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ORIGINAL ARTICLE

# Olaparib monotherapy as primary treatment in unselected triple negative breast cancer☆

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**Background:** The antitumor efficacy of PARP inhibitors (PARPi) for breast cancer patients harboring germline *BRCA1/2* (g*BRCA1/2*) mutations is well established. While PARPi monotherapy was ineffective in patients with metastatic triple negative breast cancer (TNBC) wild type for *BRCA1/2*, we hypothesized that PARPi may be effective in primary TNBCs without previous chemotherapy exposure.

**Patients and methods:** In the phase II PETREMAC trial, patients with primary TNBC >2 cm received olaparib for up to 10 weeks before chemotherapy. Tumor biopsies collected before and after olaparib underwent targeted DNA sequencing (360 genes) and *BRCA1* methylation analyses. In addition, *BRCA*ness (multiplex ligation-dependent probe amplification), PAM50 gene expression, RAD51 foci, tumor-infiltrating lymphocytes (TILs) and PD-L1 analyses were performed on pretreatment samples.

**Results:** The median pretreatment tumor diameter was 60 mm (range 25-112 mm). Eighteen out of 32 patients obtained an objective response (OR) to olaparib (56.3%). Somatic or germline mutations affecting homologous recombination (HR) were observed in 10/18 responders [OR 55.6%, 95% confidence interval (CI) 33.7-75.4] contrasting 1/14 non-responders (OR 7.1%; CI 1.3-31.5, $P = 0.008$). Among tumors without HR mutations, 6/8 responders versus 3/13 non-responders revealed *BRCA1* hypermethylation ($P = 0.03$). Thus, 16/18 responders (88.9%, CI 67.2-96.9), in contrast to 4/14 non-responders (28.6%, CI 11.7-54.7, $P = 0.0008$), carried HR mutations and/or *BRCA1* methylation. Excluding one g*PALB2* and four g*BRCA1/2* mutation carriers, 12/14 responders (85.7%, CI 60.1-96.0) versus 3/13 non-responders (23.1%, CI 8.2-50.3, $P = 0.002$) carried somatic HR mutations and/or *BRCA1* methylation. In contrast to *BRCA*ness signature or basal-like subtype, low RAD51 scores, high TIL or high PD-L1 expression all correlated to olaparib response.

**Conclusion:** Olaparib yielded a high clinical response rate in treatment-naïve TNBCs revealing HR deficiency, beyond germline HR mutations.

**Trial registration:** ClinicalTrials.gov identifier: NCT02624973.

**Key words:** triple negative breast cancer, PARP inhibitor, olaparib, homologous recombination deficiency, prediction, neoadjuvant therapy

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## INTRODUCTION

Triple negative breast cancer (TNBC) is a breast cancer subgroup defined by lack of estrogen receptors (ER) and progesterone receptors (PGR) (ER/PGR negative) and normal HER2 protein expression. TNBC constitutes approximately 15% of all breast cancers,[1,2] and despite high response rates to chemotherapy, these patients have a poor prognosis compared to patients with other breast cancer subtypes.[2-4] While early evidence indicates a potential role for immune checkpoint inhibition in selected TNBC, so far no overall survival gain has been observed either in early or metastatic disease.[5,6] Thus, as of today there are no targeted therapies with a definite role in primary TNBC.

While about 15% of unselected TNBC harbor *BRCA1* germline (g*BRCA1*) mutations,[7] the majority of TNBCs reveal a gene expression signature mirroring that observed in g*BRCA1* mutation carriers.[1,8] Moreover, TNBCs may harbor somatic *BRCA1* mutations, *BRCA1* silencing through promoter hypermethylation, or somatic/germline alterations affecting other genes related to homologous recombination (HR).[7,9] Thus, HR deficiency (HRD), defined by somatic or germline HR mutations, *BRCA1* methylation or different genomic or gene expression signatures, is observed in 50%-80% of TNBCs.[7-10] Of note, *BRCA1* methylated and g*BRCA1* mutated TNBCs share gene expression and immune profiles, and seem to have a similar outcome after adjuvant chemotherapy,[11] indicating that somatic HRD may promote the same biological phenotype and treatment response as germline HRD in TNBC.

PARP inhibitors (PARPi) impair base excision repair (BER) through direct PARP inhibition and by trapping the PARP1 complex to DNA, subsequently causing double-strand breaks (DSB).[12] Thus, PARPi are selectively cytotoxic to cells carrying defects in DSB repair due to HR defects by synthetic lethality.[13,14] Among breast cancer patients carrying g*BRCA1/2* mutations, PARPi has been shown to prolong progression-free survival in metastatic, HER2-negative disease,[15-19] but also to induce profound tumor shrinkage in the neoadjuvant setting.[20] However, no benefit was recorded among patients with metastatic TNBC not harboring g*BRCA* mutations.[17] Notably, secondary reverting mutations arising during platinum therapy may restore *BRCA1/2* function and are associated with resistance to subsequent platinum or PARP inhibitor treatment in patients with breast and ovarian cancer.[12,21-23] If treatment with DNA crosslinking agents, such as carboplatin or cyclophosphamide, induces resistance to PARP inhibitors, this could explain the lack of benefit from olaparib observed in patients with late-stage metastatic breast cancer.[17] Interestingly, PARPi was beneficial to patients with heavily pretreated metastatic prostate cancer,[24,25] a patient group typically not exposed to crosslinking agents.

