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Perspective

New Horizons of Synthetic Lethality in Cancer: Current Development and Future Perspectives

Viola Previtali,[▽] Greta Bagnolini,[▽] Andrea Ciamarone, Giovanni Ferrandi, Francesco Rinaldi, Samuel Harry Myers, Marinella Roberti,[*] and Andrea Cavalli

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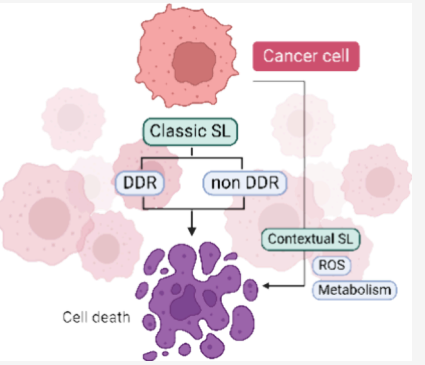
ABSTRACT: In recent years, synthetic lethality has been recognized as a solid paradigm for anticancer therapies. The discovery of a growing number of synthetic lethal targets has led to a significant expansion in the use of synthetic lethality, far beyond poly(ADP-ribose) polymerase inhibitors used to treat BRCA1/2-defective tumors. In particular, molecular targets within DNA damage response have provided a source of inhibitors that have rapidly reached clinical trials. This Perspective focuses on the most recent progress in synthetic lethal targets and their inhibitors, within and beyond the DNA damage response, describing their design and associated therapeutic strategies. We will conclude by discussing the current challenges and new opportunities for this promising field of research, to stimulate discussion in the medicinal chemistry community, allowing the investigation of synthetic lethality to reach its full potential.



1. INTRODUCTION

Synthetic lethality (SL) was first observed in fruit flies[1] and later confirmed in yeast,[2] defining it as a consequence of the combined alteration of individually viable genes. SL has emerged as a promising strategy for anticancer drug discovery, particularly for targeting challenging tumor suppressor genes.[3] Via exploitation of the concept of SL, cancer cells with mutated or inactivated driver genes can be selectively targeted, potentially reducing the side effects of therapy.[4] The successful implementation of SL in drug discovery is fueled by the identification of new SL pairs in cancer cells. Recent comprehensive reviews of this field encompass a broad spectrum of analyses, ranging from phenotypic profiling[5] to experimental methodologies[6] and predictive models facilitated by computational techniques.[7] Leveraging innovative big data strategies in SL drug discovery, researchers can now methodically scrutinize every gene across hundreds of cancer cell lines. This enables the identification of cancer-specific vulnerabilities and, consequently, synthetic lethal relationships that might otherwise remain elusive.[8] However, as our understanding of the original genetic-based definition of SL expands, newer subclasses such as "collateral SL"[9] and "metabolic SL"[10,11] have emerged, underscoring the inherent complexity of the SL definition. Moreover, the heterogeneity observed in tumor cells and their microenvironment can significantly impact genetic interactions, thereby prompting the recognition of condition-dependent forms of SL, often termed as "contextual" or "conditional" SL.[12,13]

Herein, we build upon our previously published Perspective in the *Journal of Medicinal Chemistry*[14] to describe the latest trends in drug development campaigns exploiting SL interactions. This Perspective focuses on the classical definition of SL in drug discovery, emphasizing its use in identifying novel drug targets (e.g., alteration in gene A justifies targeting gene B), developing synergistic drug combinations [e.g., drugs targeting a SL pair of genes (A and B) or synergistic drug combinations that exploit a mutation in a third gene, C], and stratifying cancer patients for tailored therapies. A majority of SL targets have been found in DNA repair pathways; we therefore dedicate a large part of this Perspective to this area of research. Nevertheless, we highlight exciting new scientific rationales, particularly SL outside the DNA repair pathways and "contextual SL", which provide promising directions for future drug discovery campaigns.

2. TARGETING SL IN THE DNA DAMAGE RESPONSE (DDR)

2.1. PARP and BRCA1/2. The concept of SL in the clinic is best exemplified by the use of poly(ADP-ribose) polymerase (PARP) inhibitors (PARPi) to specifically target tumors

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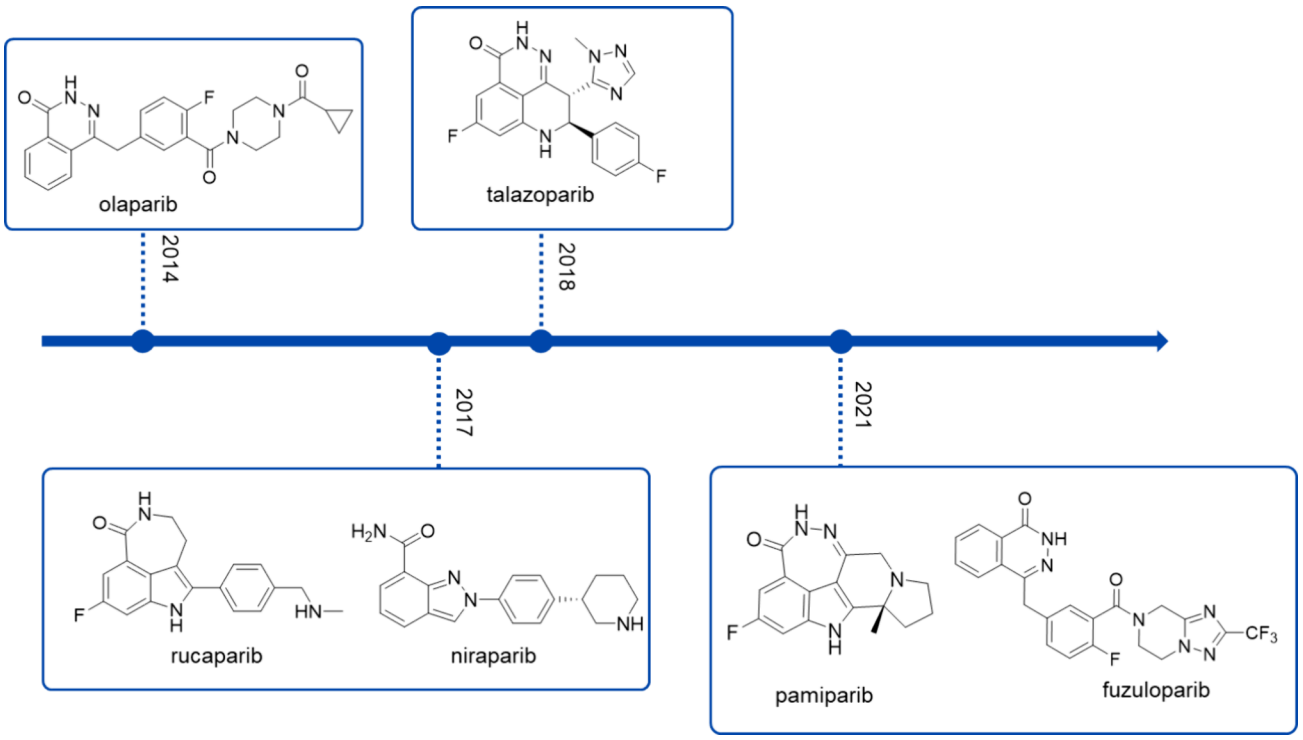

Figure 1. Representative time line of PARPi approved in clinics by global health organizations (FDA, EMA, and NMPA).

