Persistence of *BRCA1* and *RAD51C* methylation after neoadjuvant chemotherapy in high risk TNBC

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1. Background

Methylation and silencing of the promoters of the homologous recombination repair (HRR) genes *BRCA1* or *RAD51C* are observed in 20-30% of triple-negative breast cancer (TNBC) cases[1]. However, it is not known whether TNBC patients with *BRCA1* or *RAD51C* methylation would also benefit from treatments that target defective HRR, as has been shown for patients with HRR gene mutations[6].

To determine the frequency of *BRCA1* or *RAD51C* methylation in TNBC prior to neoadjuvant chemotherapy (NACT) and the degree to which this epigenetic mark is lost during therapy in those at high risk of distant recurrence due to lack of pathological complete response, we assessed promoter methylation by amplicon-based bisulphite sequencing in matched pretreatment diagnostic biopsies and post-NACT residual disease (RD) samples.

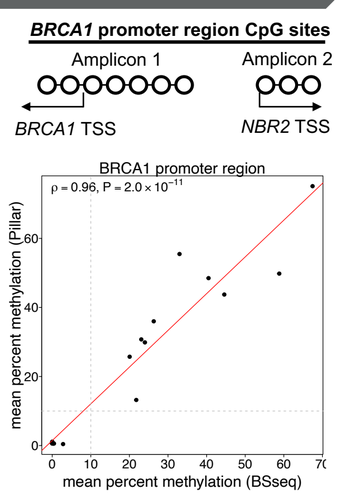
2. Study cohort

Samples from TNBC patients with residual disease after NACT were compiled from several sources, including clinical NACT trials (GeparOla[2], GeparSixto[3]; German Breast Group ("GBG") and AGO-B Study Group) as well as from observational clinical studies (BTBC, ChemoNear, FRCB) and biobanks (Breast Cancer Now Tissue Bank; BCNTB). Where status was known, patients without germline or somatic BRCA1/2 mutations (g/tBRCAm) and patients with a positive Myriad MyChoice HRD test result were selected. Patients received a range of NACT regimens that may impose an HRR-selective pressure (platinum, olaparib, anthracyclines, or cyclophosphamide)

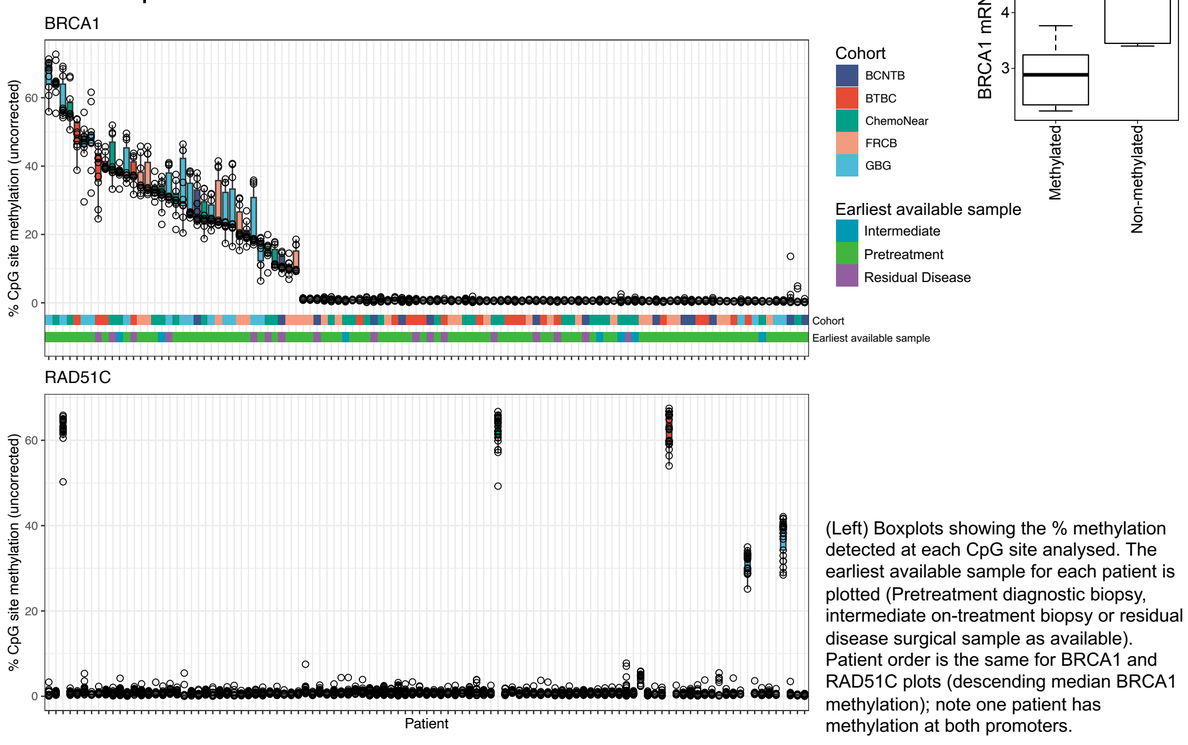
Group	Patient selection	Patients	# Patients with NACT treatment regimen containing:				
			Platinum	Olaparib	Anthracycline	Cyclophosphamide	Unknown
GBG	TNBC, no g/tBRCAm, HRD+	17	6	5	17	7	0
BTBC	TNBC, no g/tBRCAm	22	7	0	20	20	0
FCRB	TNBC, no g/tBRCAm	26	26	0	18	18	0
BCNTB	TNBC, unknown g/tBRCAm	14	5	0	11	12	0
ChemoNear	TNBC, no g/tBRCAm	30	2	0	18	18	12
Total		109					

