Targeted DNA Damage Repair CRISPR/Cas9 Knockout Screen Identifies Novel Classification of Poly-ADP Ribose Polymerase Inhibitors Based on Key Base Excision Repair Proteins

Gregory A. Breuer[1,2], Jonathan Bezney[1], Nathan R. Fons[1,2], Ranjini K. Sundaram[1], Wanjuan Feng[3], Gaorav P. Gupta[3], and Ranjit S. Bindra[1,2,*]

[1] Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT 06520-8034, USA

[2] Department of Pathology, Yale University School of Medicine, New Haven, CT 06520-8034, USA

[3] Department of Radiation Oncology, University of North Carolina, Chapel Hill, 27599

*To whom correspondence should be addressed. Email: ranjit.bindra@yale.edu

ABSTRACT

DNA repair deficiencies have become an increasingly promising target for novel therapeutics within the realm of clinical oncology. Recently, a number of inhibitors of Poly(ADP-ribose) Polymerases (PARPs) have received approval for the treatment of ovarian cancers with and without deleterious mutations in the homologous recombination proteins BRCA1 and BRCA2. Unfortunately, as over a hundred clinical trials are actively underway testing the utility of PARP inhibition across dozens of unique cancers, the mechanism of action for such inhibitors remains unclear. While many believe PARP trapping to be the most important determinant driving the cytotoxicity found in such inhibitors, clinically effective inhibitors exist which possess both strong and weak PARP-trapping qualities. Such results indicate that characterization of inhibitors as strong and weak trappers does not properly capture the intra-class characteristics of such small molecule inhibitors. Using a novel, targeted DNA damage repair and response (DDR) CRISPR/Cas9 screening library, we describe a new classification scheme for PARP inhibitors that revolves around sensitivity to key modulators of the base excision repair (BER) pathway, unrelated to trapping ability or catalytic inhibition of PARP. These findings demonstrate that inhibition of PARylation and induction of PARP trapping are not the only factors responsible for the clinical response of DDR-deficient cancers to PARP inhibition, and provide insight into the optimal choice of PARP inhibitor to be used in the setting of additional DNA repair deficiencies.

INTRODUCTION

Over the last decade, inhibitors of Poly(ADP-ribose) Polymerase-1 and -2 (PARP1; PARP2) have been described as well-tolerated and effective therapies in the presence of deleterious mutations in the homologous recombination proteins BRCA1, BRCA2, and others[1,2,3]. The PARP family of proteins are known utilize NAD+ to add one (mono-) or more (poly-) ADP-ribose chains to target proteins in response to stimulus[4]. While most proteins downstream from PARP1 and PARP-2 are known to act in

DNA damage repair and response (DDR) pathways, over 170 different interactions have been described including roles in cell cycle regulation and cell motility[5,6]. Additionally, the targets of such PARylation events are known to be stimulus-dependent[7]. Most famously, PARP proteins have been shown to bind to single strand breaks (SSBs) within the DNA and recruit proteins such as XRCC1 and others for resolution of such lesions[8]. Prevention of SSB repair can result in increased replication stress, unpaired double-strand breaks (DSBs), and difficulty with replication restart causing increased cytotoxicity in the presence of many HR defects[9,10,11].

Although the primary mechanism of PARP inhibitor synthetic lethality was originally thought to be through the conversion of SSBs to DSBs, recent evidence suggests that the effect of "trapping" PARP1 at sites of SSB repair may be more important[12,13]. Trapping has been exhibited for both PARP1 and PARP2, though PARP1 remains the most important family member regarding SSB repair and the induction of synthetic lethality[14]. Despite this new insight, clinically relevant PARP inhibitors exist across a wide spectrum of potencies and specificities - in PARP trapping ability, potency of catalytic inhibition of PARylation, and efficacy in targeting other members of the PARP family of proteins[15]. Additionally, loss of PARP function in the setting of HR deficiencies shows moderate growth inhibition independent of trapping inhibitors, indicating that both actions may be important for cell toxicity[16].

To complicate matters, recent studies suggest synthetic lethality extends beyond BRCA1 and BRCA2 to additional DDR proteins such as ATR, ATM, and others[17]. Furthermore, the relationship between PARP inhibition and cytotoxicity in the setting of HR-deficiencies is so intertwined that PARP inhibitor sensitivity has been used to determine novel functions of previously uncharacterized genes such as mutant IDH1 and ribonuclease H2[18,19]. As *in vitro* studies continue to show an ever-expanding landscape of possible uses for PARP inhibition, it is not fully understood whether these sensitivities extend across the entire class of PARP inhibitors, or only one or two of the tested agents. With the knowledge that trapping ability is functionally independent of catalytic inhibition, we set out to characterize the utility of the clinically available inhibitors -- Olaparib, Rucaparib, Talazoparib, Niraparib, and Veliparib. Using a high coverage, targeted DDR CRISPR/Cas9-based screening library, we have developed a novel assay focused solely on known modulators of DNA damage repair and response for greater sensitivity and reproducibility. In addition, we have characterized the most clinically relevant PARP inhibitors based on inhibition of PARylation and PARP1 trapping ability to look for patterns in induced sensitivity to PARP inhibition in the presence of such DDR deficiencies. We report here that clinically relevant PARP inhibitors can be functionally clustered into two unique classes based on activity in the presence of base excision repair (BER) defects, and not in PARP1 trapping ability as was previously expected. These results show that effectors of response to PARP inhibitors extend beyond the scope of HR perturbation and PARP trapping, and suggests that a better understanding of secondary targets may be critical for the optimal application of the numerous PARP inhibitors now reaching the clinic.

