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

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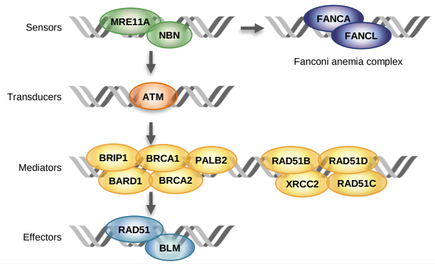
Abstract 1260

INTRODUCTION

- Rucaparib is a poly(ADP-ribose) polymerase (PARP) inhibitor approved for treatment of patients with recurrent ovarian cancer or metastatic castration-resistant prostate cancer (mCRPC). DNA repair deficiencies resulting from genetic and epigenetic alterations in breast cancer 1 and 2 genes (*BRCA1/2*) render tumor cells sensitive to rucaparib through a mechanism known as synthetic lethality
- *BRCA1/2,* and other genes such as *PALB2, RAD51C*, and *RAD51D* play a central role in homologous recombination repair (HRR) of DNA damage. Studies have shown that deficiencies in these genes can confer sensitivity to PARP inhibitors in patients[1,2]
- Biallelic and monoallelic alterations in *BRCA1* and *BRCA2* have been associated with response to PARP inhibition in clinical trials but the importance of HRR gene aberration zygosity in the context of PARP inhibition is still unclear and may be gene dependent[2,3]
- Human tumor explants transplanted from patients to immunodeficient mice have been used as preclinical models for drug development. These patient-derived tumor xenografts (PDX) retain most of the characteristics of the patient's tumors, including histology and sensitivity to anticancer drugs. Studies have shown that PDX models passaged in mice correctly replicate the response of the donor patient to standard cytotoxic anticancer drugs in >90% of cases[4]
- The goal of the studies reported here was to investigate the *in vitro* and *in vivo* synthetic lethality activity of rucaparib in multiple cancer cell types and PDX tumors harboring genetic or epigenetic alterations in non-BRCA HRR genes

Illustration of HRR Genes of Interest in DNA Repair. These genes act as sensors, transducers, mediators, or effectors of repair of damaged DNA.[2] It is unclear if *RAD54L* acts as a mediator or effector (not shown here)



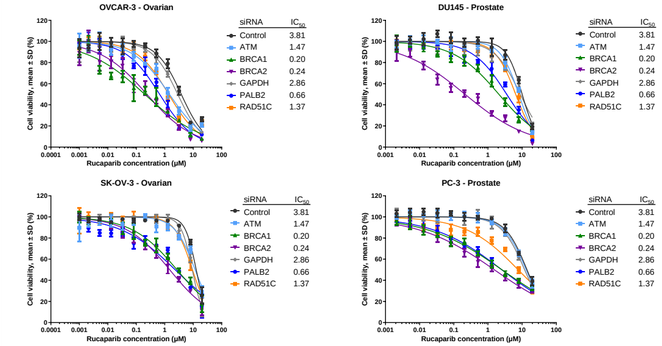
METHODS

- **Compound and cell lines:** Rucaparib camsylate salt was manufactured by Lonza (Basel, Switzerland). Cell lines were obtained from the American Type Culture Collection (ATCC) except OAW-28 which was obtained from the European Collection of Authenticated Cell Cultures (ECACC). All cell lines were cultured according to the recommended conditions
- **siRNA transfection and RNA analysis:** Small interfering ribonucleic acid (siRNA) was used to knockdown a panel of 16 HRR genes in ovarian and prostate cancer cell lines. Gene inactivation was monitored 24 hrs post siRNA transfection by qRT-PCR and an average of 80% loss of RNA expression was observed. 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 oral dosing of 50 or 150 mg/kg rucaparib twice daily (BID) or daily (QD). Body weights and tumor volume were measured twice weekly. 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
- Additional methods can be found in the supplementary material

RESULTS

In vitro siRNA knockdown of HRR genes

Rucaparib Inhibits Proliferation of Ovarian and Prostate Cancer Cell Lines with siRNA Knockdown of HRR Genes



- Rucaparib demonstrated enhanced cytotoxicity in ovarian and prostate cells with siRNA-mediated decreased expression of HRR genes such as *BRCA1, BRCA2*, and *PALB2*. Genes shown represent the range of viability curves observed post knockdown

