Published in final edited form as:

Mol Cancer Res. 2015 October ; 13(10): 1389–1397. doi:10.1158/1541-7786.MCR-15-0036.

YU238259 Is a Novel Inhibitor of Homology-dependent DNA Repair that Exhibits Synthetic Lethality and Radiosensitization in Repair-deficient Tumors

Gregory C. Stachelek^{a,b}, Elizabeth Peterson-Roth^a, Yanfeng Liu^a, Rafael J. Fernandez III^a, Luke R. G. Pike^a, Jack M. Qian^a, Laura Abriola^c, Denton Hoyer^c, William Hungerford^c, Janie Merkel^c, and Peter M. Glazer^{a,b}

^aDepartment of Therapeutic Radiology, Yale School of Medicine, 15 York St., New Haven, CT 06520

^bDepartment of Genetics, Yale School of Medicine, 15 York St., New Haven, CT 06520

^cYale Center for Molecular Discovery, 600 West Campus Dr., West Haven, CT 06516

Abstract

Radiation therapy and DNA-damaging chemotherapy are frequently utilized in the treatment of solid tumors. Innate or acquired resistance to these therapies remains a major clinical challenge in oncology. The development of small molecules that sensitize cancers to established therapies represents an attractive approach to extending survival and quality of life in patients. Here, we demonstrate that YU238259, a member of a novel class of DNA double-strand break repair inhibitors, exhibits potent synthetic lethality in the setting of DNA damage response and DNA repair defects. YU238259 specifically inhibits homology-dependent DNA repair (HDR), but not non-homologous end-joining (NHEJ), in cell-based GFP reporter assays. Treatment with YU238259 is not only synergistic with ionizing radiation (IR), etoposide, and PARP inhibition, but this synergism is heightened by BRCA2-deficiency. Further, growth of BRCA2-deficient human tumor xenografts in nude mice is significantly delayed by YU238259 treatment even in the absence of concomitant DNA-damaging therapy. The cytotoxicity of these small molecules in repair-deficient cells results from an accumulation of unresolved DNA double-strand breaks. These findings suggest that YU238259 or related small molecules may have clinical benefit to patients with advanced BRCA2-negative tumors, either as a monotherapy or as an adjuvant to radiotherapy and certain chemotherapies.

Keywords

DNA repair; synthetic lethality; translational medicine; drug development

Corresponding Author: Peter M. Glazer, Department of Therapeutic Radiology, P.O. Box 208040, New Haven, CT 06520, (203) 737-2788; peter.glazer@yale.edu.

Author contributions: G.C.S., E.P.-R., J.M., and P.M.G. designed research; G.C.S., E.P.-R., Y.L., R.J.F., L.R.G.P., J.M.Q., and L.A. performed experiments; D.H. and W.H. designed and synthesized molecules; G.C.S. and P.M.G. wrote the paper.

The authors declare no conflict of interest.

Introduction

In cancer therapy, gene expression profiling and mutational analyses are increasingly being used to inform the optimal choice of treatment and overall prognosis. Personalized cancer therapies are rapidly becoming the standard of care in clinical practice (1). Thus, novel drugs targeting molecular pathways known to be dysregulated in cancer may have utility as first-line treatments or when treatment has failed. Frequently, the inability to properly coordinate repair of damaged DNA underlies tumorigenesis and disease progression in malignancies (2–6). Human cancer syndromes have been linked to mutations in nearly every DNA repair pathway. These pathways often exhibit redundancy, and inhibition of additional repair factors may induce synthetic lethality in repair-deficient tumors while sparing healthy tissue (7–9). DNA double-strand break (DSB) repair pathways are particularly vulnerable, as shown by recent observations that inactivation of the RAD52 recombinase is lethal in the context of BRCA1, BRCA2, or PALB2 loss (10).

Synthetic lethality is not limited to defects in a single DNA repair pathway and may also occur across complementary repair pathways. This is perhaps best characterized in the relationship between the homology-dependent repair (HDR) and base excision repair (BER) pathways (11). Incompletely resolved lesions that are substrates of BER (e.g., alkylated and damaged bases) generate DNA single-strand breaks (SSBs) that persist into S-phase, leading to stalling and collapse of DNA replication forks and induction of DSBs that must be resolved by HDR. This is exemplified by the enhanced toxicity of poly (ADP-ribose) polymerase (PARP) inhibitors in BRCA1- and BRCA2-deficient cells (12). Clinical trials of PARP inhibitors are ongoing (13–16). Notably, olaparib has shown potential in the treatment of BRCA-mutant ovarian malignancies, which are often metastatic at time of diagnosis, limiting therapeutic options.

