Abstract 1716

Rucaparib Induces IFN Type 1 Regulated Genes and Enhances Immune-Associated Tumor Suppression

Minh Nguyen, Liliane Robillard, Kevin K. Lin, Thomas C. Harding, Andrew D. Simmons

Clovis Oncology, Inc., Boulder, CO, USA

BACKGROUND

The poly(ADP-ribose) polymerase (PARP) inhibitor rucaparib effectively kills homologous recombination (HR) deficient cells through inhibiting DNA repair, causing DNA damage and apoptosis. Detection of cytosolic DNA by the stimulator of interferon genes (STING) pathway mediates interferon (IFN) pathway signaling and activates the immune system. Following rucaparib treatment, the accumulation of damaged DNA in HR deficient tumors may elicit an immune response through STING signaling, and enhance rucaparib activity as a single agent or in combination with immune checkpoint blockade. To test this hypothesis, the efficacy and mechanism of action of rucaparib were evaluated using *BRCA* deficient syngeneic ovarian carcinoma models.

METHODS

***In vivo* studies:** Studies in the syngeneic ovarian cancer BrKras model were performed at Crown Biosciences in FVB mice. Tumors were established by subcutaneous implantation of 1×10^7 BrKras cells, and rucaparib was administered by oral gavage at 150 mg/kg BID. For monotherapy efficacy studies, rucaparib was given for 28 days and then tumor growth monitored in the absence of further dosing. For the re-challenge study, tumor free mice (assessed at day 125) from the original efficacy study and naïve mice (n=5 mice/group) were implanted with 1×10^7 BrKras cells. Tumor growth was monitored in the absence of drug administration. For immune depletion studies, mice were dosed for 21 days with anti-CD4 or anti-CD8 (BioXcell), and with rucaparib or anti-PD-L1 at the indicated doses. For combination studies, anti-PD-L1 (10F.9G2) and anti-PD-1 (RPMI1-14) were given at the indicated doses for 28 days, and tumor growth monitored. For PD studies, mice were dosed with 150 mg/kg BID rucaparib and tumors were collected on days 3, 6, 7, and 9.

Flow cytometry and IHC analyses: For flow cytometry, cells were dissociated using a mouse dissociation kit (Miltenyi Biotec) and a GentleMACS dissociator, and stained with CD3, CD4, and CD8 antibodies (BioLegend). For IHC, FFPE samples were stained with a CD8 antibody (Abbiotec) and scanned using the NanoZoomer-HT (Hamamatsu).

NanoString analysis: RNA was isolated from frozen tumors or cultured cell lines using RNeasy kit (Qiagen) and analyzed using NanoString nCounter Mouse PanCancer Immune Profiling Panel. Cell type scores were computed as the mean log 2 normalized expression of each cell's marker genes. Cell type scores and expression levels were analyzed in log 2, and divided by the average to obtain relative levels.

***In vitro* studies:** For siRNA knockdown, cells were seeded in 6-well plates and transfected with siRNA and DharmaFect (Dharmacon). Twenty-four hours post-transfection, 1×10^5 transfected or non-transfected cells/well were seeded in 6-well plates and treated with 5 µM rucaparib, 50 µg/mL DMXAA, or DMSO. Cells and supernatants were collected 8, 24 and 72 hours later for ELISA, qRT-PCR array, and NanoString analyses.

qRT-PCR and ELISA analysis: RNA was analyzed using the IFN type 1 PCR array (SABiosciences) or individual TaqMan assays on the ViiA7 system (ABI). For ELISA analysis, supernatants were tested for protein levels using DuoSet kits (R&D Systems).

IRF1 and ISRE luciferase reporter cell lines: ID8F3 and ID8B3.15 were transduced with lentivectors encoding IRF1 or ISRE transcriptional elements driving luciferase expression (Qiagen), and selected with puromycin for 1 week. Cells were treated with rucaparib for 96 hours, and luciferase monitored using Luciferase Assay (Promega).

