Abstract 2475

In vitro and in vivo assessment of the mechanism of action of the PARP inhibitor rucaparib

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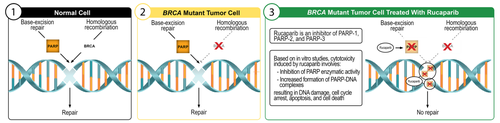
BACKGROUND

- Rucaparib is a small molecule inhibitor of poly(ADP-ribose) polymerase (PARP)-1, PARP-2 and PARP-3, and was recently approved for the treatment of patients with deleterious *BRCA* mutation (germline and/or somatic) associated advanced ovarian cancer who have been treated with two or more chemotherapies
- PARP enzymes play important roles in DNA repair, and rucaparib has shown preclinical and clinical activity in *BRCA* mutant and homologous recombination deficient (HRD) cancers through a mechanism known as synthetic lethality, where the loss of two DNA-repair pathways results in the accumulation of DNA damage and cell death (diagrammed below)
- Rucaparib-induced cytotoxicity may involve inhibition of PARP enzymatic activity and increased formation of PARP-DNA complexes resulting in DNA damage, cell cycle arrest, apoptosis, and cell death



Rucaparib is a potent and selective PARP-1, PARP-2, and PARP-3 inhibitor

- Rucaparib potently and selectively inhibits PARP-1, PARP-2, and PARP-3 in enzymatic assays

PARP Family Member

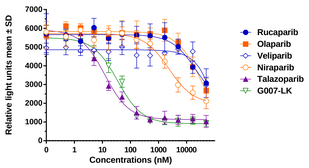
	PARP-1	PARP-2	PARP-3	TNKS1	TNKS2	PARP-6	PARP-7	PARP-8
Rucaparib	0.6	0.2	67	533	414			
Olaparib	0.9	0.2	41				750	
Niraparib	1.9	0.5						
Talazoparib	0.5	0.1	46	42	7			360
Veliparib	1.7	0.5	213					

<10 nM | 10-100 nM | 100 nM-1 µM | >1 µM

- In vitro enzymatic assays measuring the incorporation of biotin NAD+ to histones were performed using recombinant human PARP enzymes, and IC_{50} values are shown in the table (BPS Biosciences). The IC_{50} was >1 µM against PARP-10, -11, -12, -13, -14, and -15 for all of the PARP inhibitors tested (data not shown).

Rucaparib has limited cellular activity against TNKS1/2

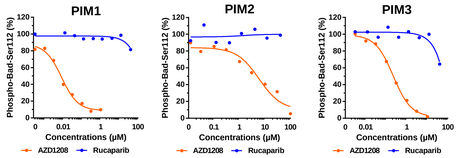
- Rucaparib has weak activity against TNKS1/2 in enzymatic assays
- TNKS1/2 play an important role in Wnt pathway signaling
- Rucaparib demonstrated limited activity in a Wnt cellular reporter assay (IC_{50} >20 µM) as compared to the positive control TNKS inhibitor G007-LK (IC_{50} = 0.036 µM)



- PARP inhibitor activity against TNKS1/2 was assessed in HEK293 cells engineered to stably express a luciferase gene under the control of TCF/LEF responsive elements. Wnt pathway signaling was stimulated by the addition of Wnt3a (BPS Biosciences).

Rucaparib has limited activity against the kinases PIM1, PIM2, and PIM3

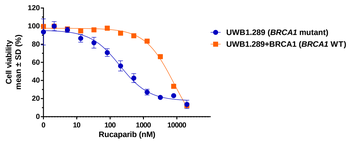
- PARP inhibitors have shown in vitro activity against the kinases PIM1 and PIM2 in enzymatic assays[1]
- Rucaparib was profiled in functional biochemical assays against PIM1, PIM2, and PIM3, and the IC_{50} values were 0.94, 8.98, and 0.24 µM, respectively
- Rucaparib showed limited activity (IC_{50} >40 µM) against PIM1, PIM2, and PIM3 in cellular reporter assays, whereas potent activity was observed with the PIM1-3 inhibitor AZD1208



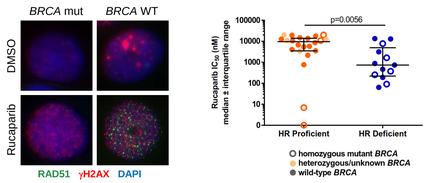
- Inhibition of the PIM substrate phospho-Bad (Ser112) was assessed in PIM1, PIM2, and PIM3 overexpressing HEK293 cells (ProQinase).

