Supplementary Online Content

Karasic TB, O'Hara MH, Loaiza-Bonilla A, et al. Effect of gemcitabine and nab-paclitaxel with or without hydroxychloroquine on patients with advanced pancreatic cancer: a phase 2 randomized clinical trial. *JAMA Oncol.* Published online May 23, 2019. doi:10.1001/jamaoncol.2019.0684

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

eAppendix. Methods

Center for Personal Diagnostics (CPD) Genomic Sequencing

Targeted next-generation sequencing (NGS) was performed at the CLIA-approved Center for Personalized Diagnostics (CPD) at the University of Pennsylvania. Genomic DNA from pancreatic tumor specimens was extracted from formalin-fixed paraffin-embedded tissues. A Hematoxylin and Eosin stained slide was used to select areas containing at minimum 10% tumor for macrodissection and isolation of DNA. Tumor DNA was sequenced on an Illumina MiSeq or HiSeq, and the data analyzed using in-house bioinformatics pipelines to identify single nucleotide variants (SNVs), insertion/deletion (indels) and amplifications. The minimum allele frequency (AF) reportable by the tumor assay was ≥4%.

For patients analyzed prior to mid-2017, the CPD version 1 panel was used, which contained 203 amplicons covering mutational hotspots of 47 cancer related genes including KRAS, p53, and SMAD4, but not p16[1]. Illumina amplicon based massively parallel sequencing panel was used (Illumina TruSeq Cancer Panel). If insufficient DNA was obtained for the full CPD panels, the Penn Precision Panel (PPP) was performed, which contains a subset of 77 amplicons covering portions of 20 cancer related genes, including KRAS and p53 but not SMAD4 or p16. Samples for CPD version 1 or PPP were sequenced on an Illumina MiSeq to a minimum mean coverage of 1000X.

For samples sequenced after mid-2017, CPD version 2 used a custom Agilent HaloPlex library preparation (Agilent, Santa Clara, CA) to cover approximately 0.5 megabases, including the entire exonic (coding) sequence of 152 genes, + 10 base pairs of intronic sequence, and including KRAS, p53, SMAD4, and p16[2]. The 152 genes sequenced on the CPD panel may be found at (https://www.pennmedicine.org/departments-and-centers/center-for-personalized-diagnostics/gene-panels). The library preparation included unique molecular identifiers to identify duplicate reads. Specimens were sequenced on the Illumina HiSeq 2500 platform (Illumina, San Diego, CA) using multiplexed, paired end reads. Analysis and interpretation were performed using a customized bioinformatics pipeline, Halo_v1.2.

Race/Ethnicity Reporting

Race/ethnicity was reported in this study so that readers could assess appropriate inclusion of under-represented clinical trial populations and determine if this study is generalizable to such groups. Study participants classified themselves according to options provided by the investigator and could decline to provide race/ethnicity data. Results of race/ethnic reporting is available in Supplemental Table 1.

Randomization Procedures

Randomization sequences were created at the Abramson Cancer Center Biostatistics Core Facility in the Department of Biostatistics & Epidemiology of the University of Pennsylvania Perelman School of Medicine. Randomization was blocked in blocks of random sizes to prevent investigators from deducing the treatment assignments of future patients. Randomization sequences were generated by a pseudo-random number generator and the randomization result was provided to the treating investigator by the Biostatistics Core after subjects were enrolled and deemed to be eligible for the study. Randomizations was not subsequently masked to either patients or clinical staff.

eTable 1. Baseline Characteristics

Characteristics	GA + HCQ N=55	GA N=57	Total N=112
Age – Years			
Median	64	65	65
Range	48-86	43-82	43-86
Distribution – No (%)			
<70 yr	35 (64)	40 (70)	75 (67)
≥70 yr	20 (36)	17 (30)	37 (33)
Gender			
Female	22 (38)	23 (42)	45 (40)
Male	33 (62)	34 (60)	67 (60)
Race/Ethnic Group – No (%)			
Caucasian	51 (93)	53 (93)	104 (93)
African-American	3 (5)	4 (9)	7 (6)
Asian	1 (2)	0	1 (1)
Hispanic	0	0	0
Other	0	0	0
ECOG Performance Status – No (%)			
0	27 (49)	30 (53)	57 (51)
1	28 (51)	27 (47)	55 (49)
Pancreatic Tumor Location – No (%)			
Head	22 (40)	29 (51)	51 (46)
Body	20 (36)	15 (26)	35 (31)
Tail	13 (24)	13 (23)	26 (23)
Site of Metastatic Disease – No (%)			
Liver	29 (53)	32 (56)	61 (54)
Lung	10 (18)	20 (35)	30 (27)
Peritoneum	5 (9)	2 (4)	7 (6)
Other or unknown	11 (20)	3 (5)	14 (13)
Carbohydrate Antigen 19-9 – Units/mL			
Median	2046	1375	1518
Range	1-113610	2-50111	1-113610

