Supplemental Methods and Figures

Immunofluorescence (RAD51, γH2AX and DMC1)

Cells were plated on 4 well culture glass slides with polystyrene (Corning, Inc.) and treated with 10 μM olaparib or 5-10 μM carboplatin and analyzed 72 hours post treatment. CD133+ cells isolated using the AutoMacs cell separator (Miltenyi Biotec) were plated on culture glass slides and incubated for 3 hours to allow the cell attachment. The immunofluorescence protocol was then performed at 3 hours to minimize differentiation of the CD133+ cells and maintain their purity. Specifically, the chamber slides were rinsed twice with PBS (37°C), fixed in 4% paraformaldehyde for 10 minutes, permeabilized with PBS-T (0.3% Triton X-100 in PBS) for 10 minutes and incubated with primary antibodies overnight at 4°C. The primary antibodies used in this study were rabbit polyclonal anti-RAD51 (H92; Santa Cruz Biotechnology, Dallas, TX, USA) at a dilution of 1:200, mouse monoclonal anti-phosphorylated histone H2AX (clone JBW301; Millipore, Billerica, MA, USA) at a dilution of 1:100 and mouse monoclonal anti-DMC1 (2H12/4; Abcam, Cambridge, MA, USA) at a dilution of 1:100. The chamber slides were rinsed twice for 5 minutes in PBS, followed by incubation with the appropriate fluorophore-conjugated secondary antibody: goat anti-rabbit IgG Alexa Fluor 488 (Abcam) and goat anti-mouse IgG Alexa Fluor 549 (Abcam) at dilution of 1:750. Nuclei staining were performed with DAPI (Vectashield Mounting Medium with DAPI; VECTORS laboratories, Burlingame, CA, USA) and the coverslips were mounted on slides. Immunofluorescence was visualized using a Zeiss LSM 150 confocal microscope and foci formation was quantified by assessing the number of stained cells for a 100-cell sampling group.

Western Blotting (PAR, PARP, RAD51 and γH2AX)

Lysates were prepared from cell lines or frozen xenograft samples with ice-cold lysis buffer Mammalian Protein Extraction Reagent (Thermo Scientific) supplemented with kinase, protease and phosphatase inhibitors (Sigma-Aldrich). Lysates were centrifuged at 12,000 rpm for 10 min at 4^0C and the resulting supernatants were then assayed for protein concentration using the *DC*™ Protein Assay kit (BioRad Laboratories, Hercules, CA, USA). Ten to forty micrograms of total protein lysate were resolved on 10%, 12%, and 4-12% Bis-Tris gels, or 3-8% Tris-Acetate gels (NuPAGE Novex, Life Technologies) and transferred to PVDF membranes (Millipore). Membranes were blocked with 5% non-fat milk in TBST and then incubated with primary antibody overnight at 4ºC. Western blots were performed using the

following antibodies: PARP at 1:1000 dilution (#9542S, Cell Signaling, Danvers, MA), pADPr at 1:200 dilution (#SC-56198, Santa Cruz Biotechnology), phospho-histone H2AX Ser139 at a 1:1000 dilution (#2577, Cell Signaling), Rad51 at 1:1000 dilution (ab133534, Abcam) and mouse monoclonal anti-DMC1 (2H12/4; Abcam, Cambridge, MA, USA). The blots were then probed with either a mouse or rabbit horseradish peroxidase (HRP) - conjugated secondary antibody at 1:2000 - 1:10,000 solutions (Santa Cruz Biotechnology) and developed using a chemiluminescent detection reagent (ECL Prime, GE Healthcare Life Sciences, Pittsburgh, PA). All images were captured using BioRad Molecular Imager ChemiDoc ™ XRS+ Imaging system and analyzed using Image J, Image Processing and Analysis in Java (National Institutes of Health, Maryland, USA, http://imagej.nih.gov/). All membranes were also probed with either a GAPDH (#5174, Cell Signaling) or β-tubulin (#2128, Cell Signaling) antibody at 1:5000 dilution to verify equal protein loading.

RNA Extraction and RT-PCR

Total RNA was isolated from all the cell lines 14 days after sphere formation, 72 hours after carboplatin treatment, and 7 days after olaparib or rucaparib treatment following the RNeasy mini kit (Qiagen) protocol. The RNAs were quantified using the Nanodrop 2000 spectrophotometer (Thermo Scientific). cDNA was synthesized from 2 μg of RNA using the SuperScript VILO cDNA synthesis kit (Invitrogen) and the Veriti Thermal Cycler (Applied Biosystems). The PCR step was performed using the BIORAD CFX96 Sequence Detection System (Biorad), with each sample run in triplicate. Results were analyzed using the comparative ΔΔCt method; ΔΔCt values were utilized to calculate the RQ=2-ΔΔCt. Data were expressed as the fold difference in gene expression (normalized to the housekeeping gene actin) relative to a reference sample.

