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# Responses to sequential sapacitabine and seliciclib in patients with BRCA-deficient solid tumors

Short Title:
Sapa/Seli in BRCA-deficient cancers

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*Abstract:*
Background: Sapacitabine is an orally administered nucleoside analogue; the active metabolite, CNDAC (2'-C-cyano-2'-deoxy-1-β-D-arabino-pentofuranosylcytosine), generates single-strand DNA breaks that are converted to double-strand DNA breaks (DSBs) during subsequent replication, resulting in cell death if unrepaired. Repair of CNDAC-induced DSBs is dependent on the homologous recombination (HR) repair pathway. Depletion or inhibition of components of the HR pathway (including ATM, BRCA1/2, Rad 51 and XRCC3) greatly sensitizes tumor cell lines to CNDAC-induced cell death in vitro. Seliciclib is an orally bioavailable inhibitor of cyclin-dependent kinases (CDKs) 2, 7 and 9. CDK2 has been shown to participate in DNA repair and to be a therapeutic target in BRCA-deficient cancers. Seliciclib inhibits DSB repair, and also reduces BRCA1 and BRCA2 mRNA levels in cancer cell lines, sensitizing tumor cells to CNDAC. This phase I study evaluates sequential sapacitabine and seliciclib. Methods: Dose escalation was conducted in patients with incurable solid tumors and adequate organ function with sapacitabine b.i.d. x 7 consecutive days (d1-7), seliciclib b.i.d. x 3 consecutive days (d8-10) followed by 11 days of rest. At least 3 patients were evaluated per dose level. MTD was the highest dose level at which less than one-third of at least 6 patients experienced cycle 1 DLT. Skin biopsies were obtained to assess DNA damage following sapacitabine (d8 vs pre-treatment) and further augmentation of DNA damage after seliciclib (d11 vs d8). Results: 38 patients were treated. The MTD is sapacitabine 50 mg b.i.d./seliciclib 1200 mg b.i.d. DLTs were reversible transaminase elevations and neutropenia. The most frequent adverse events (all cycles, regardless of causality) included, fatigue, abdominal pain, diarrhea, constipation, decreased appetite, nausea, vomiting, anemia, neutropenia, pyrexia, AST elevation, alkaline phosphatase elevation, creatinine elevation, hyperglycemia, hypophosphatemia, cough and alopecia, the majority mild to moderate in intensity. Skin biopsies showed a 2.3-fold increase in γ-H2AX staining post-sapacitabine (n=16; p=0.007) and a further 0.58-fold increase post-seliciclib (n=12; p=0.069). Four confirmed PRs occurred in patients with pancreatic, breast (2 pts) and ovarian cancer, all BRCA mutation carriers, lasting 21, 78+, 36+ and 42+ weeks, respectively. SD as best response >/= 12 weeks was observed in 8 additional patients, including two BRCA mutation carriers with ovarian and breast cancer, lasting 64 and 21 weeks, respectively. Conclusions: Sequential sapacitabine and seliciclib is safe with preliminary antitumor activity. BRCA mutation carrier status may be a potential biomarker for response across multiple tumor types. An alternative schedule with concomitant administration of sapacitabine and seliciclib is currently under evaluation.
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*American Association for Cancer Research, Annual Meeting 2013, Washington, DC*

# Responses to sequential sapacitabine and seliciclib in patients with BRCA-deficient solid tumors

Geoffrey I. Shapiro[1], John Hilton[1], James M. Cleary[1], Sara M. Tolaney[1], Leena Ghandi[1], Eunice L. Kwak[2], Jeffrey W. Clark[1], Andrew Wolanski[1], Tracy Bell[1], John Schulz[1], Sheelagh Frame[3], Chiara Saladino[3], Morag Hogben[3], Scott J. Rodig[4], Judy H. Chiao[3], and David Blake[3]

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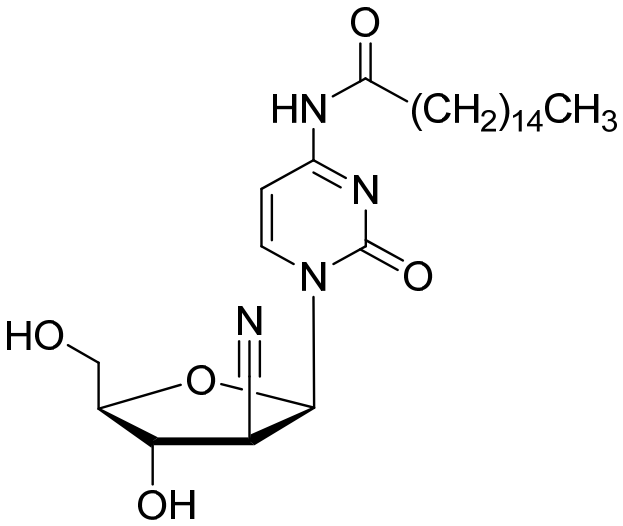
# Disclosures

Employees of Cyclacel Ltd.:

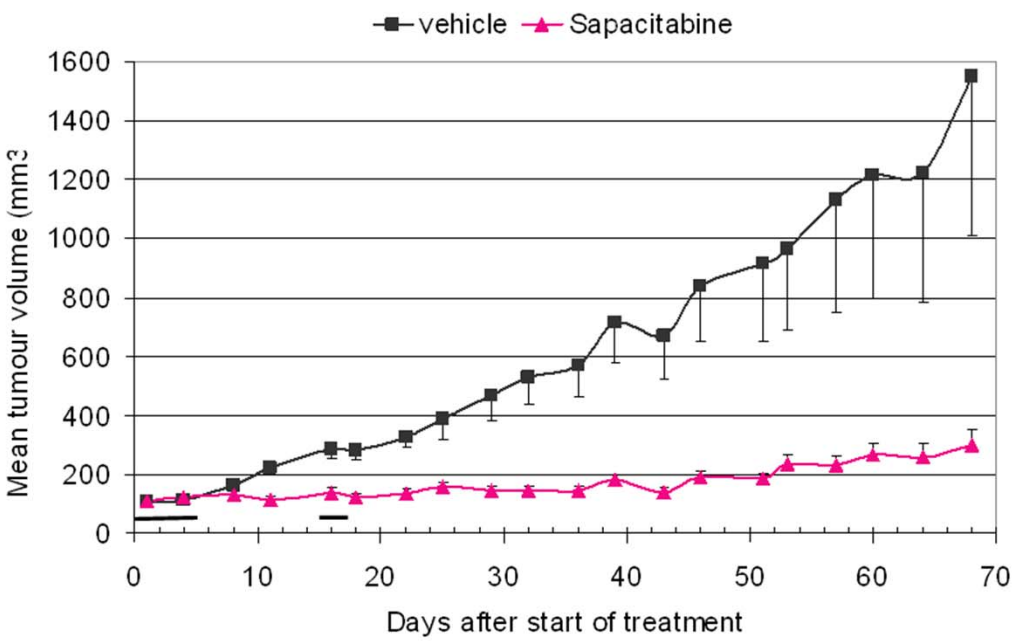
- Sheelagh Frame
- Chiara Saladino
- Morag Hogben
- David Blake (Head of Research)
- Judy Chiao (VP Clinical Development and Regulatory Affairs)

# Sapacitabine

- Orally available 2'-deoxycytidine analogue
- Converted to CNDAC *in vivo*
- CNDAC activated by deoxycytidine kinase to CNDAC-triphosphate
- CNDAC incorporated into DNA during replication or repair
- **Induces single strand DNA breaks via a β-elimination reaction**
- Resulting CNddC dideoxy terminated strand cannot be re-ligated
- In subsequent replication cycles, single strand DNA breaks are converted to double strand DNA breaks resulting in cell death
- Induces G2 checkpoint, compared to S-phase arrest by ara-C or gemcitabine
- **DNA damage repair dependent on homologous recombination repair pathway, unlike ara-C or gemcitabine**