Platinum compounds mediate DSB through DNA crosslinking and are of increased efficacy among g*BRCA1/2* mutation carriers with metastatic TNBC.[26] Furthermore, platinum compounds could be of particular benefit in primary TNBC if germline or somatic HR defects are present, although the results are at variance.[27,28] While combined platinum-based chemotherapy and PARP inhibition with veliparib improved progression-free survival in g*BRCA1/2* mutated, advanced breast cancer compared with chemotherapy alone,[29] the benefit from such combined regimens in TNBC without g*BRCA1/2* mutations is less clear.[30,31] Considering other PARP inhibitors, such as olaparib or talazoparib, which exhibit stronger PARP trapping activity than veliparib,[12] the therapeutic window for administering them in concert with platinum compounds is narrowed by bone marrow toxicity.[32,33] However, olaparib and talazoparib are effective as monotherapy in advanced breast cancer among patients harboring g*BRCA1/2* and g*PALB2* mutations.[13,16,19,20,34] Thus, an alternative approach could be to apply a PARPi with potent PARP trapping activity and chemotherapy sequentially in the neoadjuvant setting.

Based on the evidence above, we hypothesized that PARP inhibition could be effective in treatment-naïve TNBC, beyond *BRCA1/2* germline defects. Here, we report the clinical efficacy of olaparib monotherapy before chemotherapy for unselected TNBC in the neoadjuvant PETREMAC trial (Clinicaltrials.gov #NCT02624973) with predictive markers identifying patients likely to benefit from such treatment.

## PATIENTS AND METHODS

### *Study design and patients*

In the phase II PETREMAC trial, patients with stage II/III breast cancer (American Joint Committee on Cancer, Breast Cancer Staging, 7th edition, CancerStaging.org) were stratified to eight different neoadjuvant treatment regimens based on ER, PGR and HER2 expression as well as *TP53* mutation status (Figure 1). The primary aim of the trial was to implement optimal neoadjuvant therapy for high-risk breast cancers, select therapy based on predefined biological parameters and identify novel predictive biomarkers for each individual treatment strategy. Patients with TNBC received initial olaparib monotherapy 300 mg b.i.d. for up to 10 weeks, irrespective of *BRCA* and *TP53* mutation status (treatment arms G and H; Figure 1), aiming to shrink tumor size before chemotherapy. Olaparib monotherapy was halted and chemotherapy was introduced before 10 weeks for patients without evidence of tumor regression (Table 1). Chemotherapy regimens tested after initial olaparib monotherapy are described in Figure 1 and in supplementary Methods, available at https://doi.org/10.1016/j.annonc2020.11.009. Clinical and radiological evaluation of tumor size was carried out by each local investigator, blinded to knowledge of genomic aberrations, apart from *TP53* mutation status.

### *DNA and RNA analyses*

Pre-planned targeted DNA sequencing applying a 360-gene panel[35] was conducted on tumor biopsies extracted before and after olaparib treatment, as described in supplementary Methods, available at https://doi.org/10.