Rucaparib is active in homologous recombination deficient cancer cell lines

- Rucaparib was more active in *BRCA1* mutant UWB1.289 cells (blue line) as compared to *BRCA1* overexpressing UWB1.289+BRCA1 cells (orange line)



- The activity of rucaparib was assessed in a panel of 36 cancer cell lines
  - Cell lines were classified as homologous recombination (HR) proficient or deficient based on the quantification of γH2AX (DNA damage marker) and RAD51 (DNA repair marker) (left panel)
  - The zygosity of *BRCA* was determined based on the Cancer Cell Line Encyclopedia
  - Rucaparib activity was evaluated in 6-day cell viability assays (right panel)
- Cell lines with a HR deficient phenotype had a statistically significant lower median rucaparib IC_{50} as compared to HR proficient cells

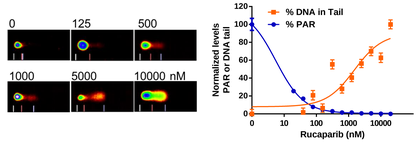


- Immunofluorescence detection of γH2AX/RAD51 nuclear foci was analyzed after the induction of DNA damage by the addition of 10 µM rucaparib for 24 hours using the UWB1.289 cell line pair (left panel).
- For 6-day cell viability assays, cells were seeded at 500-1000 cells per well in 384-well plates and treated with rucaparib for 6 days. Cell viability was measured by CellTiter-Glo (Promega) (right panel).

Rucaparib mechanism of action

1 DNA Damage

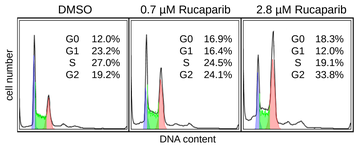
- Rucaparib treatment results in poly-ADP ribose (PAR) inhibition and increased DNA damage



- UWB1.289 cells (*BRCA1* mutant) were treated with rucaparib for 24 hours, and DNA damage was assessed in a Comet assay (Trevigen) (left), and correlated with PAR levels as determined by ELISA (right).

2 Cell Cycle Arrest

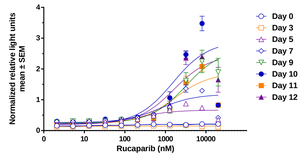
- Rucaparib treatment induces G2/M cell cycle arrest



- UWB1.289 (*BRCA1* mutant) cells were treated with 0.7 and 2.8 µM rucaparib for 72 hours, and the cell cycle was assessed by PI staining using an LSR II analyser.

3 Apoptosis

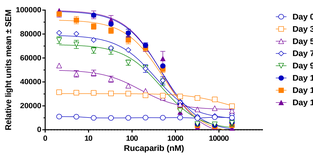
- Increased apoptosis (caspase 3/7 levels) is observed after 5-10 days of rucaparib treatment



- UWB1.289 (*BRCA1* mutant) cells were treated with rucaparib for the number of days indicated, and caspase 3/7 levels were measured using Caspase-Glo 3/7 (Promega).

4 Cell Death

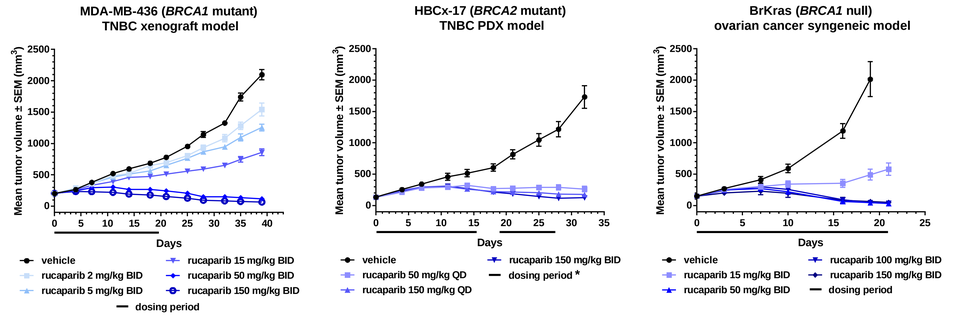
- Decreased cell viability is observed 5-12 days after rucaparib treatment



- UWB1.289 (*BRCA1* mutant) cells were treated with rucaparib for the number of days indicated, and cell viability was measured using CellTiter-Glo (Promega).