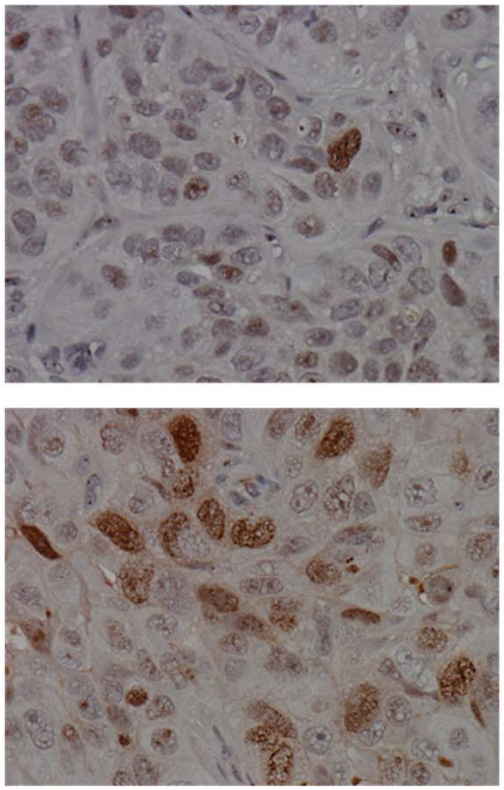


# Sapacitabine-induced double-stranded DNA damage *in vivo*




H358 NSCLC xenograft
Sapacitabine 30 mg/kg po

Tumor samples harvested after 5 days treatment



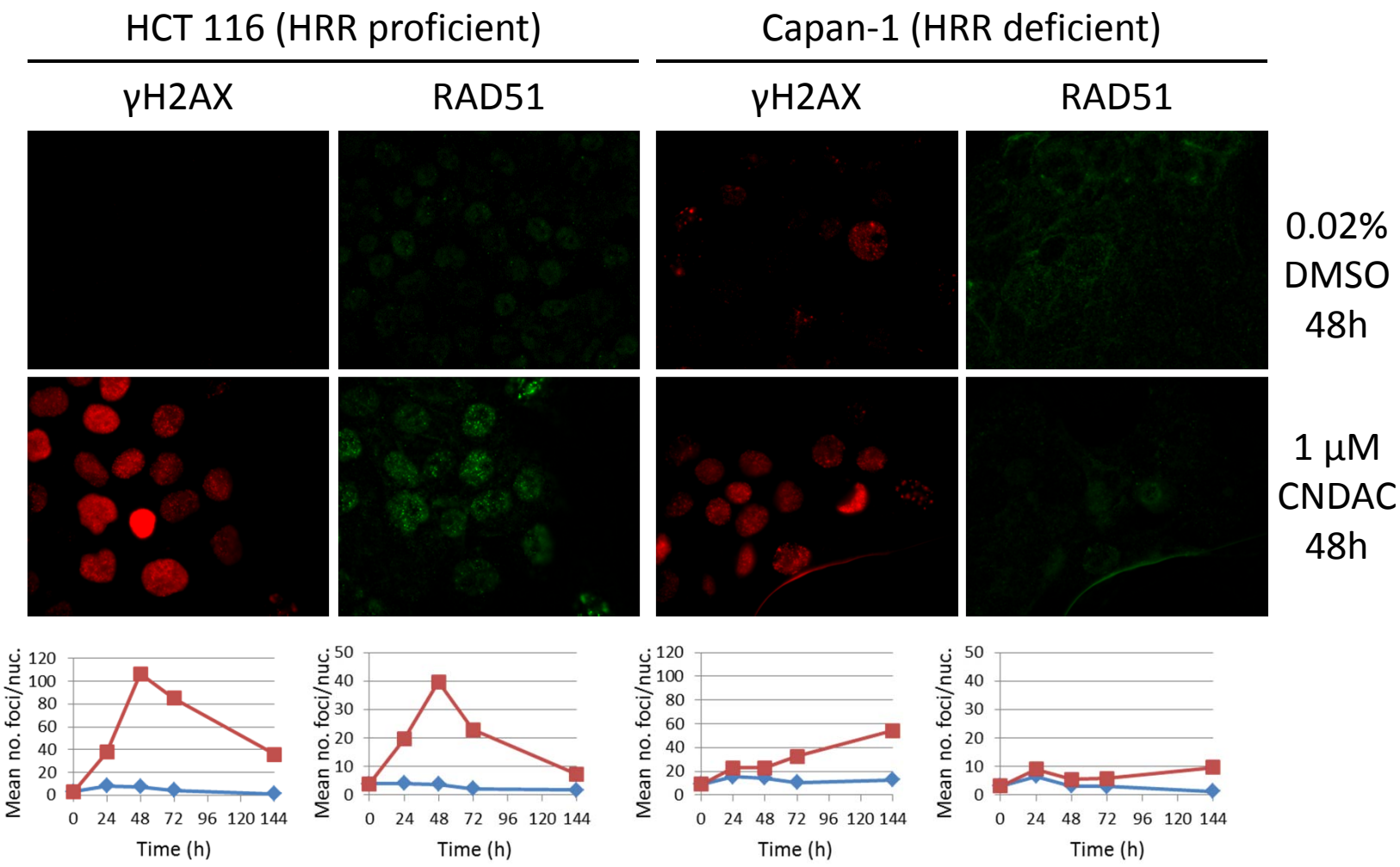
Vehicle

Sapacitabine

anti- phospho-histone H2AX

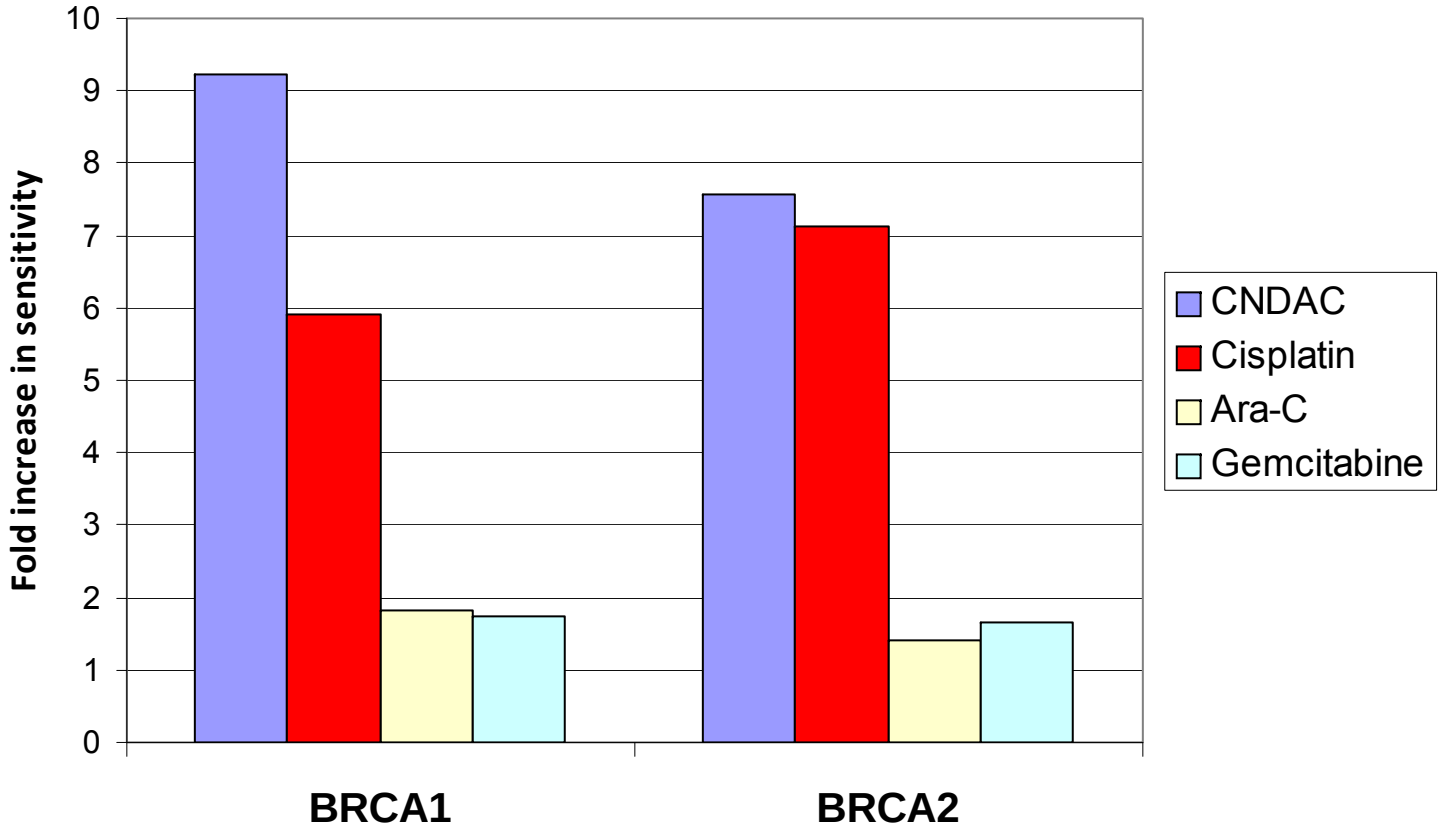
*Fleming, I. et. al. AACR 2007 Abstract 3183*

