SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Biomarker measurements

Four biomarkers including high sensitivity cardiac troponin T (hs-cTnT), N-terminal pro-B-type natriuretic peptide (NT-proBNP), placental growth factor (PIGF) and growth differentiation factor 15 (GDF-15) were assessed in all treatment groups. hs-cTnT was measured using the 4th generation Elecsys® TnT-hs assay on Cobas® platform (Roche Diagnostics, Manheim, Germany) which has a coefficient of variation (CV) of <10% at the 99th percentile upper reference limit of 14 ng/L, a limit of detection of 5 ng/L and limit of blank of 3 ng/L. NT-proBNP was measured using the Elecsys® NT-proBNP assay on Cobas® platform, which has a CV of 2.9 to 6.1% and measurement range of 5-35,000 ng/L. PIGF was measured using the Elecsys® PIGF immunoassay with a CV of <5% and measurement range of 3-10,000 ng/L. GDF-15 was measured with the Elecsys® GDF-15 immunoassay with a measurement range is 400-20,000 ng/L and CV <10%. GDF-15 reagents were available for a slightly smaller subset of individuals. In addition, myeloperoxidase (MPO) was measured in the Doxorubicin and Doxorubicin+Trastuzumab treatment groups using an ELISA assay (Cleveland HeartLab Inc, Cleveland Ohio) on a Roche Cobas® 6000 platform with a c501 module.

Table S1. **Number of available biomarker measurements in the analysis patient population.**

Biomarker	Median (IQR) number of measurements per patient	Total number of measurements within different intervals from the time of cancer therapy initiation			
		<6 months	6-12 months	12-24 months	24-36 months
hs-cTnT	5 [4 - 8]	1238	393	194	115
NT-proBNP	5 [4 - 8]	1231	392	194	115
PIGF	5 [4 - 8]	1219	391	194	115
GDF-15	3 [0 - 5]	753	247	99	38
MPO	5 [4 - 6]	974	233	174	91
GDF-15 was measured in a smaller subset of patients given limitations in the number of reagents; MPO was analyzed only in the Doxorubicin and Doxorubicin+Trastuzumab groups					

Table S2. Baseline characteristics stratified according to cancer therapy regimen in the subset of patients with quantitated echocardiograms at baseline and during at least one follow-up visit (N=254).

Variable	Doxorubicin (N=155)	Trastuzumab (N=53)	Doxorubicin+ Trastuzumab (N=46)
Age (years)	49 [41 - 57]	52 [45 - 58]	43 [38 - 53]
Race, %(N))			
Black	41 (26.5)	6 (11.3)	14 (30.4)
Caucasian/other/unknown	114 (73.5)	47 (88.7)	32 (69.6)
Breast cancer side, % (N)			
Left	71 (45.8)	21 (39.6)	21 (45.7)
Right	79 (51.0)	31 (58.5)	19 (41.3)
Bilateral	5 (3.2)	1 (1.9)	6 (13.0)
Metastases or recurrence, % (N)	1 (0.6)	3 (5.7)	0 (0)
Breast cancer stage, % (N)			
Stage 1	21 (13.5)	22 (41.5)	9 (19.6)
Stage 2	96 (61.9)	24 (45.3)	23 (50)
Stage 3	37 (23.9)	4 (7.5)	14 (30.4)
Stage 4	1 (0.6)	3 (5.7)	0 (0)
Radiation therapy, % (N)			
None	56 (36.4)	23 (43.4)	15 (32.6)
Left-sided	46 (29.9)	13 (24.5)	16 (34.8)
Right-sided	48 (31.2)	16 (30.2)	11 (23.9)
Bilateral	4 (2.6)	1 (1.9)	4 (8.7)

