Intracranial Evaluation of the In Vivo Pharmacokinetics, Brain Distribution, and Efficacy of Rucaparib in *BRCA*-Mutant, Triple-Negative Breast Cancer

Poster 3888

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INTRODUCTION

- Poly(ADP-ribose) polymerase (PARP) inhibitors, including rucaparib, niraparib, olaparib, talazoparib, and veliparib, are approved or in clinical development for many cancers. There is very limited clinical data on the effects of PARP inhibitors in the central nervous system (CNS)
- Preclinical studies have suggested that veliparib and niraparib can cross the blood-brain barrier (BBB),[1,2] whereas talazoparib and rucaparib brain penetrance is limited[3-5]
  - Niraparib has shown antitumor efficacy in an intracranial *BRCA*-mutant model[2]
- The studies presented here were performed to better understand the distribution and efficacy of PARP inhibitors in the CNS
  - In vitro plasma and brain protein binding and in vivo pharmacokinetic (PK) studies were conducted using rucaparib, niraparib, olaparib, talazoparib, and veliparib
  - Antitumor activities of rucaparib and niraparib were evaluated in a *BRCA1*-mutant, intracranial, triple-negative breast cancer (TNBC) model
  - A case report demonstrating rucaparib clinical activity in the CNS of a patient with *BRCA2*-mutant breast cancer is presented

METHODS

Reagents: Cell lines were obtained from ATCC and cultured according to their instructions. Compounds were obtained from MedChemExpress.

Cytotoxicity assays: Cells were seeded in 384-well plates and treated with PARP inhibitors for 6 days. Cell viability was measured by CellTiter-Glo (Promega).

In vivo orthotopic efficacy study: MDA-MB-436 cells (*BRCA1* c.5396+1G>A; 1×10^7) in PBS + matrigel were implanted in the right mammary fat pad of NOD/SCID female mice, and dosing began at 200 mm^3 mean tumor size. Rucaparib was given by oral gavage (PO) BID for 28 days at doses shown in the graph (n=10 mice/group). Tumor volumes were calculated as $V = 0.5\ a \times b^2$, where *a* and *b* are the long and short diameters of the tumor, respectively.

In vivo PK and PD study: Nontumor-bearing NOD/SCID female mice were given a single dose of rucaparib for PK assessment (n=3 mice/group), whereas NOD/SCID female mice bearing MDA-MB-436 tumors were given 5 doses of rucaparib BID to derive tumor to plasma ratios (n=4 mice/group). Rucaparib was given PO at the doses shown in the table. Drug levels were assessed by LC-MS/MS, and tumor poly(ADP-ribose) (PAR) levels were measured by ELISA (Trevigen).

In vitro protein-binding assay: Talazoparib (0.01 and 0.1 µM) and other agents (1 and 10 µM) were mixed with mouse plasma and brain homogenate before loading onto rapid equilibrium dialysis devices. After 4 hours at 37°C, samples were analyzed by LC-MS/MS, and the amount of protein binding was determined.

In vivo PK study: CD-1 male mice were given a single dose PO of the indicated PARP inhibitors, and plasma, brain, and CSF samples were collected at 0.5, 2, 4, 8, and 24 hours (n=3 mice/group). Drug levels were assessed by LC-MS/MS.

In vivo intracranial efficacy study: MDA-MB-436 cells were transduced with a lentivector expressing luciferase to enable tumor growth monitoring by bioluminescence imaging (BLI), and cells (5×10^5) were intracranially implanted through surgical incision over the cranium bregma of NOD/SCID female mice. Treatment started at mean 2.4×10^7 BLI photons/second, and compounds were given PO for the study duration at doses shown in the graph and table (n=11–12/group). Samples were collected 2 hours after last dose, and drug levels were assessed by LC-MS/MS.

BID, twice daily; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; LC-MS/MS, liquid chromatography with tandem mass spectrometry, NOD/SCID, nonobese diabetic/severe combined immunodeficiency; PBS, phosphate buffered saline; PD, pharmacodynamic.

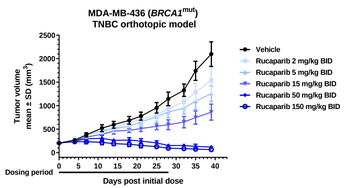
In Vitro Validation of *BRCA1*^{mut} MDA-MB-436 as a Suitable Model for PARP Inhibitor Testing

		Cytotoxicity of PARP inhibitors IC_{50}, mean ± SD, µM				
Cell line	***BRCA* status**	**Rucaparib**	**Niraparib**	**Olaparib**	**Talazoparib**	**Veliparib**
MDA-MB-231	Wild type	6.71 ± 1.89	7.15 ± 1.03	19.69 ± 0.59	2.98 ± 0.01	>20
MDA-MB-436	*BRCA1* mutated	0.09 ± 0.01	0.17 ± 0.09	0.11 ± 0.05	0.004 ± 0.001	1.86 ± 0.45

IC_{50}, half maximal inhibitory concentration; SD, standard deviation.