# Induction of Homologous Recombination Repair Foci by CNDAC



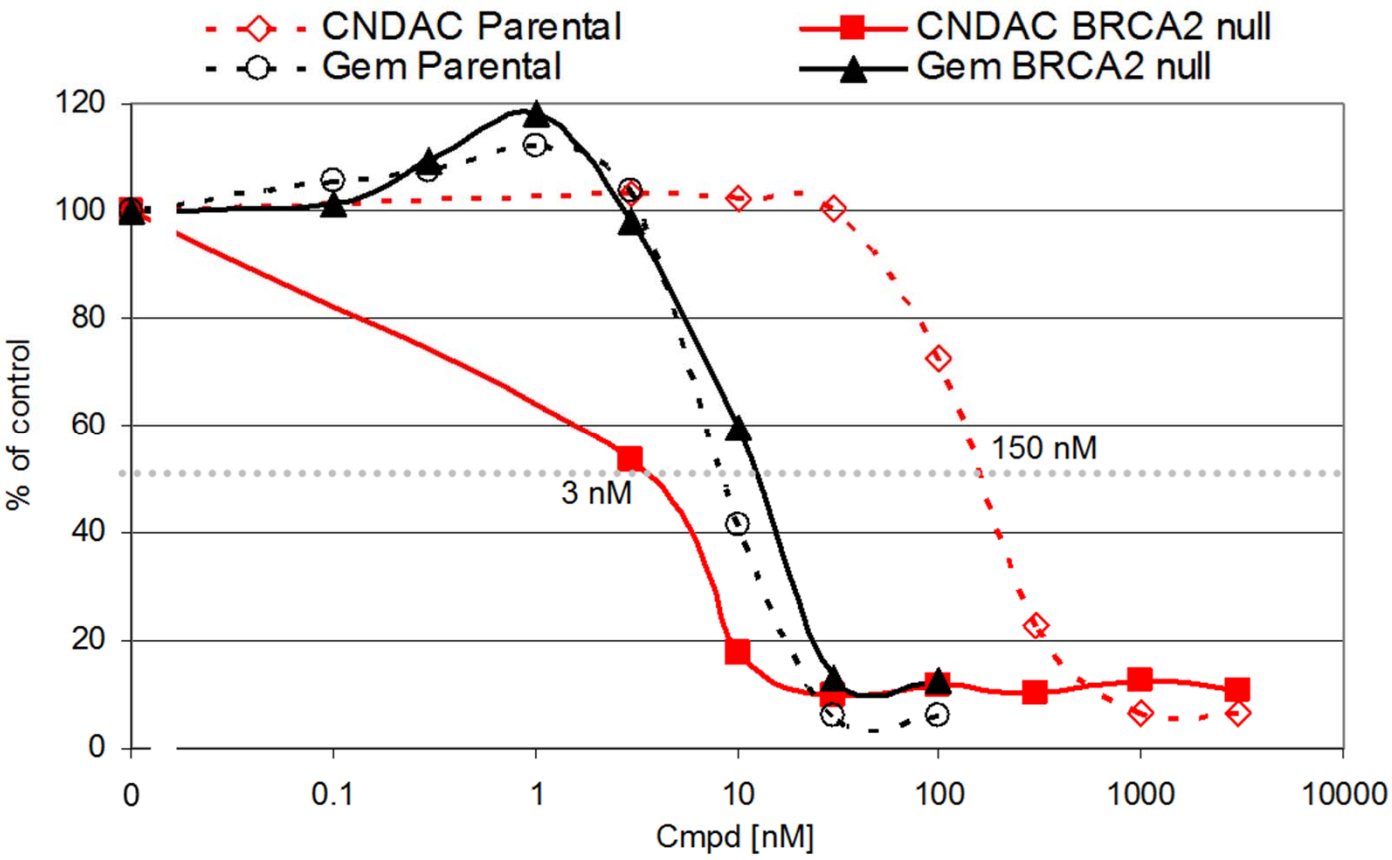

CNDAC treatment up to 48 h

# Loss of BRCA1 or BRCA2 increases cell sensitivity to CNDAC



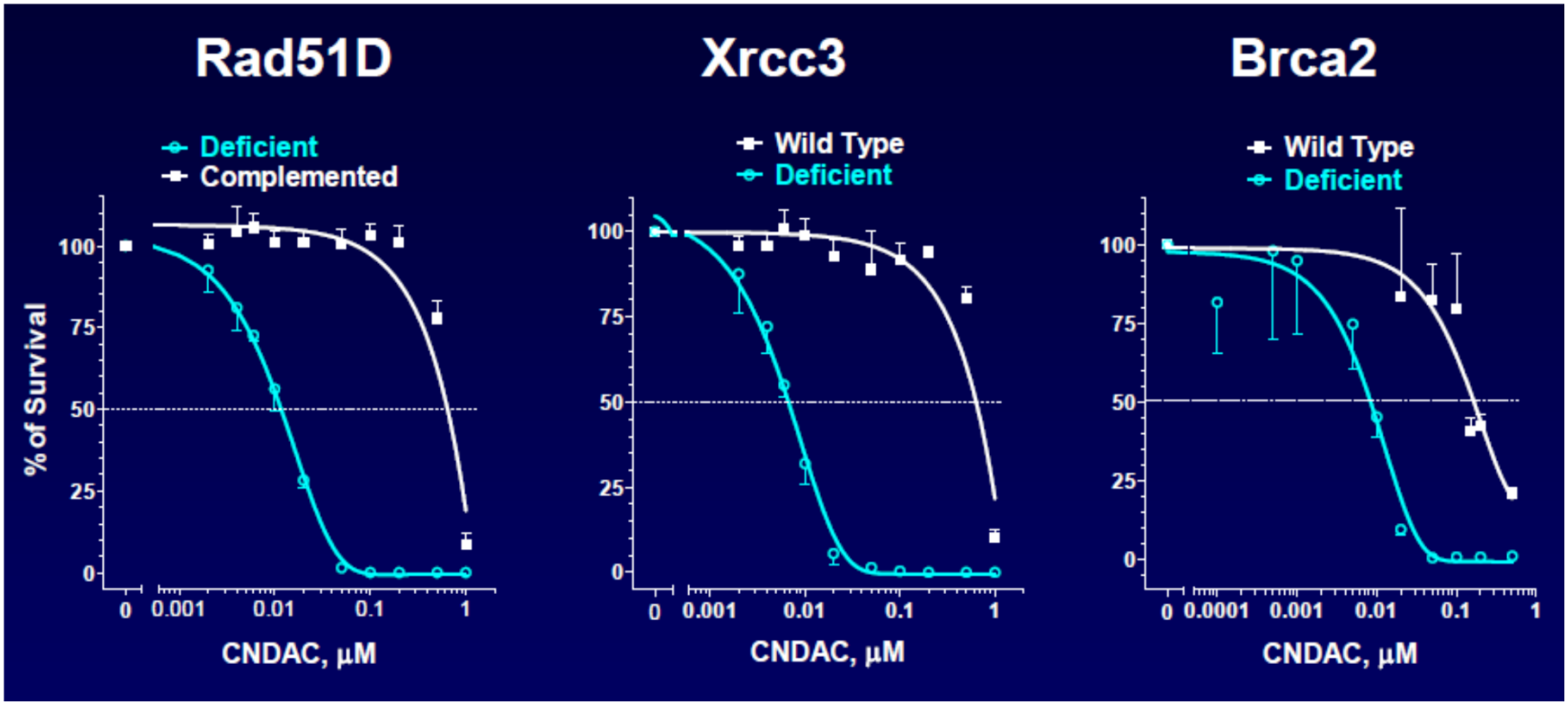

CNDAC $IC_{50}$ ratio (control siRNA/BRCA siRNA) in HCT116 cells measured by 72 h resazurin assay. siRNA knockdown confirmed > 50% by qPCR

# Enhanced CNDAC sensitivity confirmed in BRCA2 isogenic cell lines



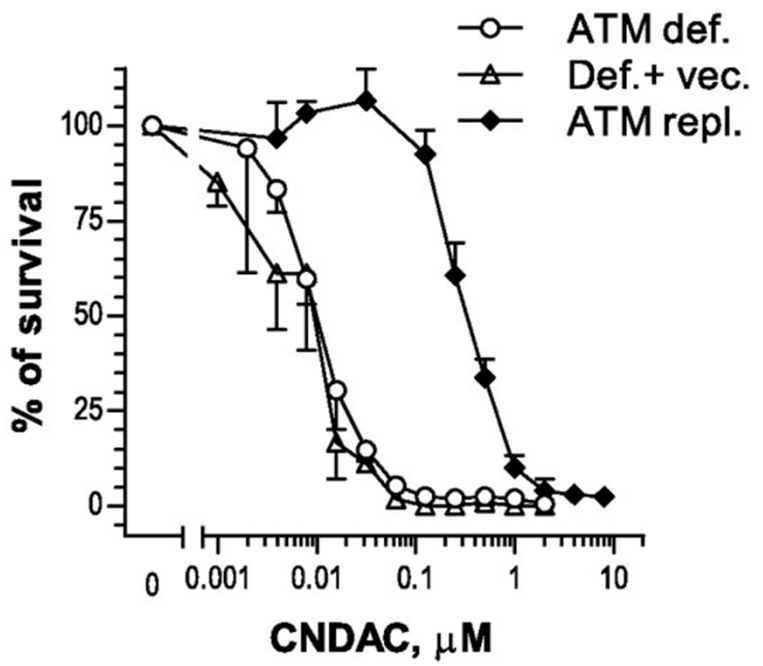

**BRCA2 mutation enhances CNDAC sensitivity (50x), but <u>does not</u> enhance gemcitabine sensitivity.**
Colony forming assay in isogenic DLD-1 colorectal cell lines BRCA2 WT and null mutated
*Frame, S. et al. AACR 2010, Abstract 3502.*