MATERIALS AND METHODS

Cell lines and reagents

Colorectal adenocarcinoma cell lines DLD-1 and DLD-1 BRCA2 -/- were used and maintained in RPMI medium with 10% fetal bovine serum (FBS; Gibco) at 37ºC with 5% CO_2. The DLD-1 BRCA2$^{-/-}$ cell line has an engineered exonic deletion as described[20]. HEK293FT (ThermoFisher) are human embryonal kidney cells maintained in Dulbecco's Modified Eagle's Medium, high glucose (DMEM; Thermo Scientific/Gibco) supplemented with 10% FBS. MCF10A cells are normal human mammary cells and were maintained in DMEM/F12 media (Gibco, #11330032) supplemented with 5% horse serum, 10 ng/ml epidermal growth factor, 0.5 mg/ml hydrocortisone, 100 ng/ml cholera toxin, and 10 ug/ml insulin. PARP inhibitors tested for the purposes of this study were obtained from the following vendors: Talazoparib (Selleckchem; #S7048), Olaparib (Selleckchem; #S1060), Rucaparib (Selleckchem; #S1098), Niraparib (Selleckchem; #S2741), Veliparib (Selleckchem; #S1004), A-966492 (Selleckchem; #S2197), KU-0058948 (Axon Medchem; #2001), NMS-P118 (Selleckchem; #S8363), E-7449 (Selleckchem; #S8419), AG-014699 (Axon Medchem; #1529), BGB-290 (BeiGene).

PARylation Immunoblot

To measure PARylation inhibition, MCF10A cells were plated on 96-well microplates (Greiner) at a density of 20k cells/well 24h prior to treatment with methyl-methanesulfonate (MMS; Sigma) and indicated PARP inhibitors. After 24h in culture, media from the plates was aspirated and a fresh 75 µl of pre-warmed media was added to each well. To this, 25 µl of media containing either 0.01% MMS, PARP inhibitors, or a combination of the two were added to each well. Cells were incubated for 30m in normal culture conditions. Following the 30m culture, media was aspirated and cells were rinsed once with PBS. Cell cultures were lysed with RIPA lysis buffer for 30m at 4ºC with occasional agitation. Lysates were spotted on nitrocellulose membrane (BioRad) and allowed to dry at room temperature for 1h. Blocking was performed in TBS-T with 5% BSA (Gold Biotechnology) for 1h at room temperature, followed by overnight incubation with anti-PAR antibody (Trevigen, #4336-BPC-100) at 4ºC. After primary incubation, three 10-minute washes with TBS-T were performed, followed by 1h incubation with HRP anti-rabbit conjugated secondary antibody (ThermoFisher; #31462) at room temperature under constant agitation. Images obtained on ChemiDoc (BioRad) following addition of Clarity Western ECL substrate (BioRad). Image quantification was done using ImageJ imaging software and normalized to no-MMS and no-PARPi control[21]. Curve fitting and data analysis performed using Graphpad Prism (Graphpad Software).

PARP1 Trapping Assay

Preparation of PARP1 dsDNA substrate was performed as previously described[22]. Briefly, single-stranded oligonucleotides were hybridized by combining in equimolar ratio of the following sequences:

5′-AlexaFluor488-ACCCTGCTGTGGGCdUGGAGAACAAGGTGAT

ATCACCTTGTTCTCCAGCCCACAGCAGGGT

This mixture was then heated to 95 ºC for 5m and slowly cooled to room temperature at 5 ºC/min. Hybridized oligonucleotide was then incubated with APE1 and UDG (NEB) at 37ºC for 1h to create a single strand break recognized by the PARP1 enzyme.

To measure inhibition of release of DNA substrate from PARP1 enzyme, 30 nM GST-Tagged PARP1 protein (BPS Biosciences) was incubated with 1 nM DNA substrate and varying amounts of PARPi or DMSO for 1h in reaction buffer containing 50 mM Tris (pH 8.0), 4 mM $MgCl_2$, 10 mM NaCl, and 50 ng/ml BSA in water at RT. After 1 hour, fluorescence polarization readings were recorded using a Cytation 3 (Biotek) multi-mode imager with fluorescence polarization filter prior to adding 1mM NAD+ and every 5 minutes after. Curve-fitting and statistical analysis was performed using Graphpad Prism (Graphpad Software).

The concentration of PARP1 to fluorescent dsDNA substrate was first titrated to optimize detection of polarization via automated plate reader in 96-well half volume microplates (**Figure S1**). To measure trapping efficiency of various PARP inhibitors, purified PARP1 protein, DNA substrate, and varying concentrations of PARP inhibitors were incubated for 1h at room temperature to ensure saturated binding capacity. After the incubation, NAD+ was added to the reaction to initiate release of DNA from PARP1, and polarization measurements were recorded in 5-minute intervals for 120 minutes. Importantly, controls lacking NAD+, PARP1 protein, and DNA substrate were included for normalization.