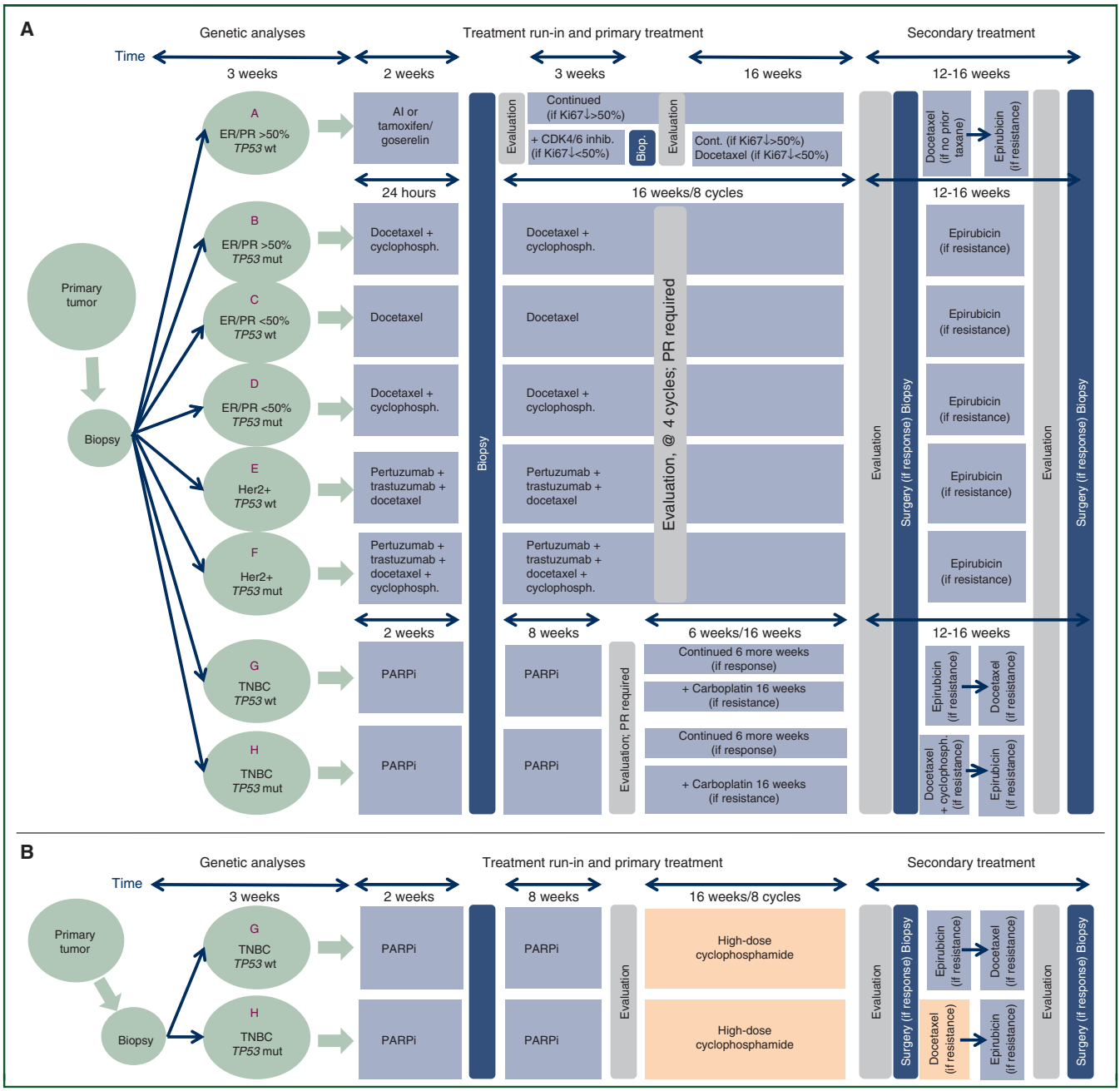


**Figure 1. Outline of study arms of the neoadjuvant PETREMAC trial (A).**
After informed consent, breast cancer biopsies were taken and examined for estrogen receptor (ER), progesterone receptor (PGR) and HER2 expression, in addition to *TP53* mutation status during the screening phase. Based on these results, patients were allocated to the eight study arms to receive personalized neoadjuvant treatment of large T2 (T >4 cm) or locally advanced breast cancers. Patients with triple negative breast cancer (TNBC) were allocated to study arms G (*TP53* wildtype; *TP53* wt) and H (*TP53* mutated tumor; *TP53* mut) and received initial olaparib monotherapy (PARP inhibitor; PARPi) with or without subsequent chemotherapy with the aim of an objective response. Due to inadequate tumor regression observed in the initial eight patients in arms G and H (Outline **A**), the protocol was amended to change the chemotherapy given after the initial olaparib monotherapy phase (Outline **B**). Chemotherapy changes are marked by orange boxes. Also, the amendment allowed for inclusion of tumors >2 cm in arms E, F, G and H.
AI, aromatase inhibitor; cyclophosph., cyclophosphamide; ER, estrogen receptor; mut, mutation; wks, weeks; wt, wildtype.

1016/j.annonc2020.11.009. Mutations identified were annotated as likely drivers, involved in HR or other DNA damage repair pathways, by predefined criteria (supplementary Table S1, available at https://doi.org/10.1016/j.annonc2020.11.009). Further, pre-planned analyses of tumor samples for *BRCA1* promoter methylation by methylation-specific quantitative PCR and *BRCA*ness by multiplex ligation-dependent probe amplification (MLPA) were carried out (see supplementary Methods, available at https://doi.org/10.1016/j.annonc2020.11.009). A post-hoc gene expression analysis was carried out on pretreatment biopsies to assign all tumors to a PAM50 breast cancer subgroup (see

**Table 1. Patient characteristics and response to olaparib monotherapy for triple negative breast cancers in the PETREMAC trial**

| ID | Study arm | T | N | Age | Response to olaparib[a] | | | | | | | | | Olaparib (weeks)[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1. Clinical measurement (caliper)[b] | | | | 2. MRI breast[b] | | | | 1 + 2[c] | |
| | | | | | TND before | TND after | % change | RECIST | TND before | TND after | % Change | RECIST | | |
| 1 | G | 2 | 0 | 37 | 35 | 0 | −100 | CR | 24 | 0 | −100 | CR | CR | 10 |
| 2 | H | 3 | 0 | 61 | 56 | 36 | −36 | PR | 75 | 39 | −48 | PR | PR | 10 |
| 3 | G | 3 | 0 | 49 | 70 | 20 | −71 | PR | 36 | 18 | −50 | PR | PR | 10 |
| 4 | H | 2 | 0 | 68 | 28 | 0 | −100 | CR | 29 | 17 | −41 | PR | PR | 10 |
| 5 | H | 3 | 0 | 42 | 56 | 30 | −46 | PR | 30 | 19 | −37 | PR | PR | 10 |
| 6 | H | 3 | 0 | 72 | 70 | 50 | −29 | SD | 33 | 23 | −30 | PR | PR | 10 |
| 7 | H | 3 | 3 | 46 | 73 | 30 | −59 | PR | 86 | NM[e] | NM | PR | PR | 10 |
| 8 | H | 3 | 0 | 46 | 62 | 33 | −47 | PR | 42 | 15 | −64 | PR | PR | 10 |
| 9 | H | 3 | 0 | 35 | 60 | 0 | −100 | CR | 38 | 23 | −39 | PR | PR | 10 |
| 10 | H | 2 | 0 | 34 | 45 | 20 | −56 | PR | 80 | 37 | −54 | PR | PR | 10 |
| 11 | H | 3 | 0 | 57 | 68 | 45 | −34 | PR | 45 | 21 | −53 | PR | PR | 10 |
| 12 | G | 3 | 2 | 72 | 75 | 55 | −27 | SD | 49 | 34 | −31 | PR | PR | 10 |
| 13 | G | 3 | 0 | 45 | 80 | 40 | −50 | PR | 73 | NM[e] | NM | PR | PR | 10 |
| 14 | H | 2 | 0 | 72 | 50 | 0 | −100 | CR | 67 | 21 | −69 | PR | PR | 10 |
| 15 | H | 3 | 0 | 41 | 80 | 40 | −50 | PR | 56 | 28 | −50 | PR | PR | 10 |
| 16 | H | 2 | 0 | 42 | 35 | 15 | −57 | PR | 28 | 14 | −50 | PR | PR | 10 |
| 17 | H | 3 | 0 | 45 | 76 | 30 | −61 | PR | 76 | 30 | −61 | PR | PR | 10 |
| 18 | H | 2 | 0 | 67 | 44 | 32 | −27 | SD | 42 | 15 | −64 | PR | PR | 10 |
| 19 | H | 3 | 0 | 60 | 53 | 40 | −25 | SD | 45 | 41 | −9 | SD | SD | 10[f] |
| 20 | H | 2 | 0 | 40 | 47 | 37 | −21 | SD | 19 | 25 | 31 | PD[g] | SD | 10 |
| 21 | H | 3 | 1 | 60 | 82 | 65 | −21 | SD | 60 | 48 | −20 | SD | SD | 6 |
| 22 | H | 2 | 0 | 45 | 50 | 56 | 12 | SD | 24 | 25 | 4 | SD | SD | 10 |
| 23 | H | 3 | 0 | 36 | 60 | 60 | 0 | SD | 105 | 95 | −10 | SD | SD | 8 |
| 24 | G | 3 | 0 | 56 | 60 | 40 | −33 | PR | 93 | 90 | −3 | SD | SD | 10 |
| 25 | H | 3 | 1 | 28 | 112 | 74 | −34 | PR | 107 | 98 | −9 | SD | SD | 10 |
| 26 | G | 3 | 0 | 66 | 55 | 45 | −18 | SD | 50 | 50 | 0 | SD | SD | 10 |
| 27 | H | 2 | 0 | 42 | 50 | 45 | −10 | SD | 40 | 41 | 3 | SD | SD | 4 |
| 28 | H | 0[h] | 2 | 58 | 25[i] | NA[j] | | NA | 39 | 31 | −21 | SD[k] | SD | 7 |
| 29 | H | 2 | 2 | 65 | 70 | 55 | −21 | SD | 32 | NA[l] | | NA | SD | 6 |
| 30 | G | 3 | 0 | 64 | 70 | 75 | 7 | SD | 60 | 80 | 33 | PD | PD | 7 |
| 31 | G | 3 | 0 | 65 | 54 | 45 | −17 | SD | 37 | 45 | 22 | PD | PD | 6 |
| 32 | H | 3 | 0 | 46 | 55 | 20 | −64 | PR | 38 | 42 | 11 | PD[l] | PD | 10 |

