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Hierarchies of resistance to DNA damage response inhibitors: From pathway restoration to replication stress tolerance

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Abstract

DNA damage response (DDR) inhibitors have reshaped precision oncology by exploiting tumor-specific vulnerabilities in genome maintenance and replication stress control. The clinical success of poly(ADP-ribose) polymerase (PARP) inhibitors—particularly in homologous recombination-deficient cancers—has validated this strategy, establishing a paradigmatic model for DDR-targeted therapies; however, both intrinsic and acquired resistance remain pervasive and limit durable benefit. In this Review, we analyze resistance mechanisms with a framework centered on PARP inhibitor biology, while extending key principles to other DDR-targeting strategies. We propose a hierarchical model that distinguishes dominant resistance drivers—mechanisms that restore DNA repair capacity or bypass the inhibited node (e.g., homologous recombination reactivation or checkpoint rewiring)—from adaptive resistance mechanisms that increase replication stress tolerance without restoring repair function, including replication-fork protection, metabolic reprogramming, cellular plasticity, and microenvironmental support. We discuss how tumor heterogeneity and therapy-imposed selective pressures shape clonal evolution and complicate biomarker development. We also critically evaluate combination strategies, highlighting why strong mechanistic rationale often fails to translate into consistent clinical benefit due to toxicity, scheduling constraints, and insufficient biomarker guidance. Finally, we outline priorities for moving beyond static genomic classifiers toward functional and longitudinal assessment of replication stress, DNA repair engagement, and tumor evolution to support more durable, biologically informed therapeutic strategies.

Introduction

The DDR is a multilayered signaling and repair network that preserves genome integrity by detecting DNA lesions, coordinating cell cycle checkpoints, stabilizing perturbed replication forks, and promoting lesion repair via distinct DNA repair pathways [1–3]. In proliferating cells, DDR activity is intrinsically coupled to DNA replication because replication forks are continuously challenged by endogenous DNA damage [4,5], replication-transcription conflicts [6,7], and oncogene-driven replication stress [8,9]. Cancer cells typically exhibit elevated replication stress and genomic instability, increasing their dependence on specific DDR components for survival and proliferation [10,11].

DDR inhibitors (DDRi) exploit this dependency by targeting key nodes of genome maintenance, thereby amplifying replication stress or disabling DNA damage tolerance beyond a viable threshold [3,11,12]. Importantly, the biological rationale and therapeutic consequences of DDR inhibition differ substantially across inhibitor classes. PARP inhibitors primarily induce cytotoxicity by trapping PARP on DNA and interfering with single-strand break repair [13], leading to replication fork collapse and the formation of double-strand breaks that require homologous recombination (HR) for resolution [14]. In contrast, ATR (ataxia telangiectasia and Rad3-related), ATM (ataxia telangiectasia mutated), CHK1 (checkpoint kinase 1), or WEE1 (Wee1 G2 checkpoint kinase) inhibition predominantly impairs checkpoint control and replication stress responses [11,15], pushing cells with unstable forks or unresolved DNA damage into mitotic catastrophe [14].

Synthetic lethality is best established for PARP inhibitors in tumors with breast cancer susceptibility gene 1/2 (BRCA1/2) mutations or other forms of homologous recombination deficiency (HRD) [15]. Extending this paradigm to other DDRi requires a more nuanced view of replication stress biology and fork-associated DNA damage rather than direct extrapolation from HRD-driven sensitivity [12]. Indeed, several DDRi show activity in tumor contexts marked by high replication stress rather than canonical HR defects [11], underscoring the need to distinguish DDR pathway inhibition, replication-fork destabilization, and checkpoint failure as mechanistically distinct therapeutic principles [16].

Despite the clinical success of DDRi—particularly PARP inhibitors in ovarian, breast, prostate, and pancreatic cancers [14]—intrinsic and acquired resistance are frequently observed, limiting durable clinical benefit [17]. Resistance arises through mechanistically distinct processes, including restoration of DNA repair capacity [18], stabilization of stalled replication forks [19], checkpoint rewiring [20], and adaptive changes driven by tumor heterogeneity and the tumor microenvironment [21,22]. Importantly, these mechanisms

differ in biological impact: some directly undermine the therapeutic principle of DDR inhibition, whereas others increase tolerance to replication stress without restoring DNA repair function [23–25].

Beyond PARP trapping, multiple complementary mechanisms contribute to PARP inhibitor-induced cytotoxicity, especially in HRD contexts. PARP inhibition can lead to the accumulation of single-stranded DNA (ssDNA) gaps during DNA replication, arising from defective processing of replication-associated lesions and impaired Okazaki fragment maturation [26,27]. These ssDNA gaps may persist and convert into double-strand breaks in later replication cycles, increasing dependence on HR for resolution. In parallel, PARP inhibition can alter replication fork dynamics, including increased fork speed and instability, further exacerbating replication stress [28]. Notably, PARP inhibitor cytotoxicity is not solely dependent on stable PARP1 trapping on chromatin, as PARP1 trapping-independent mechanisms of replication-associated damage have also been described [27,29–31]. Collectively, these findings support a model in which PARP inhibitors cause a spectrum of replication-related lesions—rather than a single dominant lesion type—requiring HR for repair, thereby explaining the synthetic lethality observed with PARP inhibitors in HR-deficient cells.

This Review examines resistance to DDRi across genetic, epigenetic, metabolic, and microenvironmental dimensions, with emphasis on mechanistically validated pathways and their translational implications. We discuss emerging biomarkers for patient stratification and resistance monitoring, critically evaluate combination strategies, and highlight constraints that contribute to inconsistent clinical benefit.

GENETIC MECHANISMS OF RESISTANCE

Genetic alterations represent the most robust and clinically validated drivers of acquired resistance to DDRi (Box 1) [25,32]. These changes can (i) restore the repair function that underpins therapeutic sensitivity [33,34], (ii) rewire damage signaling and repair pathway choice to compensate for the inhibited node [35,36], or (iii) stabilize replication-associated DNA structures to reduce dependency on the targeted pathway [37,38].

In the context of PARP inhibitor treatment, cytotoxicity arises from a spectrum of replication-associated lesions, including single-stranded DNA (ssDNA) gaps, altered replication fork dynamics, and both PARP trapping-dependent and -independent processes [27–30]. These lesions increase reliance on homologous recombination and replication stress response pathways, thereby defining the selective pressure that drives resistance evolution.

Accordingly, genetically driven resistance can be organized into three mechanistically distinct—though frequently coexisting—categories: (i) restoration of the inhibited repair pathway [39,40]; (ii) compensation through altered DDR signaling or pathway choice [41,42]; and (iii) protection and stabilization of stalled replication forks (Figure 1) [19,43]. Major DDR pathways and associated genetic resistance mechanisms are summarized in Table 1.

Restoration of the inhibited DNA repair pathway

Direct restoration of the targeted DDR pathway constitutes the most definitive resistance mechanism, as it re-establishes the molecular function whose loss underpins drug sensitivity [25]. This has been most extensively documented for PARP inhibitor (PARPi) resistance in tumors harboring BRCA1 or BRCA2 alterations [33,44,45].

Secondary ("reversion") mutations in BRCA1/2 can restore an open reading frame or partially recover protein function, thereby reactivating HR for repair of replication-associated DNA double-strand breaks [33]. Similar secondary mutations affecting other HR factors, including RAD51C and RAD51D, support the broader principle that pathway restoration is not restricted to BRCA-mutant tumors [46]. Functionally, these events reinstate RAD51 loading at sites of DNA damage—a hallmark of HR proficiency that can be measured by RAD51 foci assays [47]. Reversion mutations have been observed in ovarian, breast, and prostate cancers after PARPi or platinum-based therapy and correlate strongly with clinical resistance [39,48,49].

Signaling compensation and altered pathway choice

When direct pathway restoration does not occur, tumors may acquire resistance through rewiring of DNA damage signaling and by selecting alternative repair pathways [25]. A well-established example involves loss-of-function alterations in components of the 53BP1 pathway, including 53BP1 itself and downstream effectors such as the Shieldin complex [36,50].

Under physiological conditions, 53BP1 restricts DNA end resection at double-strand breaks, favoring non-homologous end joining over HR [50]. In BRCA1-deficient cells, loss of 53BP1 or Shieldin relieves this resection block, permitting partial restoration of RAD51-dependent HR despite continued BRCA1 deficiency [36,51]. This represents a compensatory shift in pathway choice rather than true restoration of BRCA1 function and is mechanistically distinct from reversion mutations [41]. Additional forms of signaling compensation include alterations in checkpoint control. Indeed, mutations affecting ATM, ATR, CHK1, or WEE1

signaling can reprogram cell cycle responses to DNA damage, enabling proliferation under higher levels of genomic instability during DDR inhibition [52,53]. Such adaptations reduce the cytotoxicity of DDRi without restoring canonical repair capacity [54].