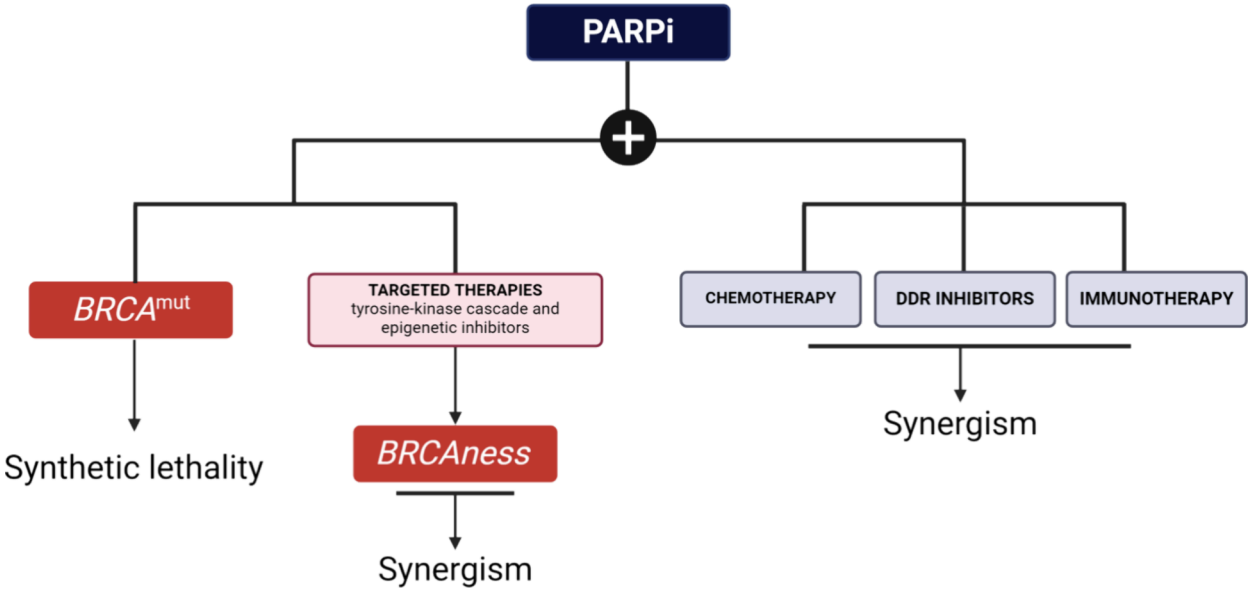

Figure 2. Application of PARPi in BRCA^{mut} tumors to achieve SL and PARPi combination strategies to enhance efficacy and overcome resistance discussed in the text (created with BioRender.com).

deficient in the homologous recombination (HR) DNA repair factors, BRCA1 or BRCA2 (BRCA1/2). PARP is a family of proteins involved in the repair of DNA single-stranded breaks (SSBs) and exerts its function through binding to the DNA damage site and using nicotinamide adenine dinucleotide (NAD^+) as a cofactor to mediate poly(ADP-ribosyl)ation (PARylation) on target proteins, including PARP itself.[15]

PARPi act by binding and trapping the PARP enzyme on the chromatin, which results in the conversion of the SSB into a DSB, a lesion that necessarily requires HR repair.[16] The use of PARPi prevents the tumor from repairing single-strand DNA breaks and increases the incidence of DSBs. Therefore, upon administration to a tumor that already has an underlying HR defect, e.g., BRCA1/2 deficiency, the tumor cells are unable to survive and die because of SL, while normal cells remain unharmed (Figure 2).[4]

Since the theory was postulated by two pivotal publications,[17,18] the number of studies of PARPi- and BRCA1/2-deficient tumors has exponentially increased. PARPi reached the first major milestone in 2014, when the Food and Drug Administration (FDA) and the European Medicine Agency (EMA) approved AstraZeneca's olaparib (Lynparza) for the

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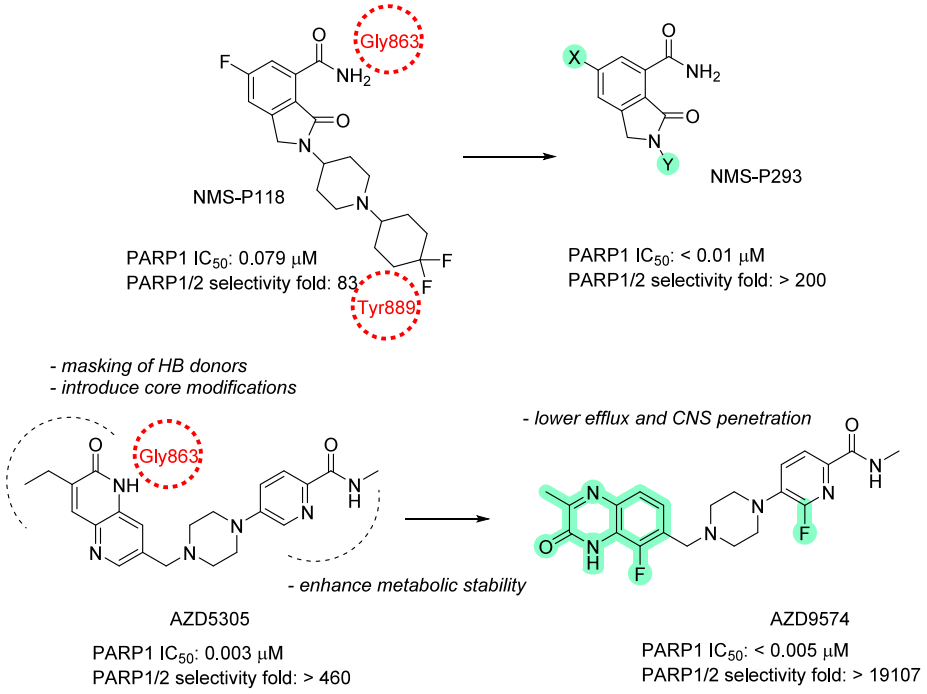

Figure 3. Structures of PARP1 selective inhibitors discussed in the text. Key structural features are highlighted in green, and amino acids that are important for the binding mode represented via the dashed red circles.

treatment of advanced ovarian cancer as a single agent in patients with germline *BRCA* mutations (gBRCAm).[19] In the past decade, the PARPi class has continued to be used successfully, as proven by the increasing number of PARPi in clinical trials or approved for the treatment of BRCA1/2- and/or somatic HR-deficient tumors, including ovarian,[20] pancreatic,[21] breast,[22] and prostate[23] cancers, either as monotherapy or in combination. The following PARPi have been authorized in the United States and/or European Union: rucaparib (Rubraca),[24] niraparib (Zejula),[25] and talazoparib (Talzenna).[26] In addition, fuzuloparib (AiRuiYi)[27] and pamiparib (Partruvix)[28] have been authorized in China (Figure 1).