CRISPR/Cas9 Screening and Analysis

A CRISPR/Cas9 DDR targeted library was assembled using available gene ontology databases and lists of genes involved in DNA damage repair and response. The top 10 suggested sgRNAs targeting each gene were selected from the http://www.genome-engineering.org/ website and supplemented with non-targeting control sgRNAs[23]. These oligos were assembled into the LentiCRISPRv2 lentivirus backbone (Addgene #52961) as described in the original protocols[24,25]. Viral production was carried out in HEK293FT cells by equimolar co-transfection of LentiCRISPRv2 library, psPAX2 (Addgene #12260), and pCMV-VSV-G (Addgene #8454) using Lipofectamine 2000 (Invitrogen; #11668027). Viral titer was assessed upon collection and concentration of lentiviral supernatant with Lenti-X Concentrator (Takara Biotech; #631231). Appropriate final concentrations were chosen to maintain MOI < 0.3 to reduce probability of coinfection with two or more sgRNA sequences. For screening, DLD-1 cells were transduced in 8 µg/ml Polybrene (Sigma-Aldrich) with the multiplexed CRISPR/Cas9 library containing 10 unique sgRNAs targeting 284 different genes involved or implicated in DDR-associated pathways along with one thousand non-targeting sgRNA controls. Cells were then selected with Puromycin (InvivoGen; #ant-pr-1) for 3 days following transduction, ensuring a MOI < 0.3 to prevent multiple sgRNA integrations per cell. After initial selection, cells were split into six treatment groups and treated with appropriate PARPi at calculated GI_{30} or DMSO as indicated to assess effects on both sensitivity and resistance to tested inhibitors. Samples were taken at Day 0 as well as every 2 days to ensure logarithmic growth while maintaining a high sample size.

Preparation and sequencing of samples was conducted using dual-indexed paired-end sequencing on MiSeq System (Illumina) using a 2x150 protocol. Library preparation was conducted two independent primer sets. Primers used in the first reaction amplify the targeted sgRNA region of the integrated vector and primers used in the second reaction allow for indexing and multiplexing during sequencing. Additional spacer sequences of 0-2 bases were inserted between the adapter and sequence-specific portions of the sequencing primers to increase library diversity during sequencing.

Analysis was performed using a rank scoring algorithm similar to one previously described[26]. sgRNAs were extracted from sequencing reads, counted, and normalized to total sample size and non-targeting control abundance. A rank score was calculated for each gene represented in the targeted library by comparing the abundance of each sgRNA to its representation in the targeting library sample. Each screen was done in duplicate and samples were prepared from multiple time points in each treatment group to reduce sampling error.

Short-Term Viability Assays

Short-term viability assays validating individual sgRNA results were performed by first transducing cells in 6-well plates with lentivirus, selecting with Puromycin for 48h, then plating into 96-well plates for 24h prior to adding appropriate concentrations of PARPi. 96h after the initiation of treatment, media was aspirated, cells were washed once with PBS and were then fixed using 4% formaldehyde (Sigma-Aldrich) in PBS solution for 15m at room temperature. Cells were then stained with Hoechst 33342 (Sigma-Aldrich; #B2261) for 45 minutes prior to imaging using the Cytation 3 multi-mode imager as described previously[27]. Cell counting was performed using a pipeline created in CellProfiler image analysis software which stitches images by well and identifies the number of cell nuclei per well by fluorescence staining[28]. Graphing and data analysis was performed using Graphpad Prism (Graphpad Software). Assays utilizing pooled siRNA (Horizon; ON TARGETplus siRNA) were conducted by first transfecting with RNAiMAX (Invitrogen; #13778100) 72h prior to exposure to individual PARP inhibitors to ensure maximum knockdown at initial treatment.

RESULTS

Clinically-relevant PARP inhibitors have varying degrees of specificity for PARP1 trapping and inhibition of PARylation

PARP inhibitors have traditionally been evaluated via quantitative immunoassay measuring downstream PARylation in the setting of induced DNA damage. We adapted this format to 96-well microplates to better accommodate high-throughput quantification and more accurately parallel the methods used in short-term viability assays using the same compounds. As expected, all PARP inhibitors showed dose-dependent inhibition of PARylation in the setting of alkylating damage, with a nearly 1000-fold difference between the most potent inhibitor of PARylation, Talazoparib, and the weakest tested, A-966492 (**Figures 1A-B**). We then tested the same panel of inhibitors in a fluorescence polarization-based assay, which measures binding of PARP1 to a fluorescently-labeled DNA substrate in the presence and absence of PARP inhibition.

As expected, measured polarization of wells containing compounds reported to have strong PARP-trapping characteristics showed increased potency when compared to compounds such as Veliparib, which have been reported to have limited trapping potency (**Supplementary Figures S1A-B**). Similar to results from measured inhibition of PARylation, Talazoparib was again found to be the most potent compound tested in the fluorescence polarization assay with a measured IC50 approximately 10-fold lower than the next most potent compound, Rucaparib (**Figure 1C**). Additional results were found to correlate well with publicly available data. Notably, potency of PARP inhibitors as measured by PARylation immunoassay was not found to be significantly correlated with trapping potency as measured by PARP1 trapping assay ($R^2 = 0.1058$, $p > 0.05$, Spearman $r = 0.3$), indicating that these two processes occur independent of one another (**Figure 1D**).

Both PARP1 trapping potency and inhibition of PARylation fail to independently predict synthetic lethality in HR-deficient cells

Synthetic lethal interactions between PARP1 inhibition and HR-deficiencies are hypothesized to be dependent on the trapping of PARP1 at sites of repair[29]. In order to quantify growth inhibition across all tested PARP inhibitors, we performed short-term viability assays in isogenic HR-proficient and HR-deficient colorectal adenocarcinoma cell lines, DLD-1 and DLD-1 BRCA2-/-, respectively. Growth inhibition in the cell lines across the spectrum of PARP inhibitors was found to vary widely relative to effect on PARylation and PARP1 trapping (**Figures 2A-B**). Growth inhibition in the HR-proficient DLD-1 cell line was found to be significantly correlated with both inhibition of PARylation ($p = 0.006$) and trapping potency ($p < 0.0001$). However, growth inhibition in HR-deficient DLD-1 BRCA2-/- cells was not found to correlate with inhibition of PARylation ($p = 0.345$) and only trended towards a significant correlation with trapping potency ($p = 0.068$). Interestingly, specific growth inhibition in HR-deficient cells relative to wild-type counterparts did not correlate with either inhibition of PARylation ($p = 0.4384$) or PARP1 trapping ($p = 0.7213$). Overall, these findings suggest that neither the inhibition of PARylation, nor the PARP trapping ability of PARP inhibitors independently predicts the induced synthetic lethality of these inhibitors in HR-deficient cell lines (**Figure 2C**).