eTable 2A. Chemotherapy Dose Reductions by Arm

	GA + HCQ	GA Alone
Mean dose intensity gemcitabine (received dose ÷ expected dose)	0.94	0.91
Mean dose intensity gemcitabine (received dose ÷ expected dose)	0.93	0.89
Subjects with gemcitabine dose reductions – N (%)	15 (32)	19 (40)
Subjects with ‘nab-paclitaxel dose reductions – N (%)	16 (34)	16 (34)

eTable 2B. HCQ Dose Delays and Reductions

Toxicity	HCQ dose reduced	HCQ held and restarted	HCQ stopped	Total
Neuropsychiatric Symptoms*	--	1	2	3
Blurry vision	2	--	1	3
Rash	--	2	--	2
Nausea/vomiting	--	--	1	1
Neutropenia + Thrombocytopenia	--	--	1	1
Total	2	3	5	10

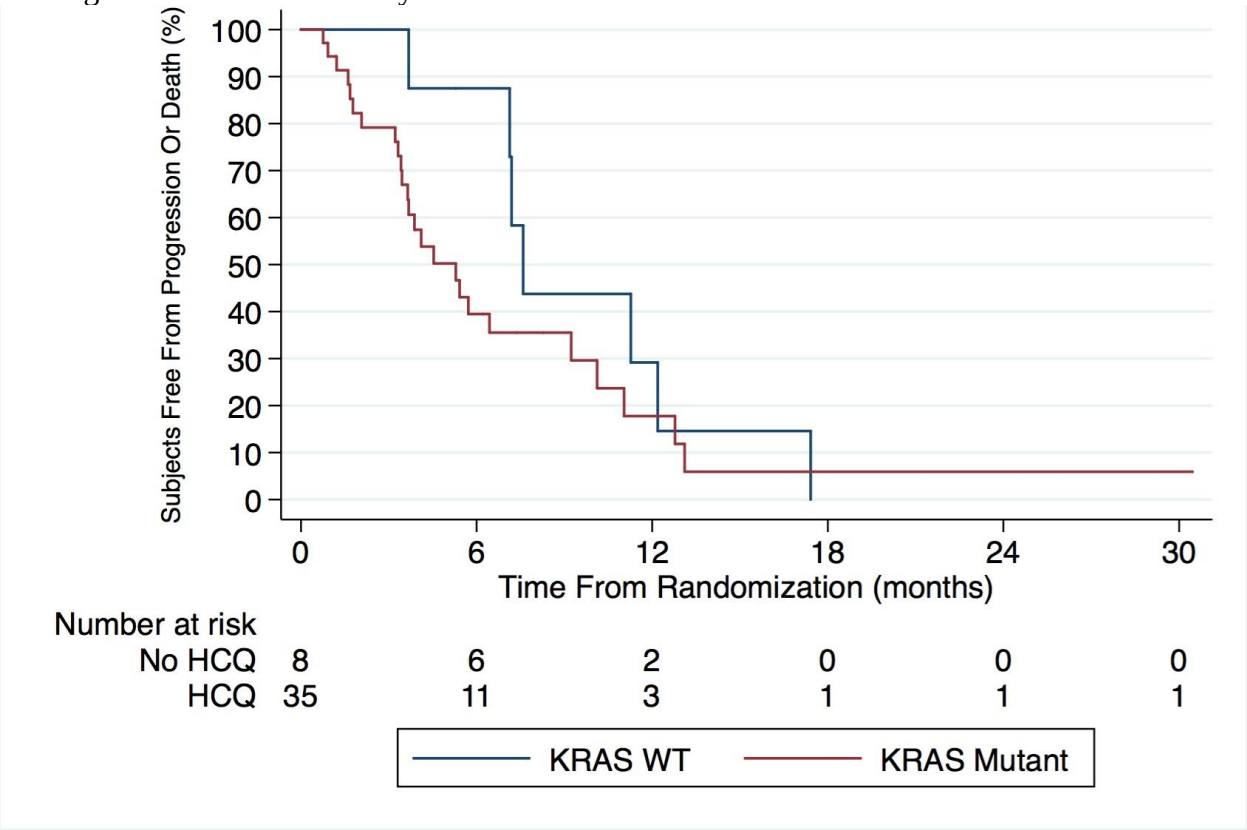
*Neuropsychiatric symptoms experienced due to HCQ included restlessness, insomnia, anxiety, cognitive dysfunction, and gait disturbance