Despite these breakthrough approvals, the success of PARPi in the clinic has been hindered by mechanisms of toxicity and resistance.[15,29] Recent research has thus turned to exploring promising combination approaches utilizing PARPi, with the aim of leveraging synergistic effects to improve efficacy and sensitize PARPi-resistant tumors to the treatment. While the combination of PARPi with DNA-damaging chemotherapy (a strategy that can be considered related to "contextual SL" that will be expanded in section 3) faces issues due to the overlapping of hematological toxicities, newer and more targeted therapies have shown promising results and are progressing in clinical development.[15]

In this context, PARPi have been studied at the preclinical level in combination with agents that induce a BRCAness phenotype or, more broadly, a homologous recombination-deficient (HRD)-like phenotype by interfering either directly or indirectly with HR (Figure 2).[15] An example is the combination of PARPi with inhibitors of receptor tyrosine kinase receptors (e.g., EGFR, VEGFR, and FLT3-ITD), which have been shown to modulate DDR genes through their downstream signaling partners, such as the RAS/RAF/MEK/ERK and PI3K/mTOR pathways.[30] Additionally, PARPi have been studied in combination with modulators of epigenetic mechanisms, as they regulate chromatin mobility and thus affect the accessibility of the DDR protein to DNA damage sites, particularly DSBs. Indeed, inhibiting certain epigenetic proteins (e.g., BRD4) has been demonstrated to downregulate fundamental DDR proteins.[30] To leverage this synergism, in the past several years various combinations of PARPi and targeted tyrosine kinase receptor or epigenetic inhibitors have been studied for their potential with promising preclinical evidence (Figure 2).[15] In addition, other combination strategies make use of inhibitors of alternative DDR pathways, which are used because of PARP inhibition (Figure 2). Examples of this approach include the long-time investigated combination of PARPi-ATR inhibitors or the newest targets Polθ and USP1.[15] Lastly, the promising synergism of PARPi and immunotherapy (Figure 2) is worth highlighting. PARPi, as result of the inhibition of DNA repair, cause the release of dsDNA fragments into the cytosol, and this in turn activates cGAS/STING signaling.[31] PD-L1 upregulation positively correlates with the accumulation of DNA breaks. Indeed, PARPi upregulate PD-L1, as a downstream effect of impairing DDR, and also correlate with cancer resistance. More broadly, this confirms the therapeutic relevance of the combination of anti-PD-1/PD-L1 therapies with the available modulators of PARP (and DDR in general). Phase 1/2 clinical trials of such combinations have been widely reviewed by Huang et al.[32]

In addition, strategies for overcoming toxicities associated with PARPi have instead focused on the development of new classes of PARP modulators (e.g., PARP selective inhibitors, dual inhibitors, PROTAC degraders, and prodrugs).[33−35] In the following section, we detail the most recent advancements in PARP selective inhibitors, which represent the forefront of progress in clinical development for overcoming toxicities associated with PARPi.

2.1.1. PARP1 Selective Inhibitors. Although first-generation PARPi have been significantly effective in clinical settings, they often cause hematological toxicities such as anemia, neutropenia, and thrombocytopenia.[36] Research suggests that

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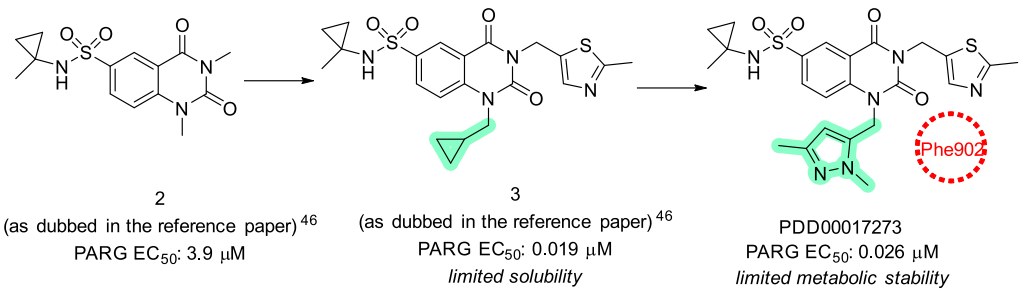

Figure 4. Structures of PARGi discussed in the text. Key structural features are highlighted in green, and the amino acid that is important for the binding mode is represented via the dashed red circle.

while PARP1, which plays a primary role in the DNA damage response (DDR), is essential for efficacy, PARP2 is linked with these side effects.[36] Existing first-generation PARPi competitively inhibit both PARP1 and PARP2; for example, the PARP1/2 selectivity fold for Olaparib is 1.[37] Despite similar enzymatic inhibition activities, they vary significantly in their ability to trap PARP enzymes and induce cytotoxic effects (with talazoparib being the most potent PARP1 trapping inhibitor and veliparib the least potent). Because the trapping capability significantly influences efficacy and toxicity, modifying the trapping ability could have impact on clinical efficacy.[37] Additional studies indicate that SL with BRCA mutations is solely mediated by PARP1, suggesting that trapping of PARP2 to DNA is unnecessary. Accordingly, a PARP1 selective inhibitor may reduce toxicity while maintaining antitumor activity, offering a higher therapeutic index and more combination treatment options. In 2015, Nerviano Medical Sciences introduced the selective PARP1 inhibitor NMS-P118 (Figure 3). This molecule featured an isoindoline-4-carboxamide core substituted with a 4,4-difluorocyclohexyl group, which better fits into the catalytic domain of PARP1, filling an induced pocket and establishing favorable hydrophobic interactions with Tyr889. NMS-P118 showed moderate potency against PARP1 (PARP1 IC_{50} = 0.079 μM) with a promising PARP1/2 83-fold selectivity.[38] As the first PARP1 selective inhibitor with *in vitro* and *in vivo* anticancer activity, NMS-P118 demonstrated that inhibiting PARP-1 alone, rather than PARP-2, is sufficient for achieving high antitumor efficacy in BRCA-deficient tumor settings.