ID: Patient study ID.
Study arm: G; TNBC; *TP53* wildtype. H; TNBC, *TP53* mutated.
T and N: tumor and nodal stage (TNM guidelines (American Joint Committee on Cancer, Breast Cancer Staging, 7th edition, CancerStaging.org)).
Age: Patient's age in years at diagnosis.
TND: tumor and nodal diameter, i.e. combined tumor diameter (longest) and nodal metastasis diameter (shortest).
CR, complete response; NA, not assessed; NM, not measurable; PD, progressive disease; PR, partial response SD, stable disease.
[a] Assessment by local principal investigator and radiologist.
[b] Size in millimeter; T and N size combined. Median pretreatment tumor diameter (clinical measurements) 60 mm for olaparib responders (CR + PR) versus 55 mm for non-responders (SD). Median pretreatment tumor diameter (MRI measurements) 44 mm for olaparib responders (CR + PR) versus 45 mm for non-responders (SD).
[c] Combined response assessment based on clinical and breast MRI evaluation per RECIST1.1. MRI response dictated the combined response, apart from patients 20 and 29 where clinical caliper measurements were used. For patient 29 an MRI had not been performed after olaparib treatment and for patient 20 the MRI result after olaparib was ambiguous. See footnote g.
[d] Olaparib therapy (tablets 300 mg BID) was pre-planned for 10 weeks, but at the discretion of the local principal investigator chemotherapy was introduced earlier if tumor regression on olaparib was not observed.
[e] NM: non-measurable tumor remnants described by the radiologist in the breast MRI exam, i.e. remaining tumor tissue is suspected, but can no longer be measured due to profound tumor regression.
[f] Patient withdrawn from trial after olaparib due to retrospective diagnosis of pre-treatment M1 disease.
[g] Diameter increase due to tumor core liquefaction/central necrosis.
[h] Prior mastectomy; inclusion failure, included in intention-to-treat analysis.
[i] Axillary recurrence; short diameter.
[j] NA: not assessed (protocol violation).
[k] Computer tomography (CT) evaluation at 4 weeks.
[l] PD due to cN1 (cN0 axilla pre-treatment).

supplementary Methods, available at https://doi.org/10.1016/j.annonc2020.11.009).

### *Immunostaining procedures and tumor-infiltrating lymphocytes*

Immunostaining for RAD51, BRCA1 and PD-L1 and quantification of immunostaining and tumor-infiltrating lymphocytes (TILs) are outlined in supplementary Methods (available at https://doi.org/10.1016/j.annonc2020.11.009). These were post-hoc assessments to examine the immune status as well as HR function in the tumors.

### *Ethics and approvals*

The study protocol and clinical trial set-up were approved by the Regional Ethical Committee of the Western health region in Norway (#2015/1493) and The Norwegian Drug Agency

