Assessment of PARPi and cisplatin resistance in *BRCA1* exon 11 mutant patient-derived xenografts

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Introduction

- 30% of pathogenic *BRCA1* mutations occur in exon 11.
- *BRCA1* exon 11 mutants are capable of expressing the BRCA1-Δ11q splice isoform which lacks most of exon 11.
- BRCA1-Δ11q supports moderate PARPi and cisplatin resistance and maintains some homologous recombination.
- **Does BRCA1-Δ11q expression influence therapy response in patient tumors?**

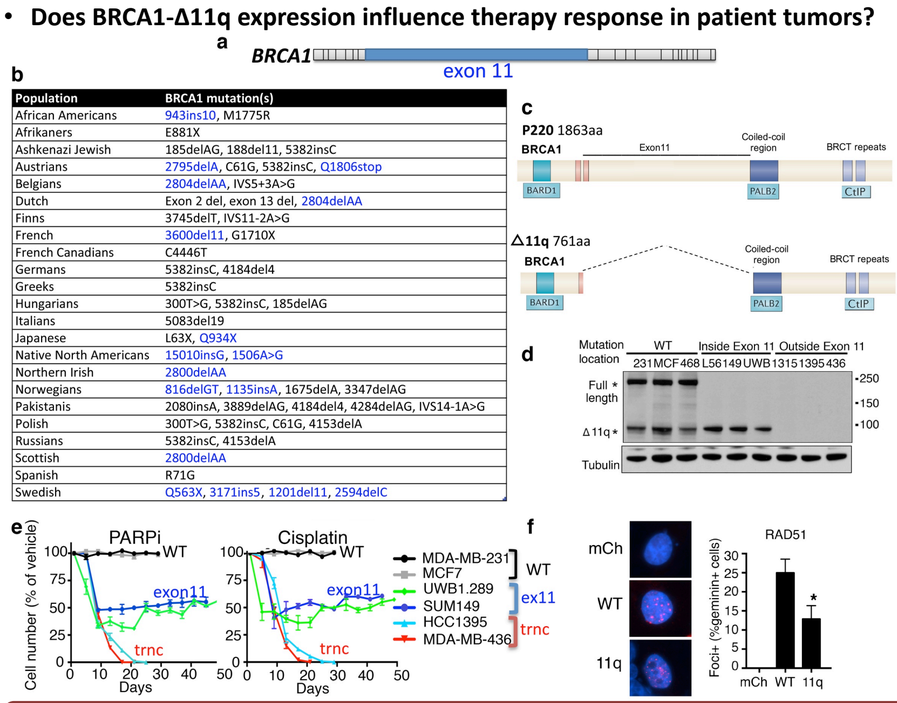


Figure 1. *BRCA1* exon 11 mutations.
(**a**) Exon 11 comprises a significant portion of the coding region of the *BRCA1* gene. **(b)** Pathogenic *BRCA1* mutations occur in exon 11 (blue) in various populations totaling approximately 30% of cases (BIC database). **(c)** Diagram of full length and BRCA1-Δ11q proteins. **(d)** Expression of full length and BRCA1-Δ11q was assessed by Western blot in cell lines with wild-type *BRCA1*, exon 11 mutations, and mutations outside exon 11. **(e)** *BRCA1* wild-type and mutant cell lines were grown in PARPi or cisplatin and counted. **(f)** RAD51 foci positive cells were quantified following irradiation in cells expressing mCherry (mCh), wild-type BRCA1 (WT), or BRCA1-Δ11q (11q). **(d, e, f)** (Wang Y, Bernhardy AJ, et al. Can Res, 2016).