Replication fork protection and stabilization

Replication-fork protection represents a genetically and mechanistically distinct resistance mechanism that reduces reliance on HR without restoring HR itself (Figure 1) [41]. In HRD cells, stalled forks are particularly vulnerable to nucleolytic degradation, which can convert transient replication stress into fork collapse and single-ended double-strand breaks [43]. PARP inhibition exacerbates this vulnerability by perturbing single-strand break repair and PARP-dependent fork-associated processes [19]. Importantly, these fork-protection mechanisms should be interpreted in the context of emerging models of PARP inhibitor-induced cytotoxicity, which extend beyond PARP1 trapping to include the accumulation of single-stranded DNA (ssDNA) gaps and altered replication fork dynamics [27–30]. These lesions increase replication-associated DNA damage and shape the selective pressure for fork stabilization as an adaptive resistance mechanism.

Genetic alterations that stabilize stalled forks can therefore confer resistance by preventing fork degradation even in the continued absence of functional HR [55]. Loss of Pax transactivation domain-interacting protein (PTIP), which recruits nucleases such as MRE11 to stalled forks, reduces nucleolytic processing and preserves fork integrity in BRCA-deficient cells [55]. In addition, components of the 53BP1-Shieldin axis can influence fork protection independently of their effects on DNA end resection at double-strand breaks [54]. Additional fork-protection mechanisms involve the CST (CTC1-STN1-TEN1) complex, which binds single-stranded DNA at stalled forks and protects nascent DNA from MRE11-mediated degradation [56]. Conversely, structure-specific endonucleases such as MUS81 play context-dependent roles in fork processing, supporting fork restart under controlled conditions but contributing to genomic instability when dysregulated [57]. Thus, the balance between fork protection and controlled processing is a key determinant of DDRi sensitivity [58]. Notably, fork stabilization does not restore HR; instead, it limits the formation of replication-associated breaks that would otherwise require HR for repair [59]. This distinction helps explain why fork protection can confer resistance in tumors that remain HRD by standard criteria [60]. Table 1 summarizes major genetic mechanisms of acquired resistance to DDRi, distinguishing pathway restoration from fork protection and signaling compensation.

EPIGENETIC MECHANISMS OF RESISTANCE

Epigenetic regulation shapes the DDR by controlling chromatin accessibility, transcriptional output of DDR genes, and the recruitment dynamics of repair factors at sites of DNA damage [61,62]. Unlike genetic alterations, epigenetic changes do not modify DNA sequence; however, they can generate stable, heritable phenotypes that influence sensitivity to DDRi by affecting HR, replication stress responses, and cell cycle control (Figure 2) [63].

Conceptually, epigenetic mechanisms most often act as adaptive resistance modifiers rather than dominant resistance drivers [64]. Their effects are highly context-dependent and frequently reversible, complicating clinical exploitation but also providing opportunities for therapeutic intervention and combination strategies [61].

Histone modifications and chromatin remodeling

Post-translational histone modifications—including methylation, acetylation, phosphorylation, and ubiquitination—regulate chromatin compaction and determine access of DDR machinery to damaged DNA [61,65]. Chromatin-modifying complexes therefore influence DDRi sensitivity by shaping both the expression of repair genes and the physical accessibility of damaged chromatin [65].

The Polycomb repressive complex 2 (PRC2), via EZH2-mediated H3K27 trimethylation (H3K27me3), can also repress HR-related genes such as BRCA1 and RAD51 and reduce HR proficiency [66]. In preclinical models, pharmacological EZH2 inhibition decreases HR capacity and can sensitize tumor cells to PARP inhibition, particularly in contexts lacking canonical genetic HR defects [67]. Conversely, loss-of-function alterations in chromatin remodeling complexes, such as SWI/SNF (e.g., ARID1A), can alter chromatin accessibility at replication-associated lesions and modulate DDR pathway engagement, thereby influencing the response to DDR inhibition [68,69].

Histone demethylases also contribute to epigenetically mediated resistance. LSD1 (KDM1A), which regulates H3K4 and H3K9 methylation, supports efficient recruitment and/or maintenance of HR programs including BRCA1/2-RAD51 axis in relevant tumor contexts [70]. Altered LSD1 activity can shift the balance between DNA end resection and end joining, indirectly influencing HR engagement and DDRi sensitivity [70]. Together, these observations underscore that chromatin state—rather than isolated changes in single DDR genes—can determine DDR pathway utilization [62,71,72].

DNA methylation and reversible homologous recombination suppression

DNA methylation at CpG-rich promoter regions is a major mechanism of transcriptional repression in cancer [73–79]. Aberrant methylation of DDR genes can create a functional HR-deficient state even in the absence of genetic mutations, thereby conferring transient sensitivity to PARP inhibitors [63,64,80,81].

Promoter hypermethylation of BRCA1 is a well-characterized example of epigenetically induced HR deficiency associated with PARP inhibitor responsiveness in ovarian cancer [80]. However, resistance can emerge through demethylation and transcriptional reactivation of BRCA1, restoring HR and reversing PARP inhibitor sensitivity [64,82]. These observations highlight a key limitation of epigenetically driven HR defects: under therapeutic pressure, they may be intrinsically unstable, allowing recovery of repair capacity without new coding mutations [82].

Because DNA methylation writers/erasers depend on metabolite availability (e.g., S-adenosylmethionine (SAM) and α-ketoglutarate-linked reactions), metabolic state becomes a direct upstream regulator of methylation dynamics—providing a mechanistic bridge between epigenetic plasticity and replication stress tolerance [73,83]. DNA methylation dynamics are governed by DNA methyltransferases (DNMTs) and ten-eleven translocation (TET) enzymes, whose activities are influenced by the cellular metabolic state [83,84]. This metabolic-epigenetic coupling provides an additional layer of adaptability and may facilitate epigenetic reprogramming of DDR gene expression during prolonged DDR inhibition [73].

Non-coding RNAs and epigenetic regulation of DDR signaling

Non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), regulate DDR pathways at transcriptional and post-transcriptional levels [85,86]. Multiple miRNAs target HR components such as BRCA1, BRCA2, and RAD51, thereby modulating PARP inhibitor sensitivity in a cellular context-dependent manner [86]. lncRNAs can further influence DDR by serving as scaffolds for chromatin-modifying complexes and regulating transcriptional repression or activation of DDR-related genes [86,87].

Although numerous non-coding RNAs have been linked to chemotherapy or PARP inhibitor resistance in preclinical models, their functional relevance in patients remains incompletely defined [85]. At present, evidence supporting non-coding RNAs as clinically actionable mediators of DDRi resistance is limited.

Epigenetic scars: an emerging and exploratory concept

DNA damage and repair can induce persistent epigenetic alterations—often referred to as “epigenetic scars”—including stable changes in DNA methylation, accumulation of repressive histone marks, and altered chromatin accessibility at DDR gene loci [63,79]. These alterations may persist beyond the initiating insult and influence long-term cellular behavior and heterogeneity [62]. Indeed, DNA damage and homologous repair modify the genome in a strand-specific manner [31], generating cell clones that express (unmethylated) or do not express (methylated) the gene(s) located in the damaged and repaired region [63,77]. Each DNA strand is specifically marked by gain or loss of methylation upon damage and HR repair. As a consequence, this strand-specific methylation of repaired DNA generates a cohort of expressing (unmethylated strand) or non-expressing (methylated strand) clones. This phenomenon greatly increases the fitness of the cell population by generating clones with distinct expression patterns and de novo methylation profiles, depending on the functional context of the repaired DNA segments [76,77].

Epigenetic scars have been proposed as contributors to adaptive resistance during sustained DDR inhibition; however, supporting evidence remains largely preclinical [62]. Long-term PARP inhibitor exposure has been associated with locus-specific chromatin remodeling and transcriptional reprogramming in ovarian cancer-relevant settings, potentially enabling alternative survival strategies [64]. Nonetheless, current clinical evidence is insufficient to support epigenetic scars as predictive biomarkers or therapeutic targets, and their translational value will depend on robust functional assays and longitudinal sampling strategies [62,74–78].

Overall, epigenetic mechanisms contribute to resistance to DDRi primarily by modulating pathway engagement, transcriptional plasticity, and replication stress tolerance rather than by directly restoring DNA repair capacity [61,64] (Figure 2 and Table 2).

METABOLIC REPROGRAMMING AND REPLICATION STRESS TOLERANCE

As with epigenetic remodeling, metabolic reprogramming most often acts as an adaptive resistance modifier rather than a dominant resistance driver, increasing tolerance to replication stress [88,89]. Cancer cells exhibit extensive metabolic rewiring that supports rapid proliferation under chronic replication stress and genomic instability [5]. Notably, metabolic alterations frequently precede therapy and contribute to baseline replication stress, rather than arising solely as a consequence of DDR inhibition [90]. In this context, resistance to DDRi can be supported by metabolic programs that sustain nucleotide availability for DNA replication, maintain nicotinamide adenine dinucleotide (NAD^+)

homeostasis, buffer oxidative stress, and meet the ATP demand of checkpoint signaling and fork-associated stress responses [91,92] (Figure 3).