Targeted CRISPR/Cas9 screen reveals a novel classification of PARP inhibitors

Although much has been published on the effects of PARP inhibition in the setting of specific DNA repair deficiencies, no attempts to date have been made to measure effects of PARP inhibition across the entire spectrum of possible DNA repair deficiencies. We performed a targeted CRISPR/Cas9-based lentiviral screen using five inhibitors that best represented the class of PARP inhibitors based on measured and clinical characteristics. Inhibitors were selected based on clinical availability as well as potency in both PARP trapping and PARylation assays (**Figures 2D-E**).

To evaluate the validity and sensitivity of our assay, analysis of all tested PARP inhibitors were combined in comparison to DMSO-treated control group with the expectation that key proteins involved in homologous recombination would be among the most sensitizing findings. Among the top single-gene knockouts conferring sensitivity to all tested PARP inhibitors were RAD51, XRCC3,

BRCA1, RNF8, ATM, ATR, and others (**Figure 3A**). Knockout of PARP1 was also shown to confer a general resistance to PARP inhibition as expected, though the size of this effect varied depending on the specific inhibitor in question. Individual PARP inhibitors were generally well-correlated with the average response to PARP inhibitors, with Talazoparib being most similar to the average ($R^2 = 0.7307$) and Veliparib and Rucaparib ($R^2 = 0.61, 0.614$) being least correlated to the average response (**Figure S2**).

In order to look for trends in response to single-gene knockouts across multiple inhibitors, dimensionality reduction was performed, using response to each gene as input. Using these techniques, compounds showing similar responses across our targeted library should cluster closer together. Principal components analysis of inhibitors based on response to single-gene knockouts revealed two groupings of clinical PARP inhibitors, with Group A consisting of Talazoparib, Olaparib, and Niraparib and Group B consisting of Veliparib and Rucaparib (**Figure 3C**). These data suggest a novel division of clinically relevant PARP inhibitors based entirely on functional classification in response to deficiencies in DNA repair, and does not appear to correlate with measured inhibition of PARylation or PARP1 trapping potency (**Figure 3D-E**).

Group-specific targets reveal response to XRCC1, LIG3, and PARP1 knockout as key predictors of overall potency of PARP inhibitors

To better evaluate the defining characteristics between Group A and Group B inhibitors, we used publicly available gene ontology data to look for differences in effect of key DDR pathways. Although Homologous Recombination and Fanconi Anemia pathways showed the strongest sensitizing phenotype to both Group A and Group B inhibitors, differences between the two groups were best exemplified by differences in sensitization to key proteins in both Base Excision Repair (BER) and Mismatch Repair (MMR) pathways (**Figure 4A**). Within BER, increased resistance to PARPi in the presence of PARP1 knockout and increased sensitivity to PARPis upon loss of LIG3 and XRCC1 were the most defining characteristics of Group A inhibitors relative to others (**Figure 4B**). Increased sensitivity to POLE4 is also noted among Group A inhibitors followed by differential sensitivities to FEN1, LIG1, and PARP3 approaching significance (**Figure 4B**).

Findings from the initial screen were confirmed first by testing selected sgRNAs from the original library by 96h short-term viability assay and then by pooled siRNA experiments to measure the effect of knockdown, rather than knockout, of each gene in the presence of PARPi. Short-term viability assays were also performed using U2-OS cells to show effects carry across unrelated cell lines, independent of tissue of origin. Both individual sgRNA experiments, as well as siRNA experiments, recapitulated the results seen by pooled CRISPR/Cas9 screening (**Figures 5A-C, S3**). XRCC1 and LIG3 knockouts and knockdowns show increased sensitivity to Group A inhibitors that are far less pronounced or absent in Group B across all assays. These findings confirm the results from our targeted CRISPR/Cas9 screen, showing that loss of function of XRCC1 and LIG3 confer increased sensitivity to some, but not all PARP inhibitors. Interestingly, sensitization to PARP inhibition has been shown previously in the setting of XRCC1 deficiency, however this study was limited only to the

Group A inhibitors, Talazoparib and Olaparib[30]. Additionally, the degree of sensitization in the setting of loss of either XRCC1 and LIG3 appears to correlate with the overall PARP1-dependence of toxicity, and may provide critical insight into better understanding the therapeutic effects of PARP inhibition in the setting of such deficiencies. Overall, the stark differences among various PARP inhibitors in response to deficiencies in PARP1/XRCC1/LIG3 highlight the importance of further research into the field to better understand the most appropriate clinical setting for each of the inhibitors.