Table 1. Cell line characterization

Cell Line	Genetic modification of ovarian cancer cell lines	Source	Rucaparib IC_{50} mean ± SD (nM)
C2Km	TP53$^{-/-}$; myc; Kras-G12D; Akt	Sandra Orsulic	12766 ± 1704
BrKras	BRCA1$^{-/-}$; TP53$^{-/-}$; myc; Kras-G12D; Akt	Sandra Orsulic	83 ± 8
ID8F3	TP53$^{-/-}$	Iain McNeish	10754 ± 686
ID8B3.15	BRCA2$^{-/-}$; TP53$^{-/-}$	Iain McNeish	936 ± 200

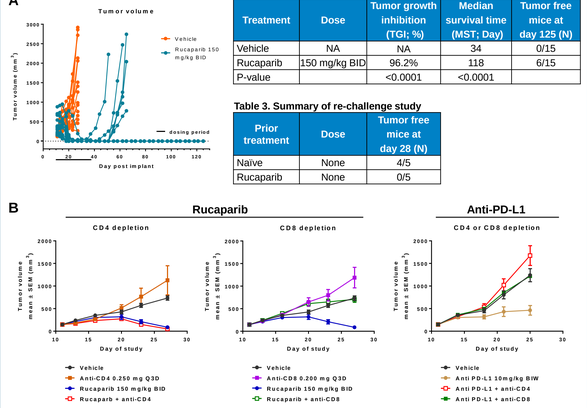
Rucaparib demonstrates potent antitumor efficacy mediated through tumor infiltrating CD8 T cells

Table 2. Summary of efficacy study

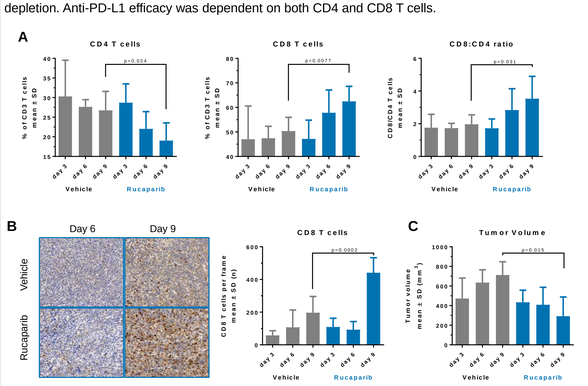
Treatment	Dose	Tumor growth inhibition (TGI; %)	Median survival time (MST; Day)	Tumor free mice at day 125 (N)
Vehicle	NA	NA	34	0/15
Rucaparib	150 mg/kg BID	96.2%	118	6/15
P-value		<0.0001	<0.0001	

Table 3. Summary of re-challenge study

Prior treatment	Dose	Tumor free mice at day 28 (N)
Naïve	None	4/5
Rucaparib	None	0/5

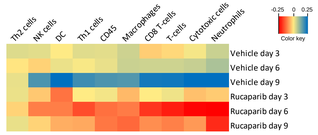


The ovarian syngeneic BrKras model was selected for evaluating rucaparib immunomodulatory activity because of its deletions in *TP53* and *BRCA1* and *in vitro* response to rucaparib (Table 1). **A.** Rucaparib showed potent efficacy, suppressing BrKras tumor growth with 96.2% TGI, 118 days MST, and complete regression in 6 out of 15 mice (Table 2). Prior rucaparib treatment provided immune protection against tumor formation upon re-challenge (Table 3). **B.** Depletion of CD8 T cells had significantly reduced the efficacy of rucaparib, whereas a limited effect was observed with CD4 depletion. Anti-PD-L1 efficacy was dependent on both CD4 and CD8 T cells.

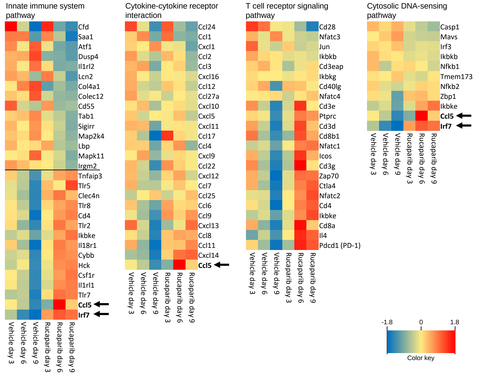


The BrKras model was used in a pharmacodynamic (PD) study to profile tumor immune infiltration following 3, 6, and 9 days of rucaparib treatment. **A.** Flow cytometry analysis of CD4 and CD8 T cells isolated from BrKras tumors showed an increase in the percentage of CD8 T cells and decrease in CD4 T cells following 9 days of rucaparib treatment. **B.** IHC analysis of tumors on day 9 was consistent with flow cytometry data showing an increase in CD8 T cells in rucaparib treated tumors. Representative IHC images are shown on the left panel and quantitative assessment of CD8 T cells are shown on the right. **C.** Consistent with the kinetics of immune cell infiltration, the tumor volume of rucaparib treated tumors was smaller than vehicle treated tumors on day 9.