Nucleotide and NAD^+ metabolism as buffers of replication stress

Sustained DNA replication requires a tightly regulated supply of deoxyribonucleotide triphosphates (dNTPs) [91]. Oncogene-driven replication stress can arise from unscheduled origin firing and accelerated S-phase progression, increasing demand for nucleotide synthesis [5]. Insufficient dNTP availability exacerbates fork stalling and collapse, promoting replication-associated DNA damage [93,94].

Consistent with an adaptive buffering model, resistant tumor cells may upregulate nucleotide biosynthesis and salvage pathways to stabilize dNTP pools and preserve replication dynamics under stress [91,95]. Increased activity of ribonucleotide reductase (RNR)—particularly via elevated expression of RRM2—has been associated with replication stress resilience and aggressive tumor behavior, consistent with reduced sensitivity to genome maintenance targeting in preclinical contexts [93,96]. Enhanced nucleotide salvage can provide additional buffering capacity, especially when origin control is compromised (e.g., under ATR or CHK1 inhibition), thereby limiting catastrophic fork collapse [97,98].

In parallel, PARP1 consumes nicotinamide adenine dinucleotide (NAD^+) during poly(ADP-ribosyl)ation reactions involved in single-strand break repair and replication-fork stability [99]. Many tumor cells upregulate NAD^+ biosynthesis pathways—most notably by increasing nicotinamide phosphoribosyltransferase (NAMPT) expression—to sustain PARP activity and broader metabolic demands [92,99]. Elevated NAD^+ availability may therefore support residual DNA damage tolerance under PARP inhibition [100]. Consistent with this concept, NAMPT inhibition can restore sensitivity to PARP inhibitors in resistant models, although clinical translation is constrained by systemic toxicity and limited therapeutic windows [100,101].

Redox homeostasis and oxidative stress adaptation

Cancer cells experience elevated basal oxidative stress driven by oncogenic signaling, mitochondrial activity, and high metabolic flux [102]. Robust redox buffering is therefore required to prevent excessive oxidative DNA damage and replication-associated lesions [103]. Rather than oxidative stress being generated exclusively by DDR inhibition, many resistant states appear to build on pre-existing redox liabilities by reinforcing antioxidant capacity through glutathione, thioredoxin, and NRF2-dependent transcriptional programs

[98,104]. Increased pentose phosphate pathway flux supports NADPH production, which sustains both redox homeostasis and nucleotide biosynthesis [91,103]. Collectively, these adaptations can reduce replication-associated damage and attenuate the cytotoxic impact of DDR inhibition [88].

Energy metabolism and replication stress resilience

Replication stress tolerance and checkpoint signaling are energy-intensive processes that depend on adequate ATP availability [89]. Many tumors display metabolic plasticity, switching between glycolysis and mitochondrial oxidative phosphorylation (OXPHOS) in response to environmental constraints [105]. Increased reliance on OXPHOS has been reported in ovarian cancer contexts and associated with therapeutic resistance phenotypes, consistent with enhanced stress tolerance [105,106].

Pharmacological inhibition of OXPHOS—including the use of metformin—reduces ATP availability and can potentiate PARP inhibitor activity in preclinical models [107,108]. One-carbon/folate metabolism also supports replication stress tolerance by sustaining nucleotide synthesis and redox balance [109,110]. Upregulation of mitochondrial folate enzymes such as MTHFD2 has been linked to redox control and replication stress phenotypes in cancer, and its pharmacological targeting can induce replication stress in tumor models [109,111]. In addition, altered levels of S-adenosylmethionine (SAM) may indirectly influence DDR gene expression through epigenetic mechanisms, linking metabolic adaptation to transcriptional plasticity [92].

Mechanistically, energy metabolism supports tolerance to replication stress by sustaining the energetic and biosynthetic demands of DNA replication and checkpoint signaling. Adequate ATP availability is required for checkpoint activation and for maintaining replication fork stability, whereas ATP depletion can impair these processes and promote fork collapse. In addition, metabolic pathways regulate the availability of key cofactors such as NAD^+, which is required for PARP activity, and dNTP pools, which are essential for replication fork progression [91,92]. Perturbation of these metabolic inputs can therefore exacerbate replication stress and sensitize tumor cells to DDR inhibition, providing a mechanistic rationale for combining metabolic interventions with DDR-targeting therapies [5,99].

Thus, metabolic rewiring does not directly restore DNA repair capacity but increases the threshold of tolerable replication stress, enabling tumor cells to survive under DDR inhibition.

Lipid metabolism and metabolic support from the tumor microenvironment

Lipid metabolic programs can contribute to ATP and NADPH production, supporting antioxidant defenses and replication stress tolerance [88]. In breast cancer models, lipid metabolic dependencies (including FA synthesis pathways) have been linked to PARP inhibitor resistance, and targeting these pathways can resensitize tumors to PARPi in preclinical settings [112]. Lipid metabolism may also influence post-translational modifications of proteins involved in replication-fork protection, further connecting metabolic state to fork stability [88].

Metabolic adaptation is shaped not only by tumor-intrinsic rewiring but also by nutrient availability and stromal interactions within the tumor microenvironment (TME) [113]. DDR inhibition can remodel immune and stromal compartments through metabolic reprogramming, influencing therapeutic response and resistance evolution [113]. Hypoxia stabilizes hypoxia-inducible factor 1α (HIF-1α) and promotes glycolytic and redox-adaptive programs; in parallel, hypoxia is linked to replication stress and altered DDR states, which can modulate the efficacy of DDRi [102,114]. These metabolic interactions reinforce adaptive resistance phenotypes and intersect with tumor heterogeneity and cellular plasticity (Figure 3 and Table 3) [114].

In summary, metabolic reprogramming supports resistance to DDRi primarily by buffering replication stress and sustaining stress-adaptive processes rather than by restoring DNA repair capacity [88,89]. This distinction has practical implications: metabolic interventions are most rationally deployed as DDRi-based combinations aimed at pushing replication stress beyond adaptive thresholds, with careful attention to toxicity, scheduling, and tumor-context dependence [88,101].

TUMOR MICROENVIRONMENT, HETEROGENEITY, AND PLASTICITY

Resistance to DDRi cannot be fully understood by focusing exclusively on tumor cell-intrinsic mechanisms [115,116]. Instead, resistance emerges from the dynamic interplay among genetic and epigenetic heterogeneity, metabolic adaptation, and selective pressures imposed by the tumor microenvironment (TME) [21]. Intratumoral heterogeneity provides the substrate, DDRi imposes selection, and the outcome is a mixture of (i) dominant clones carrying stable repair-restoring/bypass alterations and (ii) adaptive states that fluctuate with drug exposure, microenvironmental support, and plasticity—often coexisting within the same lesion [117,118]. Together, these forces shape clonal evolution and cellular plasticity

under sustained DDR inhibition, generating heterogeneous—and often transient—resistance states [119].

Intratumoral heterogeneity as a substrate for resistance

Intratumoral heterogeneity encompasses genetic, epigenetic, transcriptomic, and functional diversity within tumor cell populations and provides the substrate on which DDRi therapy exerts selective pressure [116]. While DDRi can efficiently eliminate sensitive clones (e.g., subsets with homologous recombination deficiency), pre-existing or emergent subclones characterized by enhanced replication stress tolerance, altered checkpoint control, or partial repair proficiency may expand and dominate the tumor population [115]. Genomic heterogeneity plays a central role in acquired resistance, particularly through reversion mutations or compensatory alterations that affect DDR pathway choice [115,116]. However, epigenetic and transcriptomic heterogeneity can generate phenotypic diversity even in the absence of new genetic lesions, enabling rapid adaptation to DDR inhibition [117,118]. Functional heterogeneity—including differences in replication dynamics, DDR pathway engagement, and cell cycle progression—further contributes to variable treatment responses and is not fully captured by bulk molecular profiling [120].

The tumor microenvironment as a selective and permissive niche

The TME imposes continuous selective pressure on tumor cells through gradients of oxygen and nutrients, mechanical stress, immune surveillance, and variable drug penetration [121,122]. Hypoxia, a common feature of solid tumors, stabilizes hypoxia-inducible factor 1α (HIF-1α) and promotes metabolic reprogramming; depending on context, hypoxia can modulate S-phase progression, redox balance, and replication stress burden, thereby altering the efficacy of DDRi [123,124]. Hypoxic conditions can also influence chromatin organization and transcriptional programs, indirectly shaping DDR pathway engagement [125,126]. Stromal components of the TME, including cancer-associated fibroblasts and immune cells, contribute to resistance by supplying metabolites and growth factors that sustain replication stress tolerance and DNA damage adaptation [127]. These paracrine interactions can mitigate the cytotoxic effects of DDR inhibition, particularly in tumor regions with limited drug penetration and heterogeneous exposure [122,128].

Immune components of the TME also modulate DDRi response [121]. DDR inhibition can activate innate immune signaling pathways, such as cGAS (cyclic GMP-AMP synthase)-STING (stimulator of interferon genes), through accumulation of cytosolic DNA or

micronuclei [129,130]. Depending on tumor context and immune composition, this signaling may enhance antitumor immunity or, conversely, promote immune suppression by upregulating immune checkpoints and recruiting immunosuppressive populations [131–133]. Thus, DDRi-induced immune modulation is intrinsically context-dependent and shaped by the baseline immune state of the TME [131].

