REVIEW ARTICLE

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Optimizing poly (ADP-ribose) polymerase inhibition through combined epigenetic and immunotherapy

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Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype with poor survival outcomes. Currently, there are no targeted therapies available for TNBCs despite remarkable progress in targeted and immune-directed therapies for other solid organ malignancies. Poly (ADP-ribose) polymerase inhibitors (PARPi) are effective anticancer drugs that produce good initial clinical responses, especially in homologous recombination DNA repair-deficient cancers. However, resistance is the rule rather than the exception, and recurrent tumors tend to have an aggressive phenotype associated with poor survival. Many efforts have been made to overcome PARPi resistance, mostly by targeting genes and effector proteins participating in homologous recombination that are overexpressed during PARPi therapy. Due to many known and unknown compensatory pathways, genes, and effector proteins, overlap and shared resistance are common. Overexpression of programmed cell death-ligand 1 (PD-L1) and cancer stem cell (CSC) sparing are novel PARPi resistance hypotheses. Although adding programmed cell death-1 (PD-1)/PD-L1 inhibitors to PARPi might improve immunogenic cell death and be crucial for durable responses, they are less likely to target the CSC population that drives recurrent tumor growth. Lysine-specific histone demethylase-1A and histone deacetylase inhibitors have shown promising activity against CSCs. Combining epigenetic drugs such as lysine-specific histone demethylase-1A inhibitors or histone deacetylase inhibitors with PARPi/anti-PD-1/PD-L1 is a novel, potentially synergistic strategy for priming tumors and overcoming resistance. Furthermore, such an approach could pave the way for the identification of new upstream epigenetic and genetic signatures.

KEYWORDS
cancer stem cell, immune checkpoint inhibitor, lysine-specific histone demethylase-1A, poly (ADP-ribose) polymerase inhibitor, triple-negative breast cancer

1 | BACKGROUND

Triple-negative breast cancers (TNBCs) comprise approximately 15%-20% of breast cancers and have an aggressive natural history compared to other breast cancer subtypes.[1] Triple-negative breast cancers tend to present in younger, premenopausal women and preferentially metastasize to visceral organs.[1] Despite good responses to standard cytotoxic chemotherapy regimens, recurrence is common, and retreatment with further lines of chemotherapy remains standard with suboptimal survival outcomes. Novel therapeutic drug development for TNBCs has been limited due to their heterogenous nature. There is thus an unmet need to improve therapeutic strategies for patients with TNBCs. In this review, we explore poly (ADP-ribose) polymerase inhibitor (PARPi) resistance mechanisms, hypothesize novel resistance pathways, and propose a novel combinatorial biological approach that not only overcomes PARPi resistance but also renders tumors more susceptible to immunogenic cell death and depletes the metastasis-driving cancer stem cell (CSC) population through epigenetic modulation.

Immune checkpoint inhibition and immune-mediated cytotoxicity have shown impressive durable activity in many hematological and solid organ cancers with an acceptable toxicity profile.[2] Although a large proportion of TNBCs express high levels of programmed cell death-ligand 1 (PD-L1), have a high mutational load, and are associated with high numbers of CD8$^+$ tumor-infiltrating lymphocytes, responses to immunotherapy are not as spectacular in TNBCs compared to other cancers (Table 1),[3] but numerous trials including multiple combinations are ongoing.

Poly (ADP-ribose) polymerase inhibitors are group of relatively novel drugs that cause cell cycle arrest and cell death by interfering with DNA repair.[4] Several PARPi have been tested in patients with various cancers including ovarian, breast, and prostate cancers. Impressive responses have mainly been seen in early phase trials and some phase III trials, especially in *BRCA*-mutant ovarian cancer patients (Table 2). Three PARPi (olaparib, rucaparib, and niraparib) are already approved by the US FDA for the treatment of recurrent ovarian cancer and, recently, olaparib also received approval for the treatment of *BRCA*-mutant human epidermal growth factor receptor 2-negative metastatic breast cancer. Despite promising early data, resistance develops in most patients with no significant improvement in overall survival (OS).[5,6]

2 | POLY (ADP-RIBOSE) POLYMERASE AND PARPI

The role of PARP1 in DNA damage responses is diverse and has been extensively studied (Figure S1). Poly (ADP-ribose) polymerase 1 activity is triggered by binding of PARP1 to single-strand breaks through the two zinc fingers at the N-terminal 42 kDa DNA-binding domain, which leads to enzymatic activation.[7] Using NAD as a substrate, PARP1 catalyzes the transfer of polymer of ADP ribose (PAR) molecules to glutamate, lysine, or aspartate residues on acceptor histone and/or non-histone proteins, thereby mediating the recruitment of DNA repair proteins and eventually DNA repair. AutoPARylation of PARP1 eventually enables dissociation of PARP1 from DNA to complete DNA repair.[7]

The effects of PARPi on DNA damage and the cell cycle are complex. Most PARPi are competitive NAD$^+$ inhibitors that are cytotoxic by inhibiting PARylation and by trapping PARP1 on DNA, which blocks the progression of replication forks, eventually leading to cell death.[8] In contrast, a recent report suggested that PARPi might increase the speed of fork elongation (rather than stalling it) beyond a tolerable threshold, eventually leading to cell death.[9]

Synthetic lethality is a concept in which defects in one or two genes/repair mechanisms have minimal effects on the cell, whereas defects in combinations of genes/repair mechanisms are lethal.

