

Supplemental Appendix

Paraoxonase-1 Activity in Breast Cancer Patients Treated with Doxorubicin with or without Trastuzumab

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Supplemental Table 1: Number of available measurements of paraoxonase and arylesterase at baseline, and at 1 and 2 months after doxorubicin initiation in the analytic population

Biomarker	Overall			Doxorubicin			Doxorubicin+Trastuzumab		
	Baseline	1	2	Baseline	1	2	Baseline	1	2
		month	months		month	months		month	months
Paraoxonase	225	208	198	185	169	161	40	39	37
Arylesterase	224	208	199	184	169	162	40	39	37

Supplemental Table 2A: Associations between baseline clinical variables and early changes in Pon activity levels.

Variable	Effect size* (95% CI)	P-value
Age, per 10 years	0.98 (0.96,1.01)	0.200
Black race	0.99 (0.92, 1.05)	0.670
Disease stage		
Stage 1	Ref	
Stage 2	1.06 (0.94,1.20)	0.334
Stage 3 or 4	1.05 (0.93,1.18)	0.421
Body mass index, per 5 Kg/m ²	1.03 (1.01,1.05)	0.004
Current or past smoking	1.02 (0.98,1.07)	0.327
Diabetes mellitus	1.00 (0.95,1.06)	0.952
Hypertension	0.92 (0.80,1.06)	0.260
Hyperlipidemia	1.01 (0.96,1.07)	0.606
Statin use	1.04 (0.98,1.10)	0.166
ACEI/ARB or beta-blocker use	1.07 (0.95,1.19)	0.260
<p>Log-ratio of Pon relative to baseline was included as the dependent variable.</p> <p>*Exponentiated coefficients are presented and should be interpreted as the expected multiple of change in Pon levels relative to baseline per unit difference in the independent variable under consideration. Associations were modeled using repeated measures linear regression estimated via generalized estimating equations (GEE).</p> <p>ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; CI=Confidence interval; Pon = paraoxonase; Ref = reference.</p>		

Supplemental Table 2B: Associations between baseline clinical variables and early changes in Aryl activity levels.

Variable	Effect size* (95% CI)	P-value
Age, per 10 years	0.98 (0.97,1.00)	0.050
Black race	0.99 (0.95,1.03)	0.631
Disease stage		
Stage 1	Ref	
Stage 2	1.02 (0.96,1.09)	0.514
Stage 3 or 4	1.01 (0.94,1.09)	0.749
Body mass index, per 5 Kg/m ²	1.01 (1.00,1.03)	0.076
Current or past smoking	1.04 (1.01,1.08)	0.009
Diabetes mellitus	0.98 (0.93,1.03)	0.450
Hypertension	0.95 (0.88,1.02)	0.184
Hyperlipidemia	1.05 (1.00,1.10)	0.053
Statin use	1.05 (0.99,1.11)	0.121
ACEI/ARB or beta-blocker use	1.01 (0.94,1.09)	0.709
<p>Log-ratio of Aryl relative to baseline was included as the dependent variable.</p> <p>*Exponentiated coefficients are presented and should be interpreted as the expected multiple of change in Aryl levels relative to baseline per unit difference in the independent variable under consideration. Associations were modeled using repeated measures linear regression estimated via generalized estimating equations (GEE).</p> <p>ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker, Aryl = arylesterase; CI=Confidence interval; Ref = reference.</p>		

Supplemental Table 3A: Number of available LVEF assessments at prespecified visit timepoints according cancer therapy regimen

Visit	Overall	Doxorubicin	Doxorubicin+ Trastuzumab
Baseline	184	148	36
2 months		*	31
5 months	169	138	31
8 months		*	27
12 months	151	119	32
24 months	124	97	27
≥36 months	103	74	29
*Visit is not a study timepoint in the doxorubicin group			

Supplemental Table 3B: Number of available LVEF assessments at prespecified visit timepoints according to CTRCD

Visit	No CTRCD (N=150)	CTRCD (N=34)
Baseline	150	34
2 months*	21	10
5 months	137	32
8 months*	18	9
12 months	120	31
24 months	96	28
≥36 months	80	23
*Numbers reflect available LVEF assessments in the doxorubicin+trastuzumab group only; Visit is not a study timepoint in the doxorubicin group		

Supplemental Table 4: Associations between change in Pon and Aryl levels from baseline and time to CTRCD according to race.