3. Validation and methylation in diagnostic samples

DNA from FFPE samples was analysed using the Pillar OncoReveal methylation panel. This SLIMamp multiplex bisulphite PCR assay followed by NGS is robust in FFPE samples. We validated this assay using a set of samples from the Triple Negative Trial (TNT) where *BRCA1* methylation was assessed by conventional bisulphite sequencing[4] and found a strong correlation between the two methods of detecting *BRCA1* promoter methylation (ρ = 0.96, right). The *BRCA1* CpG sites covered have previously been validated as associated with low *BRCA1* mRNA levels in TNT, and we observed the same association with Pillar-assessed methylation and *BRCA1* mRNA (P = 8.8 x 10^{-4}).



In the study cohort, 41 patients (*BRCA1*: 37/109, 34%; *RAD51C*: 5/109, 5.4%; one patient had methylation of both promoters) had >10% CpG methylation in at least one sample at at least one site.

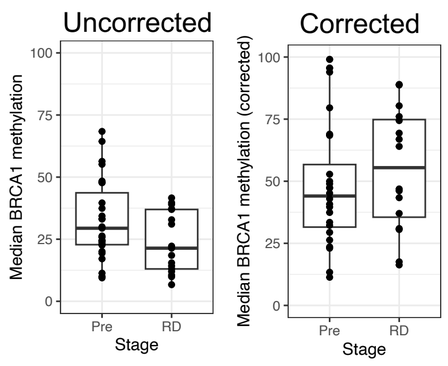


(Left) Boxplots showing the % methylation detected at each CpG site analysed. The earliest available sample for each patient is plotted (Pretreatment diagnostic biopsy, intermediate on-treatment biopsy or residual disease surgical sample as available). Patient order is the same for BRCA1 and RAD51C plots (descending median BRCA1 methylation); note one patient has methylation at both promoters.

4. Persistence of methylation in post-NACT samples

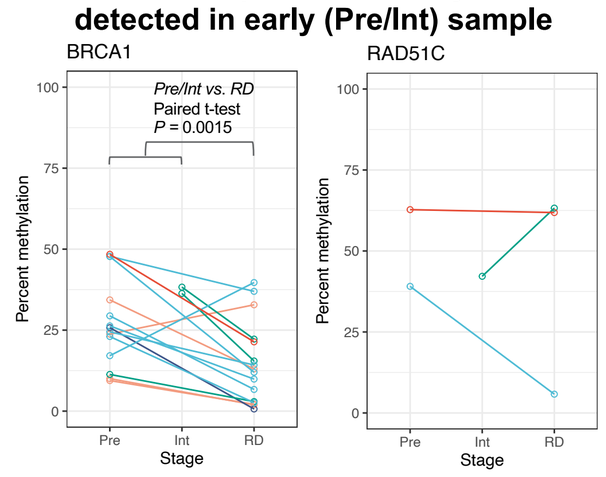
Methylation levels were generally lower in residual disease samples. However, since residual disease samples often have low tumour content, we applied a correction to all samples[6] based on whole exome sequencing (where available), or on pathologist-assessed tumour cellularity.

All samples (including unpaired)

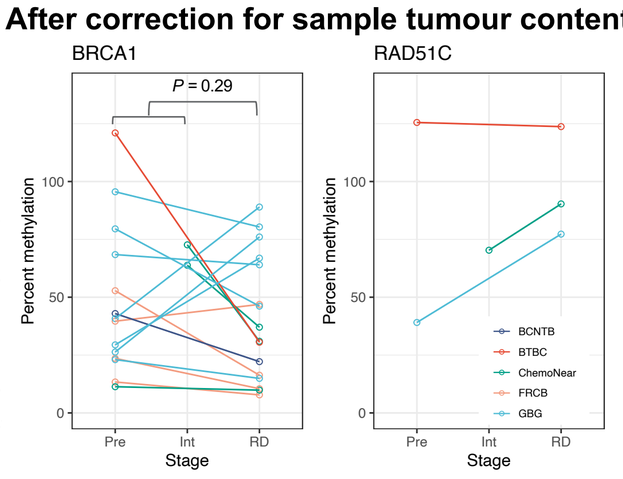


Corrected methylation values suggest that in most cases lower methylation in RD is driven by lower sample tumour cellularity. The corrected methylation level is usually less than 100%, which could suggest incomplete correction for normal cells, or presence of unmethylated alleles in tumour cells.