Expression analysis confirms potent *in vivo* induction of multiple immune compartments by rucaparib



Tumor RNA isolated from the BrKras PD study were evaluated using the NanoString PanCancer Immune Profiling Panel. Cell type scoring in rucaparib treated tumors showed an increase in immune cell type scores starting on day 3 with dendritic cells (DC) and day 6 with several immune subtypes including T cells and macrophages, while vehicle treated tumors showed a decrease in cell scores on day 9.



Plots show the relative expression levels of the genes up or down regulated by rucaparib in each of the indicated pathways. The left panel only shows the top 15 genes with the greatest change in expression. Notably, the STING pathway genes Ccl5 and Irf7 were highly induced in rucaparib treated tumors. The expression of most genes associated with T cell receptor signaling was higher on day 6 than day 3; however the expression of some of these genes decreased on day 9.

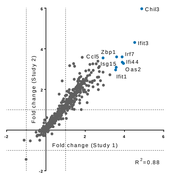
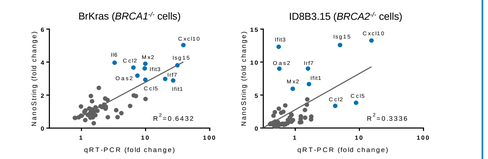


Table 4. Summary of rucaparib induction of genes

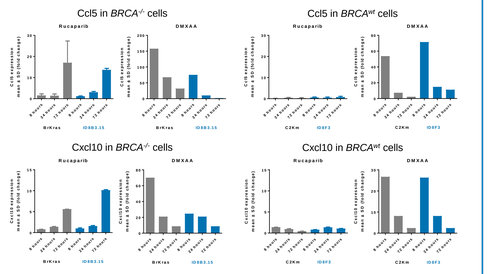
Gene	PD Study 1 Log 2 fold change	PD Study 1 SD	PD Study 1 P-value	PD Study 2 Log 2 fold change	PD Study 2 SD	PD Study 2 P-value
Ccl5	2.91	0.39	6.6E-05	3.55	0.61	2.4E-05
Chil3	4.88	1.01	1.3E-03	5.97	1.24	1.9E-04
Ifi44	3.89	0.54	9.5E-05	3.34	0.39	2.2E-07
Ifit1	3.58	0.51	1.1E-04	3.09	0.34	1.2E-07
Ifit3	4.52	0.52	2.5E-05	4.31	0.43	2.5E-08
Irf7	3.88	0.48	4.1E-05	3.60	0.45	5.1E-07
Isg15	3.55	0.41	2.5E-05	2.96	0.30	3.6E-08
Oas2	3.95	0.53	6.8E-05	3.28	0.42	7.6E-07
Zbp1	3.60	0.42	2.4E-05	3.60	0.56	7.6E-06

Two additional BrKras PD studies were performed, and the tumors were assessed by NanoString Immune Profiling panel following 7 days of rucaparib treatment. Of the ~390 genes detected in both studies, the nine genes with the highest fold change over vehicle (blue dots in left graph, Table 4) were all IFN inducible genes including Ccl5 and Irf7.

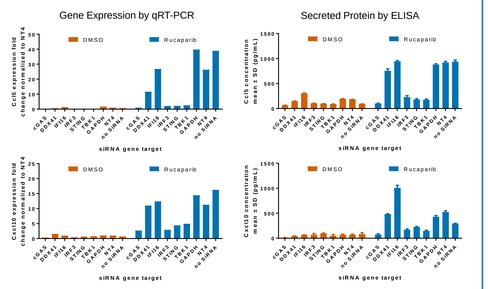
Rucaparib induces Ccl5 and Cxcl10 expression through STING-TBK1-IRF3 signaling in *BRCA*$^{-/-}$ cells



BRCA$^{-/-}$ BrKras and ID8B3.15 cells were incubated *in vitro* with 5 µM rucaparib for 72 hours. RNA levels by IFN type 1 PCR array and NanoString Immune Profiling assay were determined. Of the ~60 genes shared between PCR array and NanoString assay, several genes were upregulated including Ccl5 and Irf7. The chemokine Cxcl10 showed the greatest change in expression with rucaparib treatment.

