Olaparib in ovarian cancer xenograft model

**Tumor growth inhibition by olaparib in BRCA2 germline-mutated patient-derived ovarian cancer tissue xenografts**

Ursula Kortmann¹,², Jessica N. McAlpine³, Hui Xue², Jun Guan², Gavin Ha⁴, Sophie Tully⁵,*, Sharaz Shafait⁵,*, Alan Lau⁶, Aaron N. Cranston⁵,*, Mark J. O’Connor⁶, David G. Huntsman¹,⁷, Yuzhuo Wang²,⁸ and C. Blake Gilks⁷

**Authors’ affiliations:**

¹Department of Pathology, BC Cancer Agency and University of British Columbia, Vancouver, BC, Canada, ²Living Tumor Laboratory, BC Cancer Agency, Vancouver, BC, Canada, ³Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University of British Columbia, Vancouver, BC, Canada, ⁴Department of Molecular Oncology, BC Cancer Research Centre, Vancouver, BC, Canada, ⁵KuDOS Pharmaceuticals Ltd, Cambridge, United Kingdom, ³former KuDOS employee, ⁶AstraZeneca, Cancer Bioscience, Cheshire, United Kingdom, ⁷Department of Pathology, Genetic Pathology Evaluation Centre, Vancouver General Hospital, Centre for Translation and Applied Genomics, BC Cancer Agency and University of British Columbia, Vancouver, BC, Canada, ⁸The Prostate Centre at Vancouver General Hospital and Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada

**Running title:** Olaparib in ovarian cancer xenograft model
Olaparib in ovarian cancer xenograft model

Keywords: PARP inhibitor, olaparib, AZD2281, BRCA, ovarian cancer

Acknowledgments

Grant support: This study was supported by NCIC (C.B.G.) and funding from OvCaRe (an initiative of the Vancouver General Hospital & University of BC Hospital Foundation and the BC Cancer Foundation) (D.G.H. and Y.Z.W.), the BC Cancer Foundation and AstraZeneca Plc. (Investigator-initiated project D.G.H.).

Requests for reprints and corresponding author

C. Blake Gilks, MD, Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver General Hospital, 910 West 10th Avenue, Vancouver, BC V5Z 1M9, Canada. E-mail: Blake.Gilks@vch.ca

Disclosure of Potential Conflicts of Interest

Drs. Lau and O’Connor report being employees of AstraZeneca. Dr. Cranston as well as Sophie Tully and Sharaz Shafait report having been employed by KuDOS Pharmaceuticals at the time experiments were performed. No other potential conflict of interest relevant to this article was reported.

Statement of Translational Relevance

The efficacy of conventional platinum-based chemotherapy for high-grade serous carcinoma is limited; most patients show an initial response to treatment but upon
Olaparib in ovarian cancer xenograft model

relapse, response rates to platinum progressively diminish and they ultimately die of progressive disease. By capitalizing on the genetic defect in DNA double strand repair in serous carcinomas with BRCA loss, through targeting of DNA single strand repair with the poly(ADP)-ribose polymerase (PARP) inhibitor olaparib, selective tumour cytotoxicity can be induced while sparing normal cells. We show that olaparib therapy results in tumour cell death in a human ovarian carcinoma xenograft with a BRCA2 mutation, but not in a xenograft with normal BRCA1 and BRCA2, and the effects of olaparib are increased with concomitant carboplatin treatment. This study describes a clinically relevant model of serous ovarian carcinoma for drug testing, and provides a rationale for trials of combined PARP-inhibitor/platinum chemotherapy for serous carcinomas with BRCA loss.
Olaparib in ovarian cancer xenograft model

Abstract

Purpose: Most patients with ovarian carcinomas succumb to their disease and there is a critical need for improved therapeutic approaches. Carcinomas arising in BRCA mutation carriers display defective DNA double-strand break repair that can be therapeutically exploited by inhibition of PARP-1, a key enzyme in the repair of DNA single strand breaks, creating synthetic lethality in tumour cells.

Experimental Design: To investigate synthetic lethality in vivo, we established a BRCA2-germline-mutated xenograft model that was developed directly from human ovarian cancer tissue treated with the PARP inhibitor olaparib (AZD2281) alone and in combination with carboplatin.