Left ventricular ejection fraction (%)	53 [51 - 56]	53 [52 - 56]	54 [53 - 57]
Body mass index (kg/m^2)			
<25	60 (38.7)	23 (43.4)	17 (37)
25-30	51 (32.9)	17 (32.1)	16 (34.8)
≥30	44 (28.4)	13 (24.5)	13 (28.3)
Systolic blood pressure (mmHg)	124 [116 - 133]	127 [116 - 136]	124 [116 - 134]
Diastolic blood pressure (mmHg)	74 [69 - 81]	74 [69 - 81]	73 [70 - 79]
Heart rate (bpm)	80 [72 - 89]	78 [72- 90]	79 [73 - 89]
Current or past Smoking , %(N)	64 (41.8)	18 (34)	15 (32.6)
History of diabetes mellitus, %(N)	14 (9)	4 (7.5)	2 (4.4)
History of hypertension, %(N)	46 (29.9)	21 (39.6)	10 (21.7)
History of hyperlipidemia or statin use, %(N)	35 (22.6)	8 (15.4)	11 (23.9)
Hyperlipidemia, %(N)	34 (21.9)	8 (15.4)	11 (23.9)
Statin use, %(N)	15 (9.7)	6 (11.3)	4 (8.7)
ACEI/ARB or beta-blocker, %(N)	33 (21.3)	10 (18.9)	5 (10.9)
ACEI, %(N)	17 (11)	5 (9.4)	2 (4.3)
ARB, %(N)	8 (5.2)	5 (9.4)	1 (2.2)
Beta-blocker, %(N)	11 (7.1)	3 (5.7)	2 (4.3)
hs-cTnT (pg/mL)	3 [3 - 4]	3 [3 - 5]	3 [3 -4]
NT-proBNP (pg/mL)	61 [37 - 100]	137 [79 240]	62 [31 - 111]
GDF-15 (pg/mL)	704 [532 - 908]	585 [435 - 902]	599 [523 - 722]
PIGF (pg/mL)	13 [10 - 16]	14 [10 - 17]	13 [10 - 16]
MPO (pmol/L)	308 [212 - 499]		344 [247 - 446]
Count (%) is presented for categorical variables; median [interquartile range (IQR)] is presented for continuous variables; ACEI=angiotensin-converting enzyme inhibitor,			

ARB=angiotensin receptor blocker; GDF-15=Growth differentiation factor 15; hs-cTnT= High sensitivity cardiac troponin T; MPO=Myeloperoxidase; NT-proBNP= N-terminal pro-B-type natriuretic peptide; PIGF=Placental growth factor; MPO was not measured in the Trastuzumab group

Table S3. Associations between baseline biomarker levels and changes in LVEF.

Biomarker	Change in LVEF	
	Beta (95% CI)	**P-value**
hs-cTnT	0.1 (-0.5, 0.8)	0.73
NT-proBNP	0.2 (-0.3, 0.7)	0.38
PIGF	0.0 (-0.8, 0.9)	0.93
GDF-15	0.2 (-0.8, 1.1)	0.76
MPO	-0.2 (-0.6, 0.2)	0.39
Biomarker levels were log2 transformed; Beta estimates should be interpreted per doubling of baseline biomarker levels; Associations were adjusted for baseline variables including LVEF, age, hypertension, smoking, BMI and time since treatment initiation (modeled non-parametrically using cubic spline and its effect allowed to vary across treatment groups by including an interaction term)		

Table S4. Associations between changes in biomarker levels and changes in circumferential and longitudinal strain.

Biomarker	Circumferential strain (%)				Longitudinal strain (%)			
	Contemporaneous		Subsequent Visit		Contemporaneous		Subsequent Visit	
	Beta (95% CI)	P-value	Beta (95% CI)	P-value	Beta (95% CI)	P-value	Beta (95% CI)	P- value
hs-cTnT	0.5 (0.1,1.03	0.023	0.4 (0.1,0.8)	0.026	0.1 (-0.1,0.4)	0.267	0.2 (0,0.4)	0.101
NT-proBNP	0.4 (-0.1,0.8)	0.109	0.6 (0.2,1.0)	0.006	0 (-0.3,0.2)	0.862	0.1 (-0.2,0.4)	0.575
PIGF	0.1 (-0.5,0.8)	0.665	0.3 (-0.8,1.5)	0.559	-0.3 (-1.0,0.3)	0.320	0 (-0.6,0.7)	0.950
GDF-15	0.2 (-0.4,0.9)	0.477	0.8 (-0.1,1.6)	0.0687	0.5 (-0.1,1.2)	0.089	0.1 (-0.4,0.6)	0.663
MPO	0.4 (0.1,0.8)	0.011	0.4 (-0.2,0.9)	0.170	0.1 (-0.2,0.4)	0.570	0.4 (0.1,0.6)	0.007