BRCA1-Δ11q and 53BP1 Expression in Resistant PDX

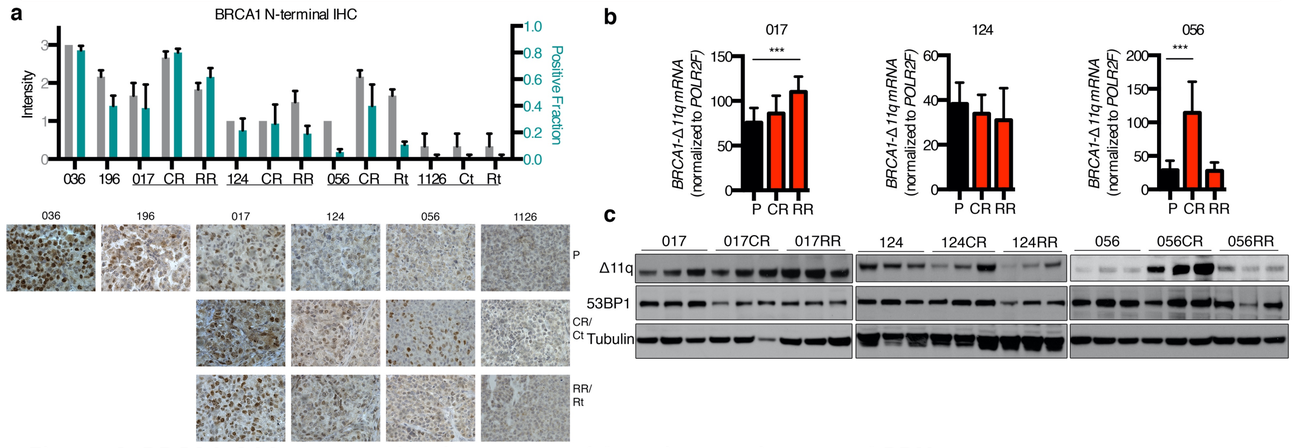


Figure 4. BRCA1-Δ11q expression increases with resistance in several PDX.
(**a**) Parental (P), cisplatin resistant or treated (CR or Ct), rucaparib resistant or treated (RR or Rt) PDX sections were stained for BRCA1 by IHC and scored 0 to 3 for intensity (grey, left axis) and fraction of positive cells assessed (blue, right axis), n=3. Representative images are shown. **(b)** *BRCA1-Δ11q* mRNA was assessed by qRT-PCR in the indicated PDX tumors, n=3. **(c)** Sets of parental, cisplatin resistant (CR), and rucaparib resistant (RR) PDX samples from (b) were evaluated for BRCA1-Δ11q and 53BP1 protein expression in triplicate by Western blot.

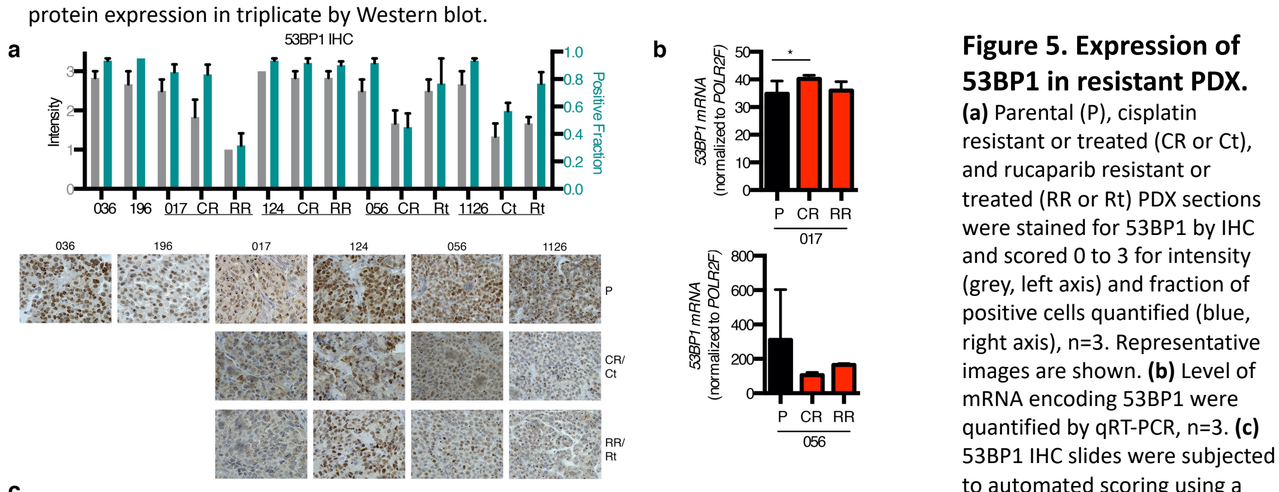
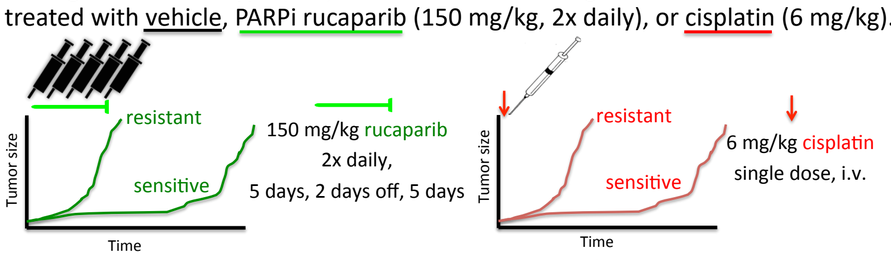