- TNBC *BRCA1*^{mut} MDA-MB-436 cells demonstrated greater sensitivity to PARP inhibitors in in vitro cytotoxicity assays than *BRCA*^{wt} MDA-MB-231 cells and were used for in vivo orthotopic and intracranial studies
- Talazoparib and veliparib had the lowest and highest IC_{50}, respectively, in MDA-MB-436 cells, whereas the IC_{50} for rucaparib, niraparib, and olaparib were comparable

Rucaparib In Vivo Efficacy in MDA-MB-436 Orthotopic Model Correlates with Drug Exposure



- Rucaparib demonstrated dose-dependent tumor growth inhibition (TGI) in the MDA-MB-436 orthotopic model. Tumor regressions of 105% and 112% TGI were observed at 50 and 150 mg/kg BID, respectively

Dose, exposure, and response relationship in the MDA-MB-436 model

Dose	PK[a]		PK/PD[a]				Efficacy
mg/kg	**Plasma C_{max}, ng/mL**	**Plasma $AUC_{0\text{-inf}}$, hr×ng/mL**	**PAR inhibition,[b] %**	**Tumor rucaparib, ng/g**	**Plasma rucaparib, ng/mL**	**T/P ratio[c]**	**TGI day 28, %**
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	20,000	96	3967	1897	2.17	112

[a]PK with nontumor-bearing mice; PK/PD with tumor-bearing mice; samples taken 2 hours after last dose. [b]PAR levels calculated as a percent of the PAR level in the vehicle-treated animals. [c]Mean of T/P ratio of rucaparib levels were calculated on individual T/P ratios. $AUC_{0\text{-inf}}$, area under the concentration-time curve extrapolated from time zero to infinity; C_{max}, maximum concentration; T/P, tumor-to-plasma.

- In an in vivo PK/PD study using the MDA-MB-436 orthotopic model, rucaparib demonstrated increased plasma and tumor concentrations with increasing doses
- Higher levels of rucaparib were observed in tumors relative to plasma at all doses evaluated. An inverse, dose-dependent correlation between rucaparib levels and PAR in the plasma and tumor was observed in female tumor-bearing NOD/SCID mice, with increased PAR inhibition correlating with greater TGI

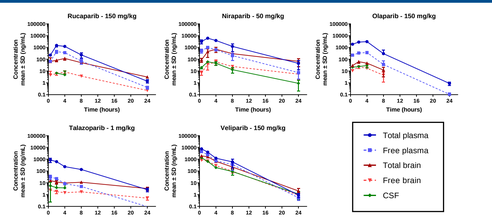
Free Fractions of PARP Inhibitors Were Determined in In Vitro Plasma and Brain Homogenate Binding Assays

	Fraction unbound of PARP inhibitors, mean ± SD, %				
	Rucaparib	**Niraparib**	**Olaparib**	**Talazoparib**	**Veliparib**
Plasma (1 µM)	30.7 ± 1.94	16.3 ± 0.93	11.6 ± 0.80	3.47 ± 0.31[a]	60.1 ± 7.53
Plasma (10 µM)	28.4 ± 0.79	16.7± 0.85	12.2 ± 1.18	3.75 ± 0.24[b]	58.5 ± 4.61
Brain homogenate (1 µM)	6.85 ± 0.27	7.76 ± 0.62	37.4 ± 6.08	7.54 ± 0.61[a]	48.9 ± 6.76
Brain homogenate (10 µM)	7.62 ± 0.54	8.62 ± 0.27	36.0 ± 2.06	23.1 ± 2.62[b]	38.6 ± 1.14

[a]0.01 µM talazoparib was used; [b]0.1 µM talazoparib was used.

- PARP inhibitors displayed concentration-independent free fraction in mouse plasma and brain homogenate, except for talazoparib, which had a higher free fraction in brain homogenate at the higher concentration evaluated
- The free fractions were used to calculate PK parameters described below

In Vivo PK Study to Evaluate Brain Penetration of PARP Inhibitors in Male CD-1 Mice



- For all PARP inhibitors, the C_{max} and $AUC_{0\text{-inf}}$ were higher in the plasma than in the brain and CSF

Pharmacokinetic parameters of PARP inhibitors

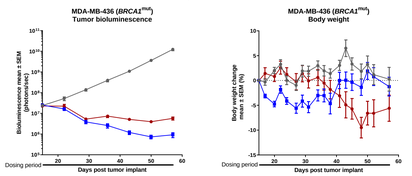
	Rucaparib	**Niraparib**	**Olaparib**	**Talazoparib**	**Veliparib**
C_{max}, plasma ng/mL[a]	1513	6140	3147	869	7603
$AUC_{0\text{-inf}}$, plasma hr×ng/mL[a]	8974	38,600	19,370	4066	24,310
C_{max}, brain ng/mL[a]	119	716	62	16	1944
$AUC_{0\text{-inf}}$, brain hr×ng/mL[a]	1122	7168	348	259	9021
C_{max}, CSF ng/mL	7	62	30	6	1343
K_p,brain	0.13	0.19	0.02	0.06	0.37
K_{puu},brain	0.03	0.09	0.06	0.27	0.27
K_{puu},CSF	0.01	0.06	0.08	0.18	0.30