Genetic instability is a hallmark of cancer and can quickly confer resistance to even targeted monotherapies or drug combinations. Inhibition of DNA repair pathways can decrease resistance by altering the response of tumor cells to DNA-damaging chemotherapy and ionizing radiation (IR). We have previously shown that inhibition of the BER pathway in a BRCA2-mutant background sensitizes cells to the DNA methylating agent temozolomide (17). Conversely, even in an HDR-defective background, intact DNA mismatch repair is necessary for certain DNA lesions to induce cytotoxicity, rendering mismatch repair-defective cells highly resistant to alkylation and crosslinking (18, 19). Efforts to identify effective radiosensitizing agents have also met with recent success and continue to represent an area of high translational potential (20). Given that improving genomic techniques will soon enable profiling of entire gene pathways in tumors, it is likely that selection of cancer therapies will increasingly be guided by elucidation of the functional status of DNA repair pathways.

We hypothesized that novel small molecules that exploit common DNA repair deficiencies in tumors and synergize with validated chemotherapeutic agents may provide the basis for rapid clinical translation. This led us to develop a synthetic lethality screen for small molecules that were preferentially toxic to cells lacking the well-studied repair proteins FANCD2 and BRCA2, but which exhibited low absolute toxicity in isogenic, repair-

proficient paired cells. Using the commercially available ChemBridge DIVERSet, we have identified several structurally related compounds that fulfill these criteria and, to our knowledge, have not been reported in the literature as anti-neoplastic agents. Each of the compounds possesses a modified sulfonamide backbone that appears essential to the cellular activity of this class. Iterative rounds of chemical synthesis and structure-activity relationship analysis of screening hits have produced a lead compound, YU238259, with increased potency and solubility.

Our work demonstrates that YU238259 is an inhibitor of the HDR pathway, yielding potent synthetic lethality in the context of additional repair defects and allowing it to synergize effectively with agents that induce DSBs in cells. Further, we show that these sulfonamide compounds do not intercalate into DNA and do not inhibit PARP activity. YU238259 and a related compound that was an early hit in our screen, YU128440, also demonstrate synthetic lethality in a mouse model using BRCA2-deficient human tumor xenografts. These characteristics make YU238259 and related molecules promising candidates for translational development.

Materials and Methods

Chemicals

Etoposide, doxorubicin, and caffeine were purchased from Sigma. Olaparib was purchased from Selleck. Rabbit-derived antibodies against PTEN (#9552), ATM (#2873), NBS1 (#3002), ATRIP (#2737), β -tubulin (#2146), and mouse-derived antibody against β -actin (#3700) were purchased from Cell Signaling Technology. Pooled siRNAs against ATRIP and NBS1, as well as non-targeted control siRNA, were purchased from Dharmacon.

Cell lines

DLD-1 and DLD-1 BRCA2-KO human colorectal adenocarcinoma cell lines (Horizon Discovery, Cambridge, UK) were cultured in McCoy's 5A medium with 10% FBS. U251 glioma cells with inducible PTEN (21) were cultured in DMEM with 10% FBS, 0.5 mg/ml G418, and 10 μ g/ml blasticidin. 1 μ g/ml doxycycline was used for induction and PTEN expression was confirmed by western blot. PD20 and PD20+FANCD2 cells were from the OHSU FA Cell Repository and were grown in MEM-alpha with 15% FBS. All other cell lines were cultured in DMEM with 10% FBS. PEO1/4 cells (22) were a gift of Dr. Toshiyasu Tanaguchi. HCC1937+vector and HCC1937+BRCA1 (23) cells were a gift of Dr. Zhong Yun. U2OS DR-GFP and U2OS EJ5-GFP cell lines have been described previously (24, 25). GM05849 ATM-deficient fibroblasts were from ATCC and were stably complemented with wildtype ATM or vector (S. Gibson, unpublished data). U2OS cell line was from ATCC. All paired lines were tested for repair protein expression by western blot.

Screening libraries

ChemBridge DIVERSet and other small molecule libraries were formatted as 10 mM DMSO stocks in 384-well plates. For dose response curves of individual screening compounds, 100 mM stocks were prepared and serially diluted by 2-fold in DMSO.