eTable 3. Genomic Analysis

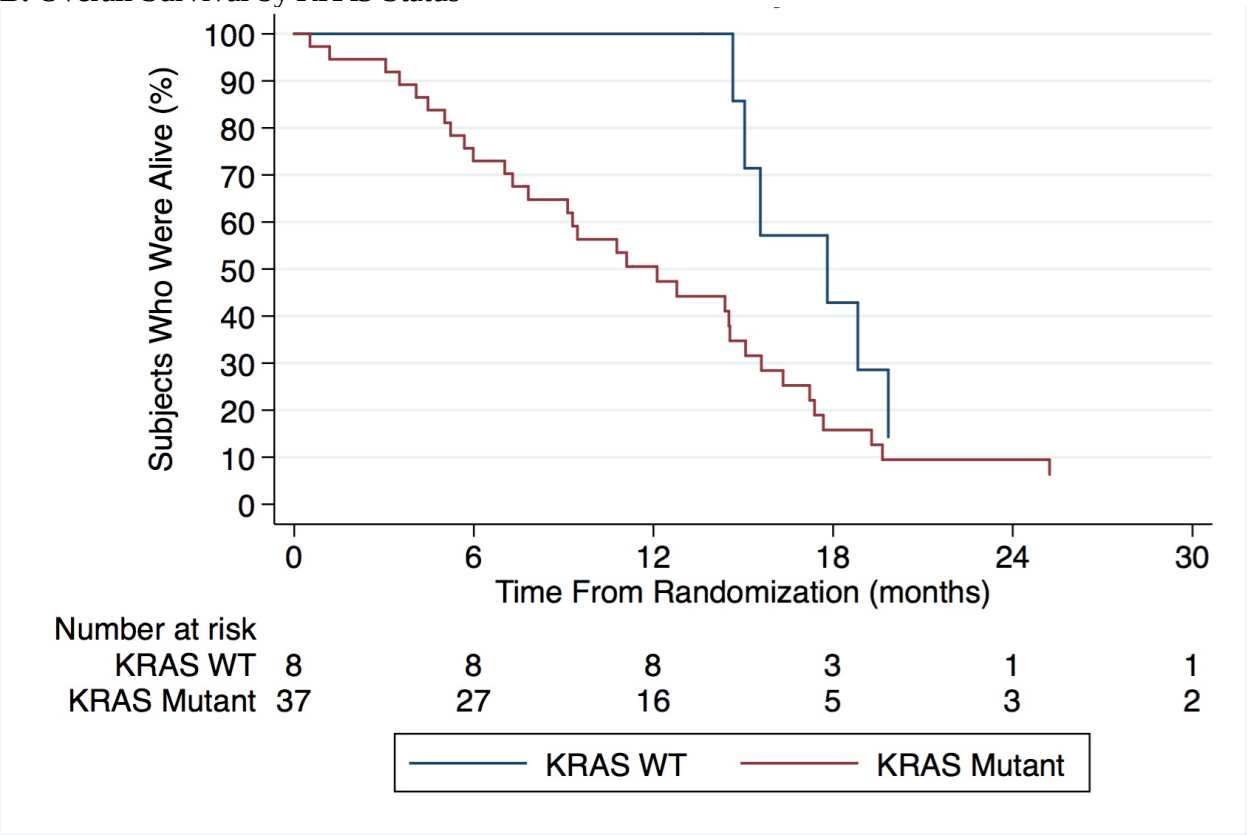
Mutation	HCQ	No HCQ
p53	17/21 (81%)	16/24 (67%)
KRAS	21/21 (100%)	16/24 (67%)
SMAD4	3/14 (21%)	5/18 (28%)
p16	0/3 (0%)	5/10 (50%)

