Supplementary Online Content

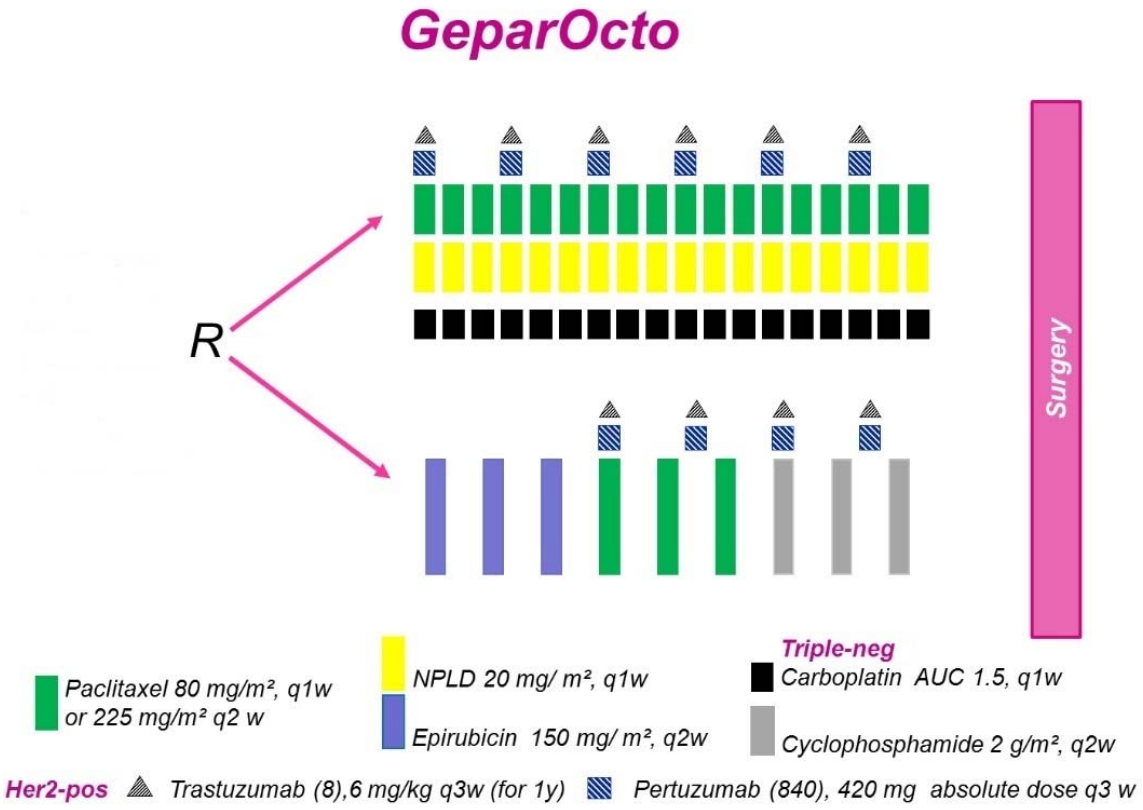
Pohl-Rescigno E, Hauke J, Loibl S, et al. Association of germline variant status with therapy response in high-risk early-stage breast cancer: a secondary analysis of the GeparOcto randomized clinical trial. *JAMA Oncol*. Published online March 12, 2020. doi:10.1001/jamaoncol.2020.0007

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

Germline variant analyses: Genomic DNA was isolated from venous blood samples using standard methods. All DNA samples were centrally analyzed in an accredited laboratory (Center for Familial Breast and Ovarian Cancer, Cologne, Germany) by targeted next generation sequencing (NGS) covering the entire coding regions and exon-flanking sequences (±15nt) of *BRCA1* (NM_007294.4), *BRCA2* (NM_000059.3), and 16 non-*BRCA1/2* cancer predisposition genes (*ATM*, NM_000051.3; *BARD1*, NM_000465.4; *BRIP1*, NM_032043.3*; CDH1*, NM_004360.5*; CHEK2*, NM_007194.4*; FANCM*, NM_020937.4; *MRE11A*, NM_005591.3*; NBN*, NM_002485.4*; PALB2*, NM_024675.4*; PTEN*, NM_000314.8; *RAD50*, NM_005732.4*; RAD51C*, NM_058216.3*; RAD51D*, NM_002878.3*; STK11*, NM_000455.5*; TP53*, NM_000546.5*; XRCC2*, NM_005431.2)[1]. For NGS, we employed a customer-tailored SureSelect gene panel (Agilent, Santa Clara, U.S.) using the QXT Target Enrichment protocol optimized for 200 ng of genomic DNA (Agilent). Sequencing was performed on a HiSeq 4000 platform (Illumina, San Diego, U.S.).

