Biomarkers associated with resistance or response to CDK4/6 treatment in patients with metastatic hormone-receptive positive breast cancer

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INTRODUCTION

CDK4/6 inhibitor (CDKi) drugs are the current standard of care for treatment of first and second-line hormone-receptor positive/HER2 negative (HR+/HER2-) metastatic breast cancers. To date, no biomarker of response has been identified. Treatment induced RB1 mutations were noted as mechanism of resistance to palbociclib and fulvestrant in about 5% of patients treated on PALOMA3 trials, whereas PI3K and ESR1 mutations emerged as potential resistance to the anti-hormonal backbone[1]. Additionally, FGFR1 amplification has been suggested as a resistance pathway to fulvestrant and ribociclib[2]. Utilizing next-gen sequencing (NGS), chromogenic in situ hybridization (CISH) and immunohistochemistry (IHC) (Caris Life Sciences, Phoenix, AZ) data from n=155 HR+/HER2- patients treated at the University of Tennessee West Cancer Center, we attempted to **retrospectively** identify a molecular signature of resistance or response as measured by PFS

METHODS

Specimens were profiled using massively parallel NGS sequencing using either a 45-gene TruSeq Amplicon panel (n=) or a 592-gene SureSelect XT panel (Agilent, Santa Clara, CA). Sequencing was performed on an Illumina MiSeq or NextSeq instrument for the 45- and 592-gene panels respectively (Illumina, San Diego, CA). Only alterations with known pathogenic potential were considered aberrant. Copy number alterations (CNA) were also explored on samples profiled with the 592-gene NGS panel. Gains ≥6 copies were considered amplified.

All IHC stains were performed using automated platforms (Benchmark, Ventana Medical Systems and DAKO Autostainer, Agilent) at CLIA/CAP/ISO15189/NYSDOH certified clinical laboratory (Caris Life Sciences, Phoenix, AZ). PD-L1 expression was evaluated in the tumor cells (TC) using SP142 (Ventana).

DEMOGRAPHICS

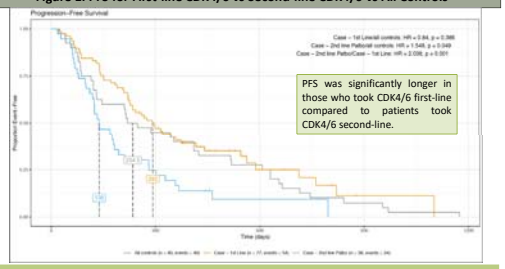
Table 1. Patient and Tumor Characteristics

	1st Line Cases (N=77)	2nd Line Cases (N=38)	All Controls (N=40)	P-value*
Age (years)				0.19
Median	61	59.5	64	
Range	37-86	32-76	37-87	
Gender				1
F	76 (98.7%)	38 (100%)	39 (97.5%)	
M	1 (1.3%)	0 (0%)	1 (2.5%)	
Race				0.74
Black	22 (28.6%)	12 (31.6%)	13 (32.5%)	
Hispanic	3 (3.9%)	0 (0%)	1 (2.5%)	
Indian	1 (1.3%)	0 (0%)	1 (2.5%)	
White	45 (58.4%)	25 (65.8%)	24 (60%)	
Unknown	6 (7.8%)	1 (2.6%)	7 (17.5%)	
Grade at Diagnosis				0.21
1	10 (13%)	7 (18.4%)	3 (7.5%)	
1 or 2	1 (1.3%)	0 (0%)	0 (0%)	
2	43 (55.8%)	13 (34.2%)	16 (40%)	
2 or 3	0 (0%)	2 (5.3%)	1 (2.5%)	
3	16 (20.8%)	8 (21.1%)	11 (27.5%)	
Unknown	7 (9.1%)	8 (21.1%)	9 (22.5%)	
Stage at Diagnosis				0.16
Early	41 (53.2%)	13 (34.2%)	19 (47.5%)	
Late	36 (46.8%)	25 (65.8%)	21 (52.5%)	
Received Adjuvant Chemo				0.12
No	15 (19.5%)	4 (10.5%)	9 (22.5%)	
Yes	27 (35.1%)	15 (39.5%)	20 (50%)	
Yes, neoadjuvant	20 (26%)	8 (21.1%)	3 (7.5%)	
Unknown	15 (19.5%)	11 (28.9%)	8 (20%)	
Endocrine DFS interval				0.72
<2 yrs	3 (3.9%)	8 (21.1%)	6 (15%)	
>5 yrs	1 (1.3%)	7 (18.4%)	9 (22.5%)	
2-5 yrs	2 (2.6%)	7 (18.4%)	11 (27.5%)	
Unknown	71 (92.2%)	16 (42.1%)	14 (35%)	