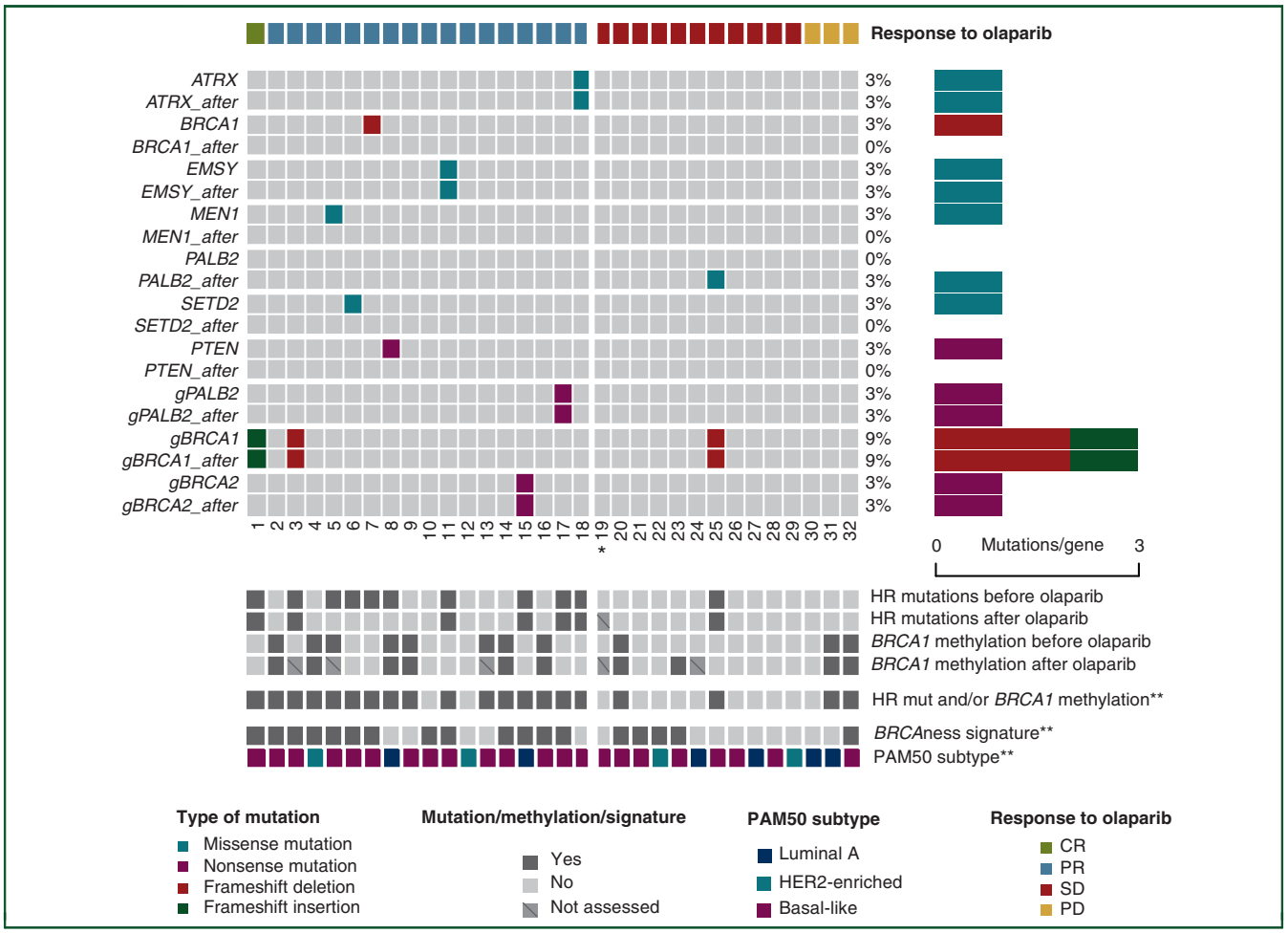


**Figure 2. OncoPrint list of mutations in homologous recombination genes in triple negative breast cancers (TNBC, $N = 32$) from the PETREMAC trial.**
HR mutations were recorded before and after initial olaparib monotherapy (4-10 weeks treatment), using targeted DNA sequencing (360-gene panel). The mutation list is sorted by olaparib response, and mutations are color-coded based on type of mutation detected. Genes are listed on the left; the letter 'g' before gene names designates germline mutations. Percentages and bars on the right indicate the prevalence of each mutation that was identified among the 32 tumors analyzed. Patient IDs are given below the columns; each column represents one tumor and one patient. Box diagrams summarize the presence of HR mutations and *BRCA1* methylation before and after olaparib, the presence of a *BRCA*ness signature and PAM50 breast cancer subtypes in pretreatment tumor samples, as well as response to olaparib. Response to olaparib was a combined assessment, clinically and by breast MRI, per RECIST1.1 guidelines. Tumors with a *BRCA1*-like profile by multiplex ligation-dependent probe amplification (MLPA) analysis were defined as having a *BRCA*ness signature.
*Only pretreatment biopsy available for Patient 19.
**Indicates analyses of pretreatment tumor biopsies.
CR, complete response; HR, homologous recombination; PD, progressive disease; PR, partial response; SD, stable disease.

(#2015/8463) and was registered at Clinicaltrials.gov (NCT02624973) and with EudraCT (#2015-002816-34). The study was conducted in accordance with the protocol, good clinical practice guidelines, provisions of the Declaration of Helsinki and all local regulations. All patients signed informed consent before inclusion.

### *Statistics*

All statistical analyses were carried out using R, version 3.5.3, or the SPSS 15.0/PASW 17.0 software package (SPSS Inc.). Statistical methods and confidence interval calculations are described in detail in supplementary Methods, available at https://doi.org/10.1016/j.annonc2020.11.009. All *P* values reported are two-tailed. No *P* value was corrected for multiple testing. However, as HR mutations and *BRCA1* methylation status were considered independent predictors of response, the *P* value threshold for statistical significance was set at $<0.025$ when these two factors were combined.

## RESULTS

Out of 222 patients screened for trial participation, 203 commenced and 200 patients completed neoadjuvant treatment in the PETREMAC trial (CONSORT diagram; supplementary Figure S1, available at https://doi.org/10.1016/j.annonc2020.11.009). Thirty-two patients with TNBC (median longest tumor diameter 60 mm; range 25-112 mm) received initial olaparib monotherapy (4-10 weeks) in treatment arms G/H (Figure 1), underwent clinical and

**Table 2. Statistical comparison of homologous recombination deficiency parameters among olaparib response groups.**

| Subgroup | HR deficiency | CR + PR[a] | SD | PD | *P* value trend | *P* value Fisher's exact[b] |
|---|---|---|---|---|---|---|
| All patients | HR mutation[c] positive | 10 | 1 | 0 | 0.006 | 0.008 |
| | Negative | 8 | 10 | 3 | | |
| | **Total** | **18** | **11** | **3** | | |
| g*BRCA/gPALB2* wt | HR mutation positive | 6 | 0 | 0 | 0.02 | 0.02 |
| | Negative | 8 | 10 | 3 | | |
| | **Total** | **14** | **10** | **3** | | |
| No HR mutation | *BRCA1* methylation positive | 6 | 1 | 2 | 0.2 | 0.03 |
| | Negative | 2 | 9 | 1 | | |
| | **Total** | **8** | **10** | **3** | | |
| All patients | HR mutation and/or *BRCA1* methylation positive[d] | 16 | 2 | 2 | 0.01 | 0.0008 |
| | Negative | 2 | 9 | 1 | | |
| | **Total** | **18** | **11** | **3** | | |
| g*BRCA/gPALB2* wt | HR mutation and/or *BRCA1* methylation positive[d] | 12 | 1 | 2 | 0.03 | 0.002 |
| | Negative | 2 | 9 | 1 | | |
| | **Total** | **14** | **10** | **3** | | |
| All patients | *BRCA*ness signature positive[e] | 13 | 4 | 1 | 0.05 | 0.07 |
| | Negative | 5 | 7 | 2 | | |
| | **Total** | **18** | **11** | **3** | | |