# CNDAC sensitivity is enhanced by defects in homologous recombination (HR) repair pathway

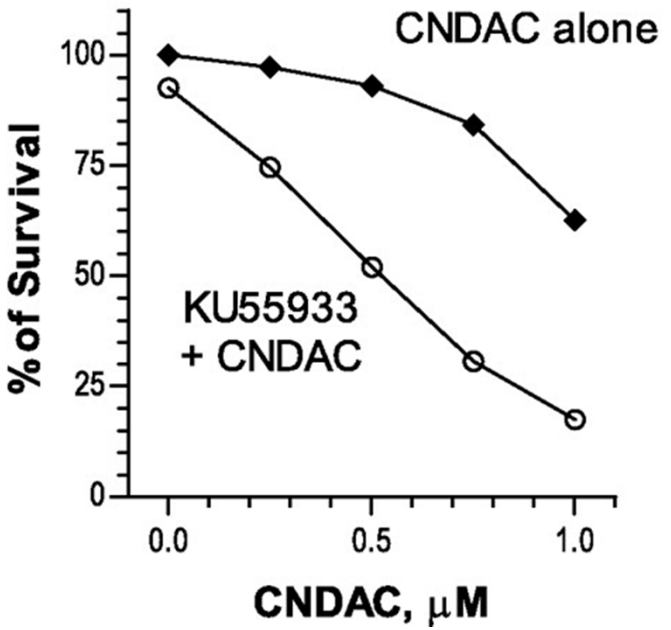



Colony forming assay in paired Chinese hamster cell lines.
*Liu, X. et al. AACR 2011, Abstract 962; Liu, X. et al. Blood, 2010, 116, 1737*

# Dependence on ATM confirmed by chemical and genetic inhibition



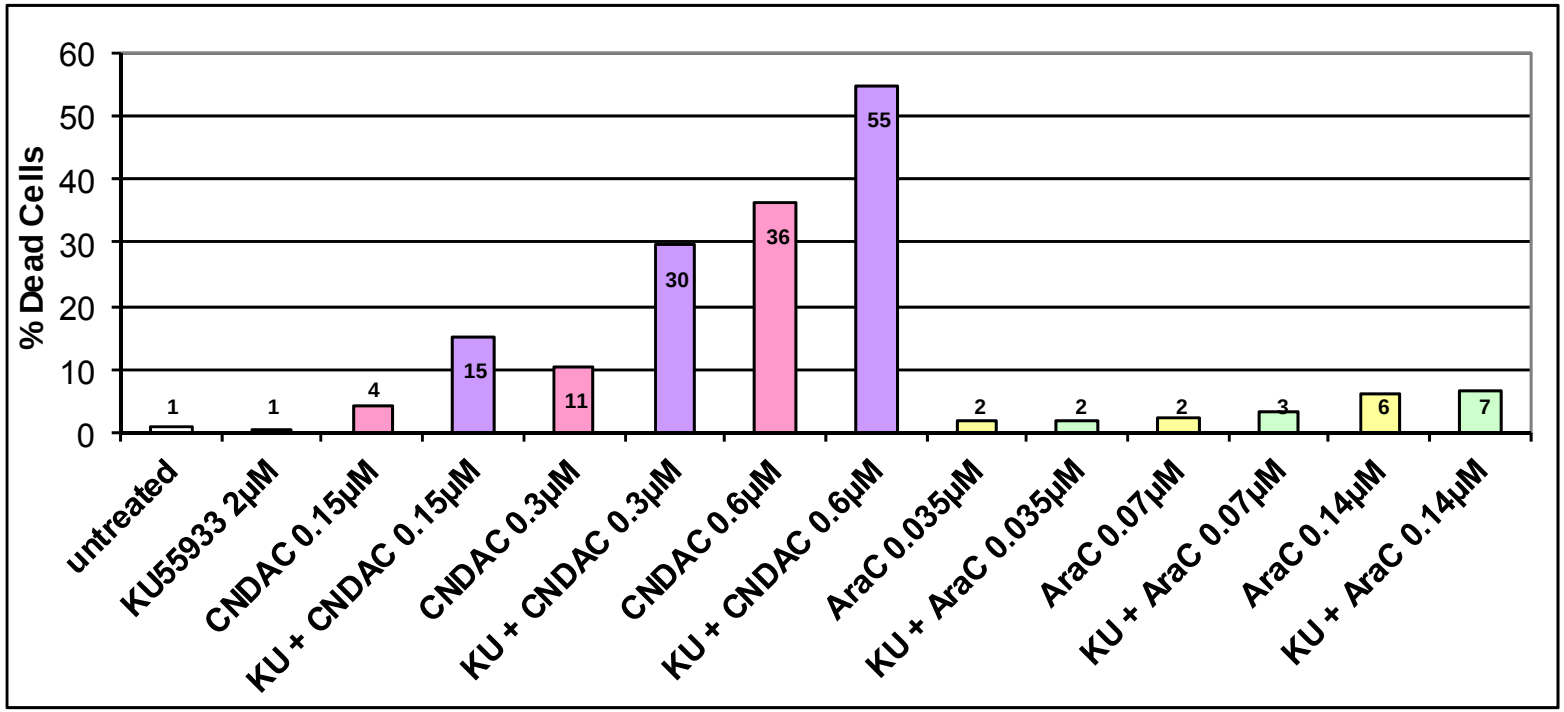

ATM def. = AT22IJE-T (AT-C), a fibroblast cell line derived from an ataxia telangiectasia patient.




AML primary samples (n=4)

*Liu, X. et al. Blood, 2010, 116, 1737*

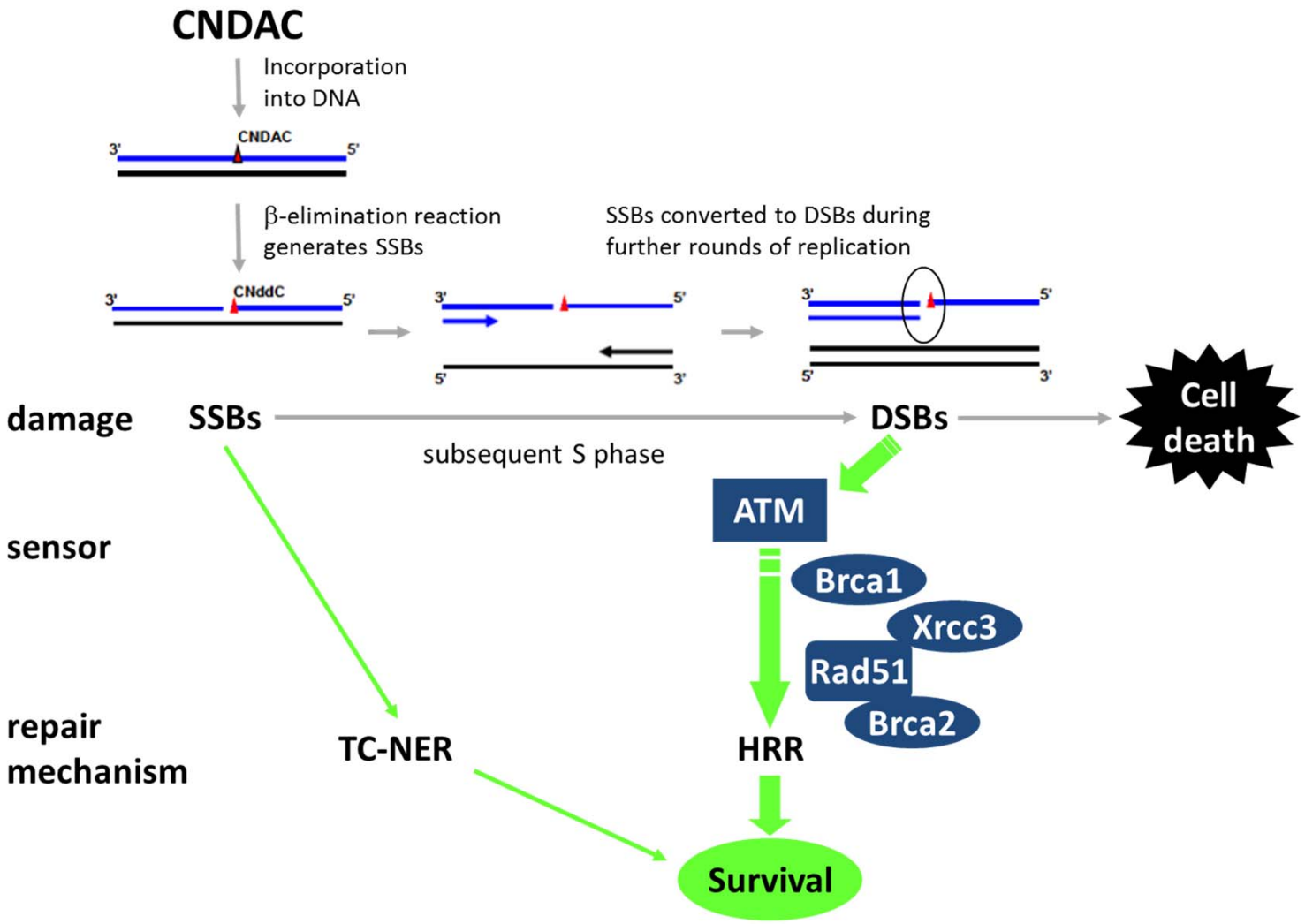
# ATM inhibition synergizes with CNDAC, but not AraC, to induce apoptosis




HL-60 AML cell line treated for 72 h with CNDAC or cytarabine (AraC) alone or simultaneously with ATM inhibitor KU55933.
Cell death indicated by sub-G1 DNA content detected by flow cytometry.