Cellular plasticity and reversible resistance states

Cellular plasticity enables tumor cells to transition between phenotypic states without acquiring stable genetic alterations, facilitating rapid adaptation to therapeutic stress [117,118]. Under DDR inhibition, plasticity can support transient drug-tolerant states characterized by altered replication kinetics, modified checkpoint engagement, and increased reliance on stress-adaptive pathways [118]. Plasticity-driven resistance is particularly relevant for DDRi because replication stress and checkpoint signaling are tightly coupled to cell-cycle dynamics [117]. Tumor cells that transiently reduce replication rates, reprogram origin usage, or adopt slow-cycling states may evade DDRi-induced lethality without restoring DNA repair activity [117,118]. These adaptive states are often reversible upon drug withdrawal, complicating resistance assessment and highlighting limitations of static biomarker strategies [118]. Epigenetic remodeling and metabolic adaptation are key enablers of cellular plasticity and interact with TME-derived signals to stabilize resistant phenotypes [117,123]. Together, these processes generate heterogeneous resistance patterns within individual tumors, even under uniform treatment conditions [21]. In this context, emerging single-cell and computational approaches—including machine learning-based analyses—may be particularly valuable for modeling clonal evolution and context-dependent resistance trajectories, rather than for immediate clinical decision-making [119,120].

Persistent DNA damage and inflammation as drivers of clonal selection

Beyond pre-existing genetic diversity, tumor heterogeneity is dynamically shaped by persistent DNA damage and chronic inflammation [129,134]. Sustained activation of the DDR, particularly through ATM and ATR signaling, not only coordinates DNA repair but also induces senescence-associated secretory phenotypes (SASP), metabolic rewiring, and type I interferon responses [135,136]. In this context, unresolved DNA lesions and replication stress can establish a self-reinforcing loop in which DNA damage promotes inflammatory signaling, which in turn exacerbates oxidative stress and genomic instability [3,131,134].

Cytosolic DNA fragments generated by replication stress, micronuclei formation, or defective repair can activate the cGAS-STING pathway, amplifying inflammatory signaling and modulating the tumor microenvironment [129,130]. While acute activation of these pathways may constrain tumor growth through senescence or immune surveillance, chronic activation can instead create a permissive niche that favors the survival of clones that tolerate high levels of genomic instability [135,137]. Importantly, persistent DDR signaling has been linked to long-term epigenetic remodeling, including DNA methylation changes and chromatin reorganization [126,138]. Such alterations may reprogram transcriptional networks and enable phenotypic plasticity, facilitating adaptive resistance to DDR-targeting agents [117]. In this framework, inflammation is not merely a bystander effect of genomic instability but an active selective force that shapes clonal evolution, promoting the expansion of subclones with enhanced DNA repair capacity, replication stress tolerance, or immune evasion traits [131,132].

COMBINATION STRATEGIES WITH DNA DAMAGE RESPONSE INHIBITORS

The limited durability of clinical responses to DDRi has prompted extensive interest in combination strategies to enhance antitumor efficacy and delay or prevent resistance [138]. Although numerous preclinical and early-phase clinical studies have investigated DDRi-based combinations, translational success has been inconsistent [139]. Many regimens are supported by strong mechanistic rationale but are frequently constrained in practice by overlapping toxicity, suboptimal scheduling, and insufficient biomarker guidance [140,141]. Importantly, toxicity is rarely a biological resistance mechanism per se; rather, it functions as a dominant clinical constraint that caps exposure and forces scheduling compromises, thereby creating ecological space for adaptive tolerance programs [142,143].

Rather than cataloging individual trials, this section highlights the biological principles underlying DDRi-based combinations and the determinants most often associated with success or failure in overcoming resistance [139]. It is important to note that DDRi comprise mechanistically distinct drug classes, including PARP inhibitors, ATR inhibitors, CHK1 inhibitors, and WEE1 inhibitors, each targeting different nodes of genome maintenance and replication stress responses [11,16]. Accordingly, their biological effects, toxicity profiles, and resistance mechanisms are not interchangeable. In the following sections, we specify the relevant DDRi class where mechanistically appropriate to improve clarity and interpretability.

DDR inhibitors and immune checkpoint blockade

The rationale for combining DDRi with immune checkpoint inhibitors (ICIs) is that DDR inhibition can promote cytosolic DNA accumulation, micronucleus formation, and activation of innate immune signaling pathways such as cGAS-STING [144]. These events may induce type I interferon responses, enhance antigen presentation, and increase tumor immunogenicity, providing a mechanistic basis for PD-1/PD-L1 blockade combinations [144].

Despite this compelling rationale, clinical outcomes have been variable [145,146]. Early single-arm studies suggested activity in selected patient populations [146,147], whereas randomized or larger prospective experiences have often shown limited improvement over DDRi monotherapy [145,148]. For example, the DORA trial reported comparable progression-free survival with olaparib plus durvalumab and with olaparib alone [149], underscoring the difficulty of translating immune activation into durable benefit and the limitations of non-randomized designs for establishing clinical added value [149]. Importantly, DDRi-induced immune modulation is context-dependent [150]. Prolonged DDR inhibition may also promote immune evasion by upregulating immune checkpoints, recruiting immunosuppressive populations, or exhausting effector responses [145,150]. Consequently, DDRi-ICI combinations are unlikely to be broadly effective and will require biomarkers that capture immune context in addition to DNA repair status [147,150].

These effects provide a mechanistic explanation for combining DDRi with immune checkpoint inhibitors targeting the PD-1/PD-L1 axis (e.g., pembrolizumab, nivolumab, durvalumab), which aim to restore antitumor T-cell activity by blocking inhibitory immune signaling pathways. These findings suggest that DDRi-ICI combinations are unlikely to be broadly effective and instead require biomarker-driven patient selection based on both immune context and DDR status.

Combination with cytotoxic chemotherapy and radiotherapy

Combining DDRi with DNA-damaging chemotherapy or radiotherapy aims to overwhelm tumor repair capacity by increasing DNA lesions while limiting repair [139]. This approach is particularly relevant for inhibitors targeting ATR, CHK1, or WEE1, which regulate replication stress responses and checkpoint control [140,141]. Although robust preclinical synergy has been observed, clinical implementation has been challenging [140]. Overlapping toxicities—most notably myelosuppression and gastrointestinal toxicity—often limit dose intensity and scheduling flexibility [141,142]. In several trials, dose reductions required to manage toxicity

compromise biological synergy, resulting in only modest clinical benefit [140,143]. These limitations reflect a fundamental constraint of DDRi-based combinations: DDRi sensitize not only tumor cells but also normal proliferative tissues to genotoxic stress [142]. Without precise patient stratification and optimized schedules that preserve target engagement while minimizing cumulative toxicity, combinations risk amplifying adverse effects without sufficient incremental efficacy [140,141]. Radiosensitization strategies with ATR-pathway targeting are also being explored, but tolerability and immune/microenvironmental context are likely to be key determinants of benefit [151].

Combination with cell cycle checkpoint abrogators (WEE1/CHK1 axis)

To avoid confusion with immune checkpoint blockade, it is useful to distinguish cell cycle checkpoint inhibition (e.g., WEE1/CHK1 pathway inhibition) as a separate combination class [152,153]. Here, the goal is to force damaged or replication stressed tumor cells through S/G2 and into mitosis, converting sublethal replication stress into mitotic catastrophe [154]. This strategy can be mechanistically rational in tumors with high baseline replication stress or impaired G1 control [142].

However, checkpoint abrogation also narrows the therapeutic window because normal proliferative tissues rely on these checkpoints under physiological stress [142]. As a result, feasibility is frequently constrained by toxicity and by the need for carefully engineered dosing schedules that maintain antitumor synergy while limiting normal-tissue injury [152–155].

Targeting metabolic dependencies

Metabolic combination strategies aim to exploit DDRi-resistant tumors' dependence on replication stress buffering and stress-adaptive pathways rather than directly increasing DNA damage [100]. Preclinical studies report synergy between DDRi and agents targeting NAD^+ biosynthesis, mitochondrial metabolism, glutaminolysis, or fatty acid metabolism [101,156].

However, most metabolic combinations remain preclinical, and translation is limited by systemic toxicity, narrow therapeutic windows, and strong tumor-context dependence shaped by microenvironmental factors [100,101]. At present, metabolic interventions are best viewed as experimental approaches to push replication stress beyond adaptive thresholds in biomarker-enriched settings rather than broadly applicable solutions [112,157,158].

Epigenetic therapies as modulators of adaptive resistance

The reversible nature of epigenetically mediated resistance provides a rationale for combining DDRi with epigenetic therapies, including histone deacetylase (HDAC) and DNA methyltransferase (DNMT), EZH2, or LSD1 inhibitors [67,159]. In preclinical models, these agents can transiently suppress homologous recombination or disrupt adaptive transcriptional programs associated with DDRi resistance [70,160].