TABLE 1 Immunotherapy in breast cancer

Citation	Year	Drug	Single agent/ combinations	Cancer subtype/ PD-L1 level	Phase	No. of patients	ORR%	Comments
Loi et al[68]	2017	Pembrolizumab	Trastuzumab	ER$^{+/-}$/PD-L1$^{+/-}$	Ib/II	58	15	ORR 39% in PD-L1$^+$, TILs >5%
Rugo et al[69]	2016	Pembrolizumab	Single agent	ER$^+$HER2$^-$/>1%	Ib	25	12	
Nanda et al[70]	2016	Pembrolizumab	Single agent	TNBC/>1%	Ib	27	18	
Schmid et al[71]	2017	Atezolizumab	Single agent	TNBC/>5%	I	115	10	17% ORR in PD-L1$^+$
Adams et al[72]	2016	Atezolizumab	Atezolizumab/ abraxane	TNBC, PD-L1$^{+/-}$	Ib	32	42	
Dirix et al[73]	2016	Avelumab	Single agent	HER2$^-$/NR	Ib	168	3	ORR; TNBC 22%, PD-L1$^+$ 16%
Santa-Maria et al[74]	2017	Durvalumab	Tremelimumab	NR	I	18	17	43% in TNBC

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; NR, not reported; ORR, objective response rate; PD-L1, programmed cell death ligand-1; TIL, tumor infiltrating lymphocyte; TNBC, triple-negative breast cancer.

TABLE 2 Poly (ADP-ribose) polymerase inhibitor trials in breast cancer

Author	Year	Drug	Single agent/ combinations	Phase	No. of patients	ORR%	PFS (mo)	OS (HR/P)	OS (mo)
Litton et al[13]	2017	Talazoparib	Single agent vs chemo	III	431	62	8.6 vs 5.6	(0.76/0.10)	22 vs 19
Robson et al[5]	2017	Olaparib	Single agent vs chemo	III	205	59 vs 28	7.0 vs 4.2	0.9 (0.57)	19.3 vs 19.6
Kaufman et al[75]	2015	Olaparib	Single agent	II	62	13	3.7	NA	11
Turner et al[6]	2017	Talazoparib	Single agent	II	84	28	4	NA	NR
Han et al[76]	2018	Veliparib	V+Cb+P, Pl+Cb+P (& V+Tem)	II	284	78 vs 61	14 vs 12	(0.75/0.1)	28 vs 26

Cb, carboplatin; chemo, chemotherapy; HR/P, hazard ratio/P value; NA, not applicable; NR, not reported; ORR, overall response rate; OS, overall survival; P, paclitaxel; PFS, progression-free survival; Pl, placebo; Tem, temozolomide; V, veliparib.

Farmer et al[10] reported that homologous recombination (HR)-deficient *BRCA1/2*-mutated human cell lines and mouse models were much more sensitive to PARPi compared to *BRCA* WT cells. Although this synthetic lethality was initially thought to be due to reliance of *BRCA*-mutant cells on the single-strand break repair (base excision repair) pathway for survival, emerging reports suggests that PARP trapping and subsequent generation of replication-associated double-strand breaks (DSBs) contribute significantly to PARPi lethality in the context of HR deficiency.[7] Clinically used PARPi differ in their cytotoxicity and ability to "trap" PARP1 on DNA, with talazoparib showing higher trapping potential and veliparib limited PARP trapping capacity.[7]

3 | POLY (ADP-RIBOSE) POLYMERASE INHIBITORS IN BREAST CANCER

Triple-negative breast cancers comprise 15%-20% of breast cancers overall and 70% of *BRCA1* and 20% of *BRCA2* mutation carriers, respectively.[11] The initial discovery of synthetic lethality in *BRCA*-mutant cancers led to massive interest in the use of PARPi in patients with breast and ovarian cancers. Response rates of 13%-59% were observed in multiple phase I and II trials (Table 2), with even higher responses of up to 88% observed when PARPi were combined with chemotherapies.[12] Subsequently, two large phase III trials reported the efficacy of PARPi in *BRCA*-mutant metastatic breast cancers. The OlympiAD trial compared olaparib 300 mg twice daily monotherapy to physicians' choice chemotherapy in patients with germ-line *BRCA*-associated breast cancer. The olaparib arm showed higher response rates and longer median progression-free survival (PFS). Although the authors reported a longer second PFS benefit with olaparib, this did not ultimately translate into an OS advantage (hazard ratio, 0.90; *P* = .57); however, OS was not the predetermined primary end-point of the study, and the survival analysis might have been affected by subsequent treatment after progression.[5]