Combined clinical and MRI evaluation ($N = 32$).
CR, complete response; HR, homologous recombination; PD, progressive disease; PR, partial response; SD, stable disease.
[a] CR and PR groups combined since there was only one CR.
[b] CR/PR versus SD/PD.
[c] HR mutations: *ATRX, BRCA1/2, EMSY, MEN1, PALB2, PTEN, SETD2*.
[d] Combined HR mutation and *BRCA1* methylation: $N = 2$; Patients #5 and #8.
[e] *BRCA*ness signature positive = *BRCA1*-like profile by Multiplex Ligation-dependent Probe Amplification (MLPA).

breast MRI evaluation (Table 1) per protocol and were included in the intention-to-treat analysis.

Responses to olaparib are detailed for individual patients in Table 1 and depicted as waterfall plots for clinical (caliper) and MRI evaluation per RECIST1.1 in supplementary Figures S2 and S3 (available at https://doi.org/10.1016/j.annonc2020.11.009). A combined clinical and MRI response to olaparib was scored for each patient, where the response category was dictated by the MRI evaluation, unless MRI data were missing or ambiguous (two patients; details in Table 1). Based on combined clinical and MRI evaluation, olaparib treatment yielded one clinical complete response and 17 partial responses from 32 patients [objective response rate; ORR 56.3% (CI 39.3-71.8)]. Response to olaparib occurred independent of tumor size (Table 1). Importantly, excluding patients harboring g*BRCA1/2* ($n = 4$) and g*PALB2* ($n = 1$) mutations, an objective response was recorded in 14 out of 27 patients (ORR 51.9%, CI 34.0-69.3, Figure 2). Olaparib monotherapy was well tolerated, with only one patient experiencing >grade 2 toxicity (fatigue; scored using Common Terminology Criteria for Adverse Events, CTCAE, version 4.03) and requiring a dose reduction (supplementary Table S2, available at https://doi.org/10.1016/j.annonc2020.11.009).

Statistical comparisons of HR deficiency parameters (HR mutations and *BRCA1* methylation) between olaparib responders and non-responders are summarized in Table 2 for combined clinical and MRI evaluation, whereas statistics based on either clinical or MRI evaluations are listed separately in supplementary Table S3, available at https://doi.org/10.1016/j.annonc2020.11.009.

Pathogenic germline (*BRCA1/2* and *PALB2*) or somatic (*ATRX*, *BRCA1*, *EMSY*, *MEN1*, *PTEN*, *SETD2*) mutations affecting genes involved in HR were present in 10 out of 18 responders (OR 55.6%, CI 33.7-75.4), contrasting 1 out of 14 non-responders (OR 7.1%, CI 0.0-31.5, $P = 0.008$, Figure 2 and Table 2). Excluding all five patients harboring g*BRCA1/2* or g*PALB2* mutations from statistical analysis, HR mutations were recorded in 6 out of 14 responders (OR 42.9%, CI 21.4-67.4) contrasting none of the 13 non-responders (OR 0%, CI 0.0-22.8, $P = 0.02$, Table 2).

Among patients not harboring HR mutations, 6 out of 8 olaparib responders were found methylated at the *BRCA1* promoter (OR 75.0%, CI 40.9-92.9), contrasting 3 out of 13 non-responders (OR 23.1%, CI 8.2-50.3, $P = 0.03$, Table 2). Taken together, pathogenic HR mutation (germline or somatic) and/or *BRCA1* promoter methylation was observed in 16 out of 18 responders (OR 88.9%, CI 67.2-96.9), contrasting 4 out of 14 non-responders (OR 28.6%, CI 11.7-54.7, $P = 0.0008$). Apart from two patients carrying somatic mutations in the *MEN1* and *PTEN* gene, *BRCA1* methylation and HR mutations (germline or somatic) were mutually exclusive (Figure 2 and supplementary Table S4, available at https://doi.org/10.1016/j.annonc2020.11.009). Notably, no tumor harbored *BRCA1* methylation and a germline/somatic *BRCA1* mutation in concert (Figure 2 and supplementary Table S4, available at https://doi.org/10.1016/j.annonc2020.11.009).

Somatic HR mutations observed in the primary biopsies disappeared after treatment in four patients (#5-8, Figure 2). The most likely explanation for this was a low tumor cell fraction after olaparib response (see supplementary Results, available at https://doi.org/10.1016/j.annonc2020.11.009). Interestingly, the only tumor where a HR mutation appeared after treatment (*PALB2*) was an olaparib non-responder (#25) harboring a germline *BRCA1* mutation. Further, the only

tumor (#23) with a change in *BRCA1* methylation status (gain of *BRCA1* methylation post-treatment) was also an olaparib non-responder (Figure 2).

In addition to HR mutation and *BRCA1* methylation analyses, we determined downstream functional ('phenotypical') HR deficiency by an MLPA-based *BRCA*ness analysis of copy number variation (CNV) to identify tumors with a *BRCA1*-like profile.[36] However, no statistically significant association between the MLPA-based *BRCA*ness signature and response to olaparib was observed ($P = 0.07$; Table 2 and supplementary Table S3, available at https://doi.org/10.1016/j.annonc2020.11.009).