*P-values calculated using t-test, chi-squared test or Fisher's exact test where appropriate. Unknowns excluded from p-value calculations.

CLINICAL OUTCOMES

Figure 1. PFS for First-line CDK4/6 vs Second-line CDK4/6 vs All Controls



BIOMARKER ANALYSIS

Figure 2. Percent Cases Mutated (SEQ) or Amplified (CNA)

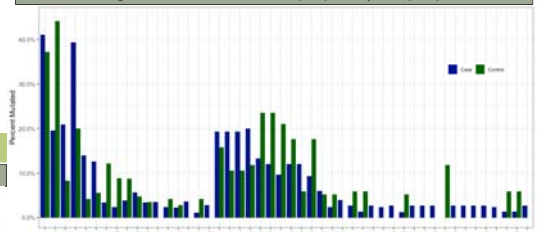


Figure 3. Percent Cases Mutated by Protein Expression IHC

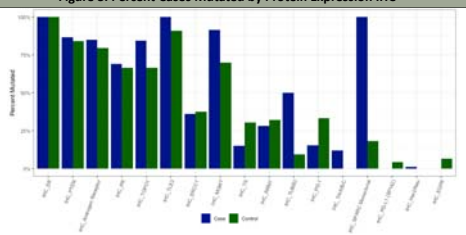


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Figure 4. PFS Based on ESR1 Mutation Status

