 0.521
NBPF10	D/G	1168	0.075	0.068	Missense_variant	Neutral, 0.304
KCNK3	S/F	83	0.350	0.491	Missense_variant	Neutral, 0.484
BSN	P/L	1894	0.379	0.544	Missense_variant	Neutral, 0.389
RAG1	S/R	609	0.324	0.478	Missense_variant	Damaging, 0.702
TCHH	E/K	669	0.160	0.273	Missense_variant	Neutral, 0.356
NUP93	Q/*	15	0.265	0.493	Stop-gain	N/A
ANKRD30B	G/V	494	0.412	0.424	Missense_variant, splice_region_variant	Neutral, 0.418
KRBOX4/ZNF673	S/L	148	0.325	0.423	Missense_variant	Neutral, 0.282
KIAA1024	A/V	897	0.292	0.528	Missense_variant	Neutral, 0.413
CBR4	M/I	122	0.225	0.590	Missense_variant	Damaging, 0.589
ASCC3	G/S	1122	0.190	0.434	Missense_variant	Neutral, 0.412
SLC45A2	P/S	241	0.199	0.503	Missense_variant	Damaging, 0.643
RAB12	V/I	241	0.269	0.495	Missense_variant	Neutral, 0.438
HEATR5B	L/S	1173	0.311	0.385	Missense_variant	Damaging, 0.623
RSPH10B2	T/M	148	0.321	0.500	Missense_variant	Neutral, 0.503

Abbreviation: N/A, not applicable.

eAppendix 1. Supplemental Methods

The patient was treated on a clinical trial (ClinicalTrials.gov identifier: NCT01723202), which was approved by The Ohio State University Cancer Center Institutional Review Board. Tissue was extracted from the dominant cervical nodal metastasis (see Figure 1) using ultrasound guidance of a 22-gauge needle. During the trial, the same nodal metastasis was sampled before drug initiation (Figure 1E), after 2 weeks on the study drug (Figure 1F), and at the time of disease progression, before drug discontinuation (Figure 1H).

Whole-Exome Next-Generation Sequencing

For the archival tumor tissue, a hematoxylin and eosin–stained section of the block was marked by a pathologist and tumor-containing regions were macrodissected from serial sections to enrich for tumor content. DNA was extracted using the Maxwell 16 FFPE Tissue LEV DNA Purification Kit (Promega, Inc.). Germline DNA was extracted from whole blood using the Maxwell 16 Blood DNA Purification Kit, and the Maxwell 16 Tissue DNA Purification Kit was used for the fine aspirate biopsy collected at progression. DNA was quantitated using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific). Whole-exome sequencing libraries were prepared using 200 ng of genomic DNA with the SureSelectXT Reagent Kit and the SureSelectXT Clinical Research Exome capture baits (Agilent Technologies). Libraries were sequenced on an Illumina HiSeq 4000 to obtain paired-end 150 bp reads. The average number of reads with a quality score >30 was 75 million for the tumor DNA samples and 25 million for the germline blood DNA, with coverage of 92% and 55% targeted bases at $>20\times$, respectively.

Raw sequence reads were processed and aligned to the human reference genome using the Genome Analysis Toolkit (GATK) workflow.[1] MuTect (v1.1.4) and VarScan 2 (v2.3.7)[2,3] were used to identify tumor-specific variants for each the archival and progression samples. The Ensembl Variant Effect Predictor was used to annotate and determine functional consequences of tumor-specific variants.[4] VarScan 2 was also used to identify copy number changes in tumor samples compared with matching normal samples.

BRAF and *KRAS* Plasma Circulating Tumor DNA Assay

Circulating cell-free DNA (ccfDNA) was purified from 4 mL of plasma using the Promega Maxwell HT ccfDNA Plasma Kit. Circulating tumor DNA analysis was performed by quantifying levels of the *BRAF* and *KRAS* mutations in ccfDNA using the Bio-Rad Droplet Digital PCR Mutation Detection Assays (Bio-Rad *BRAF* assay ID: dHsaMDV2010027; *KRAS* assay ID: dHsaMDV2510592) on the QX200 Droplet Digital PCR System (Bio-Rad). Droplets were analyzed with the QX200 Droplet Reader (BioRad) for fluorescent measurement of FAM and HEX probes. Gating was performed based on positive and negative controls, and mutant populations were identified. All reactions were run in duplicate. The digital droplet PCR data were analyzed with QuantaSoft Analysis Software (BioRad) to obtain fractional abundance of the mutant DNA alleles in the wild-type/normal background.

References

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