*Frame,S. et al. EHA 2009, Abstract 0761*

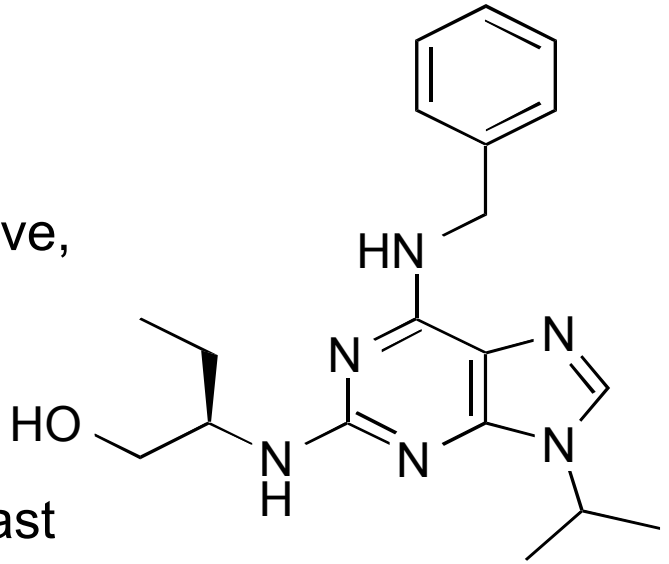
# Sapacitabine-Mechanism of Action




*Source: Adapted from Liu, X. et al. Expert Opin Investig Drugs 2012*

# Seliciclib

- Orally available tri-substituted purine
- CYC202, R-enantiomer of roscovitine
- Selective inhibitor of CDK 2, 5, 7, 9 via ATP binding sites
- Biological activities include: (1) Anti-proliferative, (2) Transcriptional inhibition and (3) Apoptosis induction
- Anti-cancer mechanisms include:
  - CDK2 inhibition in cyclin E dependent breast cancers[1-3]
  - Reduction of anti-apoptotic proteins via transcriptional inhibition (e.g. Mcl1, XIAP)[4-6]
- Inhibits DNA double strand break repair and synergizes with CNDAC *in vitro*





*1. Scaltriti, M. et al. PNAS 2011, 108, 3761*
*2. Akli, S. et al. Clin. Cancer Res. 2010, 16, 1179*
*3. Akli, S. et al. Cancer Res. 2011, 71, 3377*
*4. Hahntow, I.N. et al. Leukemia 2004, 18, 747*
*5. MacCallum, D.E. et al. Cancer Res. 2005, 65, 5399*
*6. Frame, S. et al. AACR 2010 Abstract 3886*

# Seliciclib synergizes with CNDAC *in vitro*

| Tissue of origin | Cell line | CNDAC then seliciclib | | | seliciclib then CNDAC | | | CNDAC plus seliciclib | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ED50 | ED75 | ED90 | ED50 | ED75 | ED90 | ED50 | ED75 | ED90 |
| NSCLC | ABC-1 | 1.01 | 0.94 | 0.87 | 0.67 | 0.61 | 0.56 | 1.61 | 1.27 | 1.00 |
| | A549 | 0.98 | 0.84 | 0.74 | 0.79 | 0.76 | 0.76 | 0.98 | 0.98 | 1.00 |
| | H23 | 1.01 | 1.00 | 1.00 | 0.90 | 0.82 | 0.78 | 1.35 | 1.17 | 1.03 |
| | H358 | 0.35 | 0.52 | 0.91 | 0.37 | 0.23 | 0.37 | 0.74 | 0.81 | 1.55 |
| | H441 | 0.77 | 0.63 | 1.12 | 0.39 | 0.41 | 1.14 | 1.24 | 1.20 | 2.30 |
| | H460 | 2.20 | 1.28 | 0.79 | 0.60 | 0.54 | 0.50 | 0.92 | 0.87 | 0.83 |
| | H520 | 0.71 | 0.74 | 0.78 | 0.59 | 0.59 | 0.61 | 1.57 | 1.41 | 1.30 |
| | H1581 | 0.60 | 0.66 | 0.74 | 0.68 | 0.67 | 0.67 | 0.82 | 0.83 | 0.83 |
| | H1944 | 0.28 | 0.21 | 0.23 | 0.73 | 0.49 | 0.42 | 0.75 | 0.83 | 1.15 |
| | H2122 | 0.60 | 0.68 | 0.78 | 0.37 | 0.34 | 0.40 | 0.61 | 0.66 | 0.91 |
| | LU99A | 0.59 | 0.65 | 0.73 | 0.42 | 0.45 | 0.50 | 2.31 | 1.83 | 1.46 |
| | SW1573 | 0.81 | 0.78 | 0.76 | 0.71 | 0.69 | 0.74 | 0.99 | 0.98 | 1.07 |
| colon | HCT116 | 0.88 | 0.78 | 0.70 | 0.75 | 0.70 | 0.65 | 0.57 | 0.60 | 0.63 |
| ovarian | PEO1 | 0.62 | 0.59 | 0.56 | 0.74 | 0.72 | 0.70 | 0.76 | 0.76 | 0.76 |
| | PEO4 | 0.75 | 0.65 | 0.56 | 0.68 | 0.42 | 0.26 | 3.06 | 2.03 | 1.37 |

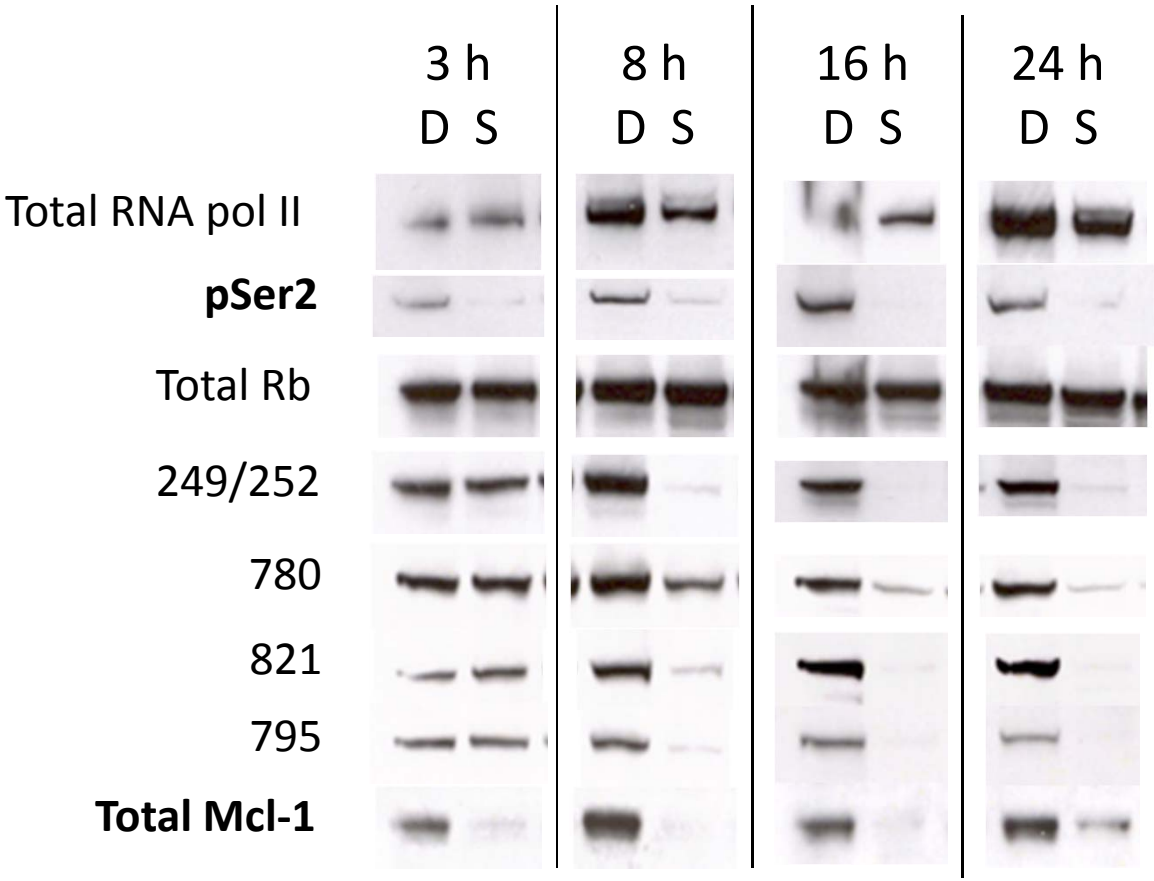
| CI value | < 0.9 | 0.9 - 1.1 | > 1.1 |
|---|---|---|---|
| Drug Interaction | synergistic | additive | antagonistic |

Cell viability data (resazurin assay) analysed by Calcusyn (Chou-Talalay method).
Combination index (CI) is a measure of the nature of the drug interaction.