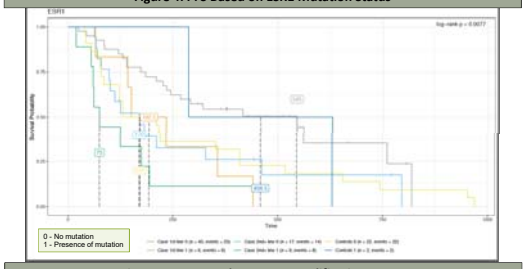


Figure 5. PFS Based on NSD3 Amplification Status

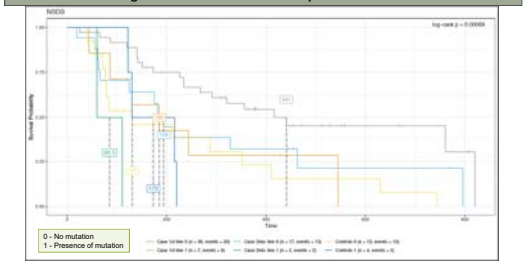


Table 2. PFS Calculated for Mutated Biomarkers

Biomarker	Therapy	Mutated			Wild Type			Mutated vs. Wildtype		
		n	median PFS	95% CI	n	median PFS	95% CI	HR	95% CI	p-value
TP53	case 1st line	9	140	25-226	35	545	321-inf	5.84	2.55-13.38	<.001
	case 2nd+ line	3	211	179-inf	26	122.5	75-182	0.34	0.08-1.44	0.142
	control	15	211	79-240	19	410	122-630	1.44	0.72-2.89	0.305
RB1	case 1st line	1	140	-inf-inf	43	357	226-562	3.14	0.42-23.47	0.264
	case 2nd+ line	0	-	-	24	125	79-194	-	-	-
	control	0	-	-	36	234.5	122-410	-	-	-
PIK3CA	case 1st line	18	339	185-562	28	416	173-inf	1.08	0.52-2.25	0.833
	case 2nd+ line	13	79	61-182	16	174.5	100-301	1.4	0.63-3.1	0.405
	control	13	287	87-518	22	171	79-424	0.78	0.30-1.56	0.482
TMB	case 1st line	1	185	-inf-inf	45	416	234-562	2.52	0.34-18.78	0.366
	case 2nd+ line	1	Not reached	-inf-inf	24	125	79-194	0	0-inf	0.996
	control	0	-	-	24	213.5	85-410	-	-	-
NSD3	case 1st line	7	185	44-inf	36	441	235-inf	2.49	0.99-6.28	0.053
	case 2nd+ line	2	85.5	60-inf	17	194	63-463	5.86	1.23-27.79	0.026
	control	4	173	122-inf	13	131	71-352	1.29	0.41-4.06	0.659
ESR1	case 1st line	6	192.5	85-inf	40	545	235-761	2.45	0.90-6.06	0.052
	case 2nd+ line	9	75	19-194	17	170	79-328	2.09	0.87-5.04	0.1
	control	2	458.5	287-inf	22	171	79-410	0.71	0.17-3.07	0.649
FGFR1 (seq)	case 1st line	0	-	-	46	357	226-562	-	-	-
	case 2nd+ line	0	-	-	29	125	79-194	-	-	-
	control	0	-	-	36	234.5	122-410	-	-	-
FGFR1 (CNA)	case 1st line	5	130	44-inf	41	416	235-761	2.7	0.93-7.82	0.068
	case 2nd+ line	2	85.5	60-inf	21	175	75-328	4.38	0.97-19.84	0.055
	control	4	173	122-inf	15	211	71-410	1.97	0.62-6.25	0.251

BIOMARKER ANALYSIS

Figure 6. Percentage of Aberrant Mutations Pre- and Post-Treatment Biopsies

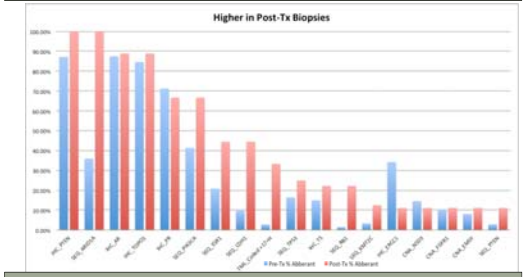
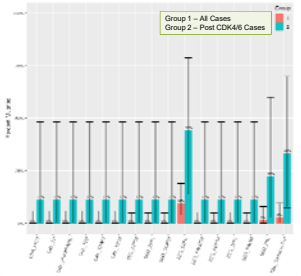


Figure 7. Percentage of Aberrant Mutations in All Cases vs Post-CDK4/6 Cases

Biomarkers of CDH1, RB1, and TML were increased in frequency post CDK4/6 treatment. However, there were only 7 specimens in this post treatment group.



CONCLUSIONS

- NSD3 gene amplification appears to be the only alteration that may significantly affect tumor CDK4/6 response. NSD3 gene is a member of DNA- methyltransferase pathway and highly correlated with ESR1 and ERα.
- Three biomarkers, RB1, CDH1, TML>17 mutations are significantly more frequent in post-palbociclib biopsies.
- FGFR1 does not appear to play a clinical role in our population.
- RB1 mutations are present post treatment, but are infrequent.
- ESR1, PI3K, ARID1A are numerically more frequently seen in post treatment biopsies.
- TP53 mutation represents poor prognosis regardless of line of therapy or treatment option.
- This study is limited by its retrospective nature and relatively small numbers. Further testing will be required to confirm the relationships observed.

**References available upon request.