

A marker of homologous recombination predicts pathological complete response to neoadjuvant chemotherapy in primary breast cancer

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Supplementary table and figure legends

Supplementary Table 1. Antibodies used for assessment of Rad51 foci in FFPE material.

Antigen	Supplier	Clone
Rad51	Abcam	ab54188
Rad51	Abcam	ab1837
Rad51	Abcam	ab20240
Rad51	Abcam	ab213
Rad51	Abcam	ab12448
Rad51	GeneTex	13E4
Rad51	GeneTex	14B4
Rad51	Calbiochem	Ab-1

Supplementary Table 2. Full data set of clinicopathological data from study

Patient number†	Age	menopausal status	Pathology	Grade	Tumour size (cm)	Chemotherapy	Tamoxifen treatment	Distal response	type of surgery	pCR	resect status	ER	HER2	Ki67% positive	Ki67% ≥4 positive	HER2/3	HER2/3 score (n)	
1	47	pre	DC	3	40	AC	yes	PR	breast conserving	1	negative	0	0	34	36.2	0	0	
2	55	post	DC	3	80	AC	yes	PR	breast conserving	0	positive	0	0	35	40	0	0	
3	40	pre	DC	2	30	ECdf	yes	PR	breast conserving	1	unknown	1	0	26.7	26.7	0	0	
4	46	pre	DC	2	60	ECdf	yes	PR	breast conserving	0	negative	1	0	36.5	36.5	0	0	
5	61	post	DC	3	80	F/C	yes	PR	mastectomy	0	positive	1	1	30	42.4	0	0	
6	53	post	DC	3	105	ECdf	yes	PR	mastectomy	0	unknown	0	0	65	75	0	0	
7	50	post	DC	3	60	ECdf	yes	PR	none	unknown	0	0	0	87.7	87.7	2	2	
8	50	pre	DC	3	50	AC	no	PR	breast conserving	0	negative	0	0	37.2	37.2	2	2	
9	45	unknown	DC	3	80	AC	no	PR	mastectomy	0	negative	0	0	44.2	44.2	2	2	
10	45	pre	DC	3	80	AC	yes	PR	breast conserving	0	positive	0	0	5.1	16.75	0	0	
11	43	unknown	DC	3	80	AC	no	PR	breast conserving	1	negative	0	1	27.1	27.1	3	3	
12	44	pre	DC	3	50	F/Cyof	yes	PR	breast conserving	0	positive	1	0	53.3	23.8	0	0	
13	45	post	DC	3	80	AC	yes	PR	breast conserving	0	negative	0	0	56.3	56.3	4	4	
14	40	pre	DC	2	80	ECdf	yes	PR	mastectomy	1	negative	1	0	36.3	36.3	0	0	
15	40	pre	DC	2	80	AC	yes	PR	mastectomy	0	negative	1	0	3.6	3.6	0	0	
16	48	pre	DC	2	100	ECyof	yes	PR	mastectomy	0	positive	0	0	21.3	13.1	0	0	
17	43	pre	L/C	2	35	ECdf	yes	DC	none	unknown	1	0	0	14.3	14.3	11	11	
18	33	pre	L/C	3	45	AC	yes	PR	breast conserving	0	negative	1	0	27	18.2	0	0	
19	49	pre	DC	3	80	ECyof	yes	DC	none	unknown	1	0	0	31	16.5	0	0	
20	57	post	DC	3	45	AC	yes	PR	breast conserving	0	positive	1	0	42.9	42.9	18	18	
21	49	pre	DC	3	80	AC	yes	PR	mastectomy	0	negative	1	0	10.2	10.2	21	21	
22	34	pre	DC	2	70	AC	yes	PR	mastectomy	0	positive	0	1	10.2	10.2	21	21	
23	41	pre	DC	2	40	ECdf	yes	DC	none	unknown	0	1	0	82.7	82.7	34	34	
24	50	pre	DC	2	40	AC	yes	PR	breast conserving	0	negative	1	0	17.8	16.9	26	26	
25	38	pre	DC	3	40	AC	yes	PR	none	unknown	1	0	0	26	27.1	27	27	
26	48	pre	DC	3	55	AC	no	PR	breast conserving	0	negative	0	0	18.5	17.5	21	21	
27	45	pre	DC	3	80	ECyof	yes	PR	mastectomy	0	negative	0	0	26	27.1	27	27	
28	47	pre	DC	unknown	50	F/C	yes	PR	none	0	unknown	unknown	unknown	unknown	unknown	unknown	26	26
29	38	pre	DC	3	21	F/C	yes	PR	none	0	unknown	unknown	unknown	unknown	unknown	unknown	38	38
30	41	pre	L/C	2	40	AC	no	PR	breast conserving	0	negative	1	0	28.5	18.8	40	40	
31	44	pre	DC	3	25	MB	yes	PR	breast conserving	0	positive	0	0	19.4	17.2	43	43	
32	41	pre	DC	3	45	AC	no	PR	breast conserving	0	positive	1	1	57.4	43	43	43	
33	28	pre	DC	2	50	ECdf	yes	DC	breast conserving	0	positive	1	0	18.5	14.8	40	40	
34	42	pre	DC	2	110	AC	no	PR	unknown	0	positive	0	1	15.5	14.8	40	40	
35	44	pre	DC	2	40	AC	yes	PR	breast conserving	0	negative	1	0	10.3	11.7	46	46	
36	47	pre	DC	2	40	AC	no	PR	mastectomy	0	positive	1	1	8.5	7.2	48	48	
37	45	pre	DC	3	80	AC	unknown	PR	unknown	0	unknown	unknown	unknown	unknown	unknown	unknown	52	52
38	45	unknown	DC	3	50	ECdf	yes	PR	mastectomy	0	negative	0	1	28.5	38.7	60	60	
39	48	pre	DC	3	80	AC	yes	PR	breast conserving	0	positive	1	0	8.1	9.44	60	60	
40	39	pre	DC	2	54	AC	no	DC	mastectomy	1	negative	1	PR4 positive	72.8	75.8	80	80	
41	41	pre	DC	3	80	F/C	yes	PR	mastectomy	0	positive	0	0	36	37	84	84	
42	85	post	L/C	2	70	ECyof	yes	PR	breast conserving	0	negative	1	0	13.8	10.8	85	85	
43	42	pre	DC	3	40	AC	yes	PR	mastectomy	0	positive	1	0	2.1	1.5	87	87	
44	48	pre	L/C	2	40	AC	no	PR	breast conserving	0	negative	1	0	5	1.4	87	87	
45	45	pre	DC	3	80	AC	yes	PR	breast conserving	0	negative	1	0	20.3	18.8	73	73	
46	38	pre	DC	3	70	ECdf	yes	PR	breast conserving	0	negative	0	0	unknown	unknown	75	75	
47	45	pre	DC	3	80	AC	yes	PR	breast conserving	0	negative	1	0	unknown	unknown	75	75	
48	38	pre	DC	2	26	NE	yes	PR	breast conserving	0	positive	1	0	22.1	15.8	77	77	
49	33	pre	DC	2	26	AC	yes	PR	breast conserving	0	positive	1	0	4.2	4.2	80	80	
50	42	pre	DC	2	85	F/C	yes	PR	breast conserving	0	negative	0	0	17.3	18.4	80	80	
51	50	unknown	DC	2	50	ECdf	yes	PR	breast conserving	0	negative	1	0	4.2	6.05	84	84	
52	45	pre	DC	2	50	AC	yes	PR	breast conserving	0	negative	1	0	1.3	2.3	84	84	
53	50	pre	L/C	2	70	ECdf	yes	PR	mastectomy	0	positive	1	0	45.8	23.5	100	100	
54	46	pre	DC	2	70	AC	no	PR	mastectomy	0	positive	1	0	15.9	1.5	100	100	
55	53	post	L/C	2	80	ECdf	yes	PR	breast conserving	0	positive	1	0	18.2	3.1	100	100	
56	47	pre	L/C	unknown	50	AC	yes	PR	mastectomy	0	positive	1	0	26.8	2.1	100	100	