Figure 5. Expression of 53BP1 in resistant PDX.
(a) Parental (P), cisplatin resistant or treated (CR or Ct), and rucaparib resistant or treated (RR or Rt) PDX sections were stained for 53BP1 by IHC and scored 0 to 3 for intensity (grey, left axis) and fraction of positive cells quantified (blue, right axis), n=3. Representative images are shown. **(b)** Level of mRNA encoding 53BP1 were quantified by qRT-PCR, n=3. **(c)** 53BP1 IHC slides were subjected to automated scoring using a Vectra Imaging System. Individual cells were scored 0-3 according to 53BP1 nuclear staining intensity. Data are presented as histograms of the scored cells and histology scores. Representative segmentation and scoring images are shown.

PDX Models and Initial Response

***BRCA1* WT Control**
PDX036
***BRCA1* exon 11 mutants**
PDX196: c.1961delA
PDX124: c.1961delA
PDX017: c.1105_1106insTC
PDX056: c.895_896delGT
***BRCA1* exon 13 mutant**
PDX1126: c.4327C>T

Dosing: *BRCA1* wild-type (WT) and mutant PDX were grown to 150 mm³ then treated with vehicle, PARPi rucaparib (150 mg/kg, 2x daily), or cisplatin (6 mg/kg).



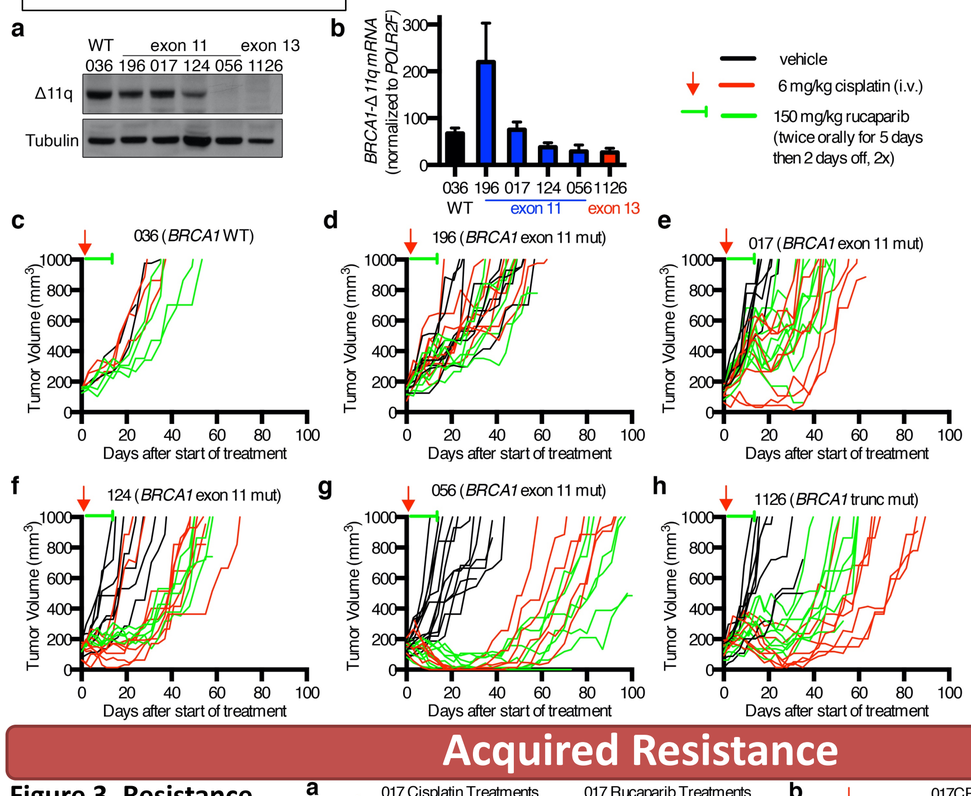


Figure 2. BRCA1-Δ11q expression and initial responses to PARPi and cisplatin.
(a) Nuclear extracts from PDX tumors were assessed for BRCA1-Δ11q expression by Western blot. **(b)** *BRCA1-Δ11q* mRNA levels were quantified by qRT-PCR, n=3. **(c)** Wild-type BRCA1 expressing PDX036, **(e-g)** *BRCA1* exon 11 mutants PDX196, PDX017, PDX124, and PDX056, and **(h)** *BRCA1* exon 13 truncating mutant PDX1126 were treated with vehicle (black), PARPi (green), or cisplatin (red) and individual tumor volumes shown.