Despite a compelling mechanistic rationale, clinical evidence supporting DDRi-epigenetic combinations remains limited [161]. Epigenetic therapies often exert pleiotropic effects, complicating attribution of clinical responses to specific DDR-related mechanisms, and cumulative toxicity remains a concern [161]. Consequently, epigenetic interventions should be regarded primarily as experimental strategies to modulate adaptive resistance states rather than to correct dominant resistance drivers [67,160].

Principles for rational combination design

Collectively, available evidence indicates that DDRi-based combinations are most likely to succeed when three conditions are met: (i) the combination targets a dominant resistance mechanism rather than a secondary adaptive modifier; (ii) patient selection is guided by mechanistically relevant biomarkers; and (iii) treatment scheduling minimizes overlapping toxicity while preserving biological synergy [139].

More broadly, the integrated influence of tumor heterogeneity, cellular plasticity, and the tumor microenvironment limits the predictive value of single biopsies and highlights the need for functional and longitudinal monitoring [139]. Effective therapeutic strategies will therefore need to couple targeting of dominant genetic resistance mechanisms with approaches that disrupt adaptive niches, metabolic support, or immune-mediated tolerance, potentially through adaptive schedules that constrain clonal outgrowth and limit plasticity [100,145] (Figures 4 and 5; Tables 2-4).

BIOMARKERS AND PRECISION APPROACHES: STEERING DDR-TARGETED THERAPY

The limited durability of clinical responses to DDRi underscores the need for biomarkers that capture dynamic, functional, and context-dependent resistance mechanisms [162]. The efficacy of DDRi depends critically on accurate patient selection and on the ability to anticipate both primary and acquired resistance [162]. Yet translating molecular features of

DNA repair into clinically actionable biomarkers has proven challenging because DDR pathways are functionally redundant, replication stress responses are inherently dynamic, and tumor evolution is shaped by heterogeneity and microenvironmental pressures [21,163]. As a result, only a limited number of biomarkers have achieved clinical validation, whereas many proposed predictors remain exploratory or context-dependent (Figure 6 and Table 5) [164,165].

Genomic biomarkers: strengths and inherent limitations

Alterations in BRCA1 and BRCA2 remain the most robust and clinically validated biomarkers predicting sensitivity to PARP inhibitors [166,167]. Both germline and somatic mutations confer HRD and underpin regulatory approvals of PARP inhibitors across ovarian, breast, pancreatic, and prostate cancers [166–169].

Efforts to extend PARP inhibitor benefit beyond BRCA1/2 mutations have focused on additional HR-related genes, including PALB2, RAD51C, RAD51D, and ATM [169]. While alterations in these genes can confer sensitivity in selected contexts, clinical responses are variable and less consistent than those observed in BRCA-mutant tumors [170]. This variability reflects a fundamental limitation of gene-centric biomarkers: the presence of a DDR gene alteration does not necessarily indicate functional pathway inactivation at the time of treatment [162].

Composite genomic HRD scores, integrating loss of heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST), were developed to capture cumulative genomic consequences of HRD [164]. Assays such as myChoice HRD and FoundationOne CDx have been used in clinical trials and regulatory decisions [164,171]. However, HRD scores primarily reflect historical genomic instability rather than current DNA repair capacity and have shown limited predictive value in several settings, particularly after prior therapy or outside ovarian cancer [162,171].

Functional biomarkers: assessing pathway activity in real time

Functional biomarkers aim to directly measure DDR pathway activity and address the static nature of genomic classifiers [162]. Among these, RAD51 foci formation assays have emerged as a leading functional readout of homologous recombination proficiency [172]. The presence or absence of RAD51 nuclear foci after DNA damage provides a real-time assessment of HR competence and correlates with PARP inhibitor sensitivity across tumor types [147,172].

Despite strong biological rationale, clinical implementation of RAD51 assays faces practical limitations, including requirements for fresh or well-preserved tissue, lack of standardized protocols, and inter-laboratory variability [172]. Similar challenges apply to biomarkers of replication stress—such as phosphorylated RPA, γH2AX, and CHK1 pathway activation—which have shown promise in predicting response to ATR and WEE1 inhibitors but remain insufficiently validated for routine clinical use [173,174].

Liquid biopsies and longitudinal monitoring

Circulating tumor DNA (ctDNA) analysis offers a minimally invasive approach to monitor tumor evolution and emerging resistance mechanisms over time [175]. Detection of BRCA1/2 reversion mutations in ctDNA provides direct evidence of homologous recombination restoration in patients progressing on PARP inhibitors [48,175].

However, ctDNA-based biomarkers for DDRi resistance remain investigational [176]. Sensitivity depends on tumor burden, disease stage, and shedding dynamics, and no ctDNA assay is currently approved to guide DDRi therapy in routine clinical practice [176]. At present, ctDNA profiling is best suited for longitudinal research studies and early-phase clinical trials rather than routine patient management [175].

Epigenetic and transcriptomic signatures

Epigenetic and transcriptomic biomarkers can capture reversible and adaptive resistance mechanisms that are not reflected by genomic alterations [73]. BRCA1 promoter methylation has been associated with PARP inhibitor sensitivity in ovarian cancer but can lack stability under therapeutic pressure, limiting predictive durability [80–82]. Transcriptomic signatures reflecting HR suppression, replication stress, or immune activation have also been proposed; however, reproducibility across cohorts remains a major challenge [162].

While current clinical workflows rely heavily on targeted panels focusing on known driver genes, emerging high-resolution technologies—such as single-cell RNA sequencing and spatial transcriptomics—hold the potential to deconvolute the functional heterogeneity and clonal dynamics depicted in Figure 4 [163,177]. Although these tools are not yet feasible for routine practice, they are pivotal for decoding the spatial organization of resistant niches and identifying actionable subpopulations that bulk or targeted profiling inevitably overlooks [177,178], ultimately informing the next generation of adaptive therapeutic strategies [163]. Their primary value lies in biomarker discovery and hypothesis generation rather than near-term clinical decision-making (Figure 6) [163].

Toward dynamic precision approaches

The limitations of individual biomarkers underscore the need for integrated precision approaches combining genomic, functional, and longitudinal data [162]. Adaptive trial designs and biomarker-enriched cohorts have improved patient stratification for DDRi in selected settings [164,169]. Nevertheless, biomarker failure remains common, emphasizing the need for rigorous validation and cautious interpretation of exploratory signals [162].

Future biomarker development should prioritize functional relevance, temporal resolution, and clinical feasibility [162]. Replication stress-based biomarkers, longitudinal monitoring strategies, and incorporation of tumor microenvironmental features are promising directions but require systematic validation [21,173]. Ultimately, precision DDR-targeted therapy is more likely to rely on integrated frameworks that account for tumor evolution under therapeutic pressure—potentially supported by computational approaches and Artificial intelligence/machine learning (AI/ML) methods for multi-omics integration in exploratory and decision-support contexts—rather than on any single static classifier (Figure 6; Table 5) [163,178].

CONCLUSIONS AND FUTURE DIRECTIONS

The clinical development of DDRi has established genome maintenance pathways as actionable vulnerabilities in cancer and has reshaped the landscape of precision oncology [16]. The success of PARP inhibitors in homologous recombination-deficient tumors provides a clear proof of principle [166]. At the same time, clinical experience indicates that resistance to DDR inhibition is not exceptional but an expected evolutionary outcome of sustained therapeutic pressure [25,179].

A central challenge moving forward is to distinguish biologically dominant resistance mechanisms from adaptive or permissive processes that modulate stress tolerance without re-establishing DNA repair capacity [25]. Genetic restoration of homologous recombination and compensatory rewiring of core DDR signaling directly undermine the therapeutic rationale for DDR inhibition and represent primary barriers to durable response [33–35]. In contrast, epigenetic remodeling, metabolic adaptation, microenvironmental support, and cellular plasticity primarily increase tolerance to replication stress and shape clonal fitness under treatment [21,61,88]. Failure to discriminate between these mechanistically distinct processes has contributed to inconsistent biomarker performance, overinterpretation of

preclinical signals, and the limited clinical success of many combination strategies [139,162].

Another key limitation is the continued reliance on static biomarkers to guide therapies targeting inherently dynamic processes [162]. Genomic scars capture historical DNA repair defects but do not reliably reflect the evolving functional state of DDR pathways during treatment [164,171]. Future progress will require greater emphasis on functional and longitudinal assessment of replication stress, DNA repair engagement, and clonal evolution, together with realistic evaluation of biomarker feasibility and robustness across clinical settings [172–175]. Finally, combination strategies will remain central to overcoming resistance to DDRi, but success will depend on biological precision rather than combinatorial breadth [139]. Effective regimens should target dominant resistance mechanisms or constrain their emergence while minimizing overlapping toxicity in normal proliferative tissues [142]. Adaptive treatment schedules and trial designs that account for tumor heterogeneity, temporal evolution, and selective pressure may offer more durable benefit than empiric escalation of combination regimens [163,169].

Ultimately, effective responses to DDR-targeted therapies will rely on the ability to identify and specifically target dominant resistance mechanisms while limiting adaptive tolerance states within an evolving tumor ecosystem.