Biomarker levels were log2 transformed; Beta estimates represent the absolute change in the outcome under consideration for each doubling of biomarker levels from baseline to the same (contemporaneous) visit; or the subsequent change in the outcome for each doubling in biomarker value from baseline to the prior visit. Associations were adjusted for baseline variables including cancer therapy regimen, baseline levels of the outcome under consideration, baseline biomarker levels, age, hypertension, smoking, BMI and time since treatment initiation (modeled non-parametrically using a cubic spline, its effect allowed to vary across treatment groups by including an interaction term); GDF-15=Growth differentiation factor 15; hs-cTnT= High sensitivity cardiac troponin T; MPO=Myeloperoxidase; NT-proBNP= N-terminal pro-B-type natriuretic peptide; PIGF=Placental growth factor.

Table S5. Associations between changes in biomarker levels from baseline to a visit and change in LVEF at the subsequent visit; Interactions with cancer therapy regimen.

Biomarker	Doxorubicin	Trastuzumab	Doxorubicin+Trastuzumab	P-interaction
	Mean change in LVEF (95% CI)	Mean change in LVEF (95% CI)	Mean change in LVEF (95% CI)	
hs-cTnT	-0.3 (-0.9, 0.2)	0.7 (-0.6, 1.9)	-0.6 (-1.6, 0.5)	0.550
NT-proBNP	-0.6 (-1.3, 0.1)	-0.2 (-0.8, 0.4)	-1.3 (-2.0, -0.6)	0.017
PIGF	-0.3 (-1.6, 1.0)	2.6 (0.7, 4.4)	1.7 (-0.6, 4.0)	0.270
GDF-15	-0.7 (-2.3, 0.9)	-0.5 (-2.4, 1.4)	0.8 (-0.7, 2.3)	0.110
MPO	0.6 (-0.1, 1.2)	-	-0.2 (-1.4, 1.0)	0.290
Biomarker levels were log2 transformed; Beta estimates should be interpreted per doubling in biomarker levels from baseline to prior visit; Associations were adjusted for baseline biomarker levels and baseline variables including LVEF, age, hypertension, smoking, BMI and time since treatment initiation (modeled non-parametrically using cubic spline)				

Table S6. Incremental predictive value of biomarker change variables when added to a baseline clinical model.

Biomarker	Clinical model + biomarker	Absolute Change in Concordance index
hs-cTnT	0.699	0.005
NT-proBNP	0.724	0.030
MPO	0.692	0.008
NT-proBNP + hs-cTnT	0.724	0.030
All biomarker values were log2 transformed; The clinical model included cancer therapy regimen, LVEF, age, hypertension, body mass index (BMI) and smoking *The clinical model had a concordance index of 0.694 in the overall group, and 0.684 in the combined Doxorubicin and Doxorubicin+Trastuzumab groups (Note: MPO is only available in these treatment groups)		

Table S7. Proportion of patients with elevated hs-cTnT and NT-proBNP at the completion of anthracycline therapy.

Biomarkers	**N (%)**
hs-cTnT > 5 ng/L	156 (91.8)
hs-cTnT >14 ng/L	71 (41.8)
NT-proBNP >125 ng/L	56 (33.1)
NT-proBNP >150 ng/L	40 (23.7)
NT-proBNP >300 ng/L	13 (7.7)
For the Doxorubicin+ Trastuzumab group, patients treated with anthracyclines then trastuzumab were included; A total of 170 and 169 measurements were available at the time of completion of doxorubicin therapy for hs-cTnT and NT-proBNP, respectively.	