DISCUSSION

While the underlying cause of synthetic lethality seen with PARP inhibition in the setting of BRCA and HR deficiencies remains disputed, our data clearly demonstrate that neither trapping potency nor strength of inhibition of PARylation fully explain the response to such inhibitors. These findings are in agreement with recent biochemical studies suggesting that inhibitors of PARP1 fit into three major classifications based on allosteric effects of PARPi binding as well as retention at sites of DNA damage[31]. Similarly, our unbiased analysis of over 280 genes known to be involved in DNA damage repair and response found unique groupings of PARP inhibitors which do not correlate solely with either the ability to inhibit downstream PARylation by PARP1 or the trapping of PARP1 to sites of damage based on widely-used biochemical assays. Across the PARP inhibitors tested in our analysis, we do not observe any correlation between synthetic lethality in the context of HR defects and strength of PARP1 trapping or inhibition of PARylation. Indeed, PARP trapping has been associated with increased toxicity in both normal tissue as well as within tumors, likely resulting in side effects seen in clinical trials such as complete bone marrow failure and other cytopenias[32]. Such findings make appropriate classification of inhibitors for use in patient populations ever more relevant, as the use of PARP inhibitors in clinic becomes increasingly common.

Within our screen, we see strong sensitization to all PARP inhibitors through knockout of key components of HR (RAD51, BRCA1, BRCA2, etc.), however only three of our tested inhibitors respond to loss of function of proteins immediately downstream of PARP1 in BER. Interestingly, loss of XRCC1 and LIG3 was found to be most toxic to cells concurrently treated with inhibitors that are dependent on PARP1 for sensitization (Group A PARP inhibitors). We hypothesize that this observation may be due to one or more of the following mechanisms. i) PARP1-independent inhibitors may be maximally disrupting downstream BER through disruption of PARP1 signaling at lethal doses, so further loss of function does not alter response to inhibition. ii) Loss of XRCC1 and LIG3 results in upregulation of PARP1, and is therefore increasing opportunities for PARP1-dependent toxicity. Loss of XRCC1 has previously been shown to cause PARP1 hyperactivation, and increased expression of PARP1 has been correlated with sensitivity to some PARP inhibitors[33]. iii) loss of XRCC1 and LIG3 results in unrepaired lesions of the DNA, which may be preferentially targeted by PARP1-dependent inhibitors. Additional work is necessary to help tease apart such mechanisms and further evaluate the utility of various classes of PARP inhibitors in specific clinical settings.

Although there are over 250 active clinical trials testing PARP inhibitors in cancer at the time of this writing, there is little information regarding appropriate selection of PARP inhibitor therapy and

utilization of PARP inhibitors in patients who have failed to respond to one or more of such inhibitors. Likewise, no head-to-head clinical trials comparing PARP inhibitors have been completed to date, making selection of PARP inhibitor treatment in the clinical setting difficult. Neither PARP trapping nor catalytic inhibition of PARylation appear to explain the efficacy of PARP inhibition in the treatment of cancers with DNA repair deficiencies. Our results indicate that the efficacy of PARP inhibitors may hinge on some combination of PARP trapping and inhibition of downstream targeting of PARP1, with a handful of inhibitors, Talazoparib > Niraparib > Olaparib, being far more dependent on the presence of PARP1 than others. Clinical trials are necessary to determine the utility of PARP1-independent inhibitors in the setting of limited PARP1 expression. Additionally, patients with mutations in XRCC1 and LIG3 may benefit from treatment with Talazoparib, Olaparib, or Niraparib over treatment with PARP1-independent inhibitors. Further studies are necessary to determine how these results may affect response to treatment in patients, and whether our findings may translate into a clinical setting. Overall, our results highlight an exciting technique in functional analysis of PARP inhibition via CRISPR/Cas9 libraries, and show the importance of functional BER in the setting of select PARP inhibitors.

ACKNOWLEDGEMENT

This work was supported in part by the US National Institutes of Health (NIH) grant R01CA215453.

CONFLICT OF INTEREST

RSB is co-founder, consultant, and equity holder of Cybrexa Therapeutics.

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TABLE AND FIGURES LEGENDS

Inhibitor	Manufacturer	FDA Approved Indication
Niraparib	Tesaro	Recurrent ovarian cancer
Rucaparib	Clovis	BRCA-mt ovarian cancer
Olaparib	AstraZeneca	Maintenance for advanced ovarian
Talazoparib	Pfizer	BRCA-mt HER2- breast cancer
Veliparib	AbbVie	Phase 3 Trials Ongoing
BGB290	BeiGene	Phase 1 Trials Ongoing
A-966492	-	-
AG-14361	-	-
E7449	-	-
KU0058948	-	-
NMS-P118	-	-

Table 1. Tested PARP inhibitors, manufacturer, and approved FDA indications, if any.