Results: We show that olaparib alone and in combination with carboplatin greatly inhibits growth in BRCA2-mutated ovarian serous carcinoma. This effect was not observed in a serous carcinoma with normal BRCA function, demonstrating a specific anti-tumor effect of olaparib in mutation carriers. Immunohistochemistry (Cleaved Caspase3 and Ki-67 stains) of remnant tissue after olaparib treatment revealed significantly decreased proliferation and increased apoptotic indices in these tumors compared to untreated controls. Furthermore, olaparib-treated tumors showed highly reduced PARP-1 activity that correlated with olaparib levels.

Conclusions: We established a BRCA2 mutated human ovarian cancer xenograft model suitable for experimental drug testing. The demonstrated in-vivo efficacy of olaparib extends on the preclinical rationale for further clinical trials targeting ovarian cancer patients with BRCA mutations.
Olaparib in ovarian cancer xenograft model
Olaparib in ovarian cancer xenograft model

**Introduction:**

Ovarian cancer is the fifth leading cause of cancer death in North American women and the most fatal gynecological cancer (1). Approximately 10% of all ovarian cancers are hereditary and of these more than 90% are associated with BRCA1 or BRCA2 germ-line mutations (2). In addition, a smaller number of non-hereditary ovarian cancers show somatic BRCA mutations, and functional loss of BRCA1 through promoter methylation is commonly observed. These changes together result in the BRCA deficiency associated with high grade serous ovarian carcinoma (HGSC), the most frequent histologic subtype in epithelial ovarian cancer (EOC) (3, 4). Patients with BRCA germline mutations treated with platinum-based therapy regimens have a survival advantage compared to non-hereditary EOC of HGSC subtype (5). Regardless, the majority of patients with HGSC relapse and ultimately die from their disease, therefore development of effective first line therapies is critical.

*BRCA1* and *BRCA2* encode tumor suppressor proteins that are part of a multi-component complex (RAD51 complex) involved in the repair of DNA double strand breaks (DSBs) and collapsed replication forks by the process of homologous recombination (HR), and are therefore essential for maintaining genomic stability (6). This genetic defect in the DNA repair pathway of affected tumors can be exploited by using poly(ADP)-ribose polymerase (PARP) inhibitors to induce selective tumour cytotoxicity whilst sparing normal cells. The most abundant member of the PARP family, PARP-1, plays a crucial role in the repair of DNA single strand breaks (SSBs) via the base excision repair (BER) pathway. Inhibition of PARPs leads to the accumulation of DNA SSBs, which can lead to DNA DSBs at replication forks. This synthetically lethal
Olaparib in ovarian cancer xenograft model

effect of BRCA1/BRCA2 mutation and interventional PARP-1 inhibition, leading to
deficiency in both the SSB and DSB pathways, has been shown to enhance the
cytotoxic effects of ionizing radiation and DNA damaging chemotherapy agents, such as
DNA cross-linking agents, alkylating agents and topoisomerase I inhibitors (7-9).

Most HGSC, regardless of the BRCA mutation status, have already spread
beyond the confines of the ovary at diagnosis and therefore management involves
surgical debulking followed by chemotherapy with carboplatin and a taxane (10, 11). To
improve response rates and treatment-free intervals and to offer treatment alternatives
to these existing therapies, PARP inhibitors have been recently introduced and tested in
early clinical trials as single agents or as part of combination therapies in patients with
advanced solid tumors (12-14). These trials were prompted by convincing pre-clinical
assays showing that cell lines lacking wild-type BRCA1 or BRCA2 were extremely
sensitive to potent PARP inhibitors compared with heterozygous mutant or wild-type
cells (15-18).

Recently, the orally active PARP inhibitor, olaparib (AZD2281), was evaluated
as a single agent therapy in humans demonstrating clinical anti-tumor activity in BRCA-
associated cancers (primarily ovarian carcinomas) (14).

In this study we present a BRCA2-mutated human ovarian cancer tissue explant
xenograft model to investigate the therapeutic response to olaparib as a single agent
and in combination with chemotherapy.

Materials and Methods

Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.
Copyright © 2010 American Association for Cancer Research
Transplantable patient-derived ovarian cancer tissue lines

Ovarian tumour tissue specimens were obtained, with informed consent, from patients undergoing surgical staging for primary ovarian cancer at Vancouver General Hospital. Fresh tumour tissue was cut into small pieces and grafted under the renal capsule of female NOD-SCID mice for subsequent serial transplantation and characterization as previously described (19). Animal care and experiments were carried out in accordance with the guidelines of the Canadian Council on Animal Care and human tissue specimens managed per protocol approved by the University of British Columbia Clinical Research Ethics Board. Treatment response (olaparib, carboplatin, and combination) was tested in LTL247, a tumour line derived from a patient with a known BRCA2 germline mutation (exon 11 c4848-4849 delAA). Pathology review of the original tumor specimen confirmed HGSC subtype. LTL258 (BRCA wild-type), developed from the HGSC tumor of a different patient was selected as a paired control.