The EMBRACA study had a similar patient cohort to that of OlympiAD and investigated the efficacy of talazoparib in metastatic *BRCA*-mutant breast cancer patients. Among 431 patients, the PFS

was 8.6 months in the talazaparib group vs 5.6 months in the control group. Despite longer PFS and higher response rates, the median duration of response was only 5.4 months and OS, at an interim analysis after 51% of the projected events, was not significantly different between groups (hazard ratio, 0.76; *P* = .10).[13] Despite impressive responses and PFS benefit, development of resistance to PARPi was almost ubiquitous, hampering the duration of response and perhaps even OS. Furthermore, PFS benefit was in the range of 2-4 months with PARPi, even in germline *BRCA*-mutant patients. Poly (ADP-ribose) polymerase inhibitors have been investigated extensively in ovarian cancers, but it is interesting to note that, despite the impressive PFS benefits, OS differences were not statistically significant in studies that have reported survival data.[14-16]

4 | RESISTANCE TO PARPI

Poly (ADP-ribose) polymerase inhibitor-treated cells acquire resistance either directly or indirectly related to restoration of HR repair or through other mechanisms. These can be broadly categorized into 7 groups (Table 3); secondary mutations that overcome the BRCA1 pathway deficiency are the best-studied mechanism of PARP resistance. Other known resistance mechanisms include partial or complete restoration of HR repair through rewiring of DNA damage response, either through loss of 53BP1 and Shieldin complex proteins that restrain DNA end resection,[17] or through regulation of HR genes.[18] More recently, *SFLN11* has emerged as a promising biomarker of PARPi sensitivity. *SFLN11* facilitates DNA replication arrest and cell death, hence potentiating the "trapping" effect of PARPi,[19,20] and *SLFN11* inactivation confers resistance to PARPi in many cancer cell lines.[21]

5 | OVERCOMING RESISTANCE BY COMBINATION APPROACHES

Poly (ADP-ribose) polymerase inhibitor combinations tested thus far have been based on 3 biological interactions.

TABLE 3 Poly (ADP-ribose) polymerase (PARP) inhibitor resistance in breast cancer

Mode of resistance	Molecular mechanism
Restoration of BRCA1/2	Secondary mutations[77]
Restoration of HR repair	Increased RAD51[78]
	HOXa9 depletion[79]
	S6 ribosomal phosphorylation[80]
	Loss of 53BP1/Shieldin[17]
Reduced access to drug	Upregulation of the *Abcb1a/b* gene and p-GP efflux pump[81]
Enrichment/increase in resistant cells	CSC enrichment[40]
	Activation of EZH2 and increased CSCs[39]
Increased PARP activity	Activated c-Met proto-oncogene[82]
	Increased PARP levels[83]
Impaired replication arrest	Downregulation of SLFN11[19,21]
Other	Upregulation of NF-κB signaling[84]

CSC, cancer stem cell; EZH2, enhancer of zeste homolog 2; HOXa9, homeobox A9; HR, homologous recombinant; NF-κB, nuclear factor-κB; SLFN11, Schlafen family member 11.

5.1 | Increasing DNA damage

Poly (ADP-ribose) polymerase inhibitors have been shown to increase the DNA damage caused by cytotoxic chemotherapy or by interrupting DNA repair pathways. This approach was limited by toxicity, predominantly myelosuppression, despite various dose and scheduling modifications.[12,22] In addition, many DNA repair pathways are upregulated to compensate for PARPi (eg CHK1/2, CDK1, Wee1, and MET1), and interfering with these can augment PARPi efficacy.[22]

5.2 | Increasing PARP trapping

Poly (ADP-ribose) polymerase inhibitors differ in their ability to trap PARP1 on DNA. Cytotoxicity was greater with olaparib/temozolomide than veliparib/temozolomide, which was attributed to the greater trapping ability of olaparib compared to veliparib. However, synergy between topoisomerase 1 inhibitors and PARPi was more dependent on catalytic activity rather than trapping.[22] Hence, appropriate selection of combinations based on PARPi biology is important to achieve synergy.

5.3 | Simulating BRCAness in *BRCA*-proficient tumors

Some tumors inherently express BRCAness despite being *BRCA* proficient and could be sensitive to PARPi. In others, silencing *BRCA* expression or depleting proteins involved in *BRCA*-associated HR creates BRCAness and increases PARPi sensitivity.[23] McCabe et al[24] reported that defects in other DNA repair genes commonly found in human cancers including those involved in DSB detection and repair rendered tumors susceptible to PARPi. Recently, inhibition of *BET* genes (in particular *BRD4*) or related proteins has been shown to sensitize cancers to PARPi by inducing BRCAness through downregulation of CTIP, BRCA1, and RAD51 transcription.[25,26] Deficiencies in several other pathways could also increase PARPi sensitivity, and drugs inhibiting the mTOR/PI3K pathway, heat shock protein 90 (HSP90), and histone deacetylase inhibitors (HDACi) are currently undergoing trials.