Synthetic lethality screening assay

Cells were seeded in 20 uL media onto white clear-bottom 384-well plates (Corning) using a MultiDrop Combi Reagent Dispenser (Thermo Scientific). Primary screening used PD20 +/- FANCD2 cells plated at 750 cells/well. Follow-up screening and dose response curves used PEO1 and PEO4 cells plated at 750 and 1500 cells/well, respectively. After seeding, the assay plates were centrifuged at 500 rpm for 10 seconds and incubated overnight. 20 nL compound was then transferred from the source plate to the assay plates using an Aquarius (Tecan) with a 384-well pin tool (V&P Scientific). Final concentration of screening compounds was 10 uM in 0.1% DMSO. Tamoxifen (final conc. 30 uM for PD20 cells and 60 uM for PEO1/4 cells) was added to positive control wells. Assay plates were centrifuged at 500 rpm for 10 seconds and then incubated for 72 h. CellTiter-Glo (Promega) was used to measure cell viability in the assay wells according to the manufacturer's instructions. 20 uL/well of CellTiter-Glo was added to each assay plate using a MultiDrop Combi. Plates were shaken on a Thermomixer R (Eppendorf) at 1100 rpm for 1 minute and incubated in the dark for 10 minutes at room temperature. Luminescence was measured using an Envision plate reader (PerkinElmer) with 0.3 second sampling time per well. Because the positive control yielded loss of signal, Z' scores were calculated by $1 - (3\sigma_{c+} + 3\sigma_{c-})/|\mu_{c-} - \mu_{c+}|$, where μ and σ represent the mean and SD, respectively, of the positive (c+) and negative (c-) control data.

PARP enzymatic assay

PARP activity was assessed using a Universal PARP Chemiluminescent Assay (Trevigen) according to the manufacturer's instructions. 3-aminobenzamide served as a positive control for PARP inhibition.

Circular dichroism spectroscopy

YU128440, doxorubicin, caffeine, or DMSO vehicle were added at 1:1000 to a 1 ug/uL solution of 8 kb plasmid DNA in TE buffer to yield drug concentrations of 10 uM, and incubated at RT for 5 minutes. Samples were read in 10 mm path length quartz micro-cuvettes (Hellma) and CD absorbance was averaged over four measurements from 230 nm to 330 nm using a Chirascan spectrometer (Applied Photophysics).

DSB repair reporter assays

U2OS reporter cell lines (DR-GFP or EJ5-GFP) were pretreated in triplicate with varying concentrations of YU238259 for 24 h, after which 4 ug of SCE-I plasmid was transfected into 1×10^6 cells/replicate using an Amaxa Nucleofector (Lonza). Transfected cells were reseeded on 6-well plates and cultured with YU238259 for an additional 72 h. The percentage of GFP-positive cells was quantified by flow cytometry. Data analysis was performed using FlowJo software (Tree Star Inc.). Error bars represent the standard deviation.

Immunofluorescence

U2OS cells were pretreated with 25 uM YU238259 or DMSO vehicle for 1 h and cells were then irradiated with 10 Gy IR. Cells were fixed at 8 h post-IR, stained with antibodies and

Hoechst dye, and imaged. Foci were quantified using the InCell Analyzer algorithm developed by YCMD. Cells were scored as foci-positive if they contained 15 foci (BRCA1, pDNA-PK) or 20 foci (53BP1).

Neutral comet assay

DLD-1 and DLD-1 BRCA2-KO cells were treated with YU238259 for 4 h and then irradiated with 5 Gy IR. Cells were then harvested either immediately or 24 h post-irradiation and a neutral comet assay was performed per manufacturer's instructions (Trevigen). Comet tail moments were analyzed using CometScore software (TriTek) for at least 50 randomly chosen cells per condition. Error bars represent the standard deviation.

Cell cycle analysis

DLD-1/BRCA2-KO cells were treated with YU238259 for 6 h and then harvested by trypsinization. Cells were fixed in 70% ethanol, treated with 100 µg/ml RNase, and stained with propidium iodide. DNA content was analyzed by flow cytometry for 10000 cells per condition.

Clonogenic survival assays

Cell lines were plated in triplicate at 500–1000 cells/well in 6-well plates. Cells were treated with YU238259 for 48 hours. For synergism assays, cells were pretreated with YU238259 for 24 h, then irradiated or treated with etoposide or olaparib for an additional 24 h in presence of YU238259. For siRNA experiments, U2OS cells were transfected with ATRIP or NBS1 pooled siRNA or non-targeting control per manufacturer instructions, incubated for 72 h to ensure maximal knockdown, and then treated with YU238259 for 48 h. Cells were cultured for 1–2 weeks until well-defined colonies had formed, replacing culture media every 2–3 days. Cells were briefly permeabilized with 0.9% saline solution and stained with crystal violet in 80% methanol. Colonies of 50 cells were then counted visually. Data points represent the average of the three values and error bars represent the standard deviation.

Mouse tumor xenografts

069(nu)/070(nu/+) athymic nude mice were purchased from Harlan. At 4–5 weeks age, mice were injected subcutaneously with 3×10^6 DLD-1 or DLD-1 BRCA2-KO cells suspended in 100 µL PBS. Tumor take rate was >80%. When tumors reached 100 mm³ geometric mean volume, the mice were injected with 3 mg/kg YU238259 or its 3:1 DMSO:PBS vehicle, or 5 mg/kg YU128440 or its 1:19 DMSO:PBS vehicle (IP, 100 µL total in each case). Treatment was repeated 3×/week (Mon/Wed/Fri) for a total of 12 doses of YU238259 and 4 doses of YU128440. Tumor growth was assessed by external caliper. Mice were euthanized when individual tumor volumes exceeded 1000 mm³.