BRCA mutation analysis

BRCA2 coding sequence mutations were confirmed by whole transcriptome RNA sequencing (RNA-seq) as previously described (20) and direct sequencing (ABI, 3130xl Genetic Analyzer) in original patient tumour and in the later passages of tumour line LTL247 (primer sequences exon 11: BRCA2 forward aaagaccctaaagtacagagagg, BRCA2 reverse cggcccgccgcccccgcccgatttattctttctggttgaccatc). Wild-type status of BRCA 1 and 2 in LTL 258 were confirmed using reference primers as described before (4).
Olaparib in ovarian cancer xenograft model

Affymetrix SNP 6.0 arrays were used to determine the copy number of chromosome 13q harbouring the BRCA2 locus for the original patient tumor and a later generation xenograft of LTL247. The arrays were normalized using CRMAv2 (21) and default settings for performing allelic-crosstalk calibration, probe sequence effects normalization, probe-level summarization, and PCR fragment length normalization. Log ratios are then computed by normalizing against a reference generated using a normal dataset of 270 HapMap samples obtained from Affymetrix. Segmentation was performed using a modified 11-state hidden Markov model derived from CNA-HMMer (20). Segment calls were generated according to 5 somatic states (homozygous deletion, hemizygous deletion, gain, amplification, and high-level amplification), 5 analogous germline states, and neutral copy number.

Olaparib efficacy studies

The structure and activity of olaparib (AZD2281, KU-0059436) has been previously described (9, 14, 17, 18). Olaparib was solubilized in DMSO and diluted to 5mg/mL with PBS containing 10%(w/v) 2-hydroxy-propyl-beta-cyclodextrin (Sigma). After two tumor doubling times (DT) (DT for LTL247: 10 days, DT for LTL258: 16 days), reaching a calculated average volume of 16.6mm³, the animals were randomized into four treatment groups (4-6 mice/group; 2 grafts/kidney): (1) olaparib alone, (2) carboplatin alone, (3) both agents, (4) vehicle control. LTL247 mice were treated for 4 weeks and LTL258 mice were treated for 5 weeks. Olaparib was dosed i.p. (50mg/Kg) once daily for 5 days x4 weeks for LTL247 and x5 weeks for LTL258 (17, 18); carboplatin (Novopharm Ltd.) was dosed i.p. (50mg/Kg) once weekly x4 weeks for
Olaparib in ovarian cancer xenograft model

LTL247 and x5 weeks for LTL258 (18). Mice were provided with food and water *ad libitum* and monitored daily for changes in general health, including body weight loss, diarrhea, food/water intake, appearance and behavior. Tumors were harvested on day 29 and 35 for LTL247 and LTL258, respectively, two hours after last dosing.

Tumor burden was determined at necropsy as previously described (19). Mean tumour volume (mm$^3$) ± standard deviation (SD) was calculated and growth inhibition (GI) reported as the percentage decrease of tumor volume compared to the vehicle control. Individual group comparisons were performed using independent student *t* tests and overall group comparisons were performed using one-way ANOVA. Results with *p*-values < 0.05 were reported as significant.

**Immunohistochemistry (proliferation and apoptotic indices)**

Immunohistochemistry was performed on 5 µm paraffin-embedded tissue sections using TUNEL assays (ApopTag® Apoptosis Detection Kit, Chemicon, Temecula, CA) and a monoclonal MIB1 antibody (Dako, Mississauga, ON, Canada, dilution 1:50) directed against the Ki-67 antigen as previously described (19).

For cleaved caspase-3 staining, 4 µm thick sections were cut and immunostained on a Ventana Discovery XT staining system (Ventana Medical Systems, AZ, USA). Sections were deparaffinized in xylene, dehydrated through three alcohol changes, and transferred to Ventana Wash solution. Endogenous peroxidase activity was blocked in 3% hydrogen peroxide. Antigen retrieval was performed in cell conditioner 1 and slides were incubated with anti-Cleaved Caspase-3 rabbit monoclonal antibody (1:100 dilution, clone 5A1E, Cell Signaling Technology, Danvers, MA, USA) for 60 min. Finally, sections
Olaparib in ovarian cancer xenograft model

were incubated with the UltraMap anti-rabbit HRP-conjugate and ChromoMap detection system, counterstained with hematoxylin, dehydrated, cleared, and mounted.