An overview of all mutations recorded by targeted sequencing of the 360-gene panel before and after olaparib monotherapy is given in supplementary Table S5 and as an oncoplot in supplementary Figure S4 (available at https://doi.org/10.1016/j.annonc2020.11.009). Besides HR mutations, we observed mutations in genes associated with other types of DNA damage repair (DDR), like *ERCC2* (germline), *MSH6*, *MUTYH* (germline) and *PARP10*. Except for a g*MUTYH* mutation found in a patient not responding to olaparib (stable disease; SD), all DDR mutations were observed in concert with either an HR mutation or *BRCA1* methylation (supplementary Table S4, available at https://doi.org/10.1016/j.annonc2020.11.009), questioning their biological relevance to olaparib outcome. Further, neither *TP53* mutations (supplementary Table S4, available at https://doi.org/10.1016/j.annonc2020.11.009) nor total mutational load (supplementary Table S6, available at https://doi.org/10.1016/j.annonc2020.11.009) predicted response to olaparib. Notably, while olaparib reduced the total number of mutations in the responder group ($P = 0.01$), no reduction was recorded among non-responders (supplementary Table S6, available at https://doi.org/10.1016/j.annonc2020.11.009).

To expand on the pre-planned HRD analyses outlined above, a set of post-hoc analyses were carried out on pretreatment samples. Functional HR deficiency, as defined by low RAD51 scores,[37] correlated to HR mutations/*BRCA1* methylation status (supplementary Table S7, available at https://doi.org/10.1016/j.annonc2020.11.009), as well as olaparib response (supplementary Figure S5 and Table S8, available at https://doi.org/10.1016/j.annonc2020.11.009). In contrast, no correlation was observed between BRCA1 foci scores and olaparib response (supplementary Figure S5 and Table S4, available at https://doi.org/10.1016/j.annonc2020.11.009). Finally, PAM50 gene expression analysis revealed 14 out of 18 olaparib responders versus 8 out of 14 non-responders expressed a basal-like subtype ($P = 0.3$; Figure 2 and supplementary Table S7, available at https://doi.org/10.1016/j.annonc2020.11.009). While there was no significant correlation between a basal-like subtype and *BRCA1* methylation/*BRCA1* mutations ($P = 0.1$) or *BRCA1* methylation/HR mutations ($P = 0.1$), four out of four patients harboring *BRCA1* mutations revealed a basal-like subtype (supplementary Table S7, available at https://doi.org/10.1016/j.annonc2020.11.009).

While we observed no clear correlation between stromal or intratumoral TIL scores and HR mutations (somatic or germline) or *BRCA1* methylation status (supplementary Table S9 and supplementary Figure S6, available at https://doi.org/10.1016/j.annonc2020.11.009), pretreatment TIL counts were higher among olaparib responders compared with non-responders (supplementary Table S9 and supplementary Figure S7, available at https://doi.org/10.1016/j.annonc2020.11.009). Similarly, despite no association between PD-L1 expression in immune cells or tumor cells and HRD parameters (supplementary Table S9 and supplementary Figure S6, available at https://doi.org/10.1016/j.annonc2020.11.009), we observed a significant correlation between PD-L1 expression in both immune cells and tumor cells and response to olaparib (supplementary Table S9 and supplementary Figure S7, available at https://doi.org/10.1016/j.annonc2020.11.009).

Chemotherapy regimens administered after olaparib and surgical outcomes after completed primary treatment are summarized in supplementary Figure S8 (available at https://doi.org/10.1016/j.annonc2020.11.009) and are not the focus of the current report. However, a key finding was the lack of pathological complete response (pCR) to olaparib monotherapy without subsequent chemotherapy, or to olaparib monotherapy followed by olaparib at a reduced dose (150 mg b.i.d. day 1-3 each carboplatin week) in concert with a low-dose carboplatin regimen (AUC2 qW; 3 out of 4 weeks per month). This caused a protocol amendment mandating more potent chemotherapy regimens without PARP inhibition after the initial 10 weeks of olaparib (see supplementary Methods, available at https://doi.org/10.1016/j.annonc2020.11.009).

## DISCUSSION

Previous studies have revealed the benefit of PARP inhibitors for g*BRCA1/2* mutation carriers across breast, ovarian, pancreatic and prostate cancer.[15,16,19-21,25,38,39] While the efficacy of olaparib in patients with advanced prostate and ovarian cancer extends beyond g*BRCA1/2* mutations,[21,24,25] olaparib was ineffective in patients with late-stage, metastatic TNBC not harboring g*BRCA* mutations.[17] Here, we present results from a phase II trial demonstrating a 56.3% objective response rate for olaparib monotherapy in patients with treatment-naïve, unselected primary TNBC and a 51.9% response rate among patients not harboring g*BRCA1/2* or g*PALB2* mutations. Of note, acquired resistance to platinum agents is associated with secondary mutations restoring HR function,[22,23,40] and may promote PARP inhibitor resistance.[12,41] Thus, prior exposure to DNA crosslinking agents such as platinum and probably cyclophosphamide may explain the discrepancy between our results in treatment-naïve patients and the negative finding observed previously in late-stage metastatic breast cancer.[17]

Similar to what was recorded in advanced prostate cancer,[24] we find somatic defects in HR to predict response to olaparib in primary TNBC. Combining HR mutations and *BRCA1* promoter methylation assessment, we identified HR defects in 16 out of 18 olaparib responders, contrasting 4 out of 14 non-responders. Of note, somatic *BRCA1*

methylation and g*BRCA1* mutations were mutually exclusive in our cohort, confirming recent findings in a population-based study of 237 patients with TNBC.[11] If a combined analysis of HR mutations and *BRCA1* methylation was used as a selection biomarker to start PARP inhibition, 16 out of 20 patients selected for olaparib monotherapy in our trial would have obtained an OR. While these findings need confirmation in larger studies, they indicate a potential for HR mutations and *BRCA1* methylation as predictive markers identifying treatment-naïve TNBCs likely to benefit from PARP inhibitor monotherapy.