eFigure 1. Survival by *KRAS* Status

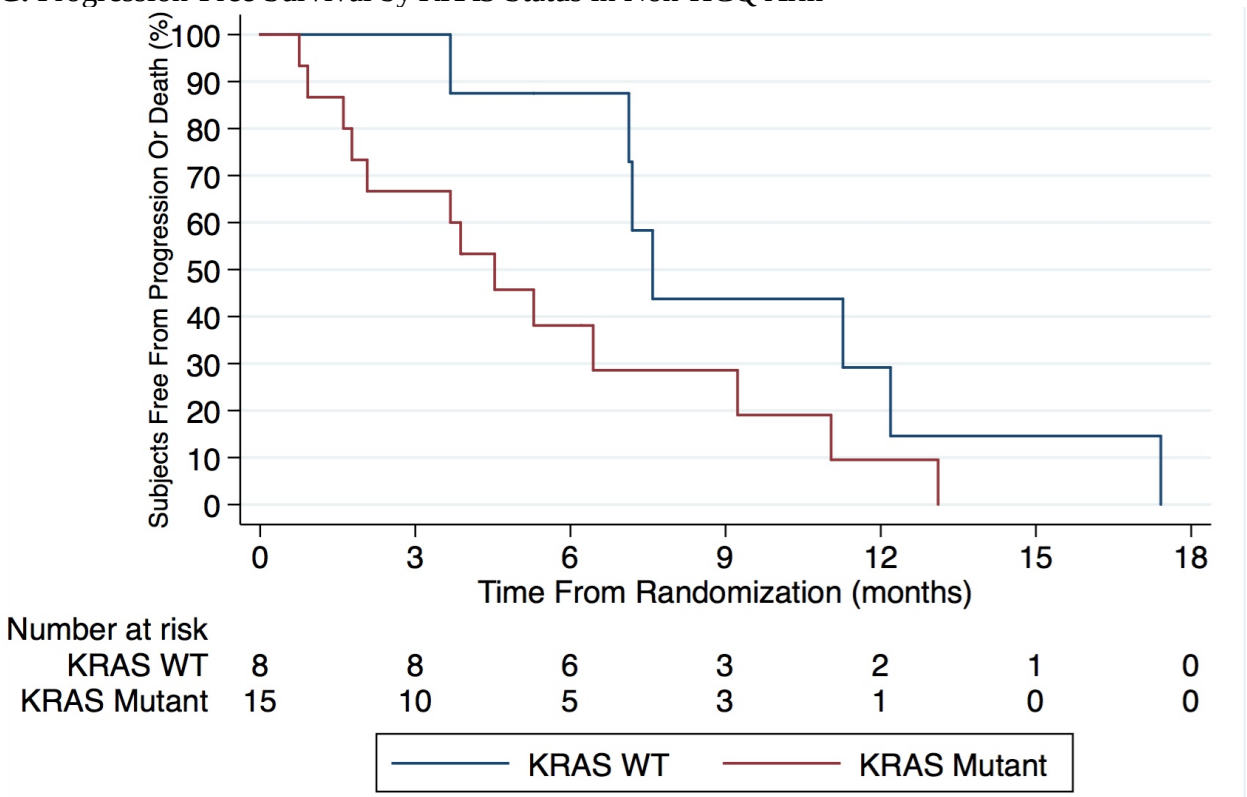
A. Progression-Free Survival by *KRAS* Status