Results

High-throughput screening reveals a class of small molecules that are synthetically lethal to HDR-deficient cells

To identify small molecules with synthetic lethality in the context of HDR defects, our screen made use of paired cell lines deficient and proficient in FANCD2 (PD20) and BRCA2 (PEO1/4) activity (Fig. 1a). Following optimization, Z' values were >0.5 , indicating excellent discrimination between positive and negative controls. Screening compounds that demonstrated increased cytotoxicity in both FANCD2- and BRCA2-deficient cells relative to repair-proficient counterparts, as well as achieving a minimum cytotoxic effect, were considered “hits.” Initial screening of 15,040 molecules in the ChemBridge DIVERSet library identified several candidates, four of which possessed a similar sulfonamide backbone (Fig. 1c). A fifth compound, YU175534, was identified through subsequent screening of structurally homologous molecules across other libraries. Multiple rounds of chemical synthesis and structure-activity relationship analysis yielded a derivative compound, YU238259 (Fig. 1d). Relative to the initial screening hits, YU175534 and YU238259 possessed equal or lower LD50s and increased maximal cytotoxicity in BRCA2-deficient cells (Fig. 1b). Given its favorable solubility profile and lower toxicity in repair-proficient cells, YU238259 was ultimately selected as the lead compound for additional study of this class of drugs.

Sulfonamide compounds do not inhibit PARP activity or intercalate into DNA

The toxicity of these novel sulfonamide compounds in cells with HDR defects and their minimal toxicity in repair-proficient cells is strikingly similar to the observed effects of PARP inhibition. An *in vitro* assay of PARP enzymatic activity, however, demonstrated no decrease in PARP activity after treatment with either YU238259 or an initial screening hit, YU128440 (Fig. 2a). Further, assessment of helical stability of duplex DNA by circular dichroism spectroscopy showed no evidence of intercalation by YU128440 as compared to doxorubicin, a known intercalating agent (Fig. 2b). Thus, the cytotoxicity of sulfonamide compounds in repair-deficient cells is unrelated to either of these established mechanisms.

YU238259 inhibits homology-dependent DSB repair

To investigate the possibility that inhibition of DSB repair pathways may underlie the activity of sulfonamides, we utilized the U2OS cell lines DR-GFP and EJ5-GFP, which contain integrated GFP genes engineered to report the repair of DNA double-strand breaks via the HDR and NHEJ pathways, respectively (24, 25). These reporters contain a restriction enzyme sequence that can be cleaved by introduction of an I-SceI expression plasmid. In each case, repair of the I-SceI-induced DSB via the respective pathway results in expression of full-length, active GFP (Fig. 3a). YU238259 was added to U2OS DR-GFP or U2OS EJ5-GFP cells 24 hours prior to DSB induction, and treatment was maintained until cell harvest for FACS analysis. YU238259-treated cells exhibited a significant, dose-dependent decrease in HDR efficiency, as quantified by percentage of cells expressing GFP, but no reduction in NHEJ frequency (Fig. 3b). The slight upward trend in NHEJ activity seen with increasing doses of YU238259 may suggest a compensatory shift to NHEJ in the context of early HDR inhibition. Similarly, analysis of IR-induced foci in U2OS cells pretreated with YU238259

showed decreased formation of BRCA1 foci, but not of the NHEJ factors 53BP1 or pDNA-PK, relative to DMSO vehicle (Fig. 3c). The percent of cells with 15 or more BRCA1 foci per cell was decreased by 30% after YU238259 treatment, and this difference was significant at the $p < 0.05$ level. There was no significant difference in the levels of 53BP1 or pDNA-PK foci-positive cells following treatment. This again demonstrates inhibition of HDR activity by YU238259 with little to no effect on the NHEJ pathway.

Using a neutral comet assay, a measure of the number of DSBs in individual cells, we observed that YU238259-treated DLD-1 cells, either BRCA2-proficient or BRCA-deficient, had significantly higher levels of DSBs 24 h after 5 Gy IR compared to DMSO-treated controls (Fig. 3d), consistent with impaired resolution of DSBs owing to HDR inhibition. In DLD-1 cells, no significant increase in DSBs was seen with YU238259 treatment in the absence of IR, though there was a significant increase in DSBs in YU238259-treated DLD-1 BRCA2-KO cells after 24 hours. Cell cycle analysis of YU238259-treated DLD-1 and DLD-1 BRCA2-KO cells demonstrated a small increase in G1 cell population and small decrease in S-phase cell population following treatment, which was heightened in the BRCA2-deficient cells (Fig. 3e), consistent with early DSB accumulation in the context of BRCA2 loss. Taken together, these data suggest that synthetic lethality of YU238259 in HDR-deficient cells results from accumulation of unresolved DSBs following additional inhibition of residual HDR pathway activity.