Rucaparib has potent activity in xenograft and syngeneic *BRCA* mutant models

- Rucaparib activity was evaluated in an established triple-negative breast cancer (TNBC) xenograft model (MDA-MB-436), TNBC patient-derived xenograft (PDX) model (HBCx-17), and ovarian cancer syngeneic model (BrKras)



- The MDA-MB-436 (*BRCA1* c.5396+G>A) and BrKras (*BRCA1* null) studies were performed by orthotopic and subcutaneous injection of tumor cells into NOD/SCID and FVB female mice, respectively. The HBCx-17 (*BRCA2* c.6033_6034delTT) study was performed by subcutaneous implantation of tumor fragments into *nu/nu* mice. *A dosing holiday occurred due to lapse in API availability. All studies had ≥10 animals per group, and rucaparib was administered by oral gavage at the doses and schedules indicated. The BrKras cell line was a generous gift from Sandra Orsulic.[2] The MDA-MB-436 and BrKras models were performed at Crown Biosciences, and the HBCx-17 study was performed at Xentech.

Rucaparib PK/PD studies

- IV and PO PK in female CD-1 mice
  - The oral bioavailability of 50 mg/kg rucaparib in 0.5% methylcellulose was 17%
  - Rucaparib showed moderate plasma binding (mean 61.9%) and high steady state volume of distribution (V_{ss}) of 6.14 L/kg
- In PK-PD relationship studies
  - Rucaparib plasma and tumor concentrations increased with dose
  - An inverse and dose-dependent correlation between PAR and rucaparib levels in the plasma and tumor was observed
  - Higher levels of rucaparib were observed in tumors relative to plasma at all doses evaluated, suggesting good distribution of rucaparib into the tumor
  - Increased PAR inhibition correlated with greater tumor growth inhibition (TGI)

Dose, Exposure and Response Relationship in the MDA-MB-436 Model

Dose	PK		PK/PD				Efficacy
mg/kg	C_{max} (ng/mL)	$AUC_{0\text{-}inf}$ (ng.hr/mL)	PAR inhibition[a] (%)	Tumor rucaparib (ng/g)	Plasma rucaparib (ng/mL)	T/P ratio[b]	TGI (%) Day 28
0	NA	NA	NA	NR	0	NA	NA
2	26	115	37	131	16	8.58	22
5	82	221	31	134	27	5.18	30
15	173	682	45	340	68	5.81	58
50	2340	6850	86	1982	975	2.63	105
150	3550	20000	96	3967	1897	2.17	112

T/P, tumor-to-plasma; NA, not applicable; NR, not reportable

[a] PAR levels calculated as a percent of the PAR level in the vehicle treated animals

[b] Mean of tumor/plasma ratio of rucaparib levels were calculated on individual T/P ratios

- A PK study was performed in non-tumor bearing NOD/SCID female mice using a single dose of rucaparib to obtain C_{max} and $AUC_{0\text{-}inf}$. PK/PD and efficacy studies were performed in the MDA-MB-436 xenograft model. For the PK/PD study, 5 doses of rucaparib were administered every 12 hours, while the efficacy study was performed using continuous twice daily dosing. Studies were performed at Crown Biosciences at the doses indicated in the table. Rucaparib levels were assessed by LC-MS/MS, and tumor PAR levels were measured by ELISA.

CONCLUSIONS

- **Rucaparib is a potent and selective PARP-1, PARP-2 and PARP-3 inhibitor with**
  - **Limited activity against other PARP enzymes including TNKS1/2**
  - **Limited activity against the kinases PIM1, PIM2, and PIM3**
- **Rucaparib inhibited the proliferation of HRD cell lines and tumors**
  - **Rucaparib demonstrated potent activity in xenograft and syngeneic mutant *BRCA1/2* TNBC and ovarian cancer models**
- **Rucaparib treatment resulted in DNA damage, cell cycle arrest, apoptosis, and decreased cell viability**
- **Rucaparib had good oral bioavailability and tumor distribution in mice**
  - **Rucaparib levels in the plasma and tumor increased with dose, and were inversely correlated with PAR levels but directly correlated with greater tumor growth inhibition**

REFERENCES

1. Antolin and Mestres. *Oncotarget*. 2014;5:3023-8
2. Xing and Orsulic. *Cancer Res*. 2006;66:8949-53