The Ki-67 proliferation index (PI) was determined on a minimum of two randomly selected high-power fields (400x magnification) containing representative sections of tumour tissue and calculated as the percentage of positively stained tumour cells to total tumour cells. The apoptotic index (AI) using cleaved caspase-3 and TUNEL stains was calculated as the percentage of positively stained apoptotic tumour cells to total tumour cells by counting at least three high-power fields (at ×400 magnification). Areas with extensive necrosis were avoided.

**Compound level quantification and PARP-1 activity determination in BRCA2⁻⁻ tumor tissues**

To demonstrate the specific PARP inhibitory effect of olaparib therapy we determined the ex vivo PARP-1 activity in relation to the drug compound levels in five BRCA2⁻⁻ xenografts of LTL247 after four weeks of treatment and six untreated controls.

Olaparib levels were analyzed by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Tumors were weighed, homogenized in three volumes of PBS and samples extracted using solid phase extraction (SPE). Chromatographic separation was by gradient elution using an Acquity BEH Phenyl (5.0x2.1mm, 1.7µm) column coupled to a Sciex API 2000 with electrospray ionization (ESI) in positive ion mode using multiple reaction monitoring of ions 435.32-281.10m/z. Calibration standards (10ng/mL-5000ng/mL) and quality control samples were prepared in mouse plasma and tumour homogenate.
Olaparib in ovarian cancer xenograft model

PARP-1 activity within tumor samples was analyzed using an ex vivo activation assay as previously described (9).

Results

Model characterization

We selected a tumor line originating from a patient with known heterozygous BRCA2 germline mutation. This mutation could be characterized as a two base pair deletion frame shift mutation in BRCA2 exon 11 using the patients constitutional DNA (peripheral blood) (Figure 1). Copy number analysis of the patient tumor and the late generation xenograft revealed a hemizygous deletion of the BRCA2 locus with loss of heterozygosity. Accordingly, direct sequencing showed a homozygous state of the frameshift mutation in the primary and xenograft tumors. RNA-seq confirmed exclusive expression of the mutated allele that was supported by two reads (data not shown). In summary, this homozygous frameshift mutation can be considered a loss of function mutation. In contrast, tumor line LTL258 did not show BRCA1 or BRCA2 mutations (data not shown).

Olaparib inhibits growth of BRCA2-/- ovarian cancer tissue xenografts

All 39 mice included in the study were available for evaluation and measurement of tumors on harvest day. On macroscopic inspection, LTL247^{BRCA2-/-} xenografts treated for four weeks with vehicle showed, on average, markedly enlarged tumor masses. This was confirmed on microscopic evaluation, with H&E staining identifying viable cancer cells (Figure 2, middle). In contrast, olaparib-treated tumors showed much
Olaparib in ovarian cancer xenograft model

smaller tumour masses, lower overall cell numbers, increased numbers of dead cells, and more cells with enlarged cellular and nuclear structures than controls (Figure 2 right). Morphometric measurements revealed significant differences in tumour mass between treatment groups tested with simultaneous comparison of means (ANOVA) ($P=9.78\text{e-14}$). Individual group comparisons demonstrated that treatment with olaparib alone markedly inhibited growth of the LTL247$^{\text{BRCA2-/-}}$ HGSC line compared to the untreated controls ($19.7\text{mm}^3\pm25.0\text{mm}^3$ vs. $97.3\text{mm}^3\pm72.6\text{mm}^3$, GI 79.8%, $P=3.1\text{e-5}$), although the tumor volume on average was still minimally larger than the calculated volume on the first day of treatment. Treatment with carboplatin alone also resulted in significant growth inhibition (GI) ($4.4\text{mm}^3\pm7.4\text{mm}^3$ vs. $97.3\text{mm}^3\pm72.6\text{mm}^3$, GI 95.5%, $P=2.1\text{e-6}$), while combination of olaparib and carboplatin showed the best treatment response ($1.2\text{mm}^3\pm1.4\text{mm}^3$ vs. $97.3\text{mm}^3\pm72.6\text{mm}^3$, GI 98.8%, $P=1.29\text{e-6}$) (Figure 3A left). Carboplatin and carboplatin + olaparib-treated tumors resulted in very small residual explant tissue consisting mostly of scar tissue (not shown). After re-grafting the 2 largest residual tumors of the olaparib alone and the combination treatment group as well as 2 tumors of the control group, different latency times were observed before re-growth: both control tumors reconstituted to palpable size after 6 weeks while one of the olaparib alone treated tumors reconstituted after 12 month. In contrast, the second olaparib-treated tumour and the combination treated tumors never expanded in size until the mice had to be euthanized after 14-18 month.