YU238259 exhibits synthetic lethality with loss of frequently mutated tumor suppressors

Although initial screening was carried out for synthetic lethality in the context of BRCA2 or FANCD2 loss, the potent inhibition of HDR by YU238259 suggested that synthetic lethality might be observed for other DNA repair- or DNA damage response-defective cells. Indeed, in paired, isogenic cell lines deficient or proficient in BRCA2 (DLD-1), ATM (GM05849), and PTEN (U251), we observed that YU238259 was significantly more toxic to DNA repair or DNA damage response-deficient cells than their repair-proficient counterparts (Fig. 4a–c). We can thus conclude that the cytotoxicity of YU238259 is not cell line-specific and that synthetic lethality exists for a variety of cells with DNA repair or damage response pathway mutations. We also investigated whether siRNA-mediated knockdown of the repair factors ATR, ATRIP, and NBS1 would sensitize A549 cells to YU238259 treatment. The overall toxicity of ATR loss precluded any determination of synthetic lethality in the case of ATR knockdown; however, siRNA against ATRIP and NBS1 rendered cells sensitive to YU238259 (Fig. 4d). Differences between the repair-deficient and proficient lines were analyzed statistically via the Wilcoxon signed-rank test and were significant at the $P < 0.01$ level.

YU238259 sensitizes tumor cells to radiation therapy and DSB-inducing chemotherapy

It has been well established that inhibition of DNA repair pathways can potentiate the cytotoxicity of radiotherapy and chemotherapy (26–28). We hypothesized that HDR inhibition by YU238259 would enable it to synergize with ionizing radiation and drugs that generate DSBs. We found that the pretreatment of DLD-1 and DLD-1 BRCA2-KO cells with YU238259 enhanced the effectiveness of ionizing radiation or etoposide (Fig. 5a–b, d–e). In DLD-1 BRCA2-KO cells, YU238259 plus IR and YU238259 plus etoposide were

mathematically proven to be synergistic at all dose combinations, using the Chou-Talalay mutually nonexclusive model (29). Interestingly, the combination of YU238259 with the PARP inhibitor, olaparib, was also synergistic in DLD-1 BRCA2-KO cells (Fig. 5c, f). This suggests that YU238259 is also able to potentiate the toxicity of endogenous lesions that subsequently progress into DSBs in the setting of BER inactivation through PARP inhibition.

Growth of BRCA2-deficient tumor xenografts in nude mice is inhibited by YU238259 treatment

DLD-1 cells have previously been shown to effectively form tumor xenografts in nude mice (30). To assess the *in vivo* synthetic lethality of our sulfonamide compounds, tumor xenografts were generated by subcutaneous injection of 3×10^6 DLD-1 or DLD-1 BRCA2-KO cells into the flanks of nude mice. The mice then received repeated doses of 3 mg/kg YU238259 or 5 mg/kg YU128440 by intraperitoneal injection. BRCA2-proficient DLD-1 xenografts were unresponsive to treatment, but DLD-1 BRCA2-KO xenografts showed a statistically significant delay in tumor growth relative to vehicle for both drugs (Fig. 6a, b). Further, there was a significant overall survival benefit (as measured by days for individual tumors to reach 4x their volume at time of treatment) in DLD-1 BRCA2-KO mice treated with YU128440 and increased survival, though not to a statistically significant degree, in mice treated with YU238259 (Fig. 6c, d). These data recapitulate the synthetic lethality of YU238259 treatment in BRCA2-deficient cultured cells. Importantly, the bioavailability of YU238259 and other sulfonamides has not yet been optimized for *in vivo* treatment. Preliminary pharmacokinetic investigation suggests that there is rapid hepatic metabolism (not shown) that potentially could be slowed through further chemical modification of YU238259. Thus, there is potential for improved *in vivo* efficacy and the possibility to achieve larger effects at lower doses.

Discussion

Exploiting deficiencies in DNA repair found in cancer cells offers a promising approach to enhance cancer therapy. Agents that exploit synthetic lethality typically have increased therapeutic ratios and the potential for synergism with existing treatment modalities. Here, we have described the identification and characterization of a novel class of DNA double-strand break repair-inhibiting compounds with antitumor activity. Our lead compound of this class, YU238259, exhibits synthetic lethality in cellular and mouse models of human BRCA2-deficient tumors. YU238259 also demonstrates synergism with ionizing radiation and etoposide, which remain in frequent clinical use. Immunofluorescence studies of IR-induced foci formation by HDR and NHEJ factors show a decrease in BRCA1 foci formation in YU238259-treated cells, but no effect on 53BP1 or pDNA-PK foci. This supports data from reporter cell lines indicating that YU238259 specifically inhibits HDR and suggests a more proximal pathway inhibition. Ongoing proteomic analysis will likely be necessary to elucidate the cellular target(s) of this class of compounds and more fully define the mechanism of action at the molecular level.