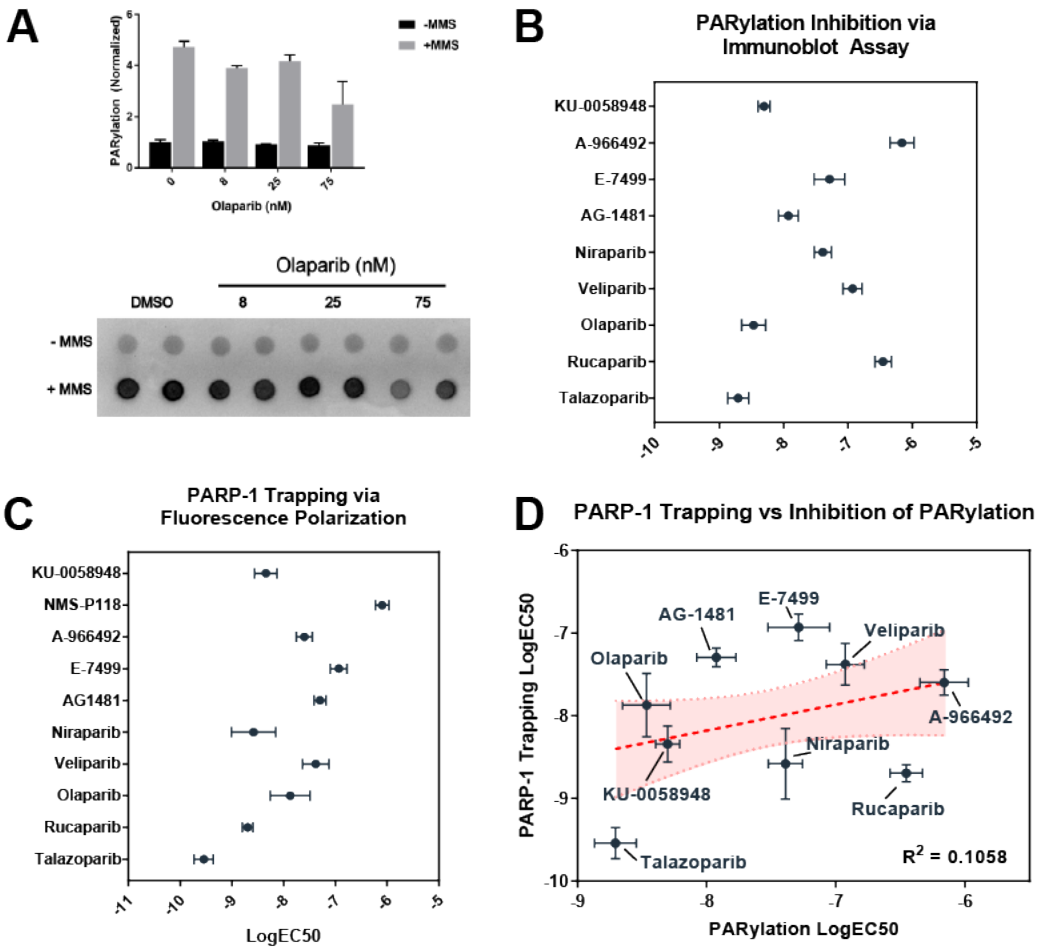
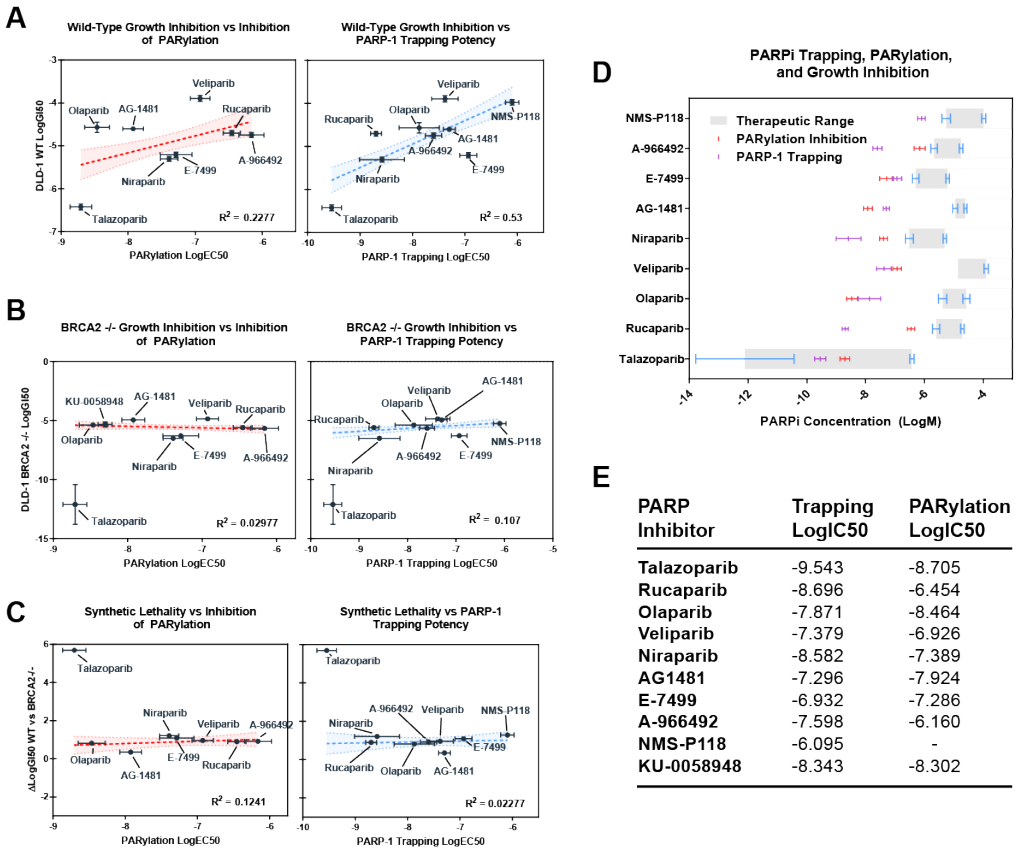


Figure 1. (A) Representative example of PARylation immunoassay in MCF10A cells in presence and absence of 0.01% MMS and increasing amounts of the PARP inhibitor Olaparib. (B) Catalytic inhibition of PARylation EC_{50} as measured by PARylation immunoassay +/- SEM. (C) PARP1 trapping EC_{50} per agent as measured by fluorescence polarization assay +/- SEM. (D) Comparison of PARP1 trapping potency and catalytic inhibition of PARylation.