BRCA wild type LTL258$^{\text{BRCA1+/+,BRCA2+/+}}$ xenografts did not show significant growth inhibition with olaparib compared to vehicle-treated mice ($90.1\text{mm}^3\pm66.4\text{mm}^3$ vs. $112.9\text{mm}^3\pm90.2\text{mm}^3$, GI 20.2%, $P=0.403$), while treatment with carboplatin
Olaparib in ovarian cancer xenograft model

(14.9mm\(^3\)±12.7mm\(^3\) vs. 112.9mm\(^3\)±90.2 mm\(^3\), GI 86.8%, P=0.0008) and combination therapy of carboplatin and olaparib (34.0mm\(^3\)±25.6mm\(^3\) vs. 112.9mm\(^3\)±90.2 mm\(^3\), GI 69.9%, P=0.004) significantly reduced tumour size (Figure 3A right).

Toxicity evaluation

Only four of all the treated animals showed substantial loss of body weight (>10%), three mice bearing tumour LTL258 and one bearing LTL247. Two of the mice received vehicle only, one carboplatin and one the combination treatment. No gross changes in food/water intake, appearance or behavior were observed, indicating that the treatments were well tolerated. Particularly the addition of olaparib to carboplatin did not significantly increase weight loss compared to carboplatin single agent treatment in the LTL247\(^{BRCA2\text{-}/\text{-}}\) tumor line (carboplatin average weight gain 0.4% vs. combination average weight loss 1.6%, P=0.5). Similarly, no statistically significant increase of weight loss was observed in the BRCA wild type line LTL258 (carboplatin average weight loss 5.7% vs. combination average weight loss 6.6%, P=0.9).

Olaparib-treated tumors exhibit decreased proliferation and increased apoptosis

Immunohistochemical analysis of Ki-67-stained tissue sections from four randomly selected LTL247\(^{BRCA2\text{-}/\text{-}}\) xenografts, treated for four weeks with olaparib (Figure 4A right), showed a significantly lower number of proliferating cells compared to four untreated LTL247\(^{BRCA2\text{-}/\text{-}}\) control xenografts (Figure 4A left). On average, the percentage of proliferating cells per high-power field was 1.9-fold lower in the olaparib-
Olaparib in ovarian cancer xenograft model

treated tumors (29.5%±14.1% (SD) vs. 55.7%±10.3% (SD), P=0.0006). In contrast, olaparib treated xenografts of the BRCA competent tumor line did not show significant reduction of proliferating cells compared to untreated control xenografts (19.1%±6.4% (SD) vs. 20.4%±5.7% (SD), P=0.7) (Figure 4B right and left).

Cleaved Caspase-3 stains of 3 representative olaparib-treated LTL247^{BRCA2-/-} tissue sections (Figure 4C right) displayed a significantly higher number of apoptotic cells than untreated control xenografts (Figure 4C left). On average, the apoptotic index per high-power field was 3.8-fold increased in the olaparib-treated tumors (3.1%±1.8% (SD) vs. 0.8%±0.5 % (SD), P=0.006). In the BRCA competent tumour line no significant difference in the apoptotic index between olaparib treated and untreated xenografts could be observed (0.7%±0.6% (SD) vs. 0.8%±0.9% (SD), P=0.8) (Figure 4C right and left).

In agreement with the caspase-3 assay, TUNEL stains of 6 representative olaparib-treated LTL247^{BRCA2-/-} tissue sections (Supplemental Figure 1 A and B) equally displayed a significantly higher number of apoptotic cells compared to untreated control xenografts (Supplemental Figure 1 C and D). On average, the apoptotic index per high-power field was 2.3-fold increased in the olaparib-treated tumors (5.4%±1.35% (SD) vs. 2.3%±1.0% (SD), P=7.6e-16).