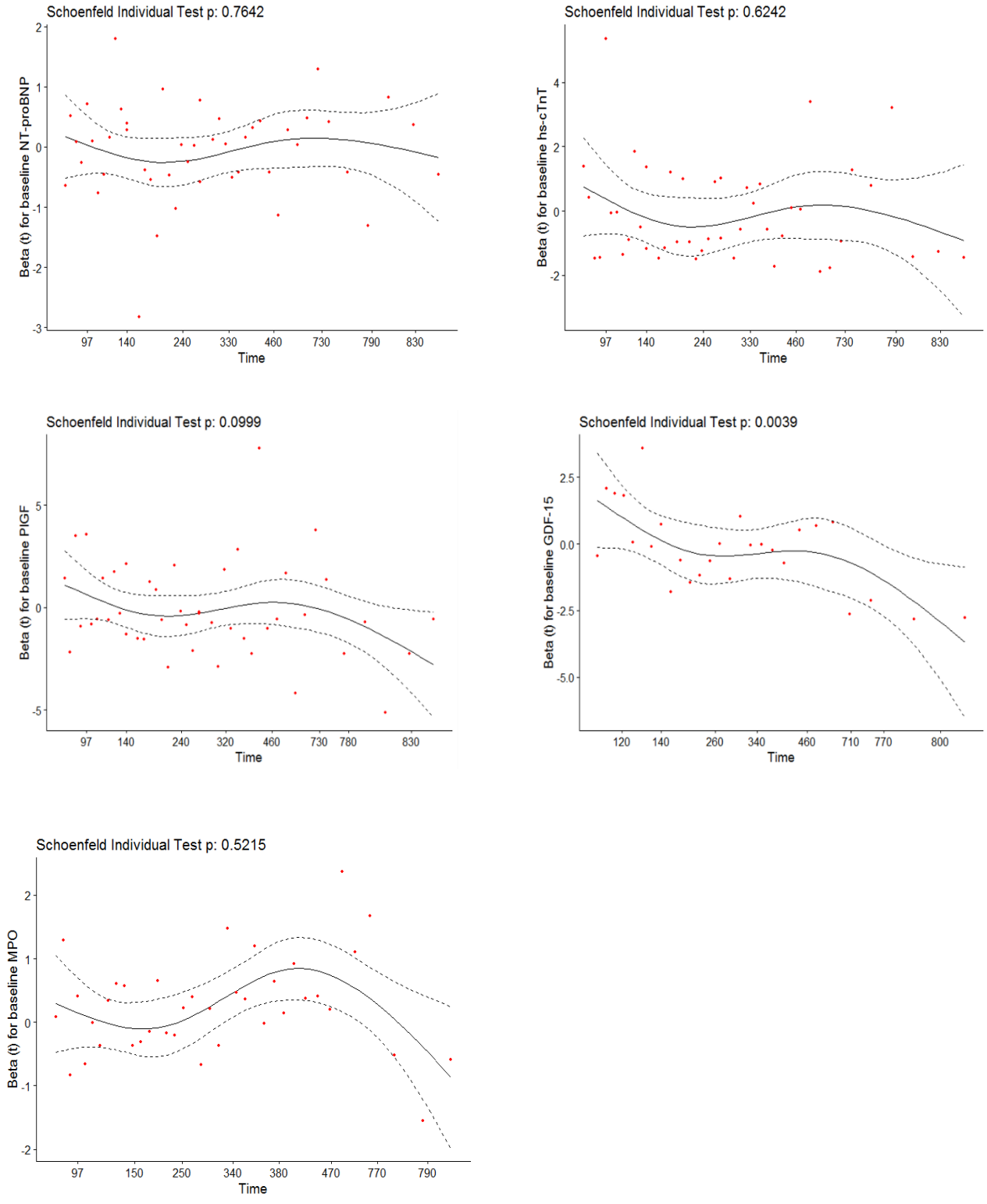

Figure S1. Plots of Schoenfeld residuals against time for the associations between baseline biomarkers and time to cancer therapy-related cardiac dysfunction.