Notably, different genomic signatures for HRD or *BRCA*-ness have been tested as potential predictive markers for platinum or PARP inhibitor sensitivity, revealing conflicting results.[12,26,36,42] Here, 69% of TNBC harbored a basal-like subtype by PAM50 analysis, but the basal-like subtype was not enriched among olaparib responders. Also, using MLPA analysis, we found the *BRCA1*-like signature not to be predictive of response to olaparib in the current patient cohort. Although we lack a definite explanation for this finding, an HRD signature could remain as a genomic 'scar' in the tumor's mutational and/or copy number profile, despite tumor cells regaining HR function from secondary reverting *BRCA* or *RAD51C/D* mutations.[13,26,43] However, while such secondary mutations have been detected in tumors developing acquired chemoresistance,[13,43] they are less likely to be present in treatment-naïve patients. Furthermore, while we observed no correlation between *BRCA1* foci and response to olaparib, a similar lack of correlation between *BRCA1* expression and platinum sensitivity was previously established for advanced TNBC.[26] A potential explanation for this is inactivation of other key HR-related genes causing HRD[44] despite normal *BRCA1* expression.

Regarding the single non-responder harboring a germline *BRCA1* mutation, this patient harbored a pathogenic mutation within a region of *BRCA1* previously shown to be potentially removed by alternative splicing,[37,45] thus rescuing *BRCA1* function. The pretreatment biopsy however revealed a low RAD51 foci score, indicating definite HR deficiency at the time the patient commenced on olaparib. In contrast, while three non-responders revealed *BRCA1* hypermethylation, two of these tumors expressed a high RAD51 score, indicating lack of effective *BRCA1* silencing. For the last non-responder, *BRCA1* methylation and a low RAD51 score were observed in the pretreatment breast biopsy, and olaparib yielded profound regression of the breast primary tumor. Still, according to the RECIST criteria this patient's response to olaparib was classified as progressive disease due to the appearance of an axillary metastasis on MRI, suggesting that HR-proficient tumor cell subclones in the breast may have metastasized to the axilla during PARP inhibitor treatment.[46]

Our findings indicate that olaparib monotherapy can be used in the neoadjuvant setting for TNBC to debulk large HR deficient tumors before implementing chemotherapy. Of note, while talazoparib monotherapy yielded a higher pCR rate in g*BRCA* mutation carriers[20] than we observed for sequential olaparib and chemotherapy in patients with unselected TNBC, the two trials are not directly comparable. In the talazoparib study patients received PARPi treatment for a longer duration. Both studies enrolled a limited number of patients, and the fraction of patients diagnosed with stage III disease, a factor predicting for a lower pCR in the neoadjuvant setting,[47] was higher in our study than in the talazoparib trial (72% versus 15%, respectively).[20] At the same time, our results demonstrate that PARP inhibition alone or followed by combined low-dose carboplatin and PARPi, may not be a substitute for established and effective chemotherapy regimens in TNBC.[10,30,48] Identifying the optimal chemotherapy regimen, potentially including immunotherapy, for patients with TNBC is an area of intensive research; yet, the results are at variance.[6,49,50] In the current TNBC cohort we observed that tumors responding to olaparib were characterized by high TIL and PD-L1 expression levels, a subset where immunotherapy may be of particular benefit.[6,50] Based on our post-hoc results showing higher TIL and PD-L1 expression levels in olaparib responders, we advocate further testing of olaparib in concert with chemotherapy and potentially immunotherapy in sequential neoadjuvant regimens for TNBCs harboring HR mutations or *BRCA1* methylation. Notably, a recent Early Breast Cancer Trialists' Collaborative Group meta-analysis demonstrated sequential administration of chemotherapy to be at least as effective as concomitant administration of the same compounds in primary breast cancer,[3] indirectly providing a rationale for sequential treatment approaches where PARPi may be tested as initial monotherapy before optimal chemotherapy regimens.

While g*BRCA1* mutations and *BRCA1* methylations are strongly associated with TNBCs, g*BRCA2* mutations are distributed across different breast cancer subtypes, mirroring spontaneous tumors. Also, in the TCGA dataset, somatic mutations affecting different HR genes are observed in all breast cancer subtypes (https://www.cancer.gov/tcga). These findings suggest that PARP inhibition may be of potential benefit in a wider selection of patients with breast cancer. Finally, the findings that PARP inhibitor monotherapy may work in breast and prostatic carcinomas harboring somatic HR mutations[24] indicate that PARPi may be effective in other types of cancer with HR deficiency as well.

## *Conclusion*

Olaparib monotherapy yielded a high response rate when administered to treatment-naïve, large TNBC, with germline or somatic HR deficiency. While the benefit of PARP inhibitor monotherapy in TNBC needs confirmation, it presents a potential sequential approach for TNBC downstaging before chemotherapy.

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## DATA SHARING

Haukeland University Hospital and the University of Bergen support the dissemination of research data that has been generated, and increased cooperation between investigators. Trial data is collected, stored and disseminated according to institutional guidelines and in accordance with national laws and regulations to ensure the quality, integrity and use of clinical data. Study protocol, including plans for statistical analyses, is available online. Signed informed consent forms are stored at each participating hospital and are available for monitoring by regulatory authorities. After publication and upon formal request, raw data, including de-identified individual participant data and a data dictionary defining each field in the data set, will be shared according to institutional procedures. Requests are via a standard pro forma describing the nature of the proposed research and extent of data requirements. Data recipients are required to enter a formal data sharing agreement that describes the conditions for release and requirements for data transfer, storage, archiving, publication and intellectual property. Requests are reviewed by the PETREMAC study team in terms of scientific merit and ethical considerations, including patient consent. Data sharing is permitted if proposed projects have a sound scientific or patient benefit rationale, as agreed by the study team and with approval from the PETREMAC co-investigators as required.

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