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Non-NAD-like PARP1 inhibitor enhanced synthetic lethal effect of NAD-like PARP inhibitors against BRCA1-deficient leukemia

Margaret Nieborowska-Skorska¹, Silvia Maifrede¹, Min Ye², Monika Toma^{1,3}, Elizabeth Hewlett², John Gordon², Bac Viet Le^{1,4}, Tomasz Sliwinski³, Huaqing Zhao⁵, Katarzyna Piwocka⁴, Peter Valent⁶, Alexei V. Tulin⁷, Wayne Childers², and Tomasz Skorski^{1,*}

¹Department of Microbiology and Immunology and Fels Institute for Cancer Research and Molecular Biology, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA

²Moulder Center for Drug Discovery Research, Temple University School of Pharmacy, Philadelphia, PA, USA ³Laboratory of Medical Genetics, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland ⁴Nencki Institute of Experimental Biology, Laboratory of Cytometry, Warsaw, 02-083, Poland ⁵Department of Clinical Sciences, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA ⁶Department of Internal Medicine I, Division of Hematology & Hemostaseology and Ludwig-Boltzmann Cluster Oncology, Medical University of Vienna, Vienna, 1090 Austria ⁷Department of Biomedical Sciences, School of Medicine and Health Sciences, University of North Dakota, Grand Forks, ND, USA

Tumor cells accumulate spontaneous and drug-induced DNA damage, but they survive because of enhanced/altered DNA repair activities [1]. The hypothesis that cancer cells are addicted to particular DNA repair pathways is supported by selective elimination of tumor cells by drugs/compounds targeting specific DNA repair mechanisms [2].

DNA double-strand breaks (DSBs), the most lethal DNA lesions, are usually repaired by BRCA1/2-mediated homologous recombination (BRCA-HR) and DNA-PK-mediated non-homologous end-joining (D-NHEJ) in proliferating cells [3]. PARP1 may prevent accumulation of potentially lethal DNA double-strand breaks (DSBs) by playing a key role in base excision repair (BER), single-strand break (SSB) repair, alternative non-homologous end-joining (Alt-NHEJ), and/or by facilitating MRE11-mediated recruitment of RAD51 to promote stalled replication fork restart [4]. The success of the PARP inhibitor (PARPi) olaparib in BRCA1/2-deficient breast tumors has established a proof-of-concept of personalized cancer therapy utilizing synthetic lethality [5]. Moreover, we have recently demonstrated that PARPi triggers synthetic lethality also in proliferating and quiescent tumor cells deficient in BRCA-HR and DNA-PK mediated NHEJ (D-NHEJ) [6].

Unfortunately, therapeutic effect of PARPi is usually short-lived and tumor cells become unresponsive due to a variety of compensatory mechanisms [7]. In concordance, we showed that BRCA-deficient breast carcinoma cells and leukemia cells could not be completely

*Correspondence: Department of Microbiology and Immunology and Fels Institute for Cancer Research and Molecular Biology, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, 19140, USA. tskorski@temple.edu.

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eradicated by PARPi [6]. Therefore, more robust and rapid targeting of PARP1 is required to reduce time-dependent emergence of PARPi-resistant/refractory clones.

Most PARP inhibitors have been designed to compete with nicotinamide adenine dinucleotide (NAD) for a binding site on the PARP1 molecule [8]. This strategy resulted in the discovery of NAD-like PARP inhibitors that not only target PARPs, but, unfortunately, other enzymatic pathways involving NAD and other nucleotides as co-factors. Using such inhibitors affects multiple NAD/nucleotide-dependent enzymatic pathways, which results in secondary toxic effects proceeding from the inactivation of other pathways, while the efficiency against the PARP1 pathway is diminished. Thus, the challenge is to design inhibitors based on other activities of PARP1.

We and others reported that DSBs-dependent interaction of PARP1 with histone 4 (H4) resulted in activation of PARP1 enzymatic activity and stimulation of Alt-NHEJ [9,10]. We identified non-NAD-like inhibitor 5F02, which interfered with H4-mediated activation of PARP1 but not PARP2 and Tankyrase-1, and was effective against several types of tumors including BRCA1-deficient MDA-MB-436 cells [11]. Here we tested the hypothesis that combinations of two structurally different PARP1 inhibitors, NAD-like olaparib or talazoparib and non-NAD-like 5F02 (Figure 1A) result in more abundant elimination of BRCA1-deficient leukemia cells and reduced toxicity to normal counterparts.