6 | NOVEL THEORIES OF PARPI RESISTANCE

6.1 | Programmed cell death-ligand 1 theory

Cancer cells can be recognized and eliminated by the innate and adaptive immune systems, especially in the early course of tumor development. T cell recognition of cancer antigens and activation are crucial to immunogenic cancer cell death, which is initiated by antigen presentation by antigen-presenting cells. Tumor antigens are presented through the MHC to T-cell receptors, facilitated by co-stimulatory molecules like B7 and CD28 to fully activate T cells. Various other co-stimulatory and co-inhibitory checkpoint molecules have been identified in the last decade that are involved in augmenting or suppressing T cell activation. Cancer cells develop a variety of mechanisms to evade immunogenic cell death such as PD-L1 expression, which can cause T cell suppression when bound to programmed cell death-1 (PD-1) (Figure 1).[27]

The combination of PARPi and immunotherapy is one novel approach currently being investigated (Table S1).[28,29] Several arguments have been proposed to rationalize such combinations. First, PARPi-related cytotoxicity could release damaged DNA, which represents a source of potential neoantigens that make the tumor more immunogenic. Many immunotherapeutic agents rely on neoantigens expressed by cancers that are recognized as non-self by T cells. Such cancers with high mutational and neoantigen load are very vulnerable to checkpoint inhibition, hence the excellent responses seen in selected cancers like melanoma, non-small cell lung cancer, renal cell carcinoma, and mismatch repair-deficient colorectal cancers.[30] Second, a recent study by Jiao et al[31] proposed that PARPi could induce PD-L1 expression. In this study, treatment of MDA-MB-231 and BT549 breast cancer cells (basal breast cancer lines) with either olaparib or talazoparib increased PD-L1 expression in vitro and in vivo. Inactivation of glycogen synthase kinase 3α/β by PARPi was noted to be a key step in the upregulation of PD-L1. Furthermore, although PARPi were associated with T cell infiltration, the proportion of infiltrating cytotoxic CD8^{+} cells was very low, thought to be due to increased PD-L1. Addition of PD-L1 inhibitors to PARPi reversed PARP resistance and increased cytotoxic CD8^{+} cells (Figure 2).