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

Summary of Percent Change in Rucaparib IC_{50} with siRNA Knockdown of HRR Genes

Gene	Freq. of mutation in HGSOC (%)[5]	% IC_{50} change from ctl			Freq. of mutation in mCRPC (%)[2,6,7]	% IC_{50} change from ctl	
		OAW-28	OVCAR-3	SK-OV-3		DU145	PC-3
ATM	0.3	36	-45*	0	6.5	-27	3
BARD1	0	-94*	-99*	-99*	0.3	-99*	-98*
BLM	0	14	-7	-38	0	-17	-48*
BRCA1	12	-74*	-96*	-93*	1.8	-84*	-94*
BRCA2	9	-81*	-92*	-88*	8.4	-98*	-97*
BRIP1	0.3	-7	25	-7	0.7	-3	-30
FANCA	2.5	31	-49*	0	1	-37	-67 *
FANCL	0	44	-2	-2	0.7	5	-12
MRE11A	0	0	-6	-9	0.2	-32	-15
NBN	0.6	42	-69*	-16	0.7	-39*	-27
PALB2	0.6	-62*	-81*	-82*	1	-72*	-90*
RAD51	1.6	59	-96*	-97*	0.2	-100*	-100*
RAD51B	0.9	-21	-44*	-48	0.5	-29	-49*
RAD51C	0.0#	29	-54*	-26	0.5	-36*	-52*
RAD51D	0.9	0	-11	-14	0.1	-14	-19
RAD54L	0	-8	na	-10	0.4	-39*	-62*

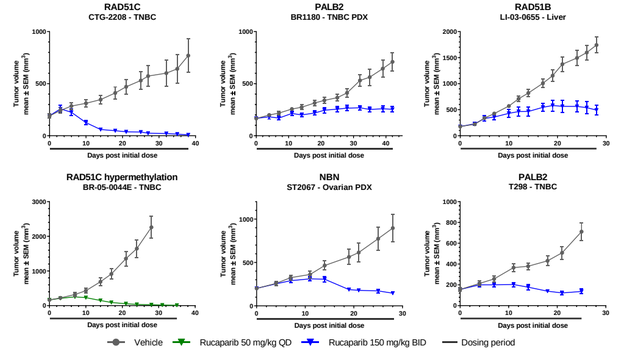
Fold change is calculated against non-targeting siRNA control treated cells. *IC_{50} significantly different than control *P*<0.05, #Deleterious mutations have been reported in HGOC at frequencies >1%.[8]
Ctl, control; Freq., frequency; HGSOC, high-grade serous ovarian cancer; na, knockdown incomplete

-100 +60
% change
Closer to -100: More sensitive to rucaparib

- siRNA was used to knockdown a panel of HRR genes in ovarian and prostate cancer cell lines to model the impact of their functional inactivation. Genes were selected based on their role in DNA repair, PARP inhibitor sensitivity, and frequency of mutations in high-grade serous ovarian cancer (HGSOC) and mCRPC
- Significantly increased potency of rucaparib was greatest in cells with siRNA-mediated decreased expression of *BARD1, BRCA1, BRCA2, FANCA, NBN, PALB2, RAD51*, *RAD51C,* and *RAD54L* where at least one cell line had <50% decrease in IC_{50}
- Lack of effect of certain genes could be due to insufficient knockdown of gene expression for some genes and in some cell lines. Threshold of inactivation for efficacy could vary by gene

In vivo validation of rucaparib in PDX models with HRR deficiencies

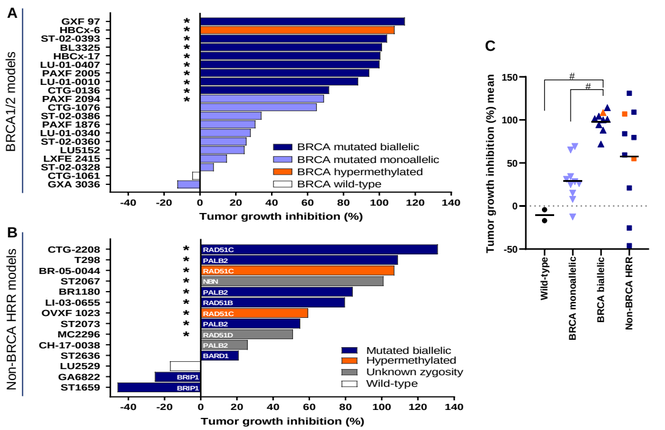
Rucaparib Demonstrates Potent Activity in Tumors with Genetic or Epigenetic Alterations of HRR Genes