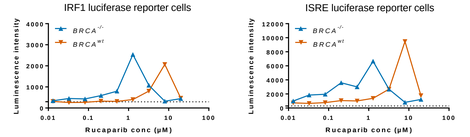


BRCA$^{-/-}$ (BrKras and ID8B3.15) and *BRCA*^{wt} (C2Km and ID8F3) cells were incubated with 5 µM rucaparib, 50 µg/mL DMXAA (STING agonist),[3] or DMSO. Following 8, 24 and 72 hours of treatment, gene expression changes were assessed by qRT-PCR using Taqman and ELISA. Rucaparib increased Ccl5 and Cxcl10 levels in *BRCA*$^{-/-}$ cells but not in *BRCA*^{wt} cells, while DMXAA increased Ccl5 and Cxcl10 in all cells.



BRCA$^{-/-}$ cells ID8B3.15 were transfected with siRNA targeting the indicated STING pathway and DNA sensing genes. Two days later 5 µM rucaparib was added. After 72 hours incubation, qRT-PCR (left graphs) and ELISA (right graphs) analyses of Ccl5 and Cxcl10 levels showed knockdown of cGAS, Irf3, STING, and Tbk1 decreased rucaparib induction of Ccl5 and Cxcl10, whereas knockdown of the other genes had no effect. qRT-PCR of each targeted gene confirmed siRNA knockdown persisted 5 days after transfection (data not shown).

Rucaparib induces transcriptional activation of IFN signaling in *BRCA*$^{-/-}$ luciferase reporter cells



BRCA$^{-/-}$ and *BRCA*^{wt} cells were engineered to express luciferase through interferon regulatory factor 1 (IRF1; left) or IFN stimulated response element consensus sequences (ISRE; right).[4] Cells were incubated with serial dilutions of rucaparib for 96 hours, and luciferase levels were measured by luminescence. Rucaparib showed activation of IFN signaling through IRF1 and ISRE sequences in *BRCA*$^{-/-}$ cells; higher concentrations were required in *BRCA*^{wt} cells.

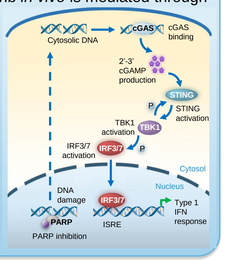
Anti-PD-1/PD-L1 combinations improve the efficacy of rucaparib in a *BRCA*$^{-/-}$ syngeneic model

Treatment	Dose	Tumor growth inhibition (TGI; %)	Median survival time (MST; Day)	Tumor free mice at day 125 (N)
Vehicle	NA	NA	34	0/15
Rucaparib	150 mg/kg BID	96.2%	118	6/15
PD-1	10 mg/kg BIW	91.4%	83	5/15
Rucaparib + PD-1	150 mg/kg BID; 10 mg/kg BIW	110.0%	NA	15/15
PD-L1	10 mg/kg BIW	78.5%	41	2/15
Rucaparib + PD-L1	150 mg/kg BID; 10 mg/kg BIW	111.6%	NA	13/13

Rucaparib and anti-PD-1 or anti-PD-L1 inhibition combinations were evaluated in the BrKras model at the schedules and doses shown. Rucaparib showed enhanced activity in combination with anti-PD-1 or anti-PD-L1.

CONCLUSIONS

- Rucaparib treatment in *BRCA*$^{-/-}$ ovarian carcinoma cells, and at a higher concentration in *BRCA*^{wt} cells, triggers IFN signaling through the STING pathway and induces expression of the chemokines Ccl5 and Cxcl10 *in vitro*.
- The single agent efficacy of rucaparib *in vivo* is mediated through the activation of immune cells including CD8 T cells and induction of IFN inducible genes.
- Rucaparib enhances the anti-tumor activity of the combination rucaparib and checkpoint inhibitors in the syngeneic tumor model.
- The combination rucaparib plus checkpoint blockade is currently being evaluated in prostate cancer (NCT03338790) and gynecologic cancer (NCT03101280).



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