PARP Inhibitor	Trapping LogIC50	PARylation LogIC50
Talazoparib	-9.543	-8.705
Rucaparib	-8.696	-6.454
Olaparib	-7.871	-8.464
Veliparib	-7.379	-6.926
Niraparib	-8.582	-7.389
AG1481	-7.296	-7.924
E-7499	-6.932	-7.286
A-966492	-7.598	-6.160
NMS-P118	-6.095	-
KU-0058948	-8.343	-8.302

Figure 2. (A) Correlation between PARP1 trapping potency or inhibition of PARylation with growth inhibition in HR-proficient and -deficient DLD-1 cells. Correlation between growth inhibition in HR-proficient cells and inhibition of PARylation was significant (R^2 = 0.2277; p = 0.006), as was correlation with PARP1 trapping potency (R^2 = 0.53, p < 0.0001). (B) In DLD-1 BRCA2$^{-/-}$ cells, there was no observed correlation between growth inhibition and inhibition of PARylation (R^2 = 0.0298, p = 0.345), and correlation with PARP1 trapping only trended towards significance (R^2 = 0.107, p = 0.068). (C) Synthetic lethality does not correlate with either strength of inhibition of PARylation or trapping potency. (D) Visual representation of logIC50 values for trapping potency, inhibition of PARylation, and growth inhibition in HR-proficient and -deficient DLD-1 cells. (E) Summary of results from D.

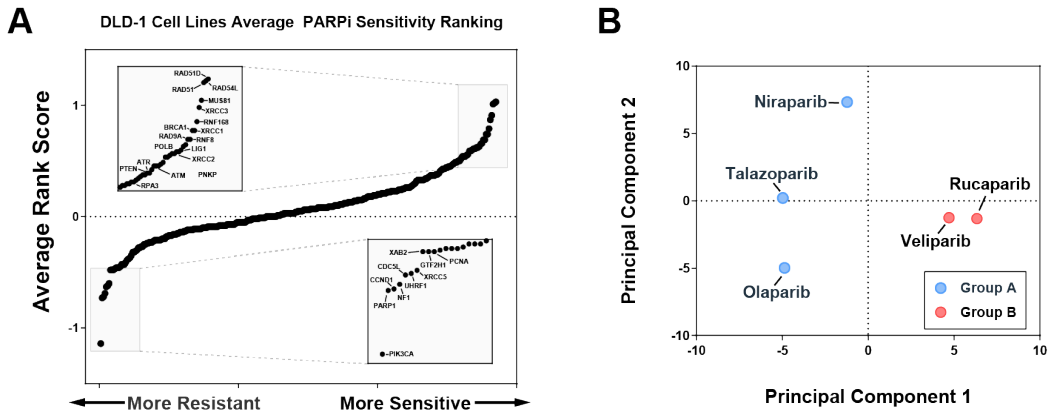


Figure 3. (A) Rank order average of tested inhibitors over entire screening set with single-gene knockouts conferring increased sensitivity or resistance highlighted at the extremes. (B) Principal components analysis of tested inhibitors reveals two distinct groups of inhibitors with Talazoparib, Olaparib, and Niraparib making up Group A and Veliparib, Rucaparib making up Group B.