H&E staining of residual tumor tissues after treatment with carboplatin alone and in combination with olaparib revealed only scar tissue but no tumor cells. Accordingly no further immunohistochemistry could be performed for those treatment groups.
Olaparib in ovarian cancer xenograft model

**PARP-1 activity is strongly inhibited in olaparib-treated BRCA2-/- tumors and correlates with compound levels**

Pharmacodynamic activity assays showed that all olaparib-treated tumors had greatly reduced PARP-1 activity compared to the average of vehicle-treated controls. On average PARP-1 activity in olaparib-treated tumors was reduced to 16.1% (range 8.16% – 33.75%, SD 10.1%) of untreated controls (100%). Untreated control tumors did not show detectable compound levels, while in olaparib-treated tumors compound concentration ranged from 198 to 3870ng/mL with compound levels correlating well with PARP-1 activity (Pearson correlation 0.89) (Figure 3B).

**Discussion**

Using a patient derived ovarian cancer xenograft model, we have demonstrated that BRCA2 germline-mutated tumors are sensitive to single agent treatment with the PARP inhibitor olaparib. Although carboplatin showed better single agent efficacy, also olaparib treatment induced a clear tumor growth inhibition. This was in contrast to BRCA wild-type tumors, supporting findings in preclinical studies and early clinical trials that BRCA deficient tumors are susceptible to a synthetic lethal therapeutic approach (14-16). Furthermore, in most of the cases, combining olaparib with carboplatin either eradicated the tumors completely or left very small tumors which showed only scar tissue on microscopical inspection.

Notably, the addition of the PARP-inhibitor to carboplatin did not increase toxicity in mice.
Olaparib in ovarian cancer xenograft model

Our results are in agreement with other studies that have tested olaparib in BRCA$^{1/-;p53/-}$ and BRCA$^{2/-;p53/-}$ genetically altered mouse models (17, 18). In these studies the authors observed \textit{in vivo} efficacy of olaparib (AZD2281) against BRCA-associated murine breast cancers with improvements in both recurrence-free and overall survival. We show, for the first time, that olaparib confers anti-tumor activity and improved survival in an experimental model utilizing human ovarian tumor tissue xenografts. Obvious differences between these models include variation of the genetic backgrounds across species, altered host immune responses, and the tissues studied. However, the comparability of the results further supports the efficacy of olaparib in these models independent of these parameters.

PARP inhibitors are currently being tested in various phase I/II clinical trials (7) and another PARP-inhibitor compound (BSI-201) is being investigated in a phase III trial of triple negative breast cancer\textsuperscript{†}. In the case of olaparib, Fong \textit{et al.} have recently reported on the pharmacokinetics and pharmacodynamics of this drug in a phase I clinical trial involving patients with cancers associated with BRCA mutations (including 16 patients with ovarian cancer) (14). The authors observed anti-tumor activity only in BRCA mutation carriers and concluded that olaparib was effective in this subgroup with few side effects compared to conventional chemotherapy. Subsequent phase II trials of olaparib in BRCA mutation carriers with chemoresistant breast or ovarian cancer have been completed and preliminary results presented showing high activity in those patients and an acceptable tolerability profile (22, 23). The suggestion has been made by others that combination therapy with PARP inhibitors amplifies cytotoxic effects of drugs such as carboplatin (17). In our study, the addition of olaparib to carboplatin in the
Olaparib in ovarian cancer xenograft model

BRCA2 deficient tumor line did result in smaller tumors than single agent therapy, but the difference did not reach statistical significance (t-test p-value 0.07). Furthermore, tumor relapse could be delayed in a mouse model by continuous treatment with olaparib alone after carboplatin chemotherapy suggesting a role for olaparib in maintenance therapy during remissions (17, 18). It is possible that development of resistance to PARP inhibitors will hamper further clinical progress. Therefore, model systems that allow for the study of drug resistance would be useful. Here, we demonstrated that a human ovarian cancer tissue explant xenograft mouse model has utility for the testing of experimental drugs in BRCA-mutated tumors. In the future, development of additional tumour tissue lines, including those that progressed under treatment, has the potential to elucidate the underlying molecular mechanisms of olaparib drug resistance.

In conclusion, this study shows the applicability of a xenograft model for experimental drug testing in human BRCA/- ovarian tumors. To our knowledge, we are the first to develop a model for BRCA2 deficiency directly derived from a patient's tumor. The efficacy of olaparib as a single agent or in combination with carboplatin warrants further investigation in clinical trials.