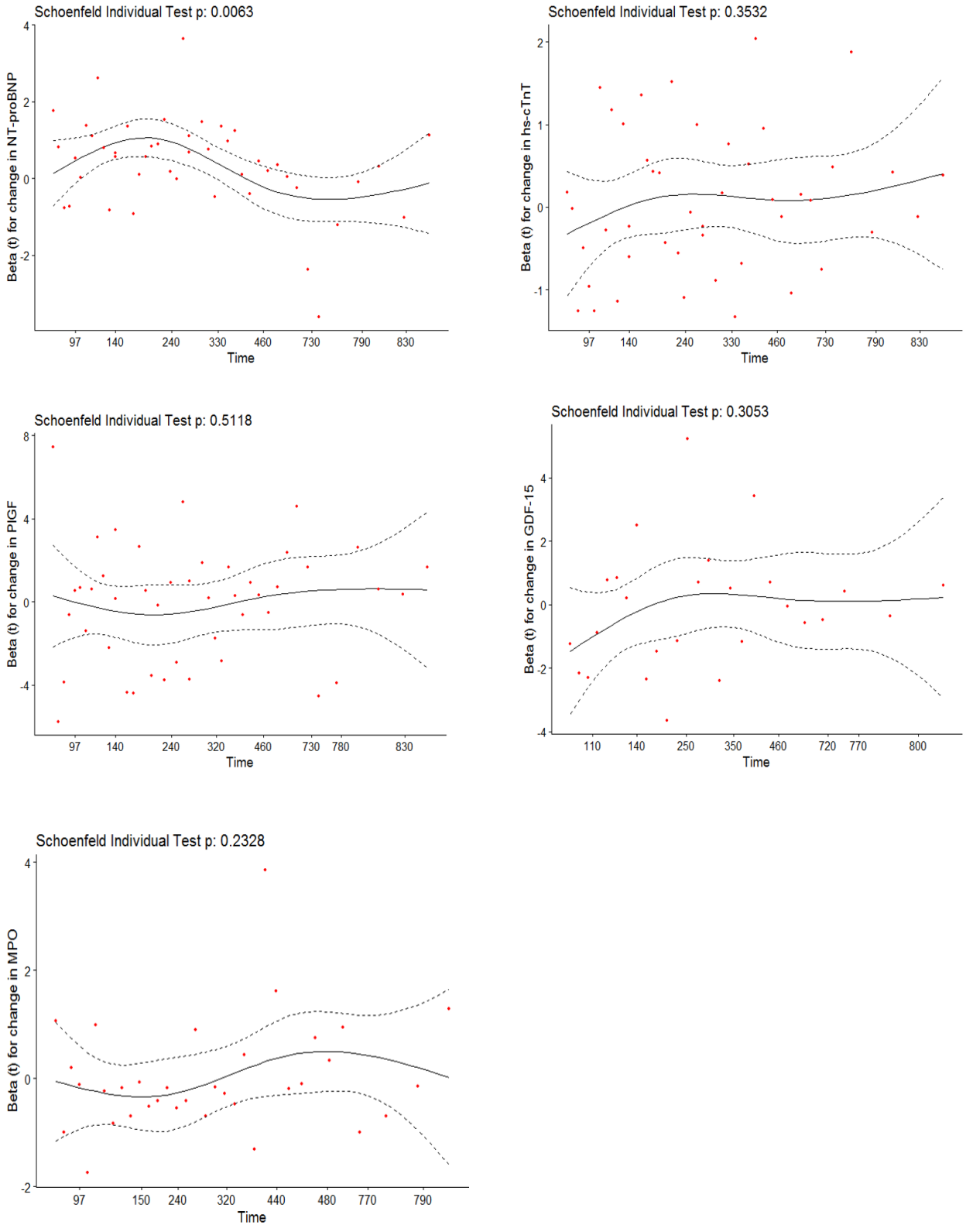

Figure S2. Plots of Schoenfeld residuals against time for the associations between changes in biomarkers and time to cancer therapy-related cardiac dysfunction.