Importantly, YU238259 does not inhibit PARP activity and so offers a novel mechanism to achieve synthetic lethality with BRCA2 deficiency. Further, we show that the divergent mechanisms of action and low toxicities of PARP inhibition and YU238259 treatment enable them to synergize effectively. The recent FDA approval of olaparib for the treatment of advanced ovarian cancer highlights the potential utility of this therapeutic approach. Additional studies will be useful in determining the degree to which co-treatment can radiosensitize tumor cells and the spectrum of DNA repair pathway mutations that confer vulnerability to these agents.

The observation of synthetic lethality with inactivation of BRCA2, ATM, and PTEN underscores the potential clinical utility of YU238259 across a variety of common tumors. Of note, BRCA1/2 mutations account for 5–13% of ovarian cancers in developed countries and are associated with higher histologic grade and poorer prognosis. Approximately 75% of ovarian cancers are metastatic at the time of diagnosis (31). For stage III and IV ovarian carcinomas, therapeutic options remain limited and five-year survival rates are low (32). Our data show increased cytotoxicity of YU238259 in the patient-derived BRCA2-deficient ovarian carcinoma line PEO1 relative to its treatment-resistant, BRCA2-wild-type equivalent, PEO4. Given the need for more effective chemotherapy and the striking lethality of ovarian cancer, novel synthetic lethal agents such as YU238259 that synergize with olaparib and other emerging therapies have significant clinical potential in this setting.

Small molecule inhibitors of many proximal HDR/NHEJ pathway enzymes, including the MRE11/RAD50/NBS1 (MRN) complex, ATM, ATR, and DNA-PKcs, have been described (33–36). Our compounds bear little structural similarity to any known DSB repair inhibitors. Given the findings, it remains highly plausible that the enzymatic target of these compounds is one for which small molecule inhibitors do not currently exist. We expect that additional characterization and development of this novel class of repair inhibitors will be a valuable avenue of inquiry with high translational potential.

Acknowledgments

The authors thank R. Bindra, J. Sweasy, and R. Jensen for helpful insights. This work was supported by a pilot project grant from the Yale Center for Molecular Discovery to PMG and by USPHS grant R01 CA168733 to PMG.

References

1. Ginsburg GS. Realizing the opportunities of genomics in health care. *JAMA : the journal of the American Medical Association*. 2013; 309(14):1463–1464. [PubMed: 23571581]
2. Leibel D, Laspe P, Emmert S. Nucleotide excision repair and cancer. *Journal of molecular histology*. 2006; 37(5–7):225–238. [PubMed: 16855787]
3. Klein TJ, Glazer PM. The tumor microenvironment and DNA repair. *Seminars in radiation oncology*. 2010; 20(4):282–287. [PubMed: 20832021]
4. Martin SA, Lord CJ, Ashworth A. Therapeutic targeting of the DNA mismatch repair pathway. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2010; 16(21):5107–5113. [PubMed: 20823149]
5. Wallace SS, Murphy DL, Sweasy JB. Base excision repair and cancer. *Cancer letters*. 2012; 327(1–2):73–89. [PubMed: 22252118]
6. Marechal A, Zou L. DNA damage sensing by the ATM and ATR kinases. *Cold Spring Harbor perspectives in biology*. 2013; 5(9)