Taking all this evidence together, a PARPi/PD-1 inhibitor (PD1i) combination is likely to show synergy. Nonetheless, Higuchi et al[32] found that, although combined CTLA4 and PARP1 inhibition was synergistic, combining a PD-L1/PD1i with a PARP1i had no effect. However, these studies have notable differences: the former used only breast cancer cell lines including MDA-MB-231, SUM149, and
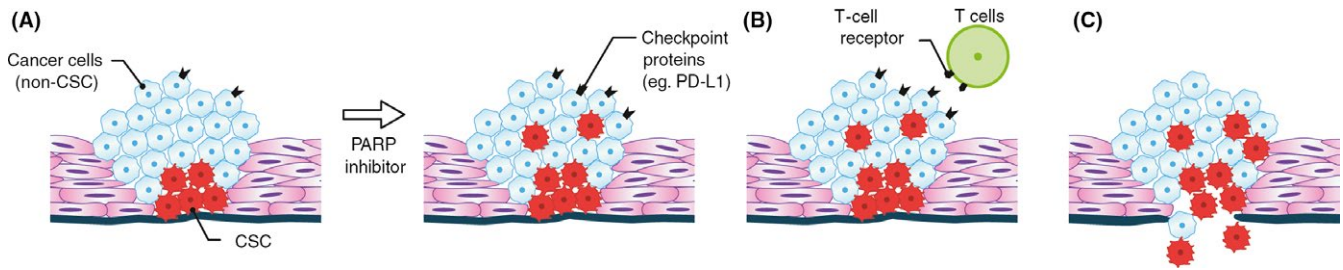
FIGURE 1 Effect of poly (ADP-ribose) polymerase inhibitors (PARPi) in the tumor microenvironment. A, Although PARPi might cause initial tumor shrinkage, they could promote epithelial-mesenchymal transition (EMT) with minimal cytotoxicity against cancer stem cells (CSCs), leading to CSC enrichment. Poly (ADP-ribose) polymerase inhibitors might also upregulate checkpoint protein expression, such as programmed cell death ligand-1 (PD-L1). B, T cells are inhibited by tumor-T cell interactions by overexpressed checkpoint proteins, for example, PD-L1-programmed cell death-1 interactions. C, Accelerated epithelial-mesenchymal transition and enrichment of CSCs with impaired immunogenic cell death leads to cancer progression and metastasis
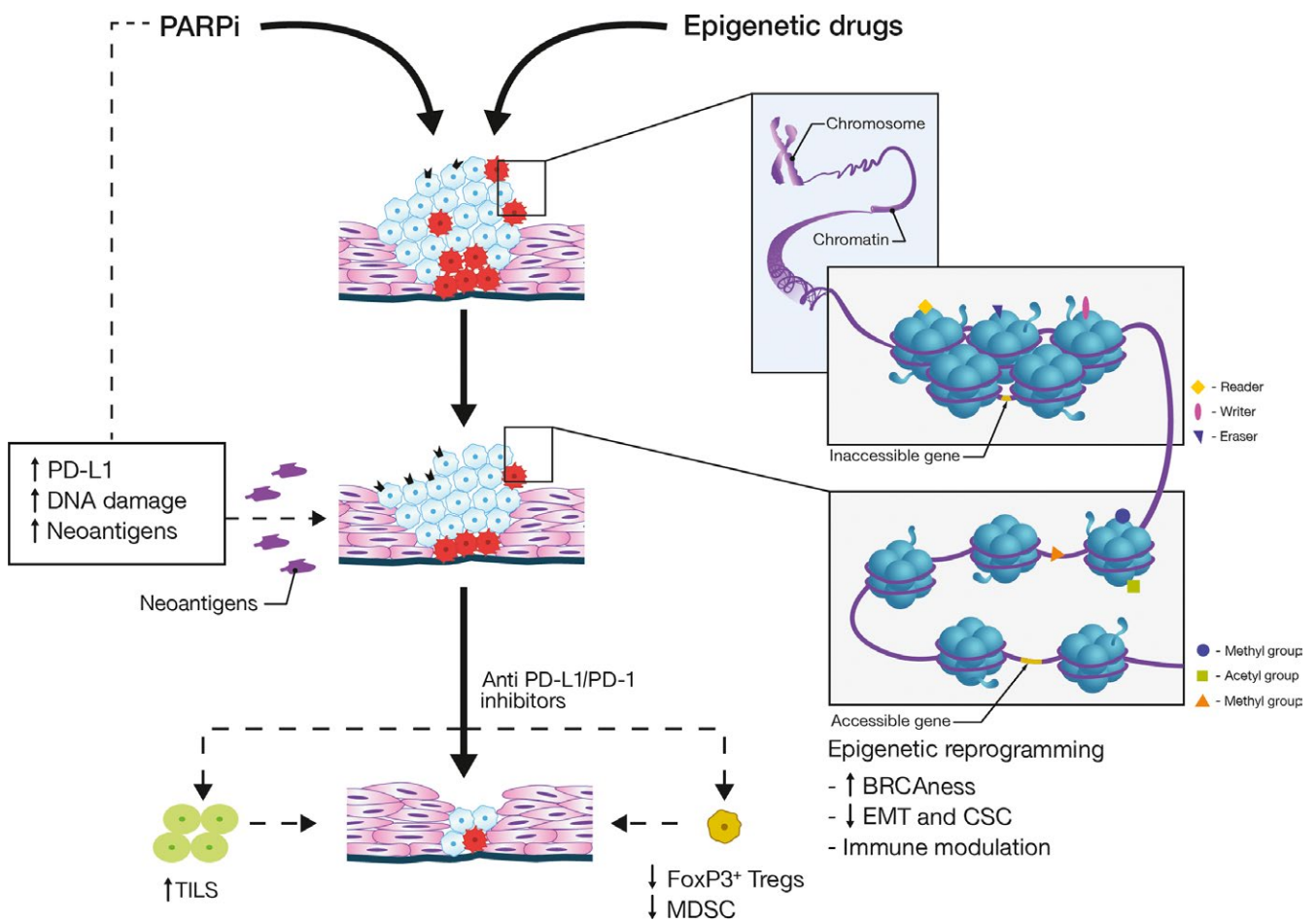

FIGURE 2 Combination strategy, specifically targeting vital resistance pathways that are likely to enhance the cytotoxicity of poly (ADP-ribose) polymerase inhibitors (PARPi). Poly (ADP-ribose) polymerase inhibitor therapy increases DNA damage, releases tumor antigens (neoantigens), and might also upregulate checkpoint proteins like programmed cell death ligand-1 (PD-L1). These PARPi-induced changes are likely to prime tumors and render them sensitive to enhanced immunogenic cell death. However, PARPi and checkpoint inhibitors are unlikely to have any effect on epithelial-mesenchymal transition (EMT) or cancer stem cells (CSCs). Many epigenetic drugs, especially lysine-specific demethylase-1 inhibitors, have shown promising activity in inhibiting CSCs and suppressing EMT. Furthermore, reprogramming of vital immune- and homologous recombination-related genes through specific epigenetic modulation might synergistically enhance the antitumor activity of a PARPi/checkpoint inhibitor combination and could identify novel targetable gene signatures. FoxP3^{+} Tregs, Forkhead box P3+ regulatory T cells; MDSC, myeloid-derived suppressor cells; PD-1, programmed cell death-1; TILs, tumor-infiltrating lymphocytes

SUM159 with olaparib and talazoparib as the PARP1i, whereas the latter used the BR5-Akt/BRCA1 deficient and T22/BRCA1 proficient ovarian cancer cell lines and veliparib.

