Nonclinical Evaluation of Rucaparib in Tumors with Mutations in Non-*BRCA1/2* Homologous Recombination Repair (HRR) Genes

Liliane Robillard[1], Kevin K. Lin[1], Andrea Loehr[1], Tanya Kwan[1], Rachel Dusek[1], Andrew D. Simmons[1], Thomas C. Harding[1], Brieuc Sautois,[2] Minh Nguyen[1]

[1]Clovis Oncology, Inc., Boulder, CO, USA, [2]University Hospital of Liege, Liege, Belgium.

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Supplementary Material

Methods

- **siRNA transfection and RNA analysis:** Cells were seeded at 1–2×10^6 cells/10 cm dish, and transfected the next day with siRNA pools using DharmaFECT reagent (Thermo Fisher) following the manufacturer's instructions. A minimum of 3 replicates/transfections per gene was performed. Cells transfected with a non-targeting siRNA were used as a control. Cells were collected 24 hours later and used for either a 6-day cell viability assay or immediate RNA analysis. RNA was extracted using the PureLink RNA kit (Invitrogen), and cDNA was generated using SuperScript III Super Mix (Invitrogen) following the manufacturer's instructions. Quantitative Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) reactions were run using Taqman Universal Master Mix II (Applied Biosystems) and performed on the ViiA 7 PCR System (Applied Biosystems). RNA expression was knocked down on average by 80% (61–96% loss when compared to control). Statistical analysis was performed using two-sided t-test, and $P<0.05$ was considered significant
- **Tumor efficacy studies:** PDX tumor fragments were implanted subcutaneously in the right flank of immunocompromised female mice, and dosing of 50 or 150 mg/kg rucaparib BID PO began when an average tumor volume of 106–234 mm^3 was reached. Body weights and tumor volume were measured twice weekly, and tumor growth inhibition (TGI) was calculated as [1-(Vt-Vi)/(Vc-Vi)]×100. Percent body weight loss (BWL) was calculated as [1-(BWLt/BWLc)]×100, where BWLc and BWLt are the BWL of control and treated groups at the time point. Statistical analysis was performed using two-sided t-test, and $P<0.05$ was considered significant
- **Genomic analysis:** DNA mutation analysis was performed using HRD Plus testing (Myriad). Methylation analysis was performed using the pyrosequencing method as per manufacturer's protocol (Qiagen)

Summary of Rucaparib Tumor Efficacy in PDX Tumors and Their *BRCA1/2* Mutations

Model	Cancer type	Genomic alteration	Biallelic	Dose (mg/kg)	Rucaparib efficacy		
					TGI (%)	*P*-value	n
BL3325	Bladder	BRCA2 c.C5750A + c.6462delTCTC; p.S1917X + p.Y2154fs	Yes	150 BID	101.45%	<0.0001	10
CTG-0136	Esophageal	BRCA2 copy loss	Yes	150 BID	72.00%	<0.0001	10
CTG-1061	Bladder	Wild-type	NA	150 BID	-4.31%	0.409	10
CTG-1076	Bladder	BRCA1 3265_3266dupTT; p.L1089fs	No	150 BID	65.13%	0.150	3
GXA 3036	Gastric Adeno	BRCA1 c.668delA; p.K223X	No	150 BID	-12.64%	0.364	3
GXF 97	Gastric Adeno	BRCA2 c.6482delACAA; p.D2161X	Yes	150 BID	114.25%	<0.0001	10
HBCx-17	TNBC	BRCA2 c.6033_6034delTT; p.F2013Qfs4aa	Yes	150 BID	100.62%	<0.0001	10
HBCx-6	TNBC	BRCA1 Hypermethylation	NA	150 BID	109.43%	<0.0001	10
LU-01-0010	Lung Adeno	BRCA1 c.C5503T; p.R1835X	Yes	150 BID	88.20%	<0.0001	3
LU-01-0340	Lung Squamous	BRCA2 c.5066_5067insA; p.A1689fs	No	150 BID	28.32%	0.266	3
LU-01-0407	Lung Squamous	BRCA2 c.C7480Tp.R2494X	Yes	150 BID	100.10%	<0.0001	10
LU5152	Lung Cancer	BRCA2 c.2951insA; p.E984fs	No	150 BID	24.87%	0.130	3
LXFE 2415	Lung Squamous	BRCA2 c.A1402T; p.R468X	No	150 BID	15.02%	0.343	3
PAXF 1876	Pancreatic	BRCA2 c.9090delA; p.T3030X	No	150 BID	30.94%	0.054	10
PAXF 2005	Pancreatic	BRCA2 c.6392delAATT; p.K2131X	Yes	150 BID	94.40%	<0.0001	10
PAXF 2094	Pancreatic	BRCA2 c.1315insAG; p.F439X	No	150 BID	69.12%	0.0008	10
ST-02-0328	Gastric Adeno	BRCA2 c.1806delA; p.G602fs	No	150 BID	7.70%	0.421	3
ST-02-0360	Gastric Adeno	BRCA2 c.3854dupA; p.E1285fs	No	150 BID	25.98%	0.192	3
ST-02-0386	Gastric Adeno	BRCA2 c.4279dupT; p.T1426fs	No	150 BID	34.28%	0.036	3
ST-02-0393	Gastric Adeno	BRCA2 c.5195delT; p.L1732fs	Yes	150 BID	104.34%	<0.0001	10