Acquired Resistance

Figure 3. Resistance resultant from serial treatments in *BRCA1* exon 11 mutant PDX.
(**a**) *BRCA1* exon 11 mutant PDX017 tumors were treated as indicated then extracted and reimplanted into mice for additional treatment. Passaging continued until therapy response was minimal. Individual volumes for each passage are shown, indicated by color. **(b)** Cisplatin resistant (CR) and rucaparib resistant (RR) PDX017 tumors from (a) were evaluated for response to vehicle (black), PARPi (green), and cisplatin (red). **(c-d)** *BRCA1* exon 11 mutant PDX124 tumors as described in (a-b). **(e- f)** *BRCA1* exon 11 mutant PDX056 tumors as described in (a-b). Note, robust resistance to rucaparib has not yet been observed. **(g)** *BRCA1* exon 13 mutant PDX1126 tumors as described in (a). Robust resistance to cisplatin or rucaparib was not observed.

Ectopic BRCA1-Δ11q Expression and 53BP1 Knockdown

Figure 6. Ectopic BRCA1-Δ11q promotes resistance to PARPi and cisplatin.
(a) *BRCA1* exon 13 mutant PDX1126 cells were infected with lentivirus encoding mCherry or BRCA1-Δ11q and implanted. Expression was assessed by Western blot and IHC. **(b)** mCherry or BRCA1-Δ11q expressing PDX1126 were serially treated with cisplatin and reimplanted. **(c)** Upon progression through cisplatin, PDX1126+BRCA1-Δ11q tumors were treated with vehicle (black), PARPi (green), and cisplatin (red) and tumor growth measured. **(d)** mCherry and BRCA1-Δ11q expressing PDX1126 sections were subject to BRCA1 IHC and scored 0-3 for staining intensity (grey, left axis) and fraction of positive cells determined (blue, right axis) for untreated (P0) and passages 1 and 2 (P1, P2) of cisplatin treatment. **(e)** 53BP1 expression was evaluated as in (d). **(f)** PDX1126 tumors expressing nontargeted (NT) or 53BP1-targeted shRNA were generated as in (a) and assessed for 53BP1 expression by IHC. **(g)** Tumors from (f) were serially treated with cisplatin as in (b). **(h)** Isogenic *BRCA1* mutant PDX1126 and cell line MDA-MB-436 with ectopic mCherry or BRCA1-Δ11q expression were subjected to RNAseq and FPKM values plotted. **(i)** Positive hits were determined based on 5-fold upregulation in BRCA1-Δ11q expressing cells, then compared between PDX1126 and MDA-MB-436 sets and consistent upregulations indicated. **(j)** Negative hits were determined as in (i) using a 5-fold downregulation in BRCA1-Δ11q expressing cells.

Summary

1. **Variable BRCA1-Δ11q expression in four exon 11 mutant PDX models**
   - PDX that express abundant BRCA1-Δ11q display minimal sensitivity
   - High BRCA1-Δ11q expression could be a potential biomarker for PARPi and cisplatin resistance
2. **Cisplatin and PARPi selection can increase BRCA1-Δ11q expression**
3. **Ectopic BRCA1-Δ11q induces robust resistance in otherwise sensitive PDX**
4. **Cross-resistance observed between cisplatin and PARPi**
5. **Determinants of BRCA1-Δ11q expression remain unknown**

Acknowledgements

We thank Clare Scott, Igor Astsaturov, Vladimir Khazak, Violeta Serra, Judith Balmaña, and Elgene Lim for providing PDX models. This work was supported by the Ovarian Cancer Academy (DoD), Ovarian Cancer Research Alliance (Phil and Judy Messing, 597484), American Cancer Society (Tri State CEOs Against Cancer, PF-19-097-01-DMC), and Ruth L. Kirchstein T32 (CA009035).