SUPPLEMENTARY METHODS

Targeted capture massively parallel sequencing

Tumor DNA from ovarian cancer patients, cfDNA from ovarian and breast cancer patients and their respective germline DNA were subjected to targeted massively parallel sequencing in the MSKCC Integrated Genomics Operation (IGO) as previously described (1,2) using a custom panel of baits encompassing all exons and introns of *BRCA1* and *BRCA2*, and all exons of 141 additional genes reported to be involved in DNA repair, drug resistance, resistance to PARP-inhibitors/ platinum-salts, and genes recurrently mutated in ovarian cancer, including *TP53* (Supplementary Table S1) (3-5). In addition, baits tiling common single nucleotide polymorphisms (SNPs) were included to allow for copy number analysis, as previously described (1). Serial plasma samples from breast cancer patients L031 and 1109 were subjected also to MSK-IMPACT sequencing targeting 410 key cancer genes, as previously described (1,6). Sequencing reads were aligned to the human reference genome GRCh37 using the Burrows-Wheeler Aligner (BWA) (7). Local realignment, duplicate removal and quality score recalibration were performed using Genome Analysis Toolkit (GATK) (8). Somatic single nucleotide variants (SNVs) were detected using MuTect (9), and small insertions and deletions (indels) were determined using Strelka and VarScan 2 (10,11). Variants with >5% global minor allele frequency in dbSNP (Build 137), or for which the tumor variant allele fraction was <5 times than that of the normal variant allele fraction were disregarded (6,12). In addition, variants in the cfDNAs and tumors were assessed using the SAMtools mpileup tool (13) and Varscan 2 (10), as previously described (6). All indels and variants detected in cfDNA were further curated by manual inspection using the Integrative Genomics Viewer (IGV) (14). The potential functional effect of each mutation was investigated *in silico* using a combination of mutation function predictors, as previously described (15,16). Copy number alterations in the tumors were identified using FACETS (17) and the cancer cell fraction (CCF) of each mutation using ABSOLUTE (v1.0.6) (18) as previously described (16). Sequence data are available at the Sequence Read Archive (SRP100525).

Targeted amplicon re-sequencing

The putative *BRCA2* reversion mutations and the somatic *SPEN* and *TGFBR1* mutations identified by massively parallel sequencing were validated in three plasma samples from case L031 using targeted amplicon re-sequencing. In case 1109 three somatic variants affecting *FAT3*, *ERCC4* and *KDM5C* were validated together with the putative *BRCA2* reversion mutations identified in the post-treatment plasma sample and in a tumor metastasis core biopsy affecting the liver obtained prior to treatment. At least 10ng of plasma DNA, microdissected tumor DNA and matching peripheral blood leukocyte-derived germline DNA were amplified using Taq Hifi polymerase (Ion AmpliSeq Library Kit 2.0, Thermo Fisher Scientific), and the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England BioLabs) was used to prepare libraries. As controls, matching normal DNA from cases 1109 and L031 and plasma DNA from two unrelated advanced breast cancer patients not treated with PARP inhibitors or platinum-based chemotherapy and two tumor DNA samples obtained from unrelated breast cancer core biopsies were included. Amplicon re-sequencing of the putative *BRCA2* reversion mutations in the cfDNA samples of L031 and 1109 was performed twice independently. PCR conditions and primers are available on request. The quality and quantity of the amplification was tested using the Agilent 2100 Bioanalyzer and the KAPA Library Quantification Kit for Illumina (Kapa Biosystems), and libraries were sequenced in one lane of an Illumina HiSeq2500 for the first run and in an Illumina MiniSeq (Mid output kit) for the second run. Reads were aligned to the human reference genome (GRCh37) using BWA mem (v0.7.12) (7) For each of the *BRCA2* amplicons sequenced, read counts for each position in the amplicon for the reference and any alternative alleles were obtained using bam-readcounts (19). Reads with a mapping quality score of <20 and bases with a base quality score of <30 were excluded from this analysis. The number of reads for each possible indel at each position was then extracted using a custom perl script and manually confirmed using IGV (14). Only *BRCA2* reversion mutations present in plasma DNA with zero counts in the germline control and in the unrelated control samples were considered validated.