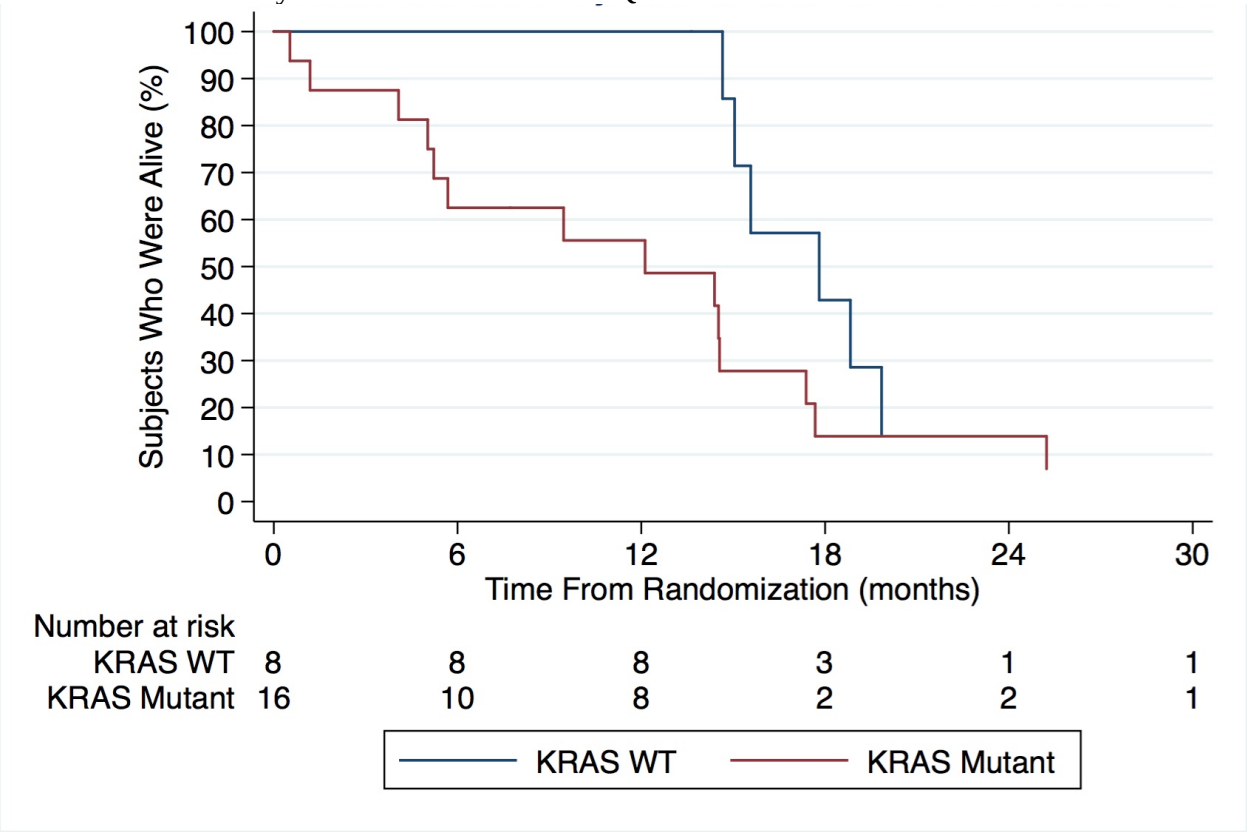
B. Overall Survival by *KRAS* Status



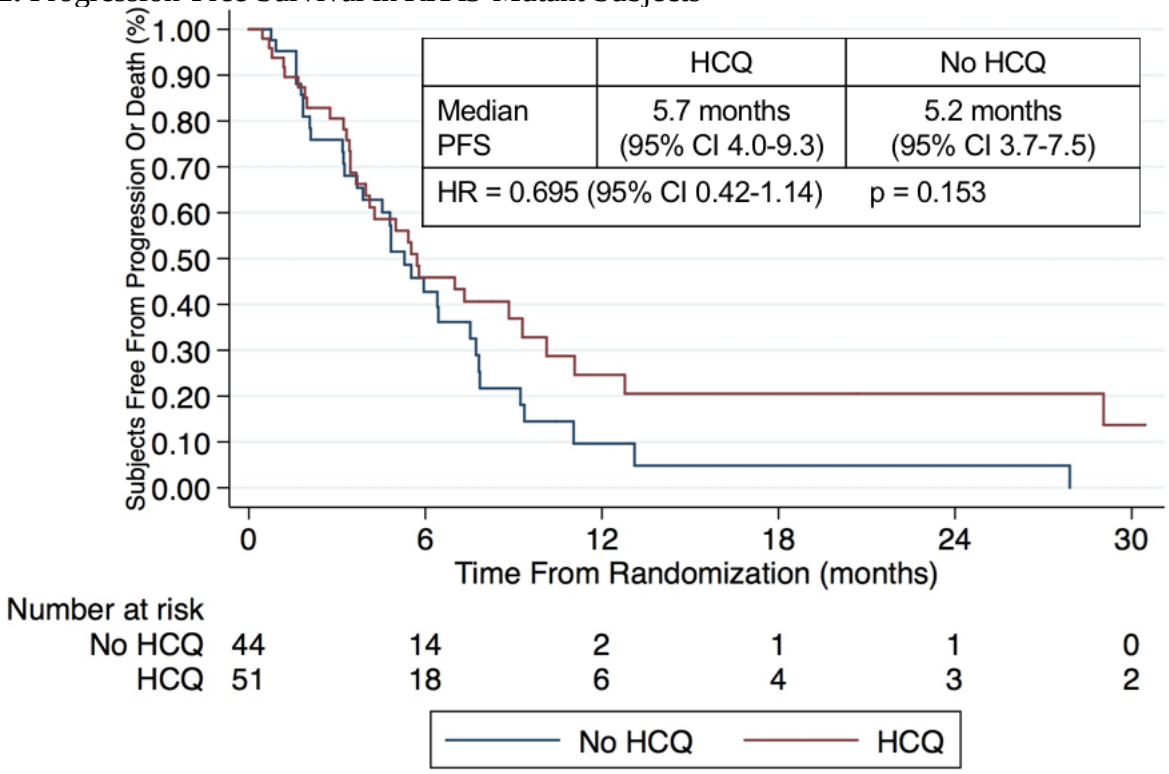