Supplementary Table 3. Multivariate analysis of clinicopathological features that are associated with RAD51 score in univariate analysis.

Model 1

	OR	95%CI_lower	95%CI_upper	P value
ER+/HER2- subtype	1	-	-	
HER2 subtype	1.76	0.25	12.64	0.57
TN subtype	5.74	0.66	50	0.11
Grade	1.74	0.23	13.38	0.59
Baseline Ki67	1.02	0.98	1.06	0.28

Model 2

	OR	95%CI_lower	95%CI_upper	P value
ER+/HER2- subtype	1	-	-	
Baseline Ki67	1.03	0.992275985	1.068583564	0.12
TN subtype	5.78	1.021599156	32.67371069	0.047

In the first model all factors significant in univariate analysis are included. No variables reach statistical significance, potentially due to the number of variables for a data set of this size. A second model was fitted using tumour subtype and baseline Ki67, identifying TN subtype as an independent predictor.

Method. Modeling of predictors of RAD51 foci was performed by fitting generalised linear models in R. Ki67 was treated as a continuous predictor; all other dependent variables were categorical and the most common group of each category was chosen as the referent. The best fitting models was selected by AIC using a backward stepwise algorithm.

Reference. R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.

Supplementary Figure 1. Flow chart of patients included in study, and reasons for exclusion.

Supplementary Figure 2. Assessment of γ H2AX in cancers with and without RAD51 foci.

Assessment of γ H2AX in baseline and 24 hours post chemotherapy biopsies, from 3 patients with high RAD51 score and four patients with low RAD51 score (RAD51 score displayed below graph). Displayed is the percentage of cancer cells positive for γ H2AX in each biopsy, and the absolute change in γ H2AX percentage. γ H2AX was assessed in the consecutive section to that assessed for RAD51. Only tumour cells that have transitioned through S phase during the 24 hour post chemotherapy are likely to be γ H2AX positive.

Supplementary Figure 3. Assessment of γ H2AX in cancers without RAD51 foci.

Illustrative images from supplementary figure 2, γ H2AX (green) and DAPI nuclear stain (Blue). γ H2AX staining was induced across tissue core. A Slide 157 (RAD51 score 0%), B Slide 97 (RAD51 score 7%), C Slide 53 (RAD51 score 0%), D Slide 159 (RAD51 score 0%).