To test the effect of 5F02 and/or olaparib against primary tumor cells we employed BCR-ABL1-positive chronic myeloid leukemia (CML) patient cells. BCR-ABL1 oncogenic tyrosine kinase induces translational repression and degradation of BRCA1 protein which makes leukemia cells sensitive to PARPi [6,12,13]. 5F02 and olaparib exerted similar anti-leukemia effect against Lin-CD34+ peripheral blood cells from CML in chronic phase (CML-CP) (Figure 1B). However, 5F02, in contrast to olaparib, exerted very limited toxicity against normal Lin-CD34+ cells obtained from healthy donors. Moreover, the combination of 5F02 and olaparib was exceptionally effective against CML-CP cells, but showed little or no toxicity in normal cells.

As expected olaparib enhanced accumulation of DSBs (detected by γ -H2AX immunofluorescence) in Lin-CD34+ CML-CP cells when compared to normal counterparts (Figure 1C). Surprisingly, 5F02 did not induce DSBs when used alone and in combination with olaparib.

Tyrosine kinase inhibitor imatinib is a standard drug for CML treatment [14]. We reported before and also show here that a combination of imatinib + olaparib and imatinib + talazoparib exerted better anti-CML chronic phase (CML-CP) and CML accelerated phase (CML-AP) effect than the drugs used individually (Figure 2A) [6]. In addition, a similar effect was exerted by imatinib + 5F02. Interestingly, imatinib combined with olaparib or talazoparib and 5F02 eradicated clonogenic Lin-CD34+ CML-AP and CML-AP cells in vitro, while causing minimal toxicity to normal counterparts. Moreover, the combination of imatinib + olaparib/talazoparib + 5F02 was extremely effective against CML-CP quiescent cells, but not against normal cells (Figure 2B).

Finally, we tested the effectiveness of talazoparib + 5F02 in humanized NRGS mice bearing primary CML-CP xenograft and treated with imatinib. Talazoparib, which in mice has favorable pharmacokinetics over olaparib, and imatinib, was already examined by us in immunodeficient mice bearing CML xenotransplants [6]. Based on *in vitro* and *in vivo* pharmacokinetics (Supplemental Table 1 and Supplemental Figure 1) we administered 2.5 mg/kg of 5F02.

Imatinib reduced leukemia burden as assessed by detection of hCD45+ cells in peripheral blood and bone marrow, and addition of talazoparib or 5F02 exerted even stronger anti-leukemia effect (Figure 2C). Remarkably, combination of both PARP inhibitors with imatinib resulted in exceptionally strong synergistic therapeutic effect.

Since patients with BRCA-deficient tumors who initially respond to PARPi often develop resistance, leading to cancer relapse [7] there is an urgent need to develop novel strategies to prevent development of drug resistance, for example by killing tumor cells more rapidly and robustly. We have shown here that combination of non-NAD-like PARPi (5F02) and NAD-like PARPi (olaparib, talazoparib) exerted synergistic effect against BRCA1 and DNA-PK - deficient proliferating and quiescent leukemia stem and progenitor cells. Enhanced anti-tumor effect of 5F02 + olaparib could be also observed in BRCA2-deficient lymphomas (Supplemental Figure S2) and in BRCA1/2-deficient solid tumor cells (Supplemental Figure S3), indicating that anti-tumor synthetic lethal effect of the combination could be achieved in a variety of BRCA1/2-deficient malignant cells. Moreover, 5F02 + olaparib eliminated quiescent D-NHEJ -deficient leukemia cells. Importantly, non-NAD-like PARPi 5F02 did not enhance the toxicity of NAD-like PARPi olaparib against normal cells. Finally, the combination exerted synergistic effect in humanized immunodeficient mice bearing BRCA1/DNA-PK-deficient primary CML xenografts. Altogether, non-NAD-like PARPi synergistically enhanced synthetic lethal effect of NAD-like PARPi in tumor cells without increasing the cytotoxic effect in normal cells.