Data availability

No datasets were generated or analysed during the current study.

Abbreviations

AI/ML: Artificial intelligence/machine learning (AI/ML)

ATM: Ataxia telangiectasia mutated

ATR: Ataxia telangiectasia and Rad3-related

BRCA1/2: Breast cancer susceptibility gene 1/2

cGAS: Cyclic GMP-AMP synthase

CHK1: Checkpoint kinase 1

ctDNA: Circulating tumor DNA

DDR: DNA damage response

DDRi: DNA damage response inhibitor

DNMT: DNA methyltransferase

FAO: Fatty acid oxidation

HDAC: Histone deacetylase

HIF-1α: Hypoxia-inducible factor 1α

HR: Homologous recombination

HRD: Homologous recombination deficiency

ICIs: Immune checkpoint inhibitors

LOH: Loss of heterozygosity

LST: Large-scale state transitions

NAD^+: Nicotinamide adenine dinucleotide

NADPH: Nicotinamide adenine dinucleotide phosphate

NAMPT: Nicotinamide phosphoribosyltransferase

OXPHOS: Oxidative phosphorylation

PARP: Poly(ADP-ribose) polymerase

PARPi: PARP inhibitor

PRC2: Polycomb repressive complex 2

PTIP: PAX transcription activation domain-interacting protein

RNR: Ribonucleotide reductase

SAM: S-Adenosylmethionine

STING: Stimulator of interferon genes

TAI: Telomeric allelic imbalance

TET: Ten-eleven translocation

TME: Tumor microenvironment

WEE1: Wee1 G2 checkpoint kinase

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Ethics declarations

Ethics approval and consent to participate

Not applicable. This manuscript does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable. This manuscript does not include details, images, or videos relating to an individual person.

Competing interests

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BOX:

BOX 1 | A hierarchical framework for resistance to DDR inhibitors: operational definitions and translational anchors

Core definitions

Dominant resistance drivers directly negate DDR-inhibitor lethality by restoring the relevant DNA repair function and/or bypassing the inhibited DDR node. Adaptive resistance

mechanisms increase tolerance to replication stress and DNA damage without restoring core repair output, often through reversible, context-dependent states.

Classification criteria

Dominant (≥1): (i) restores core repair output linked to sensitivity (e.g., regained HR proficiency and/or RAD51 loading); (ii) rewires/bypasses the inhibited DDR node, re-establishing checkpoint/repair execution at clinically achievable exposure; (iii) shows clonal fixation with strong genotype-phenotype coupling under treatment pressure (stable, selectable, trackable longitudinally).

Adaptive (predominantly): (i) buffers replication stress (fork stabilization/protection, altered origin firing, slowed cycling, dNTP/NAD^+/redox support); (ii) depends on plasticity and/or microenvironmental support with inter-lesion/region heterogeneity; (iii) is reversible or fluctuating over time, tracking treatment exposure rather than fixed genotype.

Clinical constraint

Toxicity acts as a dominant clinical constraint that caps exposure and shapes scheduling, indirectly facilitating adaptive tolerance programs.

Figure Legends:

Figure 1. Genetic mechanisms of resistance to DDR inhibitors

(**A**) Pathway restoration: in BRCA1/2-mutant tumors, secondary (“reversion”) mutations restore the open reading frame and re-enable homologous recombination (HR), reducing PARP inhibitor efficacy; reversion mutations can be detected in circulating tumor DNA (ctDNA). (**B**) Signaling compensation/altered pathway choice: 53BP1 loss relieves DNA end-resection constraints and enables residual HR activity in BRCA1-deficient settings, conferring PARP inhibitor resistance**.** (**C**) Replication-fork protection: genetic alterations such as PTIP loss or dysfunction of the Shieldin axis reduce nuclease-mediated fork degradation (e.g., reduced MRE11-mediated fork degradation), limiting fork collapse under DDR stress. Created with BioRender.com.

Figure 2. Epigenetic mechanisms of resistance and epigenetic scars

DNA damage and repair are coupled to chromatin remodeling that can generate persistent epigenetic scars. Repressive and activating histone marks and DNA methylation states modulate gene expression programs linked to adaptive resistance, with microenvironmental selection pressure shaping tumor evolution. Repressive markers: H3K9me3, H3K27me3,

methylated CpG. Activating markers: H3K4me3, H3K9ac, unmethylated CpG. Created with BioRender.com.

Figure 3. Metabolic reprogramming and resistance

Metabolic pathways support resistance to DDR inhibition by sustaining dNTP availability, redox buffering, NAD+ homeostasis, and energy production, thereby promoting replication stress tolerance. Key nodes include nucleotide metabolism/RNR and salvage, one-carbon/folate metabolism, OXPHOS/mitochondrial metabolism, and fatty acid oxidation (FAO). Abbreviations: OXPHOS, oxidative phosphorylation; RNR, ribonucleotide reductase; NADPH, nicotinamide adenine dinucleotide phosphate; SAM, S-adenosylmethionine.
Created with BioRender.com.

Figure 4. Tumor heterogeneity and clonal evolution under DDRi pressure

Genomic, epigenetic, transcriptomic, and functional heterogeneity provides the substrate for resistance. Under therapy (DDRi), selective pressure promotes the expansion of resistant subclones shaped by tumor-intrinsic programs and the tumor microenvironment (TME). Created with BioRender.com.

Figure 5. Combination strategies to overcome resistance to DDR inhibitors

Schematic overview of therapeutic combinations pairing DDR inhibitors with immune checkpoint blockade, chemotherapy/radiotherapy, metabolic inhibitors, and epigenetic therapies to target dominant resistance mechanisms or constrain adaptive resistance. The central cycle summarizes key steps of antitumor immunity (antigen release → uptake/presentation → T-cell priming → trafficking → tumor cell killing) that may be modulated by DDRi-based combinations. Created with BioRender.com.

Figure 6. Biomarkers and precision DDR-targeted therapy

Integrated use of genomic, functional, epigenetic, and transcriptomic biomarkers, together with liquid biopsy (ctDNA), supports patient stratification and longitudinal monitoring of resistance. AI/ML multi-omics integration can assist response prediction, patient stratification, and treatment selection in exploratory/decision-support settings. Created with BioRender.com.

Table 1. Genetic mechanisms of resistance to DDR inhibitors

Summary of genetically driven resistance mechanisms to DDR inhibitors, highlighting pathway restoration, altered pathway choice/signaling compensation, replication-fork protection, checkpoint adaptation, and reduced dependency on the inhibited DDR node.

Resistance mechanism	**Key molecular alterations**	**Affected DDR process**	**Impact on DDRi response**	**Clinical relevance**
Pathway restoration	BRCA1/2 reversion mutations; RAD51C/D secondary mutations	Homologous recombination (HR)	Direct restoration of DNA repair capacity	Strongly associated with acquired PARPi resistance
Signaling compensation	Loss of 53BP1, Shieldin complex components	DNA end resection and pathway choice	Partial reactivation of HR despite BRCA1 deficiency	Documented in PARPi-resistant tumors
Replication fork protection	PTIP loss; reduced nuclease recruitment	Fork stability under replication stress	Reduced fork degradation and collapse	Clinically relevant but often co-occurring with other mechanisms
Altered checkpoint signaling	ATR–CHK1 pathway rewiring	Replication stress response	Reduced dependence on inhibited checkpoint	Emerging resistance to ATR/CHK1 inhibitors
Reduced DDR dependency	Altered replication dynamics	Replication stress tolerance	Decreased reliance on targeted DDR node	Context-dependent, often adaptive

Abbreviations: DDRi, DNA damage response inhibitor; PARPi, PARP inhibitor; HR, homologous recombination.

Table 2. Epigenetic mechanisms contributing to adaptive resistance

Epigenetic and chromatin-based mechanisms that modulate DDR pathway engagement and replication stress tolerance, primarily as adaptive and potentially reversible modifiers of DDR inhibitor response.

Epigenetic mechanism	**Molecular features**	**Functional consequence**	**Role in resistance**	**Translational status**
Histone modification changes	H3K9me3, H3K27me3 accumulation	Transcriptional repression of DDR genes	Modulates adaptive resistance	Preclinical
DNA methylation	Promoter methylation (e.g., BRCA1)	Reversible HR suppression	Transient sensitization or resistance	Limited clinical durability
Chromatin remodeling	Altered accessibility at DDR loci	Modified pathway engagement	Supports plasticity	Exploratory
Non-coding RNAs	miRNAs, lncRNAs targeting DDR genes	Post-transcriptional regulation	Context-dependent adaptation	Preclinical
Epigenetic scars	Persistent chromatin alterations after DNA damage	Stabilization of stress-tolerant states	Contributes to long-term adaptation	Conceptual, not validated

Abbreviations: DDR, DNA damage response; HR, homologous recombination; miRNA, microRNA; lncRNA, long non-coding RNA.

Table 3. Combination strategies to overcome resistance to DDR inhibitors

Representative DDR inhibitor–based combination strategies, the vulnerabilities they target, expected benefits, and major translational constraints (toxicity, scheduling, and biomarker limitations).