6.2 | Cancer stem cell theory

Cancer stem cells are a small subpopulation of cancer cells found in the tumor mass that express unique cell surface marker profiles like CD44^{high}/CD24^{low}, and epithelial cell adhesion molecule (EPCAM) in breast cancer, and CD133 in colon, brain, and lung cancers.[33] There is increasing evidence that they play crucial roles in tumorigenesis, invasion, and metastasis.[33] Cancer stem cells are inherently more resistant to standard chemotherapy and radiotherapy, so are implicated in the development of tumor progression, resistance, metastasis.[34] Many features of CSCs confer the drug-resistant phenotype: reduced proliferation rate/quiescence, adaptation to hostile conditions such as inflammation and low nutrient availability, metabolic reprogramming, marked resistance to oxidative stress, the ability to rapidly activate detoxifying strategies by ATP-binding cassette transporters, enhanced and quick DNA damage responses, and impaired apoptotic machinery.[35]

6.2.1 | Epithelial-mesenchymal transition (EMT) and CSCs

Epithelial-mesenchymal transition is a latent embryonic program implicated in cancer invasion and metastasis.[36] In EMT, epithelial cancer cells lose their adhesive properties and acquire a mesenchymal trait. Epithelial-mesenchymal transition is a complex process that involves many transcription factors including, but not limited to, SNAIL, SLUG, TWIST, ZEB1, SIP1, and E47. Many pathways play crucial roles in EMT such as Wnt, Notch, nuclear factor-κB, Hedgehog, and transforming growth factor-β (TGF-β).[37] Cells undergoing EMT can acquire stem cell-like features to become CSCs.[38] The tumor microenvironment also plays a crucial role in exerting

selective pressure during the development of CSCs with metastatic properties, a process attributed to multiple cytokines participating in the various pathways mentioned above and derived from the surrounding stroma and stromal cells including tumor-associated macrophages and cancer-associated fibroblasts.[35]

6.2.2 | Effect of PARPi on CSCs

The role of PARPi on CSCs is unknown; however, there is indirect evidence to suggest CSCs are resistant to PARPi. Yamaguchi et al[39] reported that inhibition of PARP1 with olaparib activated EZH2 and increased the formation of breast cancer CSCs. In another study of human TNBC cell lines, even though tumor bulk reduced when treated with a PARPi, there was CSC sparing.[40] Various other features of CSCs are described that confer resistance to standard therapeutics (Table 4). Poly (ADP-ribose) polymerase inhibitors share many of these mechanisms, and it is reasonable to presume that PARPi are very unlikely to exert significant cytotoxicity on CSCs (Figure 1).

6.2.3 | Effect of PD-1/PD-L1 inhibitors on CSCs

The direct impact of immunotherapy on the CSC population is currently unknown; however, it is unlikely to have a significant impact on its own based on current evidence.

Hugo et al[41] examined the genomic and transcriptomic features of responders and non-responders in pretreatment tumor specimens of metastatic melanomas from patients treated with PD1i. Resistant signatures in nonresponders included genes involved in angiogenesis, immunosuppression, monocyte/macrophage chemotaxis, and EMT. Gene ontology enrichment and gene set variant analysis also confirmed that these tumor specimens were enriched in EMT and TGF-β pathway genes, both of which are implicated in CSC development. In addition, Wu et al[42] reported high PD-L1 levels in breast and colon CSCs compared to non-CSCs. Expression profiling of large breast cancer datasets revealed a positive

TABLE 4 Potential pathways and therapeutic strategies against cancer stem cells (CSCs)

Target	Mechanism
Canonical pathways	Inhibitors of Src and FAK tyrosine kinases
	Inhibitors of PI3K/Atk/mTOR
	STAT3 inhibitors
Signaling cascades in EMT	1. Stemness signaling pathway • EGFR TKIs like icotinib, which can convert CSCs to non-CSCs • Inhibiting Wnt/β-catenin or Notchb. 2. EMT signaling pathway • Hedgehog, TGF-β
Surface markers	CD133, CD44, ESA, ALDH1
Manipulation of miRNA expression	miR-21, miR-24
Epigenetic manipulation	See text

ALDH1, aldehyde dehydrogenase 1; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; miR, microRNA; TGF-β, transforming growth factor-β; TKI, tyrosine kinase inhibitor.