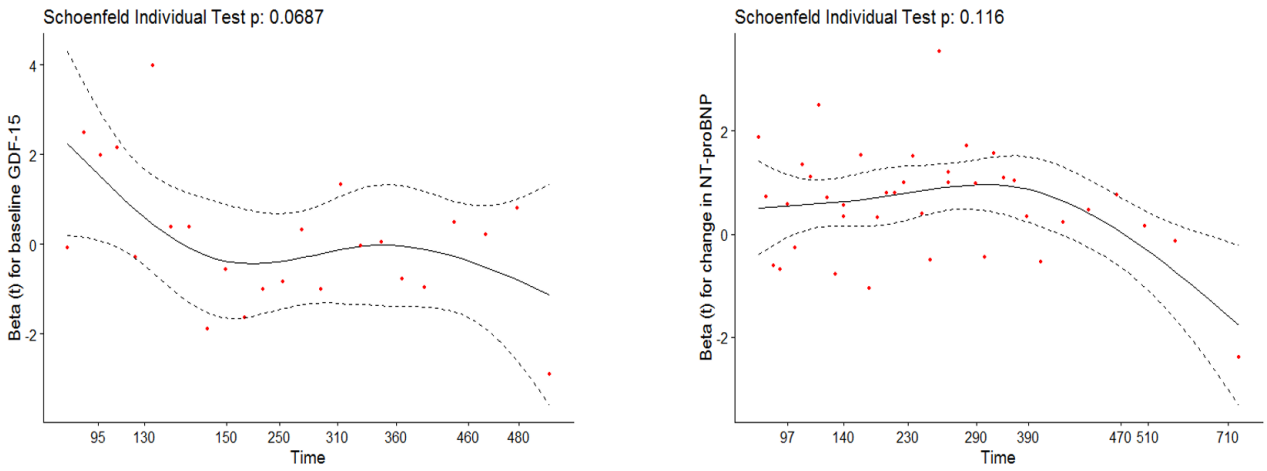

Figure S3. Plots of Schoenfeld residuals against time for the associations between baseline GDF-15 and change in NT-proBNP, and time to cancer therapy-related cardiac dysfunction limiting maximum follow-up time to 2 years.