The development of NMS-P118 led to the development of NMS-P293 (Figure 3), which exhibited potent *in vitro* activity (PARP1 IC_{50} < 0.01 μM, and PARP1/2 selectivity fold > 200) and *in vivo* efficacy in HRD tumors (BRCA- and PTEN-deficient) and did not induce DNA trapping. Although the structure of NMS-P293 is undisclosed, a patent by the same authors describes a series of isoindoline-4-carboxamide derivatives with different linear/cyclic chains replacing the 4,4-difluorocyclohexyl substituent (Patent WO2014/064149A1). Remarkably, NMS-P293 is not a substrate of PgP efflux, a mechanism of resistance attributed to current PARPi, and can penetrate the blood−brain barrier. Moreover, this molecule demonstrates potent synergistic efficacy and tolerability in combination with temozolomide (TMZ) in glioblastoma (GBM) tumor models, thereby paving the way for the use of PARPi in central nervous system (CNS) tumors. These favorable properties led to NMS-P293 entering a phase 1 clinical study in adult patients with selected advanced and metastatic solid tumors (NCT04182516).

Building upon these findings, AstraZeneca introduced AZD5305 (Figure 3), a more potent (PARP1 IC_{50} = 0.003 μM) and even more selective (PARP1/2 selectivity fold > 460) PARP1 inhibitor with excellent *in vivo* pharmacokinetic (PK) and efficacy properties in a BRCA mutant PDX model. Although AZD5305 partially mimicked NMS-P118's binding pose (interaction with Gly863), it demonstrated the ability to trap PARP1-DNA complexes.[39] Nevertheless, data from its ongoing phase 1/2 PETRA trial (NCT04644068) demonstrated compelling and promising evidence of safety.[40]

In its latest generation of PARP1 inhibitors, AstraZeneca managed to lower efflux for CNS penetration by masking hydrogen bond (HB) donors and introducing core modifications, resulting in the development of AZD9574 (Figure 3). This molecule represents the first brain penetrant PARP1 selective inhibitor (PARP1 IC_{50} < 0.005 μM, and PARP1/2 selectivity fold > 19 107) and PARP1-DNA trapping agent in clinical trials (NCT05417594).[41]

Concurrently with the advancement of PARP1 inhibitors, it is necessary to mention the controversial studies emerging about previously unexpected toxicity in normal tissues. These include evidence of toxicity in human healthy bone marrow cells,[42] linked to the trapping activity of PARP1 inhibitors, and the embryonic lethality due to PARP1 loss in a dominant-negative manner in a mouse model.[43] Ultimately, this may weaken the therapeutic potential and safety of the trapping mechanism mediated by PARP1 inhibitors, eventually hampering their future in the clinic.

2.2. PARG. Poly(ADP-ribose) glycohydrolase (PARG) is an enzyme that preserves genome integrity from replication stress by catalyzing de-PARylation, offering an exciting and intriguing point of therapeutic intervention within DDR.[44] Interestingly, PARG is often upregulated in cancer and positively correlates with resistance to PARPi.[44] PARG inhibitors (PARGi) were initially predicted to be active in BRCA2-deficient tumor cells,[45] hinting at a possible SL parallelism with PARPi. However, recently it has been reported that the consequences following inhibition of PARG or PARP may suggest different pharmacology and genetic dependencies linked with HRD status.[46] The application of the majority of reported small molecules targeting PARG is limited due to their modest low-micromolar IC_{50} and low cell permeability.[44] Recently, a potent and cell-permeable PARGi, PDD00017273 (Figure 4), was identified through a homogeneous time-resolved fluorescence (HTRF) high-throughput biochemical screen (1.4 million compounds), conducted by AstraZeneca.[46] This discovery was followed by iterative chemical modifications and optimization of its chemotype. Although the addition of a cyclopropyl group improved the *in vitro* potency of the initial hit (EC_{50} values of 3.9 and 0.019 μM for compounds dubbed **2** and **3**, respectively, in the reference paper), it hindered its solubility (<10 μM in 1% DMSO/PBS). Further optimization

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by substituting the cyclopropyl ring with a dimethyl-pyrazol led to the development of the potent (EC_{50} = 0.026 μM) and soluble compound PDD00017273, allowing investigation into PARG cellular pharmacology. Despite these promising results, PDD00017273 could not be studied *in vivo* due to its poor metabolic stability [intrinsic clearance (CL_{int}) of 211 and 78 $\mu L\ min^{-1}\ mg^{-1}$ in mouse and human microsomes, respectively]. Ideaya built upon these discoveries and developed IDE161, an orally bioavailable PARGi. While the structure of IDE161 is undisclosed, it is likely a 4-substituted indole and indazole sulfonamide derivative, as outlined in a company patent (Patent WO2021/055744A1). Biochemical and cellular assays have demonstrated that IDE161 effectively inhibits PAR chain hydrolysis and exhibits antiproliferative activity in breast and ovarian cancer cell lines, including those resistant to PARPi.[47] Consistent with these *in vitro* findings, IDE161 has shown antitumor activity in PARPi-resistant xenograft models, which has facilitated its entry into clinical trials as the first PARGi (NCT05787587). According to the results from this first clinical trial, it will be possible to evaluate the therapeutic relevance of PARG in tackling BRCA1/2-deficient and HR-defective tumors.

2.3. USP1. Ubiquitination and deubiquitination are recognized as key mechanisms for maintaining cellular homeostasis, and their alterations contribute to tumor development. The enzymes that participate in these processes, in particular E3 ubiquitin ligase and deubiquitinase (DUBs), have been regarded as potential targets for anticancer therapy. One of the best characterized human DUBs is ubiquitin-specific protease 1 (USP1), which plays an important role in the cellular response to DNA damage.[48] Indeed, the complex of USP1 with USP1-associated factor 1 (UAF1) acts in two important DDR pathways, namely, translesion synthesis (TLS) and interstrand cross-links (ICLs), through the deubiquitination of proliferating cell nuclear antigen (PCNA) and Fanconi anemia complementation group 2D (FANCD2). Inhibition of USP1 causes the impairment of the reversible protein ubiquitination regulation mechanism, resulting in increased levels of monoubiquitinated PCNA and FANCD2, leading to DNA repair damage (Figure 5).[48] Importantly, USP1 is frequently overexpressed in certain human tumor types such as cervical and gastric cancer, non-small cell lung cancer (NSCLC), melanoma, and sarcoma, suggesting that USP1 could represent a valid target in cancer therapy.[48]

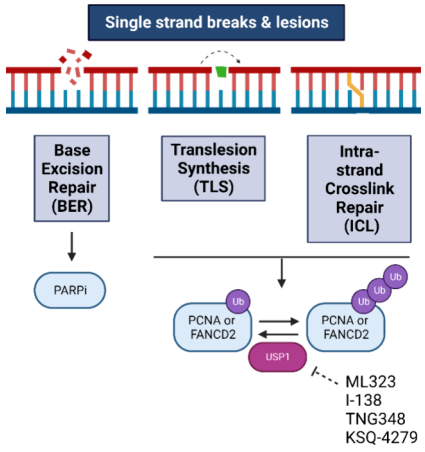

Figure 5. Schematic representation of DNA lesions associated with the USP1 function and UPS1 inhibitors discussed in the text (created with BioRender.com).