Bioinformatic analyses and variant classification: Bioinformatic analyses were carried out using VARBANK 2.0 as previously described[2]. For the detection of copy number variations (CNVs), three publicly available *in silico* approaches, namely panelcn.MOPS[3], ExomeDepth[4], and our in-house tool OpaCNAT (https://bitbucket.org/CorinnaErnst/opacnat/), were employed. All tools were executed run-wise and with default parameters. Validation of predicted CNVs was performed by either Multiplex Ligation-dependent Probe Amplification (MLPA; SALSA® MLPA probe mixes P002 and P087 for *BRCA1*, P045 for *BRCA2*, and P190 for *CHEK2*; MRC-Holland, Amsterdam, The Netherlands) or quantitative real-time polymerase chain reaction (*FANCM*). MLPA data were analyzed using the Coffalyzer.Net v.140429.1057 software (MRC-Holland). Variant classification was performed in accordance with the regulations of the international ENIGMA consortium (https://enigmaconsortium.org) as previously described in detail[5]. All genetic variants were classified using a 5-tier variant classification system as proposed by the International Agency for Research on Cancer (IARC) Unclassified Genetic Variants Working Group, namely, deleterious=class 5, likely deleterious=class 4, variant of uncertain significance (VUS)=class 3, likely benign=class 2, and benign=class 1. Variants reported to occur in large outbred control reference groups at an allele frequency of >1% were generally considered benign. Class 4/5 germline variants were subsequently defined as 'variants'. All variants identified by this approach were verified by Sanger sequencing.



eFigure. GeparOcto study design. GeparOcto compared the efficacy of two neoadjuvant treatment regimen: Sequential intense dose-dense epirubicin, paclitaxel, and cyclophosphamide (iddEPC) versus weekly paclitaxel and non-pegylated liposomal doxorubicin (PM), plus carboplatin (PMCb) in triple-negative BC (TNBC, abbreviated as ´Triple-neg´). In both study arms, patients with ERBB2/HER2 (MIM# 164870)-positive breast cancer (abbreviated as ´Her2-pos´) received trastuzumab and pertuzumab. A description of the study cohort, randomization, clinical procedures, and statistical analyses is given in the initial GeparOcto publication[6]. NPLD = non-pegylated liposomal doxorubicin; AUC = area under the curve.

eTable 1. Pathological complete response (pCR) rates (ypT0/is ypN0 definition) according to germline (g) variant status (*BRCA1/2* and non-*BRCA1/2* breast cancer predisposition genes), overall and by treatment arm. The odds ratios (ORs) refer to the comparison of variant positive versus variant negative patients (univariate logistic regression). CI = confidence interval; pos = positive; neg = negative.

A: all patients (n=914)	**both treatment arms**	**PM(Cb)**	**iddEPC**
pCR overall	48.1 (440/914)	47.9 (222/463)	48.3 (218/451)
pCR g*BRCA1/2* pos (n=96)	60.4 (58/96)	68.1 (32/47)	53.1 (26/49)
pCR g*BRCA1/2* neg (n=818)	46.7 (382/818)	45.7 (190/416)	47.8 (192/402)
OR (95%CI; P-value)	1.74 (1.13-2.68; .01)	2.53 (1.33-4.83; .005)	1.24 (0.68-2.24; .48)
TNBC (n=393), pCR overall	50.1 (197/393)	51.8 (103/199)	48.5 (94/194)
pCR g*BRCA1/2* pos (n=69)	69.6 (48/69)	74.3 (26/35)	64.7 (22/34)
pCR gBRCA1/2 neg (n=324)	46.0 (149/324)	47.0 (77/164)	45.0 (72/160)
OR (95%CI; P-value)	2.69 (1.54-4.69; .001)	3.26 (1.44-7.39; .005)	2.24 (1.04-4.84; .04)
ERBB2+ (n=365), pCR overall	60.3 (220/365)	58.5 (107/183)	62.1 (113/182)
pCR g*BRCA1/2* pos (n=5)	60.0 (3/5)	100 (2/2)	33.3 (1/3)
pCR g*BRCA1/2* neg (n=360)	60.3 (217/360)	58.0 (105/181)	62.6 (112/179)
OR (95%CI; P-value)	/	/	/
ERBB2-/HR+ (n=156), pCR overall	14.7 (23/156)	14.8 (12/81)	14.7 (11/75)
pCR *gBRCA1/2* pos (n=22)	31.8 (7/22)	40.0 (4/10)	25.0 (3/12)
pCR *gBRCA1/2* neg (n=134)	11.9 (16/134)	11.3 (8/71)	12.7 (8/63)
OR (95%CI; P-value)	3.44 (1.22-9.72; .02)	5.25 (1.22-22.69; .03)	2.29 (0.51-10.30; .28)
B: *gBRCA1/2*-negative patients (n=818)	**both treatment arms**	**PM(Cb)**	**iddEPC**
pCR non-*gBRCA1/2* pos (n=76)	43.4 (33/76)	41.3 (19/46)	46.7 (14/30)
pCR non-*gBRCA1/2* neg (n=742)	47.0 (349/742)	46.2 (171/370)	47.8 (178/372)
OR (95%CI; P-value)	0.86 (0.54-1.39; .55)	0.82 (0.44-1.53; .53)	0.95 (0.45-2.01; .90)
TNBC (n=324)			
pCR non-*gBRCA1/2* pos (n=30)	40.0 (12/30)	45.0 (9/20)	30.0 (3/10)
pCR non-*gBRCA1/2* neg (n=294)	46.6 (137/294)	47.2 (68/144)	46.0 (69/150)
OR (95%CI; P-value)	0.76 (0.36-1.64; .49)	0.91 (0.36-2.34; .85)	0.50 (0.13-2.02; .33)
ERBB2+ (n=360)			
pCR non-*gBRCA1/2* pos (n=36)	58.3 (21/36)	52.6 (10/19)	64.7 (11/17)
pCR non-*gBRCA1/2* neg (n=324)	60.5 (196/324)	58.6 (95/162)	62.3 (101/162)
OR (95%CI; P-value)	0.91 (0.45-1.84; .80)	0.78 (0.30-2.03; .62)	1.11 (0.39-3.15; .85)
ERBB2-/HR+ (n=134)			
pCR non-*gBRCA1/2* pos (n=10)	0.0 (0/10)	0.0 (0/7)	0.0 (0/3)
pCR non-*gBRCA1/2* neg (n=124)	12.9 (16/124)	12.5 (8/64)	13.3 (8/60)
OR (95%CI; P-value)	/	/	/