Combination strategy	Targeted vulnerability	Biological rationale	Expected benefit	Translational limitations
DDRi + immune checkpoint inhibitors	Innate immune activation	cGAS–STING signaling, increased immunogenicity	Enhanced antitumor immunity	Context-dependent immune effects, limited biomarkers
DDRi + chemotherapy/radiotherapy	DNA repair overload	Increased DNA damage with impaired repair	Synergistic cytotoxicity	Overlapping toxicity, dose limitations
DDRi + metabolic inhibitors	Replication stress buffering	Disruption of nucleotide, redox, or energy balance	Exacerbated replication stress	Systemic toxicity, tumor-context dependence
DDRi + epigenetic therapies	Transcriptional plasticity	Reversible suppression of adaptive resistance states	Delayed resistance emergence	Pleiotropic effects, limited clinical validation
DDRi + cell cycle checkpoint inhibitors (e.g., WEE1i/CHK1i)	Cell cycle dysregulation	Forced mitotic entry under damage	Increased tumor cell death	Narrow therapeutic window

Abbreviations: cGAS–STING, cyclic GMP–AMP synthase–stimulator of interferon genes.

Table 4. Selected clinical trials of DDR inhibitor–based combinations

Selected clinical trials evaluating DDR inhibitor–based combination regimens, summarizing trial phase, cancer type, endpoints, and key limitations relevant to durability and clinical deployment.

Trial	Phase	Combination	Cancer type	Primary endpoint	Key limitation
DORA	II	Olaparib + Durvalumab	Ovarian cancer	PFS	No clear benefit vs monotherapy
CAPRI	I/II	Ceralasertib + Chemotherapy	ATM-deficient tumors	ORR, PFS	Hematologic toxicity
FIRST	III	Olaparib + Bevacizumab	Ovarian cancer	PFS, OS	Biomarker-restricted benefit
MAGNITUDE	III	Niraparib + Abiraterone	mCRPC	PFS, OS	HRD-dependent efficacy
ATRi + chemotherapy trials	I/II	ATRi + cytotoxics	Solid tumors	Safety, ORR	Dose-limiting toxicity

Note: Most trials highlight the importance of patient selection and scheduling, rather than universal efficacy. Abbreviations: ORR, objective response rate; PFS, progression-free survival; OS, overall survival; mCRPC, metastatic castration-resistant prostate cancer; HRD, homologous recombination deficiency.

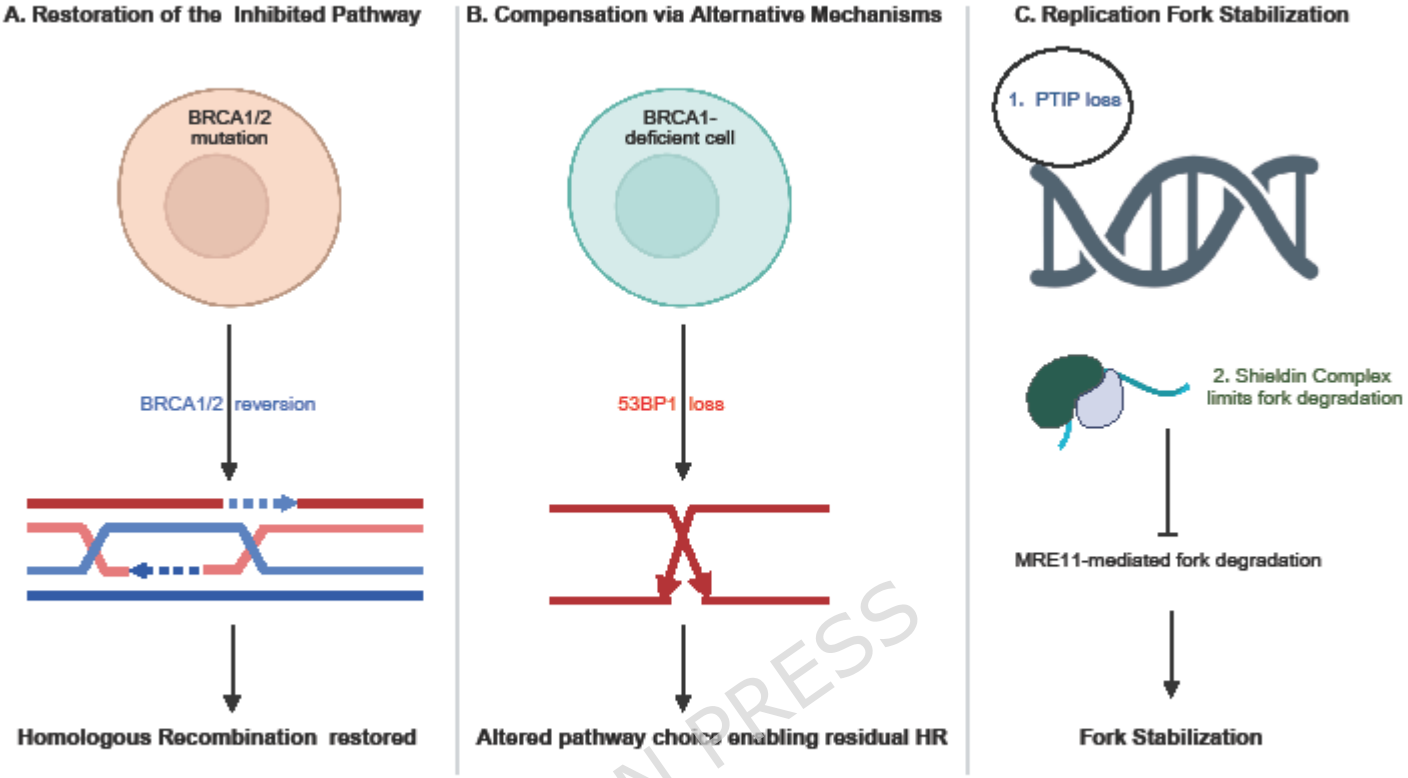
Table 5. Biomarkers for DDR-targeted therapy and resistance monitoring

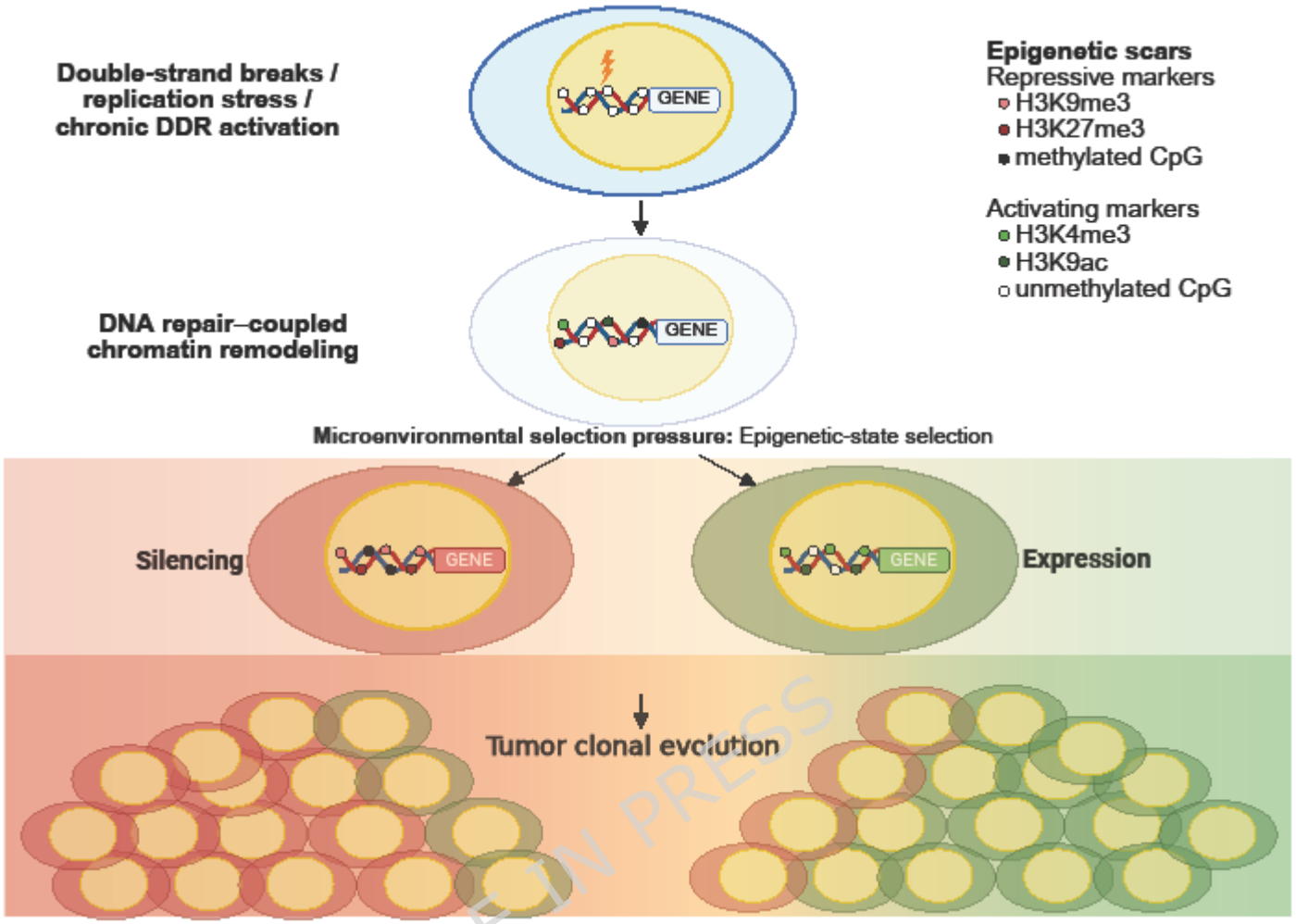
Biomarker classes for DDR-targeted therapy, distinguishing static genomic classifiers from functional and longitudinal approaches, with strengths and limitations relevant to clinical implementation and resistance monitoring.