7. Lin ZP, Ratner ES, Whicker ME, Lee Y, Sartorelli AC. Triapine disrupts CtIP-mediated homologous recombination repair and sensitizes ovarian cancer cells to PARP and topoisomerase inhibitors. *Molecular cancer research : MCR*. 2014; 12(3):381–393. [PubMed: 24413181]
8. Origanti S, Cai SR, Munir AZ, White LS, Piwnica-Worms H. Synthetic lethality of Chk1 inhibition combined with p53 and/or p21 loss during a DNA damage response in normal and tumor cells. *Oncogene*. 2013; 32(5):577–588. [PubMed: 22430210]
9. Konstantinopoulos PA, Wilson AJ, Saskowski J, Wass E, Khabele D. Suberoylanilide hydroxamic acid (SAHA) enhances olaparib activity by targeting homologous recombination DNA repair in ovarian cancer. *Gynecologic oncology*. 2014
10. Lok BH, Carley AC, Tchang B, Powell SN. RAD52 inactivation is synthetically lethal with deficiencies in BRCA1 and PALB2 in addition to BRCA2 through RAD51-mediated homologous recombination. *Oncogene*. 2013; 32(30):3552–3558. [PubMed: 22964643]
11. Illuzzi JL, Wilson DM 3rd. Base excision repair: contribution to tumorigenesis and target in anticancer treatment paradigms. *Current medicinal chemistry*. 2012; 19(23):3922–3936. [PubMed: 22788768]
12. Farmer H, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature*. 2005; 434(7035):917–921. [PubMed: 15829967]
13. Lee JM, et al. Phase I/Ib Study of Olaparib and Carboplatin in BRCA1 or BRCA2 Mutation-Associated Breast or Ovarian Cancer With Biomarker Analyses. *Journal of the National Cancer Institute*. 2014; 106(6)
14. Sandhu SK, et al. The poly(ADP-ribose) polymerase inhibitor niraparib (MK4827) in BRCA mutation carriers and patients with sporadic cancer: a phase 1 dose-escalation trial. *The lancet oncology*. 2013; 14(9):882–892. [PubMed: 23810788]
15. Kummar S, et al. A phase I study of veliparib in combination with metronomic cyclophosphamide in adults with refractory solid tumors and lymphomas. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2012; 18(6):1726–1734. [PubMed: 22307137]
16. Plummer R, et al. A phase II study of the potent PARP inhibitor, Rucaparib (PF-01367338, AGO14699), with temozolomide in patients with metastatic melanoma demonstrating evidence of chemopotential. *Cancer chemotherapy and pharmacology*. 2013; 71(5):1191–1199. [PubMed: 23423489]
17. Stachelek GC, et al. Potentiation of temozolomide cytotoxicity by inhibition of DNA polymerase beta is accentuated by BRCA2 mutation. *Cancer research*. 2010; 70(1):409–417. [PubMed: 20028873]
18. Aebi S, et al. Loss of DNA mismatch repair in acquired resistance to cisplatin. *Cancer research*. 1996; 56(13):3087–3090. [PubMed: 8674066]
19. Friedman HS, et al. Methylator resistance mediated by mismatch repair deficiency in a glioblastoma multiforme xenograft. *Cancer research*. 1997; 57(14):2933–2936. [PubMed: 9230204]
20. Hehlhans S, Storch K, Lange I, Cordes N. The novel HDAC inhibitor NDACI054 sensitizes human cancer cells to radiotherapy. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology*. 2013; 109(1):126–132. [PubMed: 24060178]
21. Levitt RJ, Georgescu MM, Pollak M. PTEN-induction in U251 glioma cells decreases the expression of insulin-like growth factor binding protein-2. *Biochem Biophys Res Commun*. 2005; 336(4):1056–61. [PubMed: 16154532]
22. Sakai W, et al. Functional restoration of BRCA2 protein by secondary BRCA2 mutations in BRCA2-mutated ovarian carcinoma. *Cancer research*. 2009; 69(16):6381–6386. [PubMed: 19654294]
23. Scully R, et al. Genetic analysis of BRCA1 function in a defined tumor cell line. *Molecular cell*. 1999; 4(6):1093–1099. [PubMed: 10635334]
24. Gunn A, Stark JM. I-SceI-based assays to examine distinct repair outcomes of mammalian chromosomal double strand breaks. *Methods in molecular biology*. 2012; 920:379–391. [PubMed: 22941618]

25. Pierce AJ, Johnson RD, Thompson LH, Jasin M. XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. *Genes & development*. 1999; 13(20):2633–2638. [PubMed: 10541549]
26. Chernikova SB, Wells RL, Elkind MM. Wortmannin sensitizes mammalian cells to radiation by inhibiting the DNA-dependent protein kinase-mediated rejoining of double-strand breaks. *Radiation research*. 1999; 151(2):159–166. [PubMed: 9952300]
27. Morgan MA, et al. Mechanism of radiosensitization by the Chk1/2 inhibitor AZD7762 involves abrogation of the G2 checkpoint and inhibition of homologous recombinational DNA repair. *Cancer research*. 2010; 70(12):4972–4981. [PubMed: 20501833]
28. Taverna P, et al. Inhibition of base excision repair potentiates iododeoxyuridine-induced cytotoxicity and radiosensitization. *Cancer research*. 2003; 63(4):838–846. [PubMed: 12591735]
29. Chou TC, Talalay P. Generalized equations for the analysis of inhibitions of Michaelis-Menten and higher-order kinetic systems with two or more mutually exclusive and nonexclusive inhibitors. *European journal of biochemistry / FEBS*. 1981; 115(1):207–216. [PubMed: 7227366]
30. Lin C, et al. Combination of quercetin with radiotherapy enhances tumor radiosensitivity in vitro and in vivo. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology*. 2012; 104(3):395–400. [PubMed: 22119371]
31. Lengyel E. Ovarian cancer development and metastasis. *American journal of pathology*. 2010; 177(3):1053–1064. [PubMed: 20651229]
32. Matsuda A, Katanoda K. Five-year Relative Survival Rate of Ovarian Cancer in the USA, Europe, and Japan. *Japanese journal of clinical oncology*. 2014; 44(2):196. [PubMed: 24496206]
33. Hickson I, et al. Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. *Cancer research*. 2004; 64(24):9152–9159. [PubMed: 15604286]
34. Leahy JJ, et al. Identification of a highly potent and selective DNA-dependent protein kinase (DNA-PK) inhibitor (NU7441) by screening of chromenone libraries. *Bioorganic & medicinal chemistry letters*. 2004; 14(24):6083–6087. [PubMed: 15546735]
35. Dupre A, et al. A forward chemical genetic screen reveals an inhibitor of the Mre11-Rad50-Nbs1 complex. *Nature chemical biology*. 2008; 4(2):119–125. [PubMed: 18176557]
36. Reaper PM, et al. Selective killing of ATM- or p53-deficient cancer cells through inhibition of ATR. *Nature chemical biology*. 2011; 7(7):428–430. [PubMed: 21490603]