† http://compbio.bccrc.ca/?page_id=401
‡ www.clinicaltrials.gov NCT00938652
Olaparib in ovarian cancer xenograft model

References


Olaparib in ovarian cancer xenograft model


Olaparib in ovarian cancer xenograft model


Olaparib in ovarian cancer xenograft model

Olaparib in ovarian cancer xenograft model

**Figure Legends**

**Figure 1**
A: Direct sequencing of the two basepair deletion frame shift mutation in BRCA2 exon 11. Upper panel: Patient germline gDNA showing a heterozygous deletion AA; Middle panel: Primary patient tumor gDNA showing a homozygous deletion with likely contamination by stromal cells; Lower panel: Matching late generation xenograft gDNA showing a homozygous deletion without contamination (xenograft tumor with less than 1% mouse stroma cells)

B: Affymetrix SNP6.0 data comparing primary tumour and xenograft samples. Each probe is represented as a point in the plot, where the y-axis is logR and the x-axis is chromosome position (bp). HMM discrete copy number predictions are represented by colour coding: homozygous deletion (light green), hemizygous deletion (dark green), neutral (blue), gain (dark red), amplification (light red). In both samples, BRCA2 (dotted black line) is contained within a hemizygous deletion region.

**Figure 2**
H&E staining (400x magnification) of tumor sections from
A: BRCA2−/− xenograft LTL247. Left: Primary patient tumour; Middle: untreated control xenograft; Right: Olaparib-treated xenograft
B: BRCA1+/+,BRCA2+/+ wildtype xenograft LTL258. Left: Primary patient tumor; Middle: untreated control xenograft; Right: Olaparib-treated xenograft

**Figure 3**
A: Tumor growth inhibition. Left: BRCA2−/− xenograft LTL247; Right: BRCA1+/+,BRCA2+/+ wildtype xenograft LTL258

B: TVD1: calculated averaged tumour volume on first treatment day = 16.64 mm³

**Figure 4**
Ki-67 staining (400x magnification) of tumor sections.
A: BRCA2−/− xenograft LTL247 Left: untreated control; Right: olaparib-treated
B: BRCA1+/+,BRCA2+/+ wildtype xenograft LTL258 Left: untreated control; Right: olaparib-treated

Cleaved Caspase-3 staining (400x magnification of tumor sections.
C: BRCA2−/− xenograft LTL247 Left: untreated control; Right: olaparib-treated
D: BRCA1+/+,BRCA2+/+ wildtype xenograft LTL258 Left: untreated control; Right: olaparib-treated
Figure 1

A. Primary patient germline gDNA

B. Primary patient tumour gDNA

Xenograft LTL247 gDNA

B. Primary patient tumour

Copy number (Log ratio)

Chromosome 13
Figure 3

A. LTL247: BRCA2 germ-line mutation

<table>
<thead>
<tr>
<th>Tumor Size mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Olaparib</td>
</tr>
<tr>
<td>Carboplatin</td>
</tr>
<tr>
<td>Olaparib+ Carboplatin</td>
</tr>
</tbody>
</table>

B. LTL258: no BRCA mutation

<table>
<thead>
<tr>
<th>Tumor Size mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Olaparib</td>
</tr>
<tr>
<td>Carboplatin</td>
</tr>
<tr>
<td>Olaparib+ Carboplatin</td>
</tr>
</tbody>
</table>

B. Olaparib Concentration (ng/mL)

<table>
<thead>
<tr>
<th>% PARP-1 Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Averaged Controls</td>
</tr>
<tr>
<td>Treated 1</td>
</tr>
<tr>
<td>Treated 2</td>
</tr>
<tr>
<td>Treated 3</td>
</tr>
<tr>
<td>Treated 4</td>
</tr>
<tr>
<td>Treated 5</td>
</tr>
</tbody>
</table>

- □ % PARP-1 Activity
- ■ Compound Concentration ng/mL
Clinical Cancer Research

Tumor growth inhibition by olaparib in BRCA2 germline-mutated patient-derived ovarian cancer tissue xenografts

Ursula K J Kortmann, Jessica N McAlpine, Hui Xue, et al.

Clin Cancer Res  Published OnlineFirst November 19, 2010.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-10-1382

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2011/02/17/1078-0432.CCR-10-1382.DC1

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.