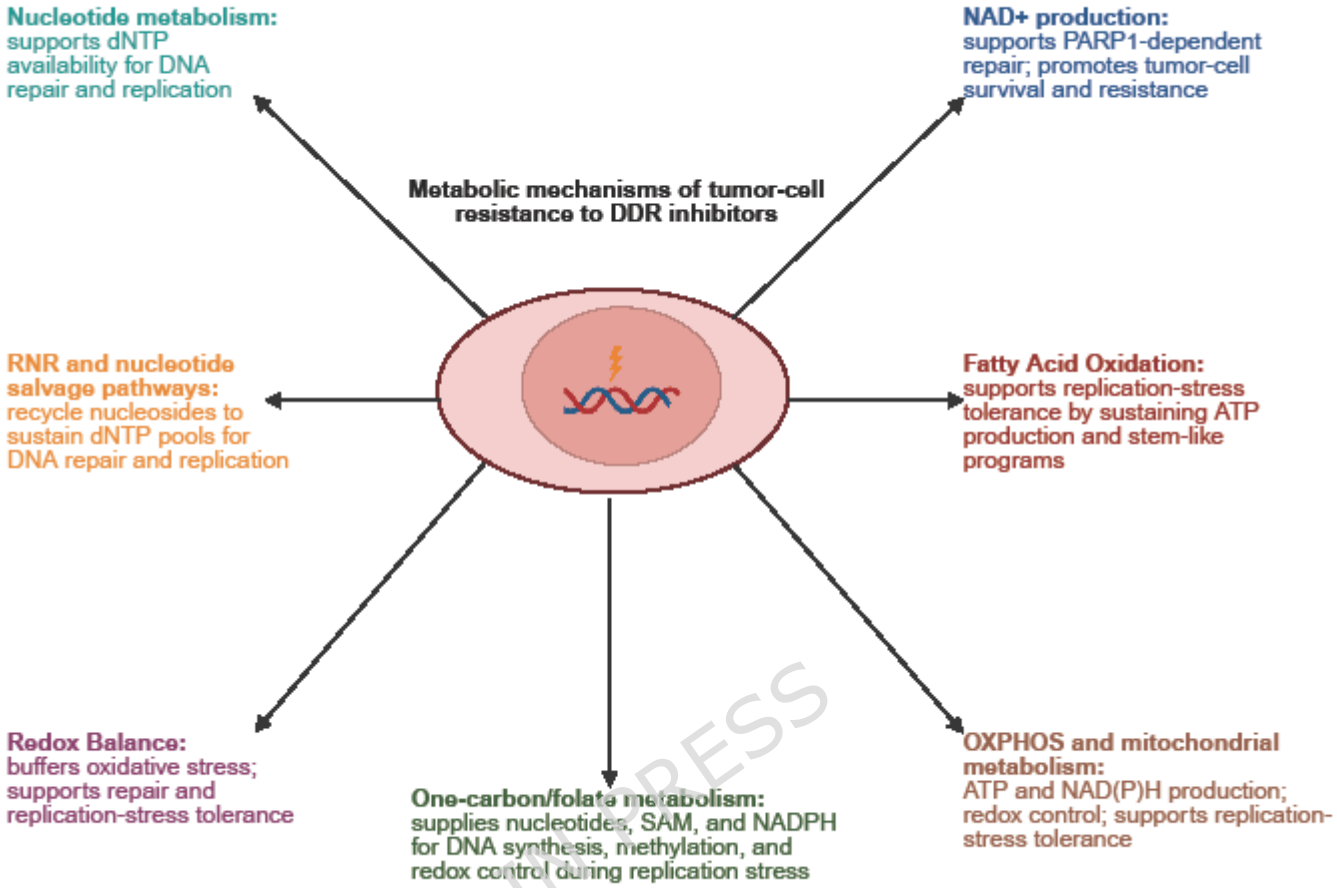
Biomarker class	Example	What it measures	Strengths	Limitations
Genomic	BRCA1/2 mutations	HR deficiency	Clinically validated	Static, not dynamic
Genomic scars	HRD score (LOH, TAI, LST)	Historical genomic instability	Broad applicability	Poor prediction after therapy

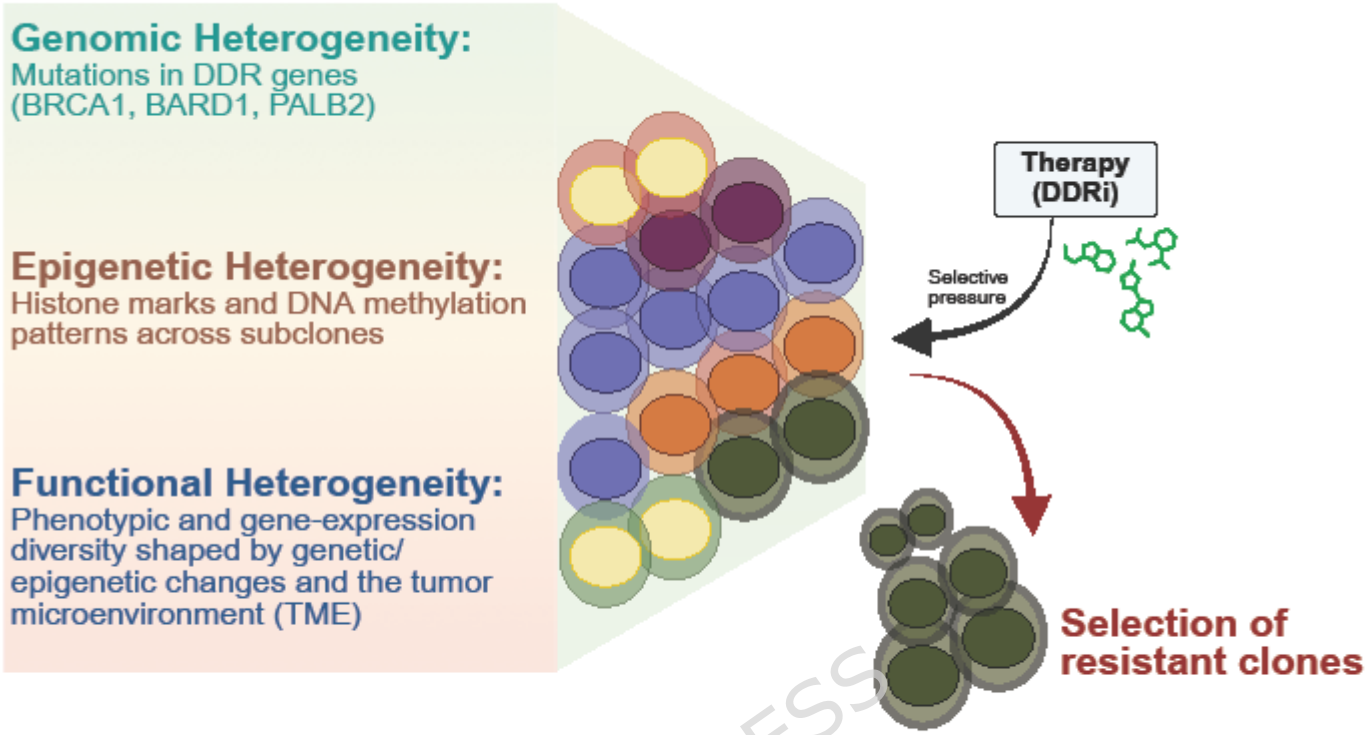
Biomarker class	Example	What it measures	Strengths	Limitations
Functional	RAD51 foci	Real-time HR activity	Mechanistically informative	Technical complexity
Replication stress markers	γH2AX, pRPA	Ongoing replication stress	Relevant for ATR/WEE1i	Limited standardization
Liquid biopsy	ctDNA reversion mutations	Clonal evolution	Longitudinal monitoring	Investigational
Epigenetic and transcriptomic	DDR gene expression signatures	Adaptive states	Captures plasticity	Low reproducibility

Abbreviations: HR, homologous recombination; HRD, homologous recombination deficiency; LOH, loss of heterozygosity; TAI, telomeric allelic imbalance; LST, large-scale state transitions; ctDNA, circulating tumor DNA.









Clonal evolution under drug pressure