

Sensitizing HR-proficient cancers to PARP inhibitors

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Homologous recombination (HR) is a major DNA repair pathway that is deficient in a small subset of cancers. The best characterized of these are cancers associated with a germline or acquired mutation in the breast cancer 1 and 2 tumour suppressor genes (*BRCA1* and *BRCA2* respectively). HR-deficient cells rely on non-HR DNA repair pathways that are dependent on poly (ADP-ribose) polymerase (PARP), so that inhibition of PARP is an Achilles' heel in these cancers. PARP inhibitors have emerged as an effective therapeutic strategy in HR-deficient tumours (1), and several are currently in clinical trials in ovarian, *BRCA*-mutated breast cancer, and other cancers.

In spite of the promise that this class of therapies holds, it is increasingly apparent that tumours may possess intrinsic and/or acquired resistance mechanisms involving restoration of HR, presenting a pressing clinical problem (2-5). Thus, strategies targeting HR may potentially confer or restore PARP inhibitor sensitivity. One such strategy involves the drug dinaciclib, an inhibitor of cyclin-dependent kinases (CDKs) 1, 2, 5, and 9 (6). Additionally, we have recently demonstrated that dinaciclib is the most potent inhibitor of CDK12 known thus far, and therefore a transcriptional regulator of HR genes (7-10).

We comprehensively examined the combination of dinaciclib and PARP inhibitors, such as veliparib and olaparib, in preclinical models of triple-negative breast cancer (TNBC), including *BRCA* wild-type cells, *BRCA*-mutated cells, and patient-derived xenograft (PDX) models of primary and acquired resistance to PARP inhibition (10). First, pathway analyses demonstrated that the genes down-regulated by dinaciclib in *BRCA* wild-type TNBC cells were significantly enriched for those involved in HR repair and DNA damage-sensing, including *BRCA1* and *RAD51*. Consequently, dinaciclib disrupted HR repair in cell-based assays, and the IC₅₀ of veliparib was consistently reduced in the presence of dinaciclib in a panel of *BRCA* wild-type TNBC cell lines. These results were phenocopied by CRISPR/Cas9-mediated knockout of CDK12 in TNBC cells. Importantly, dinaciclib had minimal effects on the cell cycle of breast cancer cells. These data point to a pivotal role of dinaciclib in disrupting HR, mediated through inhibition of CDK12.

In light of this, we hypothesized that *BRCA*-mutated TNBC with acquired PARP inhibitor resistance might be resensitized to PARP inhibition with dinaciclib. Using a *BRCA1*-mutated cell line with acquired resistance after exposure to increasing PARP inhibitor concentrations *in vitro*, and an *in vivo* TNBC PDX model derived from a *BRCA2* carrier, who had progressed on olaparib and cisplatin, we demonstrated that the combined dinaciclib and veliparib therapy restored tumour growth inhibition.

We next evaluated this strategy in *BRCA1*-mutated TNBC cell lines with *de novo*

PARP inhibitor resistance, and a TNBC PDX model derived from a 185delAG *BRCA1* carrier, whose tumor demonstrated primary resistance to olaparib. Despite *BRCA1* mutation, these cell lines and the PDX demonstrated residual HR activity, accounting for *de novo* resistance. Dinaciclib resulted in a concentration-dependent reduction of RAD51 Recombinase (RAD51), BRCA1, and BRCA2 protein levels, and similarly sensitized tumour cells to PARP inhibition both *in vitro* and *in vivo*. Dinaciclib suppressed expression of RAD51 and formation of RAD51 foci, and in combination with PARP inhibition, caused greater induction of H2A histone family, member X (γ -H2AX) foci compared with PARP inhibitor alone.

Finally, we modeled the *in vivo* activity of these treatments in a PDX model derived from a patient with an early-stage TNBC harboring a somatic R1443* *BRCA1* mutation. In this model, veliparib monotherapy produced prolonged stable disease. In contrast, combined dinaciclib and veliparib resulted in substantial and durable tumor regression.

Importantly, histological analyses of the bone marrow and organs harvested at the end of the experiment (156 days) from combination-treated mice revealed no abnormalities, and minimal γ -H2AX staining, indicating that the treatment combination was well tolerated and selective for tumour cells. It is possible that dinaciclib-mediated inhibition of CDKs 1 and 2 induced greater cell cycle arrest in non-transformed cells, protecting them from PARP inhibitor-mediated cytotoxicity. This produces the critically important therapeutic window for this combination strategy.

Residual HR or restoration of HR plays a major role in *de novo* and acquired PARP inhibitor resistance, and may occur by varied and complex mechanisms. The ability of CDK12 inhibition to transcriptionally downregulate multiple components of the HR pathway suggests that it may be therapeutically effective as a sensitizer to PARP inhibitors even when the precise mechanism of HR restoration is unknown. Our work has demonstrated the efficacy of combined CDK12 and PARP inhibition in multiple *BRCA*-mutated models in reversing PARP inhibitor resistance, and conferring improved therapeutic efficacy in PARP inhibitor-sensitive TNBC. The latter finding is important, as even in this setting, tumour regression is rarely complete and durable with PARP inhibitors alone.

This therapeutic strategy may also have broader implications beyond reversing PARP inhibitor resistance in *BRCA*-mutated tumours. In the setting of breast cancer, there is a larger subset of *BRCA1/2* wild-type tumours; combinatorial strategies that render tumour cells HR-deficient have the potential to sensitize these tumours to PARP inhibitors, and therefore increase the utility of PARP inhibitors to a larger patient population beyond germline *BRCA1/2* carriers.

Building on our findings, a phase 1 trial of dinaciclib and veliparib is currently in progress (NCT01434316). Once recommended phase 2 treatment doses are determined, the trial will enroll expansion cohorts assessing preliminary activity in both *BRCA* wild-type and *BRCA*-mutated TNBCs, including those that are PARP inhibitor resistant and PARP inhibitor-naive.

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