# Possible Mechanisms of Synergy of Sapacitabine and Seliciclib

- CDK2 inhibition during S phase enhances E2F-1 activity, overcoming a threshold required to induce apoptosis
- CDK2 inhibition disrupts both HR and NHEJ repair pathways
- CDK9 inhibition results in reduced levels of Mcl-1 and other anti-apoptotic proteins with short half-lives enhancing sapacitabine-induced cell death
- CDK9 inhibition reduces expression of components of DNA repair pathways (BRCA proteins and RAD50)

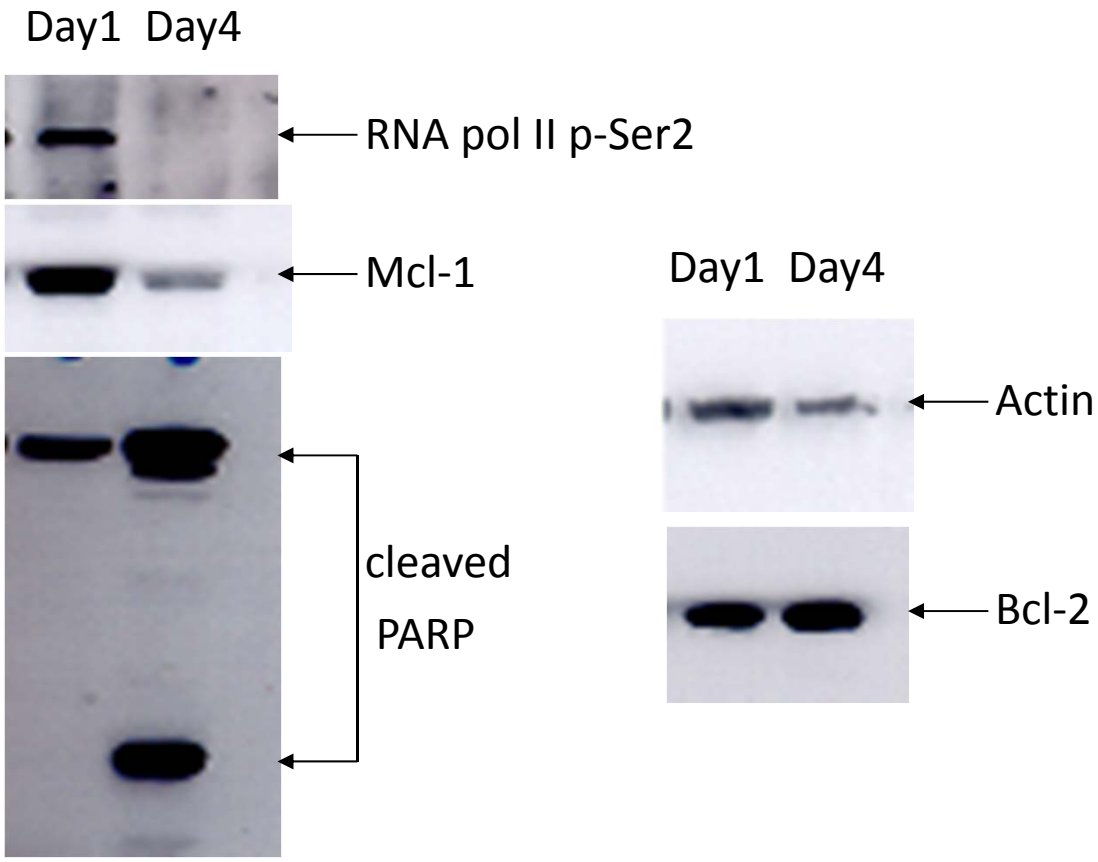
# Seliciclib decreases Mcl-1 levels *in vitro*




COLO 205 cells treated for up to 24 h
D = DMSO
S = 2x$IC_{50}$ seliciclib (26 μM)

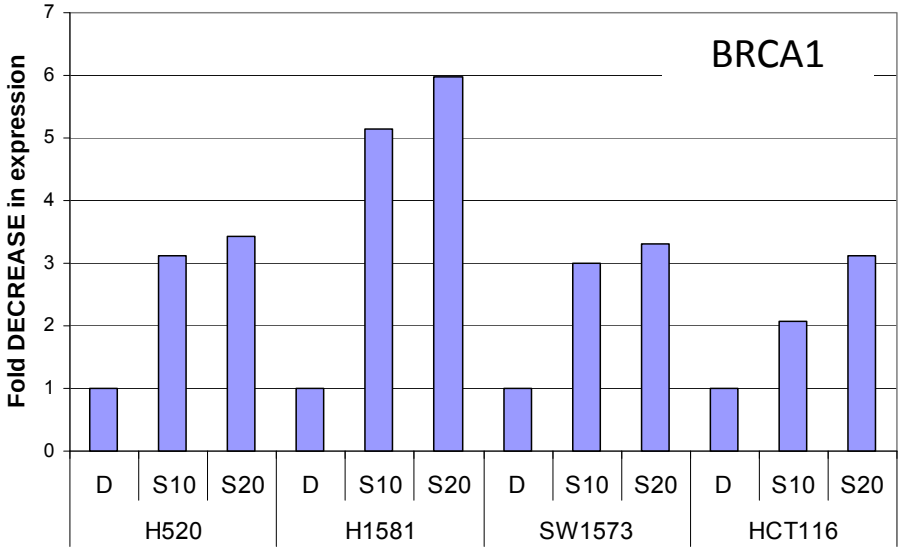
*Green, S.R. et al. AACR 2009 Abstract 3863*

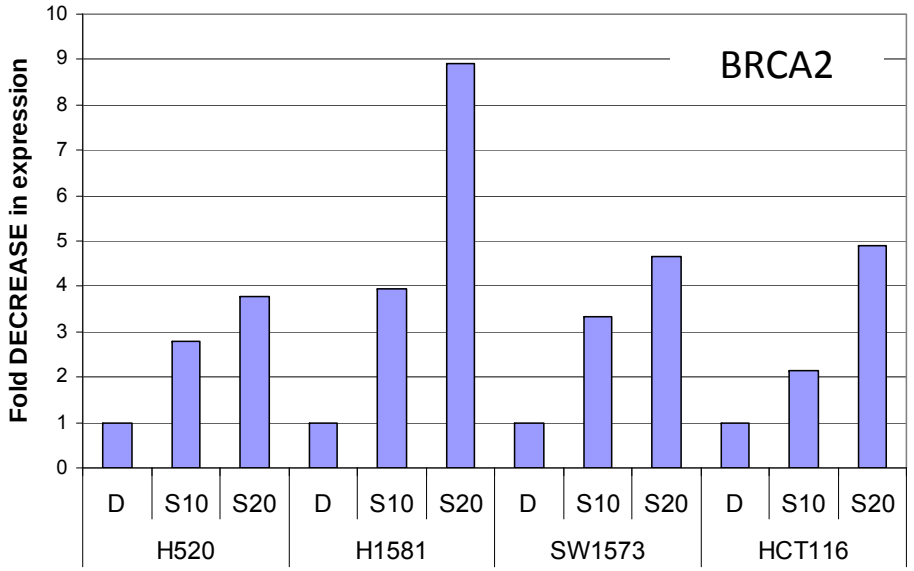
# Seliciclib decreases Mcl-1 levels *in vivo*




Protein extracts isolated from WBCs of a B-CLL patient treated with 1600 mg bid seliciclib for 3 days Q2W (cycle 2)