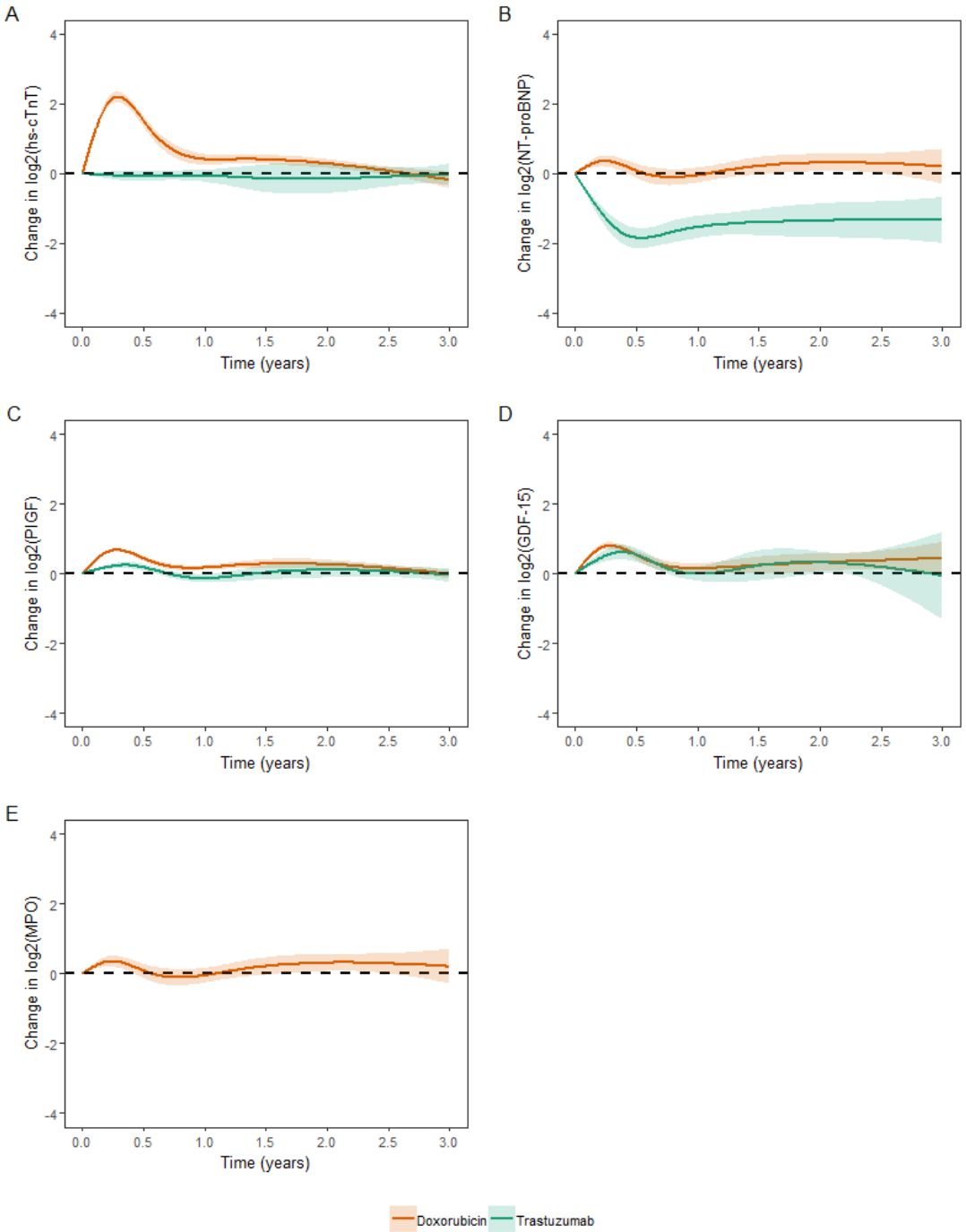

Figure S4. Mean estimated changes in biomarkers over time according to cancer therapy regimen; the Doxorubicin group includes both those treated with doxorubicin alone and sequential doxorubicin and trastuzumab therapy. The solid line represents mean estimated changes over time and the width of the surrounding band represents the corresponding 95% confidence interval; biomarker levels were log2 transformed (a unit increment from baseline should be interpreted as doubling); (A) hs-cTnT, (B) NT-proBNP, (C) PIGF, (D) GDF-15, (E) MPO; GDF-15=Growth differentiation factor 15; hs-cTnT= High sensitivity cardiac troponin T; MPO=Myeloperoxidase; NT-proBNP= N-terminal pro-B-type natriuretic peptide; PIGF=Placental growth factor

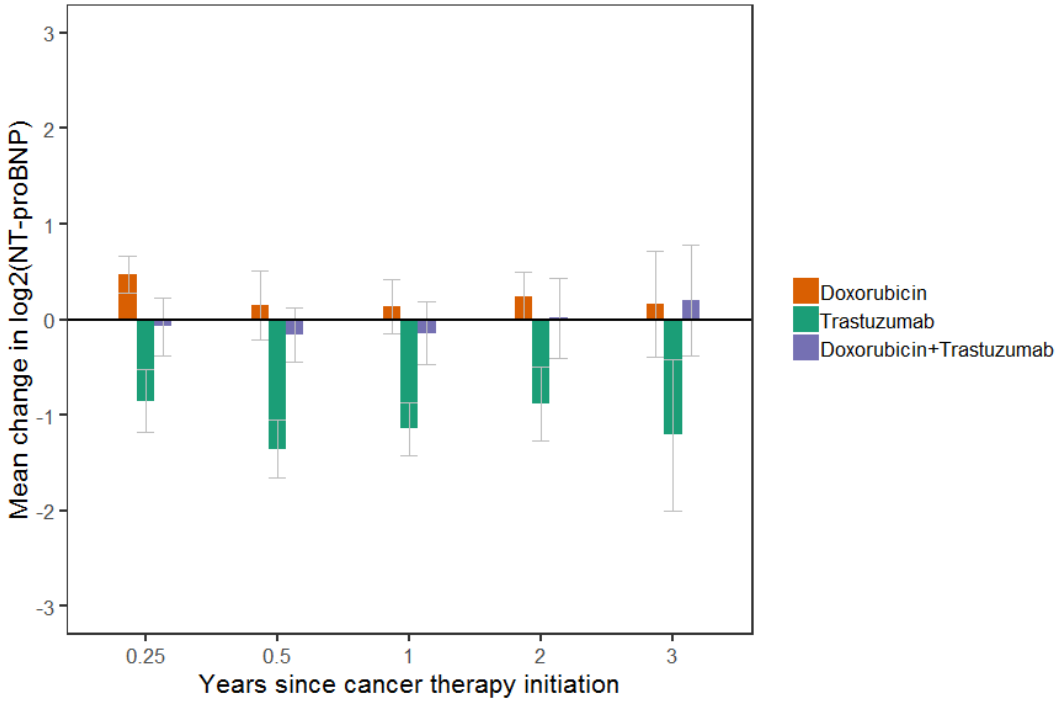

Figure S5. Covariate-adjusted marginal mean estimates of NT-proBNP change at 3, 6, 12, 24 and 36 months after initiation of cancer therapy according to cancer therapy regimen. Covariates include baseline NT-proBNP and baseline variables including age, hypertension, BMI, smoking; the effect of time was allowed to differ according to cancer therapy regimen by including a time*treatment interaction term