- Tumor-bearing mice were treated with rucaparib at doses of 50 mg/kg QD or 150 mg/kg BID, and representative tumor growth graphs are shown for the 6 PDX models with the greatest rucaparib tumor growth inhibition

SEM, standard error of mean; TNBC, triple negative breast cancer

Equivalent Rucaparib Response in PDX Tumor Models with HRR or *BRCA1/2* Genetic or Epigenetic Alterations

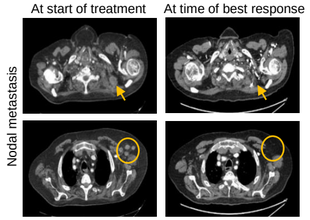


- Rucaparib tumor growth inhibition (TGI) for *BRCA1/2*-mutated or hypermethylated models (A)
- Rucaparib TGI for non-*BRCA1/2* HRR PDXs (B): Of the 13 PDX models with HRR gene mutations, 10 have confirmed biallelic mutations and 3 models have unknown zygosity
- Efficacy was associated with biallelic inactivation of HRR genes (C) with a combined mean TGI for *BRCA1/2* and non-BRCA genes of 77% vs 29% mean TGI for monoallelic *BRCA1/2* alterations (*P*<0.05). Only confirmed biallelic non-*BRCA* HRR genes were included in this analysis
- No significant difference was observed between PDX models with *BRCA1/2* vs non-*BRCA1/2* biallelic alterations

HBCx6, LU-01-0010, CTG-1076 and GXA-3036 models carry *BRCA1* alterations; all other *BRCA1/2* models have *BRCA2* alterations. Mutations are detailed in the supplemental material.
*P<0.05 from vehicle control; #P<0.05

Response to Rucaparib in a Patient with *RAD51B*-Mutated mCRPC

- A 75-year-old man with mCRPC was treated with rucaparib in 2018, 12 years after his initial diagnosis (2006) of adenocarcinoma of the prostate (pT3a Nx M0, Gleason Score 7)
  - Prior treatments included radical prostatectomy (2006), androgen-deprivation therapy (2014), abiraterone (2016), docetaxel (2017), and radiotherapy (2018)
- Next-generation sequencing using Foundation Medicine[9,10] assays detected a deleterious truncating rearrangement of *RAD51B* in a bone lesion and in the plasma of this patient
  - No other drivers of disease or response were detected*
  - No family history of cancer



- Rucaparib treatment was initiated at the recommended starting dose of 600 mg BID with subsequent dose reductions
- The patient had both a confirmed radiographic partial response with a maximum sum of target lesion decrease of 81%, and a prostate-specific androgen (PSA) response with a maximum decrease of 99%
- The total time on treatment was 107 weeks
- Post-rucaparib, the patient received enzalutamide, and died in 2021

*The presence of undetected somatic driver mutations such as homozygous BRCA loss cannot definitively be ruled out. They may have developed after the archival tumor tissue was collected in or missed in the plasma sample due to low (10%) tumor content.
rPD, radiographic progressive disease; rPR, radiographic partial response

SUMMARY

- ***In vitro* siRNA knockdown of a subset of HRR genes showed synthetic lethality with rucaparib treatment in ovarian and prostate cancer cell lines**
- **Rucaparib efficacy observed in PDX models with deleterious alterations in a core group of non-BRCA HRR genes was similar to the efficacy observed in *BRCA1/2* altered models across different solid tumors, with enhanced sensitivity in biallelic altered tumors**
- **Phase 2 trial LODESTAR (NCT04171700) has been designed to evaluate rucaparib efficacy in a wide range of solid tumors associated with deleterious alterations in *BRCA1*, *BRCA2*, *PALB2*, *RAD51C,* and *RAD51D* HRR genes**

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