[a]Total $AUC_{0\text{-inf}}$ and total C_{max}.
K_p,brain, $AUC_{0\text{-inf}}$ (total brain)/$AUC_{0\text{-inf}}$ (total plasma); K_{puu},brain, $AUC_{0\text{-inf}}$ (free brain)/$AUC_{0\text{-inf}}$ (free plasma); K_{puu},CSF, C_{max} (free CSF)/C_{max} (free plasma).

- Brain-to-plasma ratios K_p,brain, K_{puu},brain, and K_{puu},CSF were determined to evaluate brain penetration of the PARP inhibitors
- Of the 5 PARP inhibitors tested, veliparib consistently had the highest brain and CSF exposures, whereas the other 4 showed limited CNS penetration in mice with an intact BBB

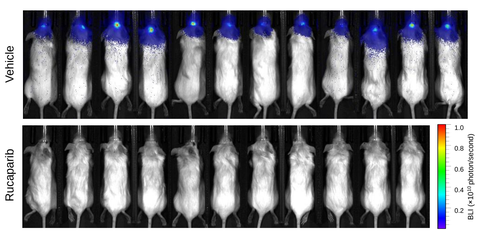
Rucaparib Demonstrates In Vivo Efficacy in the Intracranial *BRCA1*^{mut} MDA-MB-436 Model

- An intracranial MDA-MB-436 efficacy study was performed to evaluate rucaparib activity in the CNS based on prior reports of PARP inhibitor activity in this model[2]



QD, once daily.

- Rucaparib and niraparib demonstrated statistically significant (P<0.05 2-way analysis of variance; day 57) tumor suppression relative to control tumor volumes in the MDA-MB-436 intracranial model, with rucaparib showing greater inhibition than niraparib (P=0.0017)
- Mice treated with rucaparib and niraparib showed maximum body weight loss of 5.7% and 9.5% on days 27 and 48, respectively (P<0.01)



- BLI of mice from an intracranial efficacy study on day 57 confirmed the presence of tumors in the vehicle control mice (12/12 mice, top panel), but not in rucaparib-treated mice (0/11 mice, bottom panel)

Exposure and response in the intracranial MDA-MB-436 model

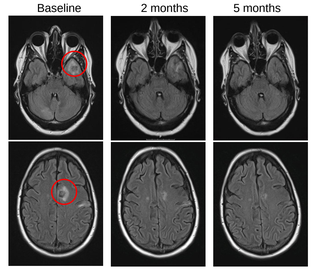
Drug	Dose	Efficacy	Body weight loss		Drug concentrations		
	mg/kg	**T/C day 57, %**	**Max average BWL, %**	**Day**	**Plasma, ng/mL**	**Brain, ng/mL**	**B/P ratio[a]**
Rucaparib	150 BID	0.01	-5.7	27	2206 ± 653	41 ± 21	0.019
Niraparib	50 QD	0.05	-9.5	48	3760 ± 940	100 ± 57	0.027

[a]Mean of B/P ratio was calculated by dividing mean brain level (ng/mL) by mean plasma level.
B/P, brain-to-plasma; BWL, body weight loss; T/C, treated tumor volume to control tumor volume ratio.

- At the end of the intracranial efficacy study, rucaparib and niraparib were measured in the brain and plasma at 2 hours post dose. Levels of the inhibitors and brain-to-plasma ratios were lower than the C_{max} and K_p derived from the PK study with nontumor-bearing mice

Rucaparib Shows Resolution of Neurological Symptoms in a Patient with TNBC

- Case study of a 51-year-old patient with *BRCA2 c.6225dupA (p.Val2076fs)* mutation. Patient experienced progressive CNS disease 18 months after whole brain radiation therapy, with headaches, slurred speech, facial pain, and numbness in right side of face and right hand
- Following 1 cycle of rucaparib, complete resolution of neurological symptoms was observed. Magnetic resonance images at baseline and on rucaparib (600 mg PO BID) treatment are shown below; red circles indicate lesions. The patient progressed after 9 months (overall survival, 25 months)



CONCLUSIONS

- In vivo PK studies confirmed the limited brain penetration of all PARP inhibitors evaluated in a murine model with an intact BBB. However, rucaparib and niraparib demonstrated antitumor efficacy in a *BRCA1*-mutant, intracranial, TNBC mouse model
- Clinical activity has been observed in a patient with germline *BRCA2*-mutant breast cancer and CNS involvement who was treated with rucaparib
- Additional studies are warranted to investigate the multiple factors that play a role in CNS activity, including potency, distribution, efflux ratio, and apparent permeability

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