C. Progression-Free Survival by *KRAS* Status in Non-HCQ Arm



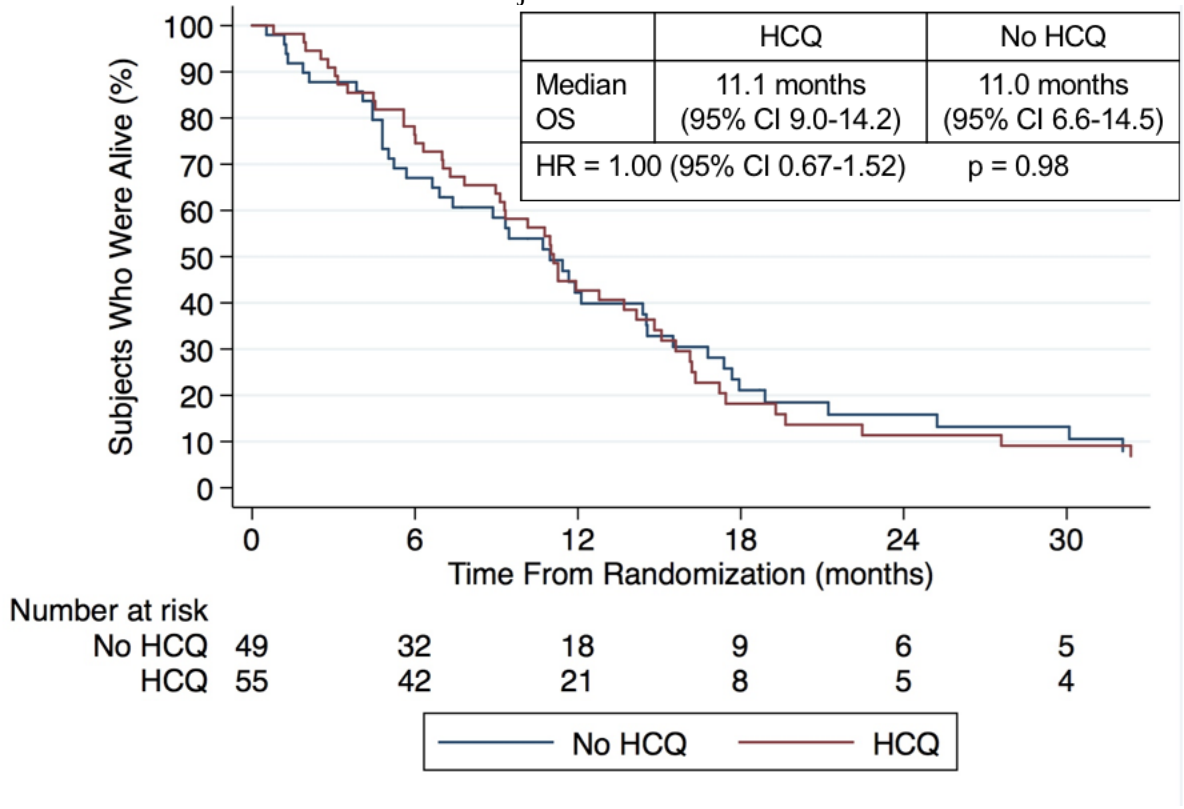
D. Overall Survival by *KRAS* Status in Non-HCQ Arm