correlation between the stemness score of the breast cancer and PD-L1 levels.[43] Furthermore, PD-L1 knockdown decreased expression of embryonic stem cell transcription factors like octamer-binding transcription factor-4A, Nanog, and the stemness factor BMI1. Wnt signaling might also be associated with PD1i resistance, and Wnt is a crucial pathway in EMT and CSC formation.[44]

7 | EPIGENETICS

Epigenetics is defined as heritable modifications to DNA without alteration in the nucleotide sequence, resulting in altered gene transcription and chromatin structure.[45] Epigenetic changes include DNA methylation and post-translational histone modifications involving methylation or acetylation. A hallmark of epigenetic changes is their reversibility, which contrasts with the irreversible nature of the gene sequence. Aberrations in DNA methylation and histone methylation and acetylation, often involving tumor promotors or suppressors, are associated with tumorigenesis so are potential drug targets.[45]

7.1 | Lysine-specific histone demethylase-1A (LSD1) and LSD1 inhibitors

Lysine-specific histone demethylase-1A specifically demethylates mono- or dimethylated histone3 lysine4 (H3K4) or histone3 lysine9 (H3K9). Lysine-specific histone demethylase-1A is required for normal stem cell differentiation and maintenance. It is overexpressed in some cancers including bladder, prostate, and lung cancers.[46] *LSD1* knockdown reduces LSD1 expression and inhibits cancer growth, migration, and invasion. In breast cancer, LSD1 expression increases when ductal carcinoma in situ progresses to invasive ductal carcinoma.[47] In addition, LSD1 is overexpressed in TNBCs, and their stemness properties proportionately increase with LSD1 expression.[48] High LSD1 levels are seen during EMT in MCF-7 breast cancer cells following stimulation with Phorbol 12-myristate-13-acetate/TGF-β and in fully dedifferentiated mesenchymal MDA-MB-231 breast cancer cells. Decreased LSD1 levels have also been reported during the opposing biological process, mesenchymal-epithelial transition.[49]

Boulding et al[49] showed that treatment of induced MCF-7 cells with LSD1 siRNA completely abolished the CD44+/CD24− CSC population, and pharmacological inhibition with a known LSD1 inhibitor pargyline partially inhibited CSC formation. In addition, LSD1 inhibition reduced EMT and stemness-like resistance signatures induced by chemotherapy.[49] In vivo inhibition of LSD1 in combination with chemotherapy reduced tumor growth compared to chemotherapy alone. Given these findings, LSD1 inhibitors have the potential to act as anticancer agents in breast cancer due to the pathway's clear role in EMT and CSC formation.

7.2 | Histone deacetylase and HDACi

Histone deacetylase is a family of hydrolases that remove acetyl groups from lysine residues on histones, and they play important

and varied roles in tumorigenesis, including regulation of numerous genes responsible for tumor initiation and progression, angiogenesis, and cell migration.[50] Therefore, HDACi have emerged as potential anticancer drugs that inhibit DNA repair. In addition, HDACi seem to be cytotoxic to breast cancer CSCs. Hsieh et al[51] showed that HDAC3 was linked to CSC homeostasis by increasing β-catenin expression through the Akt/glycogen synthase kinase 3β pathway. Other preclinical models also have shown the impact of HDACi on CSCs.[51,52]

However, single-agent epigenetic drugs have failed to show clinical activity in many solid organ malignancies despite impressive activity in multiple cell lines in preclinical models, perhaps because they are not DNA damaging on their own and due to the development of resistance.[53] Hence, there is growing evidence and interest in their use in combination with other anticancer drugs, especially with PARPi and immunotherapies.

7.3 | Do epigenetic drugs synergize with PARPi and PD1i?

7.3.1 | Epigenetic drugs and PARPi

Epigenetic drugs might synergize with PARPi in several ways. As discussed above, PARPi exert their cytotoxicity by increasing DNA damage and generating lesions that require HR, hence the synthetic lethality seen with defective HR. Epigenetic drugs might affect the DNA repair pathways directly or indirectly. Histone deacetylase inhibition downregulates genes involved in DNA damage response and repair pathways. Prostate cancer cells inhibited with HDACi showed increased sensitivity to DNA-damaging agents,[54] an effect thought to be related to downregulation of the transcription factor E2F1. Further supporting this, PCI-24781, an HDACi, decreased RAD51 and HR pathway expression.[55] In this background, many epigenetic drugs have been combined with PARPi in the hope of enhancing DNA damage and inducing BRCAness.[56]

Muvarak et al[57] described a different mode of interaction, noting that low-dose DNA methyltransferase inhibitor (DNMTi) combined with talazoparib enhanced tight binding of talazoparib to DNA and increased DSB formation and cytotoxicity in MDA-MB-231 TNBC stem cell-like cell lines. Bhalla et al[58] reported that HDACi blocked the deacetylation of HSP90, which led to hyperacetylation and inhibition of HSP90 and many of its chaperone proteins RAD52, Atr, and checkpoint kinase 1, all of which are involved in HR and again potentially inducing BRCAness.