# Seliciclib decreases BRCA mRNA expression

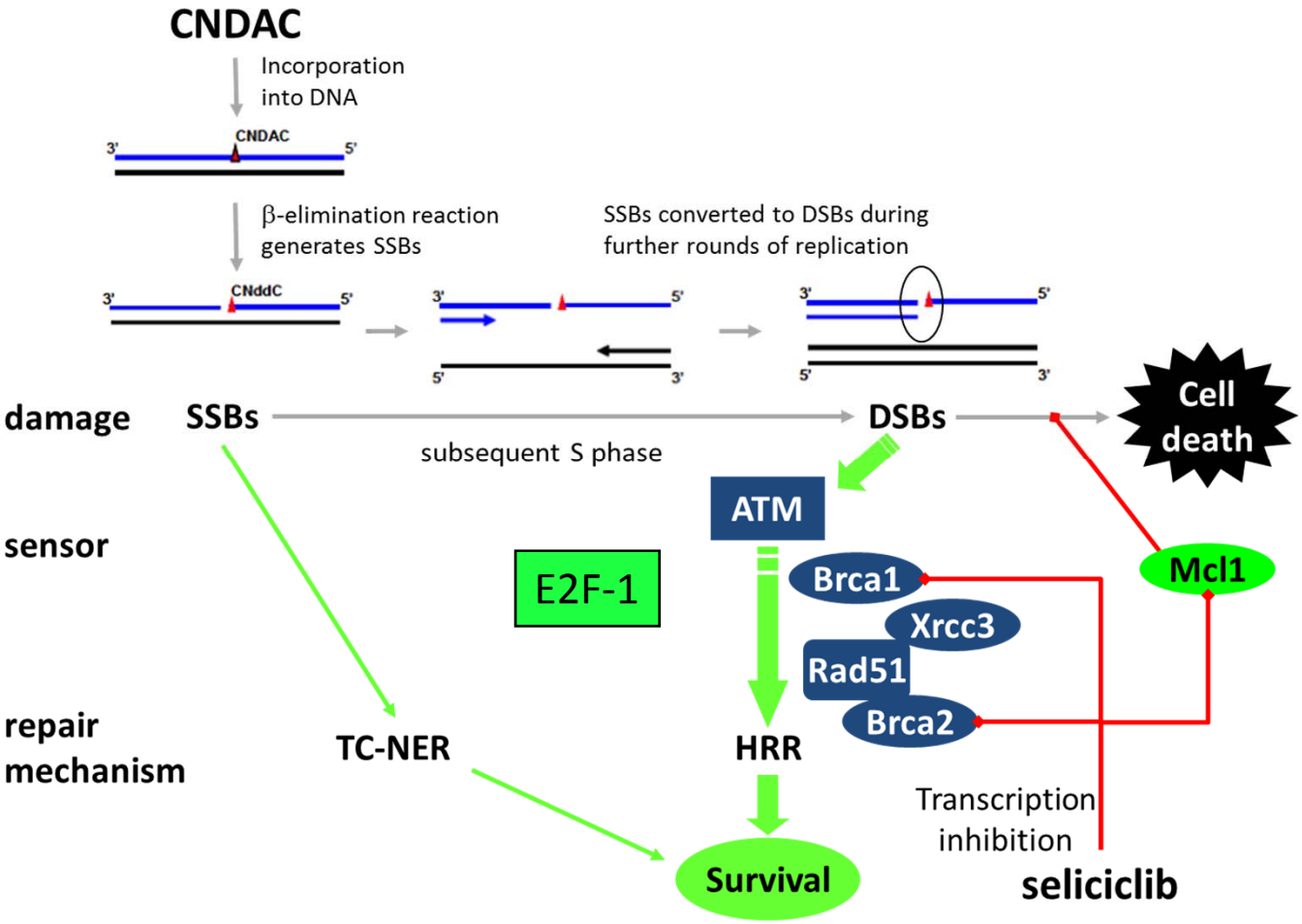



Fold decrease in BRCA1 or BRCA2 mRNA

qPCR analysis of cell lines treated with seliciclib for 5 h
D: DMSO; S10: 10 μM seliciclib; S20: 20 μM seliciclib

# Mechanism of Sapacitabine-Seliciclib Synergy




BRCA associated cancers have activated CDK2, overexpress cyclin E and are 2-4-fold more sensitive to CDKi (Deans et al., Cancer Res, 2006)

# Study Objectives and Design

- Open label, single arm, phase I dose escalation study in patients with advanced solid tumors
- Primary: To determine the MTD or recommended phase II doses of sapacitabine and seliciclib administered sequentially.
- Secondary : To evaluate antitumor activity of sequential treatment and to explore the pharmacodynamic effect of this treatment in skin.

- Sapacitabine twice daily for 7 days (Day 1-7);
- Seliciclib twice daily for 3 days (Day 8-11)
- Skin biopsies at baseline and on Day 8 and 11
- One treatment cycle is 3 weeks
- The first repeat tumor imaging study is conducted after 2 cycles of treatment and subsequently every 3 cycles
- At least 3 patients were enrolled at each dose level
- Dose escalation proceeds if $\leq$ 1/3 or $\leq$ 2/6 patients experience DLT during cycle 1
- $\geq$ 6 patients may be enrolled at recommended phase II dose level to confirm safety

# Eligibility and DLT/MTD Definitions

Incurable advanced solid tumors unresponsive to conventional therapy or for which no effective therapy exists

ECOG performance status 0 – 2

Evaluable disease

Adequate organ function

- Absolute neutrophil count $\geq$ 1.5 x $10^9$/L, platelets $\geq$ 100 x $10^9$/L
- Total bilirubin $\leq$ ULN, ALT $\leq$ 1.5 x ULN, serum albumin $\geq$ 2.8 g/dL, prothrombin time (PT) < 4 seconds prolonged over the ULN in patients not receiving chronic anticoagulation treatment
- Creatinine $\leq$ ULN or creatinine clearance > 60 mL/minute by the Cockcroft formula

Patients with brain metastases must have completed appropriate therapy and be stable for $\geq$ 4 weeks after the last treatment

Signed informed consent

DLT is the occurrence of any of the following events during cycle 1 when judged to be clinically significant and related to sapacitabine and/or seliciclib

- Grade 3 or 4 non-hematological toxicity (except alopecia, inadequately treated nausea, vomiting and diarrhea)
- Neutropenic fever or grade 4 neutropenia lasting longer than 5 days
- Grade 3 thrombocytopenia associated with bleeding or grade 4 thrombocytopenia
- Treatment delay > 2 weeks due to drug-related adverse events

MTD is the dose level at which at least 2/3 or 3/6 patients experience DLT in cycle 1

# Patient Characteristics

| | *BRCA mutation carriers (n=16)* | *Others (n=22)* |
|---|---|---|
| **Median age (range)** | 55 (31-75) | 56 (32-80) |
| **Gender** | | |
| Male | - | 15 |
| Female | 16 | 7 |
| **ECOG** | | |
| 0 | 7 | 5 |
| 1 | 9 | 17 |

# Disease Characteristics

| | *BRCA mutation carriers (n=16)* | *Others (n=22)* |
|---|---|---|
| **Tumor Type** | | |
| Breast | 8 | - |
| Colorectal | - | 3 |
| Non-small cell lung | - | 6 |
| Ovary | 7 | 1 |
| Pancreas | 1 | 3 |
| Other | - | 9 |
| **Prior systemic therapy** | | |
| 1- 3 | 5 | 7 |
| ≥ 4 | 11 | 15 |
| **Prior gemcitabine** | 8 | 13 |
| **Prior CDK inhibitor** | 0 | 3 |
| **Prior PARP inhibitor** | 5 | - |

# Dose-Limiting Toxicities

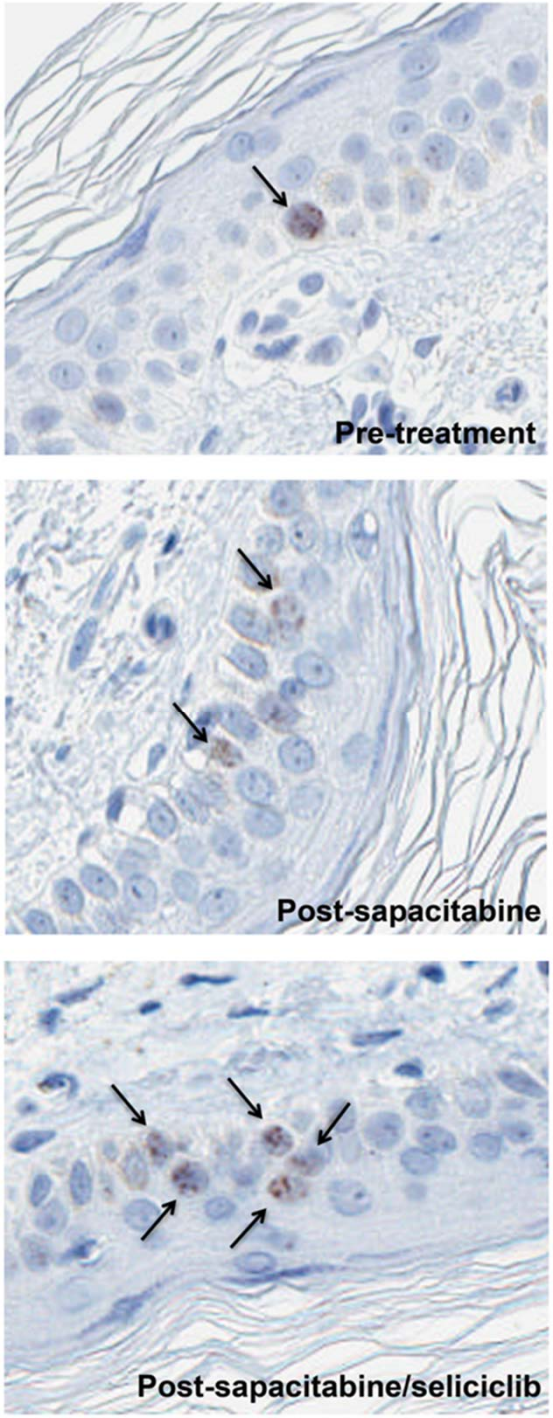
| ***Sapacitabine b.i.d. (mg) x 7 days*** | ***Seliciclib b.i.d. (mg) x 3 days*** | ***Treated (n=38)*** | ***Dose-Limiting Toxicity*** |
|---|---|---|---|
| 50 | 400 | 10 | Gr 3 elevation in AST and Gr 4 neutropenia (n=1) |
| 75 | 400 | 3 | Gr 4 neutropenia (n=2) |
| 50 | 800 | 6 | - |
| 50 | 1200 | 19 | Gr 4 neutropenia (n=1)<br>Gr 3 fatigue (n=1)<br>Gr 4 elevation in AST and total bilirubin (n=1)<br>Gr 3 elevation in ALT/AST (n=1)<br>Gr 3 elevation in AST, ALT and total bilirubin (n=1)<br>Gr 3 abdominal pain (n=1) |