BRCA1 foci formation

The U2OS-double-strand break (DSB) reporter system was employed to define the ability of putative *BRCA1* somatic reversion mutations to recognize DSBs, as previously described (20-22). U2OS cells were grown on cover slips overnight, and transfected with pcDNA-BRCA1(Δ510-1283) and *BRCA1* mutant plasmids (i.e. c.68-69delAG, c.68-69delAG/c.108delC, c.68-69delAG/c.113delA, c.68-69delAG/c.85delG, c.3908dupT, c.3908dupT/c.3951delG, c.3908dupT/3926delA, c.4724delC, c.4724delC/c.4786-4787delTC). After 48 hr, cells were subjected to 8 Gy irradiation (IR) using a Cs-137 irradiator. Six hr post IR, cells were fixed using 3% paraformaldehyde/ 2% sucrose solution. Immunofluoresence was performed using anti-HA (Santa Cruz) and anti-γ-H2AX (Novus Biologics) antibodies. Images were captured with a QImaging RETIGA-SRV camera connected to a Nikon Eclipse 80i microscope. Images were analyzed using the ImageJ software (NIH). IR-induced foci-positive cells were defined as cells harboring more than 10 BRCA1 foci colocalized with γ-H2AX foci, and an average of 100 cells was counted to define the IR-induced foci-positive percentage of cells, as previously described (21,22).

Supplementary References

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SUPPLEMENTARY LEGENDS

Supplementary Methods

Supplementary Figure S1: Repertoire of non-synonymous somatic mutations in tumors and plasma DNA derived from ovarian cancer patients with *BRCA1/2* germline mutations resistant/ refractory to platinum-based chemotherapy.

Cancer cell fractions of the somatic non-synonymous mutations identified in the ovarian tumors are shown on the left. Presence of loss of heterozygosity of the wild-type allele of a mutated gene is represented by a diagonal bar, and mutations found to be clonal by ABSOLUTE are indicated by a yellow box. Variant allele fractions of the non-synonymous somatic mutations identified in the tumors and cfDNA from patients with ovarian cancer are shown on the right. The number of mutant reads over total reads is shown for each mutation in each sample. T, tumor; C, cfDNA.

Supplementary Figure S2: Putative *BRCA1* somatic reversion mutations identified in plasma DNA derived from ovarian cancer patients with *BRCA1* germline mutations resistant/ refractory to platinum-based chemotherapy.

Nucleotide and amino acid sequences for the affected genomic location shown are based on ENSEMBL transcript no. ENST00000357654.3. Representation of the predicted nucleotide and protein sequences for *BRCA1* wild-type (WT), germline mutation (red) and reversion mutations (blue) from ovarian cancer patients OCT1C, OCT5C, OCT15C and OCT10C. The aberrant amino acids produced by a given mutation are marked in red. Black arrows indicate the BRCA1 transcription direction. AA, amino acid; MAF, mutant allele fraction; ORF, open reading frame; WT, wild type.

Supplementary Figure S3: Repertoire of non-synonymous somatic mutations in plasma DNA derived from breast cancer patients with *BRCA1/2* germline mutations previously treated with platinum-based chemotherapy and/or PARP inhibitors.

Mutant allele fractions of the non-synonymous somatic mutations identified in the cfDNA of breast cancer patients are presented. The number of mutant reads over total reads is shown for each mutation in each sample.

Supplementary Figure S4: Variant allele frequencies of somatic mutations in the longitudinal plasma samples from breast cancer patient L031 as defined by amplicon re-sequencing.

Supplementary Table S1: List of 143 genes included in the targeted capture massively parallel sequencing assay.

Supplementary Table S2: Sequencing statistics and non-synonymous somatic mutations identified in cfDNA and tumor tissue of ovarian and breast cancer patients included in this study.

Supplementary Table S3: Putative *BRCA1/2* reversion mutations identified in cfDNA of ovarian and breast cancer patients using targeted massively parallel sequencing and results of amplicon re-sequencing of putative *BRCA2* reversion mutations and other somatic mutations in cfDNA and tumor tissue of metastatic breast cancers.