Epigenetic drugs might also improve the clinical efficacy of PARPi through their impact on CSCs. In addition to the effect of LSD1i on CSCs, other studies have shown the effect of DNMTi and HDACi on CSCs. Azacytidine, a DNMTi, reduced the CD44+/CD24−/ALDH+ population in T47D breast cancer cells. Furthermore, there was downregulation of key EMT regulatory pathway genes like *TWIST*, *SLUG*, and *SNAIL*.[59] Liu et al[40] reported that PARPi with olaparib caused a 1.9-fold increase in CSCs in *BRCA*-mutant SUM149 and HCC-1937 cells without changes in absolute CSC numbers. Addition of vorinostat, a

pan-HDAC inhibitor, reduced the absolute number of CSCs in SUM149, SUM159, and HCC1397 cultures and sensitized the TNBC CSCs to PARPi irrespective of the BRCA status. The researchers also found reduced formation of RAD51 foci at sites of DNA damage with the addition of vorinostat, hence creating a BRCAness phenotype.[40]

7.3.2 | Epigenetics and immunotherapy

Epigenetic drugs could potentiate the antitumor activity of immunotherapy by either reducing F Forkhead box P3+ regulatory T cells, reducing circulating and tumor-infiltrating myeloid-derived suppressor cells, or by upregulating PD-L1 and/or PD-L2 expression, thereby increasing T cell infiltration of tumors and augmenting antigen presentation by MHC.[60-63] The immune-priming effect of epigenetic drugs was observed in a clinical trial setting in 2011, when 6 patients were treated with dual epigenetic therapy (azacytidine and entinostat) but failed to show any clinical responses. Subsequently, these patients were enrolled into an immunotherapy trial and showed remarkable responses, with 5 of them experiencing a PFS of over 6 months and 2 living for over 4 years.[64] However, it should be noted that not all the results in this field are consistent. A number of clinical trials are now prospectively investigating the utility of combined epigenetic drugs and immunotherapy.

8 | FINAL DISCUSSION AND FUTURE STRATEGIES

The combination of PARPi with a PD-1 or PD-L1 inhibitor has a very promising biological rationale for synergy; however, as discussed above, both drugs are unlikely to have a significant impact on CSCs. This could lead to residual tumors enriched with CSCs that might eventually relapse, progress, and metastasize despite initial tumor shrinkage. Hence, it is of the utmost importance that future therapeutic strategies should incorporate drugs targeting CSCs.

Many therapeutic approaches are being investigated for targeting CSCs, with potential targets including signaling cascades or transcription factors involved in EMT. It is apparent that there is significant crosstalk between multiple signaling cascades and transcription factors, so combination therapies are likely to be needed. Occasionally, such crosstalk may be beneficial, as inhibition of one factor might also result in downregulation of the other; however, such close interaction could also lead to upregulation of an alternative pathway, as seen with PARPi. Kwon et al[65] reported an interaction between membrane-associated Notch and β-catenin, showing that inhibition of the Notch pathway (with PF-03084014) inhibited the Wnt pathway as well as decreasing β-catenin levels post-translationally. However, prolonged inhibition of Notch has also been shown to cause compensatory activation of Hedgehog signaling in the skin.[66] Hedgehog signaling was found to inhibit Wnt signaling through upregulation of secreted frizzled-related protein 1, so inhibition of the Hedgehog pathway could potentially activate Wnt signaling.[67]

We, therefore, believe that combination strategies targeting multiple signals in the same cascade are less likely to provide an acceptable clinical yield, especially with respect to long-term survival, due to the numerous genes, signaling pathways, and effector proteins involved. The effect of epigenetic drugs on CSCs is an upstream effect that is likely to have controlled inhibition/regulation of all corresponding downstream cascades.

Although toxicity is always a concern with combination therapy, as seen in PARPi/chemotherapy trials, it is possible that low doses of epigenetic drugs could be adequate to induce durable responses, hence the risk of added toxicity is expected to be lower when used in this way.[59] In addition, the rate of serious toxicity is low with PD1i and the spectrum of toxicity is distinct to the other two drug classes.

We propose the addition of epigenetic drugs to a PARPi/PD1i combination might be synergistic and could potentially overcome different resistance mechanisms, causing cytotoxic cell death by DNA damage, suppression of CSCs, and potentiating immunogenic cell death (Figure 2). Furthermore, such novel combination strategies could uncover new epigenetic or gene signatures related to DNA repair, PD-L1 expression, and/or CSC formation, which could be potential targets for future therapies. Correction of such upstream targets or aberrations might avoid the need to use multiple drugs to target multiple downstream signals.

CONFLICT OF INTEREST

All authors have declared no conflicts of interest.

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SUPPORTING INFORMATION

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