In 2001, Chen et al.[49] identified pimozide and GW7647 as the first inhibitors of the human USP1-UAF1 complex with low micromolar affinity. These compounds demonstrate the possibility of inhibiting the USP1-UAF1 complex with small molecules and synergize with cisplatin in cisplatin-resistant cells.[49] However, both compounds are known to bind several DUBs, deSUMOylase, and cysteine proteases, which has limited their utility as USP1-UAF1 chemical probes. For this reason, the same authors developed ML323 through quantitative high-throughput screening (qHTS) and subsequent medicinal chemistry optimization of the hit compound (Figure 6 and Table 1). This nanomolar inhibitor of the USP1-UAF1 deubiquitinase complex demonstrated remarkable selectivity and was reported to exert its effect through an allosteric mechanism, binding far from the USP1 active site (Figure 6).[50]

A recent report of the cryo-EM structure of USP1 bound to ML323 [Protein Data Bank (PDB) entry 7ZH4] shed light on the binding of the inhibitor to the enzyme for the first time.[51] This structure showed the interaction of the inhibitor in the USP-1 binding site with many hydrophobic residues and through two HBs mediated by the triazole ring with glutamine 160 (Q160) and the pyrimidine ring with glutamine 97 (Q97) (Figure 6). This potent and selective effect against USP1 translated into the inhibition of TLS and Fanconi anemia (FA) pathways in cells, making ML323 the best-in-class chemical probe for investigating the role of USP1 in the cellular response to DNA damage.[50] Moreover, ML323 potentiates cisplatin cytotoxicity in NSCLC H596 cells and U2OS osteosarcoma cells. This finding suggested that USP1-UAF1 is a key regulator of DDR and a potential target for overcoming resistance to the platinum-based anticancer drugs.[50]

Recently, a study published by Tango Therapeutics showed that USP1 inhibition is effective in BRCA1/2 mutant tumors and in a subset of BRCA1/2 wild-type lung and ovarian tumors, underlying the *USP1* dependency on PCNA ubiquitination and consequent protein degradation.[52] They demonstrated that, in BRCA1 mutant cells, the inhibition of USP1 by I-138 (Figure 6) causes the accumulation of mono- and polyubiquitinated PCNA, associated with decreased levels of DNA synthesis and DNA damage. I-138 is a patented (Patent WO2017/087837A1) small molecule structurally related to ML323. In I-138, the benzyl and isopropylphenyl groups of ML323 are kept in place, the aminopyrimidine is cyclized in a purinone core, and the triazole substituent is converted into a methyl-imidazole substituted with $-CF_3$ at position 4 (Figure 6). These characteristics instilled I-138 with improved potency and physicochemical properties (Table 1). Further studies suggested that I-138 binds to USP1-UAF1 at an allosteric site, thought to be the same ML323 binding site (Figure 6). To validate USP1 as an SL target, the authors determined the effect of USP1 inhibition on the viability of BRCA1/2 mutant and BRCA1/2 wild-type tumor cells. The inhibition of USP1 by I-138 selectively reduced the viability of BRCA1 mutant breast cancer cell line MDA-MB-436, but not that of wild-type breast cancer cell line HCC195.[52] Similarly, USP1 inhibition caused the loss of viability of BRCA1 mutant ovarian cancer cell line UWB1.289. BRCA1 re-expression in UWB1.289 cells rescued USP1 inhibitor sensitivity, consistently with an SL interaction. Moreover, the combination was tested across a panel of BRCA1/2 wild-type and mutant cells

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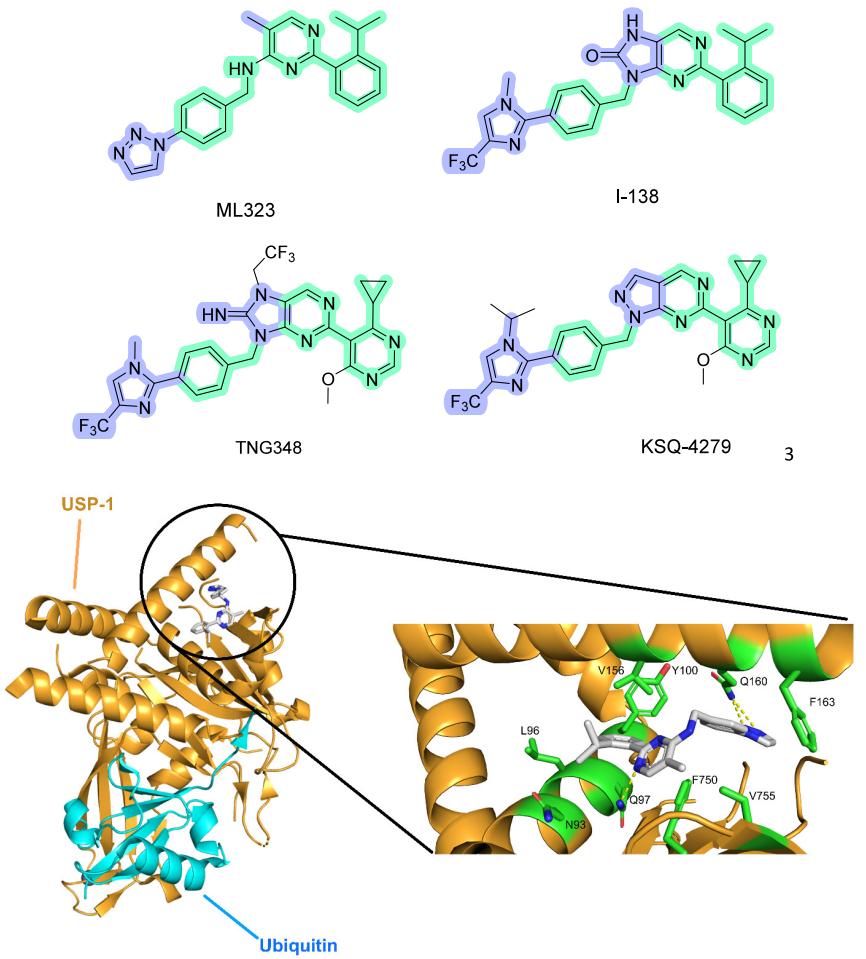