While synthetic lethality mediated by NAD-like PARPi is usually associated with enhanced accumulation of potentially lethal DSBs, non-NAD-like PARPi 5F02 did not induce DSBs when used as a single agent and also in combination with olaparib. Thus, the mechanistic aspect of the synergistic effect of NAD-like and non-NAD-like PARPis needs to be elucidated. Nevertheless, our data suggest that combining NAD-like and allosteric non-NAD-like PARPi's may represent a viable therapeutic strategy for enhancing tumor response to PARP inhibition and reducing the resistance that inevitably results from treatment with NAD-like PARPi's alone.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. 5F02 enhanced the effect of olaparib against BRCA1-deficient CML cells.

(A) Scheme illustrating anti-PARP1 activity of NAD-like (olaparib and talazoparib, both from Selleckchem) and non-NAD-like (5F02, synthesized as described in Supplemental Methods) inhibitors. (B) BCR-ABL1 –positive CML primary samples (n=3) and samples of normal hematopoietic cells (n=3) were characterized before [6]. Lin[–]CD34⁺ cells were obtained from mononuclear fractions by magnetic sorting using the EasySep negative selection human progenitor cell enrichment cocktail followed by human CD34 positive selection cocktail (StemCell Technologies) as described before [6]. Cells were incubated for 72 hrs with the indicated concentrations of olaparib (triangles), 5F02 (circles) and olaparib + 5F02 (squares) in IMDM supplemented with 10% FBS and growth factors (100 ng/ml SCF, 20 ng/ml IL-3, 100 ng/ml Flt-3 ligand, 20 ng/ml G-CSF, 20 ng/ml IL-6) followed by plating in Methocult (Stemcell Technologies). Colonies were counted after 7 days. Results represent mean % of clonogenic cells $\pm$ SD surviving the treatment (from triplicates); *p<0.005 when compared to individual treatment using the response additivity approach to study synergistic effects [15]. (C) Lin[–]CD34⁺ cells from CML-CP patient (black bars) and from healthy donor (grey bars) were incubated for 24 hrs with 2.5 μ M olaparib, 2.5 μ M 5F02, and olaparib + 5F02. DSBs were detected by γ -H2AX (H2AX phosphorylated on serine 139) immunofluorescence as described before [6]. Results represent relative increase of γ -H2AX immunofluorescence $\pm$ SD in comparison to normal cells (from triplicates); *p<0.05 when compared to healthy donors using unpaired two-tailed Student t test.

Figure 2. 5F02 enhanced anti-CML effect of imatinib + talazoparib.

(A, B) Lin⁺CD34⁺ CML-CP/AP cells (black bars; n=3 patients of each) and normal counterparts (grey bars; n=3 healthy donors) were stained with cell trace violet (CTV) (eBioscience) and incubated for 5 days with 1 μ M imatinib (IM), 2.5 μ M 5F02, 2.5 μ M olaparib (Ola), 25nM talazoparib (Tala) or with indicated combinations in StemSpan®SFEM medium (Stem Cell Technologies, Vancouver, Canada) supplemented with the cocktail of growth factors (see Figure 1 legend). (A) Lin⁺CD34⁺ clonogenic cells were detected after plating in Methocult. (B) Lin⁺CD34⁺CD38⁺CTV^{max} cells were detected by flow cytometry using fluorochrome-conjugated anti-Lin (#340546), anti-CD34 (#347203, #555821) and anti-CD38 (#355790, #555460) antibodies (all from BD Pharmingen). Results represent mean number of colonies or quiescent cells $\pm$ SD from triplicates; *p<0.001 in comparison to IM+Ola, and IM+5F02 using the response additivity approach to study synergistic effects. (C) Sub-lethally total-body irradiated (600 Gy) NOD.*Rag1*^{-/-};*γc*^{null} mice expressing human IL-3, GM-CSF and SCF (NRGS) were injected with 10⁷ Lin-CD34⁺ CML-CP cells and treated for 7 days with imatinib (IM) (100 mg/kg twice daily by oral gavage [6]) and indicated combinations with 5F02 (2.5 mg/kg i.p.) and/or talazoparib (Tala) (0.33 mg/kg i.v. [6]). Anti-CML effect was assessed by detection of hCD45⁺ cells in peripheral blood leukocytes (PBL) and bone marrow cells (BMC) at the end of treatment as described before [6]. Results represent mean $\pm$ SD percentage of hCD45⁺ in PBL and BMC (3–4 mice/group); *p<0.01 in comparison to IM+Tala, and IM+5F02 using the response additivity approach to study synergistic effects.