Primer sequences:

PROM1	forward	ACTACCAAGGACAAGGCGTT
	reverse	TGGTCTCCTTGATCGCTGTT
KIT	forward	CCCACTGCAATCCTGTCTTT
	reverse	TAGGATGGTGGCTGATGACA
SOX2	forward	TCCCATCACCCACAGCAAATGA
	reverse	TTTCTTGTCGGCATCGCGGTTT

POU5F1	forward	TGCCCGAAACCCACACTG
	reverse	CTCGGACCACATCCTTCTCG
ACTB	forward	CATGTACGTTGCTATCCAGGC
	reverse	CTCCTTAATGTCACGCACGAT

PCR Array of DNA Repair Pathways

Total RNA was isolated from UWB1.289 WT and UWB1.289 MUT cells harvested 72 hours after treatment with 10 μM olaparib and sorted for CD133+ and CD133-CD117- cell populations. RNA from sorted cells was extracted following the protocol of the RNeasy micro kit (Qiagen). The RNAs were quantified using the Nanodrop 2000 spectrophotometer (Thermo Scientific) and RNA integrity was evaluated with Agilent Genomics Platform. cDNA was synthesized from 0.1 μg of total RNA with Reverse transcription mix (Qiagen) and hybridized to Qiagen Custom RT2 Profile PCR Arrays (Qiagen) designed for 25 genes:

Double-strand break (DSB) repair: *BRCA1, BRCA2, DMC1, FEN1, LIG4, MRE11A, PRKDC, RAD21, RAD50, RAD51, RAD51C, RAD51B, RAD51D, RAD52, RAD54L, XRCC2, XRCC3, XRCC4, XRCC5, XRCC6.*

BRCA-ness genes: *ATM, ATR, CHK2, CHK1, PALB2*

The PCR step was performed using the BIORAD CFX96 Sequence Detection System (Biorad), with each sample run in triplicate. Results were analyzed using the comparative ΔΔCt method. Data were expressed as the fold difference in gene expression (normalized to different housekeeping genes included in the PCR array) relative to a reference sample. For both cell lines, PCR analysis was performed in both vehicle and olaparib treated cells. Gene expression relative to vehicle control in either CD133+ or CD133- CD117- olaparib treated cell populations was determined and compared between the UWB1.289 *BRCA1* wild type and mutant cell lines.

Supplementary SA: Relevant characteristics of the analyzed ovarian cancer cell lines including histology, platinum response, *TP53* and *BRCA* gene status and frequency of cells expressing the CSC markers CD133 or CD117.

Supplementary Figure SB: 1 MTT cell metabolism assay after olaparib and rucaparib treatment *in vitro* with serial dilutions of the drugs to determine the drug concentration required to cause a 50% reduction in cell metabolic activity in the different cell lines. **2** Annexin/PI staining and relative frequency of live, apoptotic and necrotic cells harvested 7 days after treatments: vehicle (black), olaparib (green) or rucaparib (purple) (n=3, mean $\pm$ SEM).

Supplementary Figure SC: RT-PCR analysis of *PROM1* (CD133), *KIT* (CD117), *SOX2* and *POU5F1* (OCT4) genes in the indicated cell lines harvested 7 days after olaparib treatment. Gene expression in olaparib treated cells (green) was normalized to that in vehicle treated cells (black).

Supplementary Figure SD: Flow cytometric analysis of ALDH+ cell frequency in the indicated cell lines harvested 7 days after treatment with 10 μM olaparib (n=3, mean $\pm$ SEM, p value < 0.05).

Supplementary Figure SE: 1 PAR levels in the indicated cell lines 7 days following rucaparib treatment. The relative level of PAR in each sample was quantitated by scanning densitometry and normalized to the β-Tubulin loading control. **2** Flow cytometric determination of the frequency of viable (left panels), $CD133^+$ (center panels) and $CD117^+$ (right panels) cells in the indicated cell lines 7 days following treatment of rucaparib *in vitro* (n=3, mean $\pm$ SEM, p value < 0.001). **3** Relative cell viability of CD133- CD117-, CD133+, CD117+ and CD133+CD117+ cells was determined 7 days post *in vitro* treatment with the indicated concentrations of rucaparib as determined by flow cytometry (n=3, mean $\pm$ SEM, p value < 0.001). **4** Extreme limiting dilution analysis (ELDA) of the indicated cell lines was determined 7 days following treatment with rucaparib *in vitro*. The data are expressed as sphere forming frequency of cells exposed to rucaparib normalized to the corresponding vehicle control and suggest the relative frequency of cancer stem cells in each sample (n=3, mean± SEM, p-value < 0.001).

Supplementary Figure SF: Western analysis of γH2AX and RAD51 levels in UWB1.289 WT (left panel) and MUT (right panel) cell lines. Cells were harvested after 7 days of olaparib treatment. The WB analysis showed an increase in γH2AX (signal of DNA damage), and RAD51 (signal of HR DNA repair activation), after PARPi treatment, regardless of *BRCA* gene status (n=3, mean ± SEM, p-value < 0.01).

Supplementary Figure SG: Comet assay of DNA damage in the UWB1.289 WT and UWB1.289 MUT cell lines was assessed 72 hours following treatment with 10 μM olaparib. The presence of a comet tail indicates damaged and unrepaired DNA in the olaparib treated cells.

Supplementary Figure SH: Analysis of RAD51 and DMC1 foci formation 3 days after treatment with carboplatin (5 μM) or rucaparib (5 μM) in UWB1.289 WT (upper panel) and UWB1.289 MUT (lower panel) cell lines. Representative images of confocal immunofluorescence showing nuclear expression of RAD51 (green) and DMC1 (red). Nuclei were stained with DAPI (blue). Quantification of DMC1 foci in Figure 6E.

Supplementary Table S1: p-values of RT-PCR analysis of *PROM1* (CD133), *KIT* (CD117), *SOX2* and *POU5F1* (OCT4) genes (Supplementary Figure SC).

Supplementary Table S2: p-values of RT-PCR analysis of RAD51 gene family in CD133+ cells and CD133- CD117- cells FACS purified (Figure 6A and 6B).