eTable 2. Prevalence of germline variants in BRCA1/2 and 16 non-BRCA1/2 genes in 914 breast cancer patients. For each gene, the variant prevalence is given overall and according to biological subtype (TNBC, ERBB2+, ERBB2-/HR+). Percentages of patients carrying a variant in the respective subgroup are shown in parentheses. No variants were identified in *CDH1, MRE11A, PTEN, RAD51D,* and *STK11*. Four patients carried copy number variations (CNVs) in the *BRCA1* gene (three deletions, one duplication), two patients carried deletions in the *CHEK2* gene, and another two patients carried deletions in the *FANCM* gene. * = in this subgroup, one patient carried two different variants in the *ATM* gene. # = in this subgroup, one patient homozygously carried the *CHEK2* c.1100del variant. / = no variant identified.

	overall (%)	TNBC (%)	ERBB2+ (%)	ERBB2-/HR+ (%)
***BRCA1/2*-positive patients**	**96/914 (10.5)**	**69/393 (17.6)**	**5/365 (1.4)**	**22/156 (14.1)**
BRCA1 only	69 (7.5)	56 (14.2)	3 (0.8)	10 (6.4)
BRCA1 and *NBN*	1 (0.1)	1 (0.3)	/	/
BRCA1 and *RAD50*	1 (0.1)	1 (0.3)	/	/
BRCA1 and *XRCC2*	1 (0.1)	1 (0.3)	/	/
BRCA1 and *BRCA2*	1 (0.1)	1 (0.3)	/	/
BRCA2 only	21 (2.3)	9 (2.3)	1 (0.3)	11 (7.1)
BRCA2 and *CHEK2*	1 (0.1)	/	/	1 (0.6)
BRCA2 and *XRCC2*	1 (0.1)	/	1 (0.3)	/
***BRCA1/2*-negative patients with variants in non-*BRCA1/2* genes**	**76/818 (9.3)**	**30/324 (9.3)**	**36/360 (10.0)**	**10/134 (7.5)**
ATM only	9* (1.1)	/	6 (1.7)	3* (2.2)
ATM and *CHEK2*	2 (0.2)	1 (0.3)	1 (0.3)	/
ATM and *TP53*	1 (0.1)	/	1 (0.3)	/
BARD1 only	4 (0.5)	2 (0.6)	/	2 (1.5)
BRIP1 only	5 (0.6)	5 (1.5)	/	/
CHEK2 only	23# (2.8)	1 (0.3)	19# (5.3)	3 (2.2)
FANCM only	14 (1.7)	9 (2.8)	5 (1.4)	/
NBN only	1 (0.1)	1 (0.3)	/	/
PALB2 only	10 (1.2)	8 (2.5)	1 (0.3)	1 (0.7)
PALB2 and *XRCC2*	1 (0.1)	1 (0.3)	/	/
RAD50 only	1 (0.1)	1 (0.3)	/	/
RAD51C only	1 (0.1)	1 (0.3)	/	/
TP53 only	3 (0.4)	/	3 (0.8)	/
XRCC2 only	1 (0.1)	/	/	1 (0.7)

eReferences

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