Figure 6. USP1i discussed in the text (top). Key and common structural features are highlighted in green and violet, respectively. Cryo-EM structure of ML323 (gray) in complex with USP1 (orange) bound to ubiquitin conjugated to FANCD2 (PDB entry 7ZH4) (bottom). Amino acids interacting with the inhibitor are colored green, while hydrogen bonds (HBs) are represented as dashed yellow lines (prepared using PyMol).

to study whether USP1 and PARPi synergize in cells. The USP1-PARPi combination was preferentially synergistic in BRCA1/2-mutated compared to wild-type cells. To test the efficacy of USP1-PARPi combination *in vivo*, I-138 was dosed in mice with MDA-MB-436 tumors, either alone or in combination with niraparib. Administration of both compounds resulted in a complete tumor regression and an improved efficacy compared to that of niraparib or I-138 alone (Table 1). In summary, these findings demonstrated the benefit of combining USP1 and PARPi in BRCA1/2 mutant tumors *in vitro* and *in vivo*.[52]

On the basis of these findings, Tango Therapeutics has also been focusing on the development of TNG348 (Figure 6), a highly selective allosteric USP1 inhibitor (USP1i) for the treatment of BRCA1/2 mutant tumors and other HRD tumors. TNG348 strongly synergizes with PARPi in patient-derived xenograft (PDX) cancer cell models of BRCA1/2 mutant breast and ovarian cancers. The synergy between USP1i and PARPi was observed in both PARPi-sensitive and -resistant models. Additionally, TNG348 showed strong antitumor activity in HRD BRCA1/2 wild-type xenograft models, thus potentially expanding USP1i indication. TNG348 is now being evaluated in a phase 1/2 clinical trial as a monotherapy or in combination with olaparib in patients with BRCA1/2 mutant or HRD solid tumors (NCT06065059) (Table 1).[53]

Meanwhile, KSQ Therapeutics has also developed a series of small molecules, potent, and highly selective USP1i. Notably, among these molecules, compound KSQ-4279, structurally related to both I-138 and TNG348 (Figure 6), triggered the accumulation of monoubiquitinated substrates of USP1 in cell lines with BRCA mutations or HRD alterations (Table 1).[54] The activity of KSQ-4279 was evaluated in both ovarian- and TNBC-derived tumor xenograft models, in which it showed a dose-dependent inhibition of tumor growth. Combining KSQ-4279 with olaparib resulted in greater and more durable tumor regression than monotherapy in tumors with only partial sensitivity to PARPi. KSQ-4279 exhibited favorable *in vitro* ADME properties and a favorable PK profile in different preclinical species, with no evidence of dose-related blood toxicity. These results led to KSQ-4279 entering a phase 1 clinical trial for patients with tumors carrying BRCA mutations or other HRD impairment, either as a single agent or in combination with PARPi (NCT05240898).

2.4. Polθ. In the search for additional SL interactions involving BRCA mutations, DNA polymerase θ (Polθ, or POLQ) emerged as an exciting target. Polθ is a versatile DNA repair enzyme that is central in the error-prone Polθ-mediated end joining (TMEJ) pathway, serving as a vital backup pathway to repair DSB when NHEJ or HR is compromised.[55] Polθ consists of an N-terminal helicase-like domain (HelD) and a C-terminal DNA polymerase domain (PolD) linked by a

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Table 1. USP1i Discussed in the Text and Their Preclinical/Clinical Evaluation

inhibitor	target	tumor type	*in vitro* preclinical data	*in cellulo* preclinical data	*in vivo* efficacy	clinical trial	ref
ML323	USP1	–	IC_{50} = 76 nM	combination of cisplatin and ML323 synergistic in H596 cells and U2OS	NA	NA	50
I-138	USP1	BRCA1/2 mutation tumors	IC_{50} = 4.1 nM	cell viability reduction in BRCA1/2 mutation cell lines vs wt; synergic in combination with olaparib in BRCA1/2 mutation cells (UWB1.289 and SUM149PT)	tumor regression with niraparib (15 mg/kg QD) and I-138 (50 mg/kg QD) in mice bearing MDA-MB-436 tumors	NA	52
TNG348	USP1	BRCA1 mutation and HRD breast tumors	NA	synergic with niraparib in PARP-resistant patient-derived organoids	decrease in tumor volume with PARPi in PDX models of BRCA1 mutation and HRD breast cancer	NCT06065059	53
KSQ-4279	USP1	BRCA1 mutation tumors	IC_{50} = 11 nM	decrease in viability for a subset of cell lines, including with BRCA1/2 mutation (clonogenic assay)	activity in monotherapy and with olaparib in ovarian and TNBC PDX BRCA1 mutation and p53 mutation models	NCT05240898	54

large unstructured central region.[56] This unique structure enables Polθ to perform DNA synthesis through its polymerase function and to compete with HR through its helicase domain in an ATPase-dependent manner.[55] Interestingly, Polθ is overexpressed in a variety of human cancers, including colorectal, breast, ovarian, lung, and gastric cancer, whereas in most normal cells, it is expressed at low level or completely absent, making this enzyme an ideal target for oncology.[57−60] Depletion of Polθ by siRNA and shRNA was effective in *BRCA1*-deficient cells in combination with PARPi, leading to a synergistic antitumor effect and a possible delay of PARPi resistance.[61] Additionally, Polθ depletion improved the radiosensitization of different HR-proficient tumor cells[62−64] and sensitized cancer cells to other chemotherapeutic agents.[65,66]

Considering this evidence, Polθ inhibition could provide a novel therapeutic strategy in several tumors, including HR- and NHEJ-deficient cancers. On the basis of these considerations, the identification of Polθ inhibitors (Polθi) and their application with SL interactions is a research field that is currently attracting a great deal of interest from the scientific community, as thoroughly reported in the recent perspective of Pismataro and co-workers.[67] Herein, we therefore report on only the latest updates in the field. Compounds are classified according to their mechanism of action, i.e., Polθ-Hel or Polθ-Pol domain inhibitors.

Novobiocin is described as a Polθ HelD inhibitor, but despite the promising preclinical results (Table 2),[68] caution should be used when assessing its suitability as a Polθi for SL strategies, as its phase 1 clinical trial in solid tumors has recently been suspended (NCT05687110). In 2023, Ideaya announced clearance of an IND application for GSK101 (IDE705), another Polθ HelD inhibitor, for the treatment of patients with HRD tumors in association with niraparib (PARPi).[69] The disclosure of the structure is currently limited to patent applications that describe the development of thiadiazolyl derivatives (Patents WO2020/243459A1 and WO2022/118210A1). In addition, Repare Therapeutics has recently announced the start of a clinical trial in the second half of 2024 for RP-3467 (structure undisclosed), described as a potential best-in-class Polθ HelD inhibitor, which is SL with BRCA loss and capable of synergism in PARPi resistance models.[70]

In 2021, Lord and colleagues reported the discovery of the Polθ-Pol domain inhibitor ART558 (Figure 7), after a screening of approximately 165 000 inhibitors of Polθ polymerase activity.[71] ART558 is a small molecule-substituted pyrrolidine with an IC_{50} of 7.9 nM against the full-length enzyme and binds to the allosteric binding site of the catalytic domain of Polθ polymerase with high specificity and selectivity (Table 2). The authors obtained the cocrystal of Polθ-ART558 (PDB entry 7zx1) and were able to rationalize the binding pose of the inhibitor (Figure 7). Interestingly, ART558 interacted through its hydroxyl group at position 4 with glutamic acid 2365 (E2365) via a HB interaction (Figure 7), which the authors suggested as being crucial for explaining its improved potency.