Biomarker Activity Level	Change from baseline at 1 month				Change from baseline at 2 months			
	Black Race		White/Other Race		Black		White/Other	
	HR (95% CI)	P- value	HR (95% CI)	P- value	HR (95% CI)	P- value	HR (95% CI)	P- value
Pon	1.03 (0.94,1.13)	0.537	1.11 (0.85,1.45)	0.432	1.24 (0.89,1.74)	0.200	1.15 (0.99,1.35)	0.072
Aryl	1.15 (0.89,1.49)	0.283	1.05 (0.84,1.30)	0.677	1.18 (0.87,1.61)	0.292	1.10 (0.90,1.34)	0.371

Hazard ratios should be interpreted per 10% increase in biomarker value from baseline to 1 or 2 months after doxorubicin initiation; Associations were modeled using Cox proportional hazards models; associations were adjusted for age, cancer therapy regimen and hypertension. Aryl = Arylesterase; CI = confidence interval; HR = hazard ratio; Pon = paraoxonase.

Supplemental Table 5: Associations between change in Pon and Aryl levels from baseline and time to CTRCD according to age (cohort median age was used to define categories)

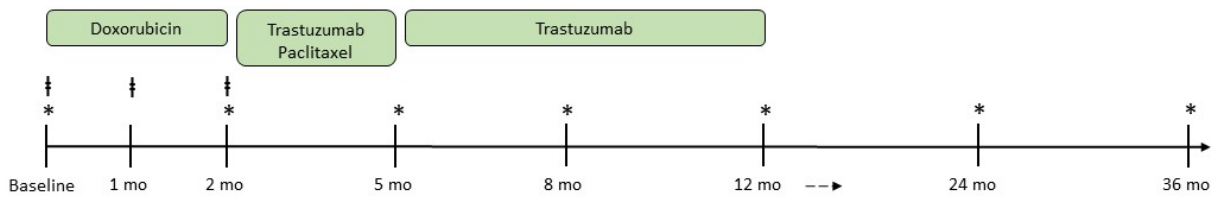
Biomarker Activity Level	Change from baseline at 1 month				Change from baseline at 2 months			
	Age <49		Age ≥49		Age <49		Age ≥49	
	HR (95% CI)	P- value	HR (95% CI)	P- value	HR (95% CI)	P- value	HR (95% CI)	P- value
Pon	1.09 (0.87,1.36)	0.447	0.98 (0.83,1.16)	0.826	1.20 (0.95,1.52)	0.121	1.15 (0.96,1.36)	0.122
Aryl	1.13 (0.92,1.39)	0.248	1.05 (0.85,1.31)	0.635	1.21 (0.96,1.60)	0.103	1.02 (0.81,1.28)	0.873

The median age across the cohort was 49 years. Hazard ratios should be interpreted per 10% increase in biomarker value from baseline to 1 or 2 months after doxorubicin initiation; Associations were modeled using Cox proportional hazards models; associations were adjusted for race, cancer therapy regimen and hypertension. Aryl = Arylesterase; CI = confidence interval; HR = hazard ratio; Pon = paraoxonase.

Doxorubicin



Doxorubicin + Trastuzumab



† Blood sample collected at visit

* Transthoracic echocardiogram performed at visit

Supplemental Figure 1: Biomarker sampling protocol according to treatment regimen. Patients enrolled in the study received either Doxorubicin or Doxorubicin+Trastuzumab. Blood samples were collected at baseline, 1 month, and 2 months. Echocardiograms were performed periodically as indicated by asterisks in the figure.