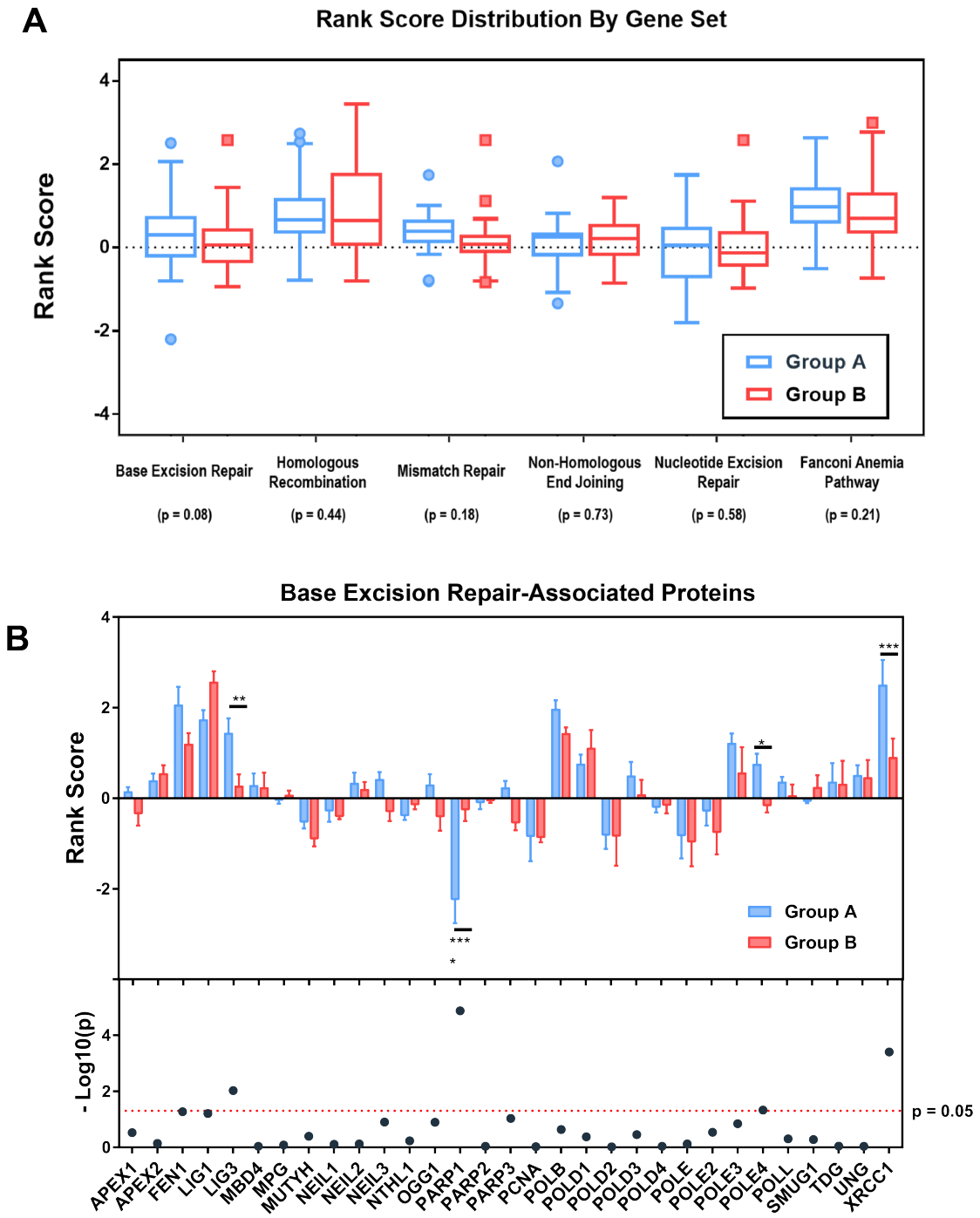


Figure 4. (A) Comparison of group-averaged rank score by associated pathway. Higher rank scores are associated with increased sensitivity to loss of function of proteins within each reported pathway. A single gene may appear in more than one pathway. (B) Per-gene rank scoring by PARPi group reveals significant differences in PARP1, LIG3, XRCC1, and POLE4 response to inhibitors ($p<0.0001$, $p=0.0094$, 0.0004, and 0.047 via student's t-test). Additional genes involved in base excision repair approaching significance include FEN1, LIG1, and PARP3 ($p=0.054$, 0.062, 0.093).

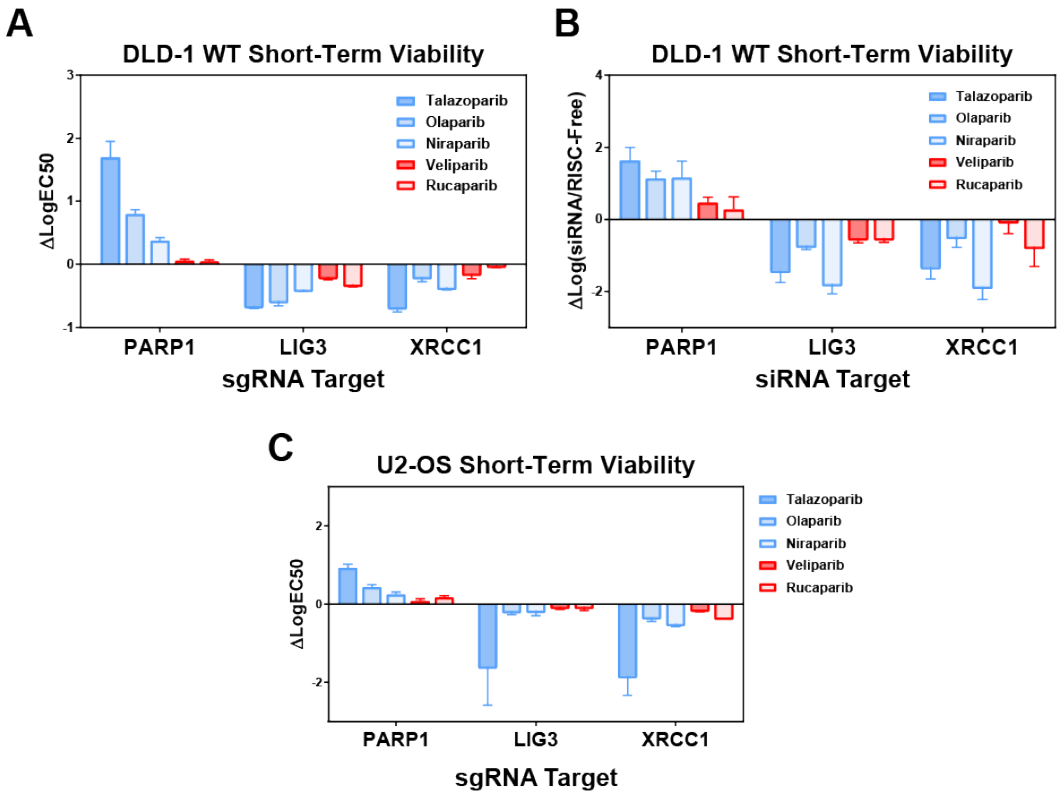


Figure 5. (A) Short-term viability assays reveal specific response to PARP1, LIG3, and XRCC1 seen from CRISPR/Cas9 screen. Strong resistance in presence of PARP1 knockout also associated with increased sensitivity in presence of XRCC1, LIG3 knockout. (B) Pooled siRNA knockdown of each of the reported genes shows similar phenotype to CRISPR/Cas9 lentiviral knockout; again showing increased sensitivity to Talazoparib, Olaparib, and Niraparib in the presence of XRCC1/LIG3 disruption. (C) Short-term viability assays in U2-OS cell line shows similar phenotype with lentiviral CRISPR/Cas9 knockout of reported genes.