Inhibition of Polθ by ART558 shows SL with *BRCA1/2* mutant tumor cells, resulting in more DNA damage in knockout cells than in wild-type cells and enhanced effects of PARPi. Moreover, the authors showed that defects in the 53BP1/Shieldin complex, known to cause PARPi resistance, resulted in *in vitro* sensitivity to ART558 (Table 2). Despite

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Table 2. Polθi Discussed in the Text and Their Preclinical/Clinical Evaluation

inhibitor	target	tumor type	*in vitro* preclinical data	*in cellulo* preclinical data	*in vivo* efficacy	clinical trial	ref
novobiocin	Polθ	HRD tumors	IC_{50}: 24 μM	antiproliferative activity and apoptosis in BRCA1/2$^{-/-}$ cells, TMEJ inhibition in U2OS cells	TGI[a] in the xenograft model TOV21G (HR-deficient) (100 mg/kg/BID) and in the PDX model DF83 (RAD51C-deficient) (75 mg/kg/BID) in combination with olaparib	NCT05687110[b]	68
ART558	Polθ	BRCA1/2 mutation and defect in Shieldin DNA repair complex	IC_{50}: 7.9 nM	antiproliferative activity in BRCA1/2$^{-/-}$ cells, inhibition of TMEJ activity in HEK-293T cells, olaparib sensitivity in KCL014BCPO BRCA1 mutation breast cancer *ex vivo* organoids	NA (poor *in vitro* metabolic stability)	NA	71
ART812	Polθ	BRCA1/2 mutation and defect in Shieldin DNA repair complex	IC_{50}: 7.6 nM	inhibition of TMEJ activity in HEK-293 cells (EC_{50} = 240 nM)	good oral bioavailability and moderate to high exposure after a single oral dose in both mice and rats, single agent TGI in a BRCA1$^{-/-}$ SHLD2$^{-/-}$ rat breast cancer xenograft model dosed orally at 100 mg/kg	NA	72
ART4215	Polθ	HRD tumors	NA	NA	NA	NCT04991480	–
RP-6685	Polθ	BRCA2 mutation tumors	IC_{50}: 5.8 nM	antiproliferative activity in HCT116 BRCA2$^{-/-}$ cells (IC_{50} = 0.32 μM), DSB HCT116 wild type (IC_{50} = 0.45 μM)	TGI in HCT116 BRCA2$^{-/-}$ mouse tumor xenograft model (80 mg/kg/BID), metabolic stability issues	NA	73
C1	Polθ	BRCA2$^{-/-}$	IC_{50}: 23.5 nM	antiproliferative activity in BRCA1/2$^{-/-}$ cells in DLD-1 colorectal cancer cells (IC_{50} = 8.1 μM), inhibition of TMEJ activity in HEK-293T cells (IC_{50} = 0.14 μM)	favorable ADME profiles and pharmacokinetic properties in mice		74

[a]TGI, tumor growth inhibition. [b]Study completed.

the ART558 good solubility (0.16 mg/mL) and moderate logD (pH 7.4 = 3.5), it showed poor metabolic stability in rat microsomes. Therefore, a second Polθ-Pol inhibitor, ART812, was developed, which possessed better drug-like properties (Figure 7). ART812 resulted in significant *BRCA1/SHLD2* (Shieldin complex subunit 2)-defective tumor inhibition and was found to be efficacious *in vivo* in a model of PARPi-resistant TNBC (Table 2).[72] Further studies have led to ART4215 (undisclosed structure), the first Polθi to enter clinical evaluation that is currently undergoing a phase 2 clinical trial by Artios Pharma in combination with talazoparib (PARPi) in advanced solid tumors (NCT04991480).

Recently, Repare Therapeutics published a study reporting another Polθi that targets the polymerase domain, namely RP-6685 (Figure 7).[73] To identify a small molecule inhibitor, a high-throughput screen (HTS) was carried out, leading to a fused N2-substituted pyrazole that proved to be an interesting starting point for further development, with the aim of optimizing ADME properties (Figure 8). Reducing the size of the fused cyclohexyl to cyclopentyl was well tolerated, and the replacement of the amide with a thioamide group led to a significant enhancement in the potency, without an improvement in the metabolic stability. Replacing the fused N2-substituted pyrazole ring with a 2,4-bis-trifluoromethyl aryl moiety improved the potency 10-fold compared to that of the hit compound. Moreover, this aryl replacement enabled more effective SAR exploration due to the accessibility of the chemistry. Introduction of a fluorine atom on a phenyl group and substitution of the methyl with a propargyl group increased the microsomal stability and potency. Replacing the bistrifluoromethyl phenyl core with a bistrifluoromethyl pyridyl core optimized the logP value of the compound. Finally, the introduction of a basic amino group on the propargyl function led to RP-6685, the best candidate for further profiling in xenograft studies. RP-6685 showed promising *in vivo* efficacy in HCT116 BRCA2$^{-/-}$ mouse tumor xenograft models (Table 2).[73]

Very recently, Ding and colleagues reported the development of 3-hydroxymethyl-azetidine derivatives as a novel class of Polθ-Pol domain inhibitors.[74] To explore new cores and optimize the ADME profile of previously developed Polθi, the authors employed their proprietary molecular generative AI platform (Chemistry42) followed by a structure-based drug design. Deuterated compound C1 showed the best profile. It exhibited robust cellular target engagement and antiproliferative effects in BRCA-compromised cells. Moreover, promising drug-like properties, e.g., solubility, permeability, and metabolic stability exhibited by C1, corroborates its potential as a therapeutic agent (Figure 7).[74]

2.5. RAD51. RAD51 recombinase is a central HR factor, and its activity is critical for maintaining genome stability and cancer prevention.[75] Once loaded by BRCA2 onto the ssDNA strand, RAD51 catalyzes strand invasion on a homologous DNA template initiating HR repair.[76] In addition to its role in the repair of DSBs, RAD51 exerts its function also at stalled or collapsed replication forks during the replication stress response.[75] RAD51 is overexpressed in several cancers, including pancreatic, breast, prostate, non-small cell lung cancer, leukemia, and glioblastoma.[77] Overexpression of RAD51 enhanced DNA repair, helping cancer cells to survive and develop resistance to DNA-damaging agents,[75] while its depletion or inhibition increases sensitivity to DSB-inducing agents, including ionizing radiation (IR).[78]