# Common Adverse Events

*(all cycles, maximum grade, regardless of causality, n=38)*

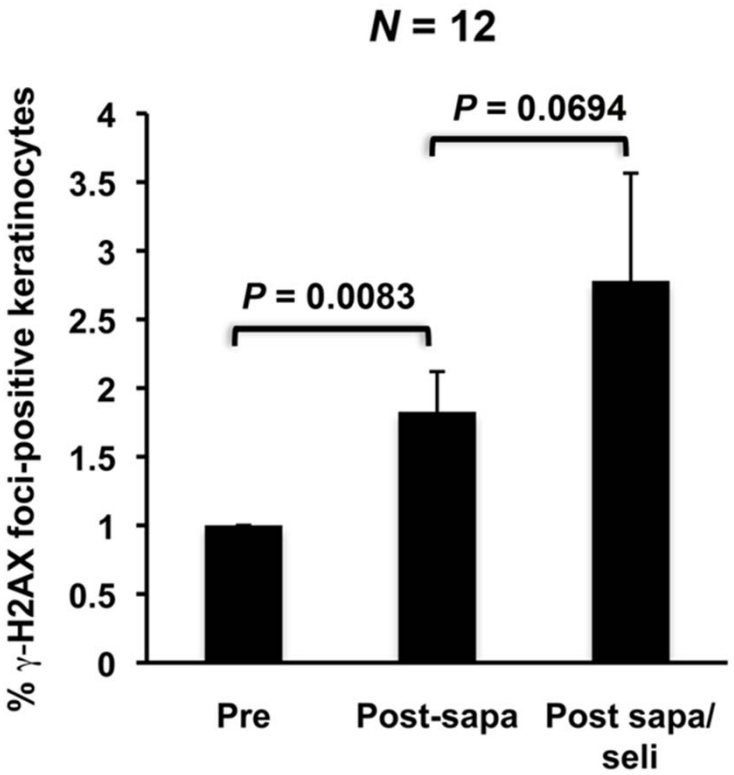
| | Grade 1 – 2 | Grade 3 - 4 |
|---|---|---|
| **Anemia** | **14** | **-** |
| **Neutropenia** | **1** | **6** |
| **Photophobia, photopsia, vision blurred, vision impairment, floaters** | **13** | **-** |
| **Abdominal pain** | **9** | **5** |
| **Constipation** | **11** | **2** |
| **Diarrhea** | **17** | **-** |
| **Nausea** | **24** | **-** |
| **Vomiting** | **16** | **-** |
| **Alkaline phosphatase elevation** | **5** | **3** |
| **ALT elevation** | **6** | **3** |
| **AST elevation** | **10** | **6** |
| **Hyperbilirubinemia** | **5** | **3** |
| **Creatinine elevation** | **11** | **-** |
| **Hyperglycemia** | **4** | **3** |
| **Hypokalemia** | **6** | **3** |
| **Hypophosphatemia** | **6** | **2** |
| **Decreased appetite** | **13** | **1** |
| **Dehydration** | **10** | **-** |
| **Alopecia** | **7** | **-** |
| **Fatigue** | **18** | **3** |

# Seliciclib enhanced sapacitabine-mediated DNA damage




***Patient 16***

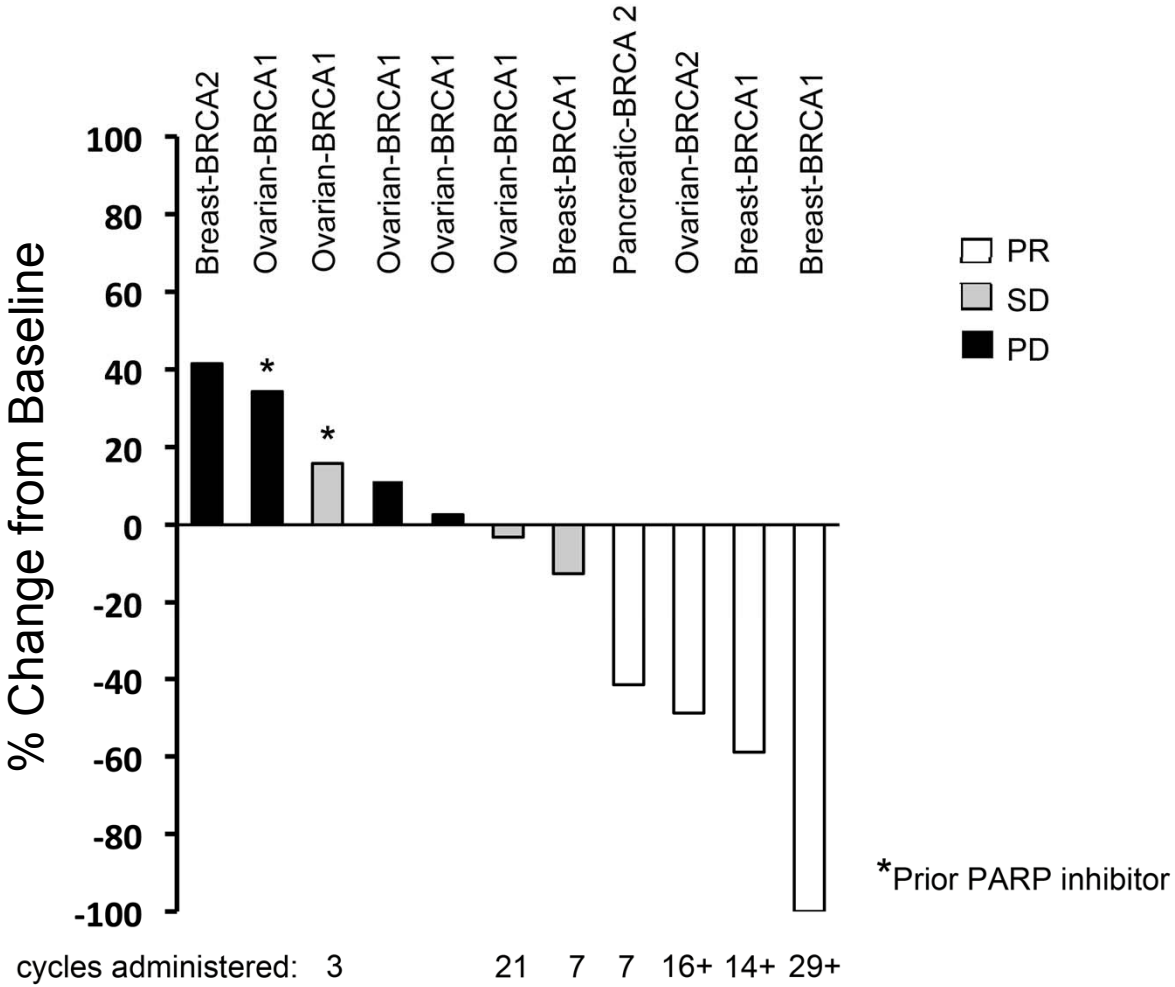
## γ–H2AX foci



# Investigator Assessment of Best Responses

| ***Cancer*** | ***Best Response*** | ***Prior Treatment*** | ***Total cycles*** |
|---|---|---|---|
| ***BRCA mutation carriers (n=16) (***** PARP inhibitor naïve BRCA mutation carriers)* | | | |
| Pancreas* | **PR** | gemcitabine, 5-FU, oxaliplatin | **7** |
| Ovary* | **SD** | paclitaxel, carboplatin, gemcitabine | **21** |
| Ovary* | **PR** | paclitaxel, carboplatin, gemcitabine, topotecan, iniparib | **>16** |
| Breast* | **SD** | tamoxifen, raloxifen, anastrozole, adriamycin, Cytoxan, paclitaxel, carboplatin, navelbine | **7** |
| Breast* | **PR** | adriamycin, Cytoxan, paclitaxel, carboplatin | **>14** |
| Breast* | **PR** | adriamycin, cyclophosphamide, paclitaxel, cisplatin | **>29** |
| ***Others (n=22)*** | | | |
| Nasopharyngeal (squamous) | **SD** | docetaxel, paclitaxel, cisplatin, carboplatin, 5-FU, pemetrexed, cetuximab, SCH727965 | **6** |
| Uterine leiomyosarcoma | **SD** | gemcitabine, flavopiridol | **9** |
| Non-small cell lung (poorly differentiated) | **SD** | paclitaxel, carboplatin, pemetrexed, navelbine, erlotinib, cetuximab | **6** |
| Sigmoid colon | **SD** | 5-FU, folfox, irinotecan, cetuximab, bevacizumab | **7** |
| LE sarcoma | **SD** | doxorubicin, ifosfamide, dacarbazine, vinorelbine, trabectedin, ALB-109564, Ro4929097, PF-04554878 | **4** |
| Pancreas | **SD** | folfox, gemcitabine, erlotinib, Abraxane, 5-FU | **4** |

# RECIST Evaluable BRCA Carriers




1 pt: SBO after 2 doses of sapacitabine;
1 pt: rapid disease progression in cycle 1 (prior veliparib);
1 pt: withdrew consent in cycle 1 (prior olaparib);
1 pt: brain metastases in cycle 1 (prior veliparib);
1 pt: clinical progression during cycle 1

# Summary and Future Directions

**Recommended Phase 2 dose for sequential administration of sapacitabine and seliciclib:**

- Sapacitabine 50 mg b.i.d. followed by seliciclib 800 mg b.i.d.

**Pharmacodynamic effect of sapacitabine and seliciclib observed in skin biopsies**

**Partial responses and prolonged stable disease observed in BRCA mutation carriers**

- 4 PRs and 2 SD among 11 PARP inhibitor naïve BRCA mutation carriers

**Future evaluation in BRCA mutation carrier patients warranted to address the following questions:**

- Role of other drug administration schedules
- Additional biomarkers beyond BRCA mutation carrier status to predict response?
- Role of CDK inhibition in BRCA mutation carriers

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