Implications

We have identified a novel series of compounds that demonstrate synthetic lethality in DNA repair-deficient cell and animal models and have strong potential for clinical translation.

Fig. 1. High-throughput screening reveals a novel class of compounds with synthetic lethality in the context of HDR defects

(A) Methodology of synthetic lethality screen. (B) LD50 values and maximal percent effect values for given sulfonamide compounds were calculated in cells proficient or deficient in either BRCA2 or FANCD2 (C) Structures of sulfonamide compounds identified in screen of ChemBridge DIVERSet small molecule library or through structural homology screening. (D) Structure of lead agent, YU238259.

Fig. 2. Sulfonamide compounds do not inhibit PARP activity or intercalate into DNA

(A) PARP activity in the presence of YU128440, YU238259, or vehicle was assessed by *in vitro* enzymatic assay. 3-aminobenzamide, a known PARP inhibitor, was a positive control. (B) Plasmid DNA was incubated with YU128440, caffeine (a negative control), or a known intercalating agent, doxorubicin. Disruption of the duplex DNA helical structure was analyzed by circular dichroism spectroscopy.

Fig. 3. YU238259 specifically inhibits HDR activity

(A) Schematic of DR-GFP and EJ5-GFP cell-based reporter assays for HDR and NHEJ, respectively. (B) Effect of YU238259 on the frequency of HDR and NHEJ, as assessed by FACS analysis of GFP+ cells. (C) Relative percent of cells positive for DNA repair foci at 8 h post-irradiation with 10 Gy IR, following pretreatment with 25 μM YU238259 or vehicle. (D) Neutral comet assay of DLD-1 or DLD-1 BRCA2-KO cells pretreated with YU238259 and irradiated with 5 Gy IR. DSB levels, as assessed by comet tail moment, were measured at 0 h and 24 h post-irradiation. (E) Cell cycle analysis of DLD-1 and DLD-1 BRCA2-KO cells treated with YU238259 for 6 h and stained with propidium iodide.

Fig. 4. YU238259 demonstrates synthetic lethality with loss of HDR proteins

Paired, isogenic human cell lines deficient and proficient in (A) BRCA2, (B) ATM, and (C) PTEN were treated with increasing doses of YU238259 for 48 h and toxicity assessed by clonogenic survival assay. (D) U2OS cells were transfected with siRNAs against ATRIP, NBS1, or non-targeting control, or mock-transfected. 72 h later, cells were treated with YU238259 as above and survival assessed by clonogenic assay. Differences between repair-deficient and proficient lines were analyzed statistically via the Wilcoxon signed-rank test and were significant at the $p < 0.01$ level.

Fig. 5. YU238259 exhibits synergism with radiotherapy and DNA-damaging chemotherapy that is potentiated by BRCA2 loss

Clonogenic assay of DLD-1 and DLD-1 BRCA2-KO cells pretreated with YU238259 for 24 h and then co-treated with (A, B) ionizing radiation, (C, D) etoposide, or (E, F) olaparib for an additional 24 h in the presence of YU238259. Analysis using the Chou-Talalay model yielded CI values < 0.9 for all dose combinations in the DLD-1 BRCA2-KO cell line, mathematically proving synergism. Higher doses of YU238259 were also synergistic with IR in the DLD-1 cell line.

Fig. 6. YU238259 inhibits growth of BRCA2-deficient tumor xenografts in nude mice
 Growth curves of subcutaneous DLD-1 or DLD-1 BRCA2-KO tumor xenografts in athymic mice treated with (A) four doses of 5 mg/kg YU128440 or (B) twelve doses of 3 mg/kg YU238259. Survival rate of mice, as measured by number of days required for tumors to reach 4x their volume at time of treatment, for (C) YU128440 and (D) YU238259.