E. Progression-Free Survival in *KRAS*-Mutant Subjects

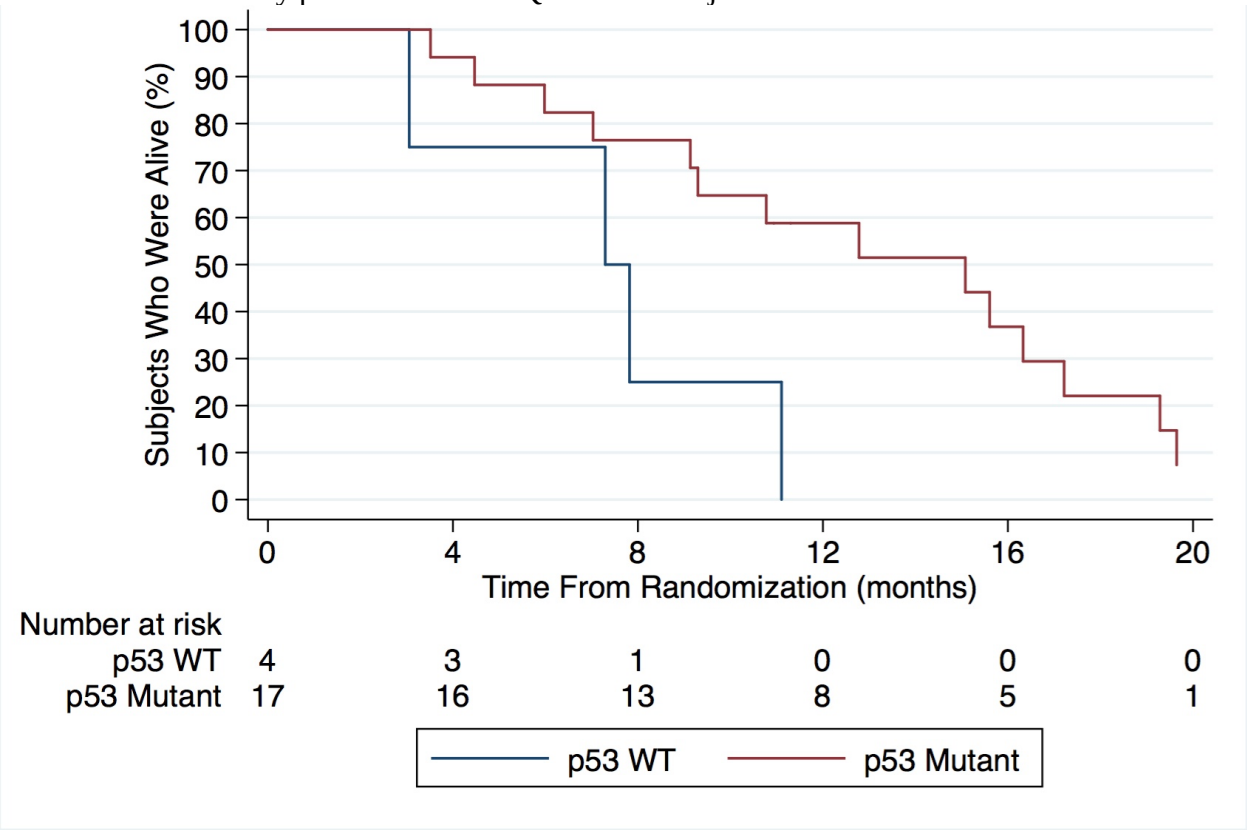


F. Overall Survival in *KRAS*-Mutant Subjects

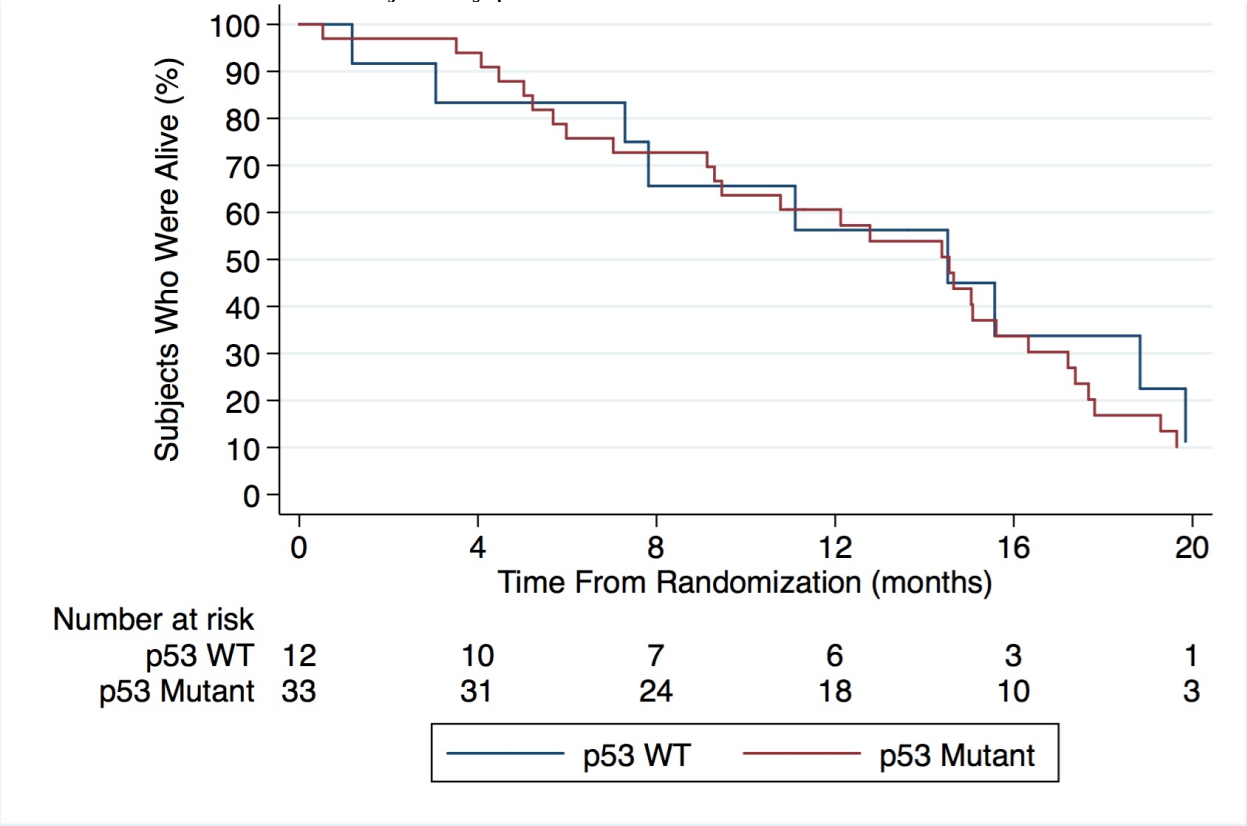


eFigure 2. Overall Survival by p53 Status

A. Overall Survival by p53 Status in HCQ-Treated Subjects



B. Overall Survival in All Subjects by p53 Status



eReferences

1. Maxwell KN, Soucier-Ernst D, Tahirovic E, et al. Comparative clinical utility of tumor genomic testing and cell-free DNA in metastatic breast cancer. Breast Cancer Res Treat. 2017 Aug;164(3):627-638.

2. Viaene AN, Santi M, Rosenbaum J, Li MM, Surrey LF, Nasrallah MP. SETD2 mutations in primary central nervous system tumors. Acta Neuropathol Commun. 2018 Nov 12;6(1):123.