TGI, tumor growth inhibition; n, number of animals per treatment group; TNBC, triple-negative breast cancer

Summary of Rucaparib Tumor Efficacy in PDX Tumors and Their HRR Mutations

Model	Cancer type	Genomic alteration	Biallelic	Dose (mg/kg)	Rucaparib efficacy		
					TGI (%)	*P*-value	n
BR-05-0044	Breast	RAD51C Hypermethylation	NA	50 QD	107	<0.0001	10
BR1180	Breast	PALB2 c.758dupT; p.S254fs	Yes	150 BID	84	0.0004	10
CH-17-0038	Cholangiocarcinoma	PALB2 c.1444_1448del p.L482fs	Unknown*	150 BID	26	0.169	3
CTG-2208	Breast	RAD51C c.653_654del; p.E218Vfs	Yes	150 BID	131	0.001	10
GA6822	Gastric	BRIP1 c.2507_2508del; p.R836Kfs	Yes	150 BID	-25.4	0.275	5
LI-03-0655	Liver	RAD51B c.G40T; p.E14X	Yes	150 BID	79.7	<0.0001	10
LU2529	Lung (Clear)	Wild type	NA	150 BID	-17	0.335	3
MC2296	Neuroendocrine carcinoma	RAD51D c.C898T; p.R300X	Uncertain#	150 BID	51	0.0005	10
OVXF 1023	Ovarian	RAD51C Hypermethylation	NA	50 QD	59.4	0.0018	10
ST1659	Colon	BRIP1 c.2392G>A p.R798X	Yes	150 BID	-46	0.506	3
ST2067	Ovarian	NBN c.1645T>A p.K549X	Uncertain#	150 BID	101	0.001	10
ST2073	Endometrial	PALB2 c.2257G>A p.R753X	Yes	150 BID	55	0.013	10
ST2636	Breast	BARD1 c.614dupA; p.Q206Afs	Yes	150 BID	21	0.215	3
T298	Breast	PALB2 c.1424C>G; p.S475X	Yes	150 BID	109	<0.0001	10

*DNA sample unavailable for confirmation; #LOH cannot be determined due to splice variants.
TGI, tumor growth inhibition. n, number of animals per treatment group

Disclosures

- Liliane Robillard, Kevin K. Lin, Andrea Loehr, Tanya Kwan, Rachel Dusek, Andrew D. Simmons, Thomas C. Harding, and Minh Nguyen are employees of Clovis Oncology, Inc. and may own stock or have stock options in that company.
- Brieuc Sautois has served in a consulting or advisory role for Clovis Oncology, Astellas, BMS Belgium, and Janssen; and received honoraria from Janssen and MSD.