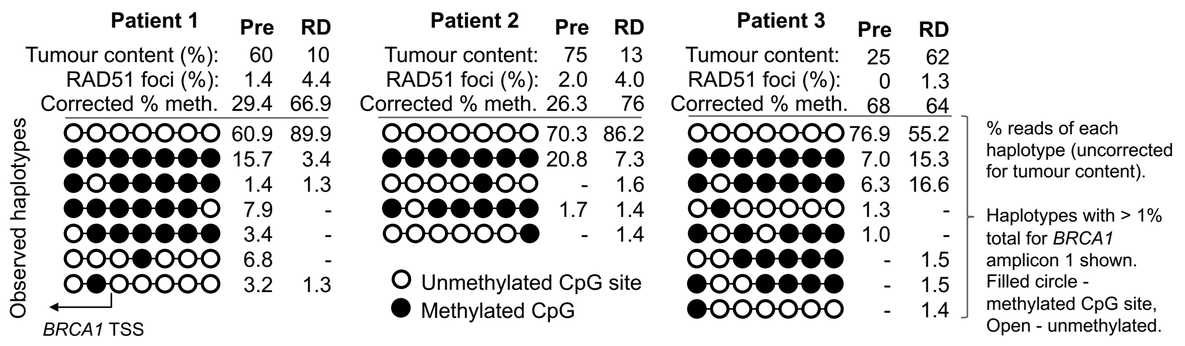
Paired samples with methylation detected in early (Pre/Int) sample



After correction for sample tumour content



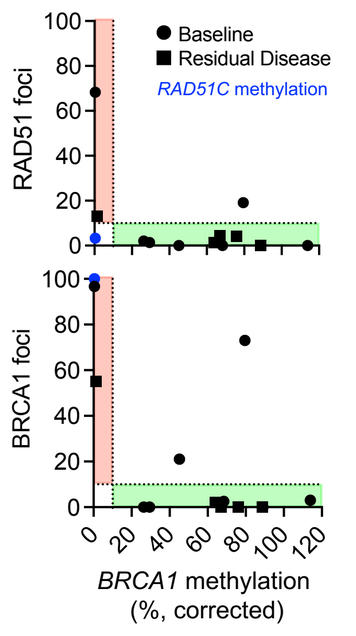
In residual disease samples, as in pretreatment samples, most reads were either fully methylated or fully unmethylated, and there was no increase in partially methylated reads. Methylation haplotypes for three example cases are shown below:



5. Observed methylation levels are sufficient for HRD

To assess whether the methylation levels detected reflect a functional HR defect, we conducted RAD51, Geminin and BRCA1 staining in tumour material from matched samples where this was available[7]. In almost all cases, including in all RD samples assessed (n = 3 with methylation), *BRCA1* or *RAD51C* methylation detected using the Pillar assay was associated with a functional HR defect assessed by RAD51 foci negativity (11/12 samples analysed with > 10% corrected median CpG methylation, 92%). There were two cases where BRCA1 foci were still observed despite detectable *BRCA1* methylation, one of which was also positive for RAD51 foci. One patient with *RAD51C* methylation was positive for BRCA1 foci but negative for RAD51 foci, as expected.

These data suggest that methylation detected using the oncoReveal assay is associated with a functional HR defect.



6. Conclusions

BRCA1 and *RAD51C* methylation in triple negative breast cancer generally persists chemotherapy at levels sufficient for functional HRD in residual disease after neoadjuvant chemotherapy. This population of high-risk patients who retain BRCA1 or RAD51C methylation after NACT may benefit from adjuvant treatments targeting HRD such as PARP inhibitors, as seen for gBRCAm TNBC[2]. This study identifies a significant population of high risk HRD TNBC, beyond gBRCAm, in which the potential benefits of adjuvant PARPi might be tested

References

1. J. Staaf *et al.*, *Nat. Med.* **25**, 1–23 (2019).
2. A. N. J. Tutt *et al.*, *N. Engl. J. Med.* **384**, 2394–2405 (2021).
3. P. A. Fasching *et al.*, *Ann. Oncol.* **32**, 49–57 (2021).
4. S. Loibl *et al.*, *Ann. Oncol.* **29**, 2341–2347 (2018).
5. A. Tutt *et al.*, *Nat. Med.* **24**, 628–637 (2018).
6. O. Kondrashova *et al.*, *Nat. Commun.* **9**, 3970–3916 (2018).
7. C. Cruz *et al.*, *Ann. Oncol.* **29**, 1203–1210 (2018).

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