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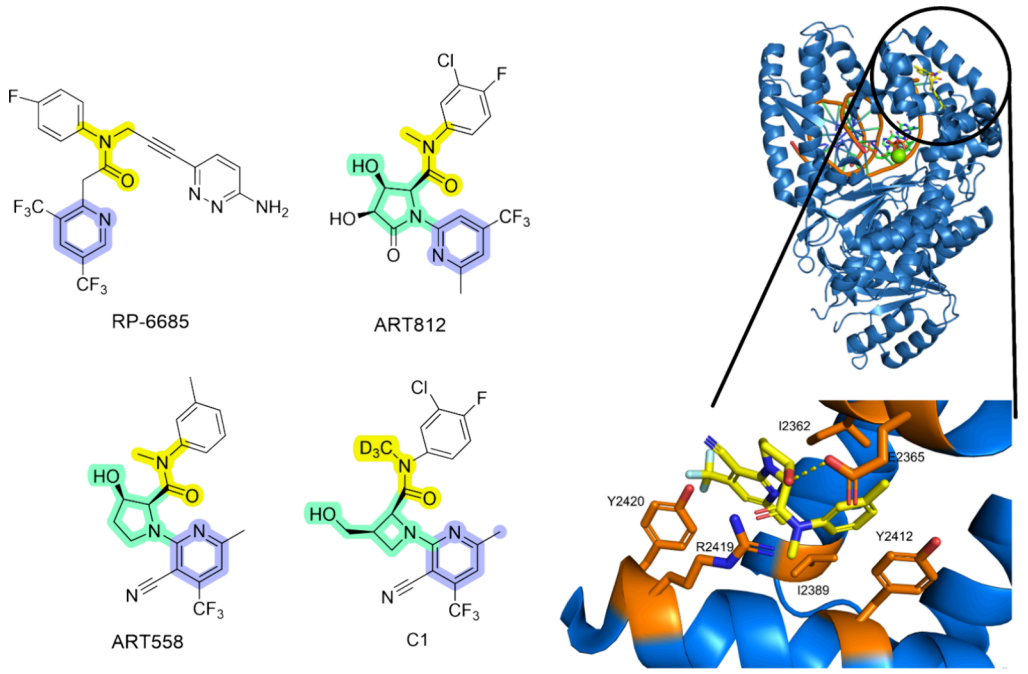

Figure 7. Polθi discussed in the text (key and common structural features are highlighted in green, violet, and yellow) (left) and cocrystal structure of Polθ in complex with ART558 (yellow, PDB entry 7zx1) (right). Amino acids interacting with the inhibitor are colored orange. A dashed line highlights the HB taking place between glutamic acid (E) 2365 of Polθ and the hydroxyl group in position 3 of ART558 (prepared using PyMol).

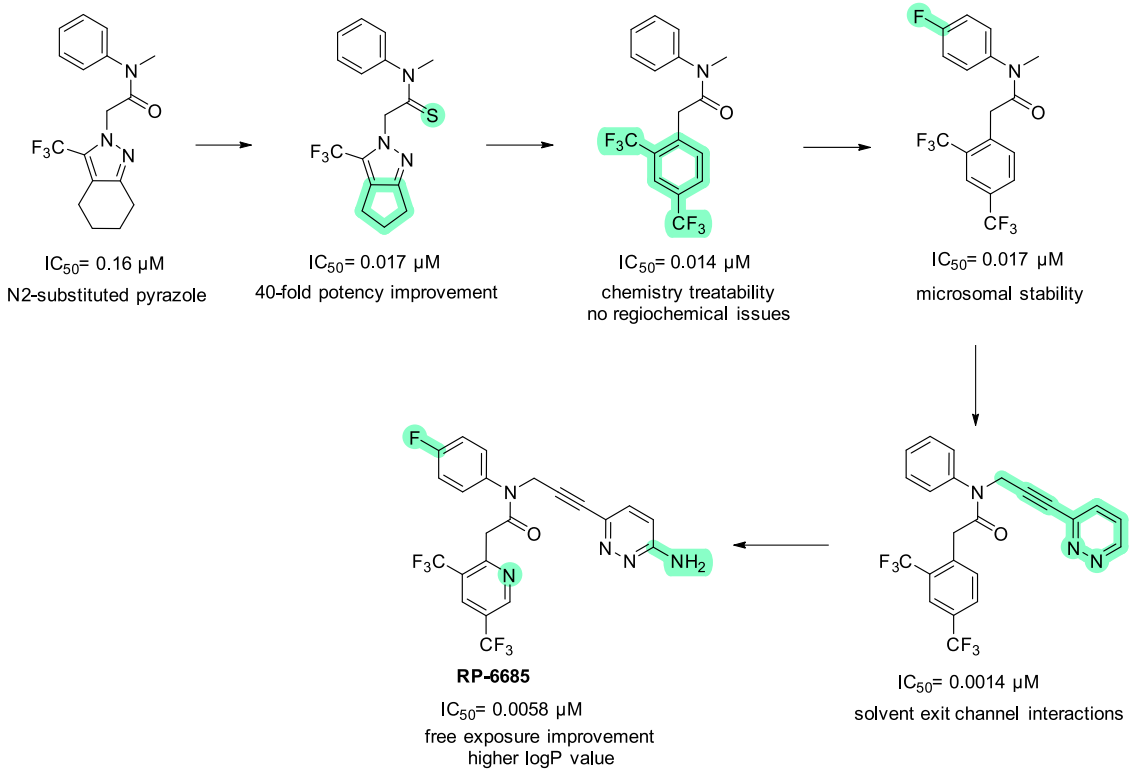

Figure 8. Medicinal chemistry optimization that led to the generation of RP-6685.[73] Key structural features are highlighted in green.

RAD51 impairment proved to be SL with PARPi.[79] Specific miRNAs were found to sensitize tumor cells to olaparib by weakening the expression of RAD51, thus highlighting the importance of RAD51 inhibition to achieve SL outside BRCA deficiencies. Furthermore, this also raised the possibility of using such miRNAs and RAD51 expression levels to identify patients that might benefit from treatment with PARPi.[80] Interestingly, functional assays, measuring the formation of RAD51 foci as an indirect measurement of HR activity, have been shown to predict the PARPi response, thus expanding their clinical application.[81−83]

Beyond the potential of RAD51 as a biomarker for HRD and PARPi sensitivity, pharmacological RAD51 inhibition has also been investigated as a strategy for inducing SL with PARPi.

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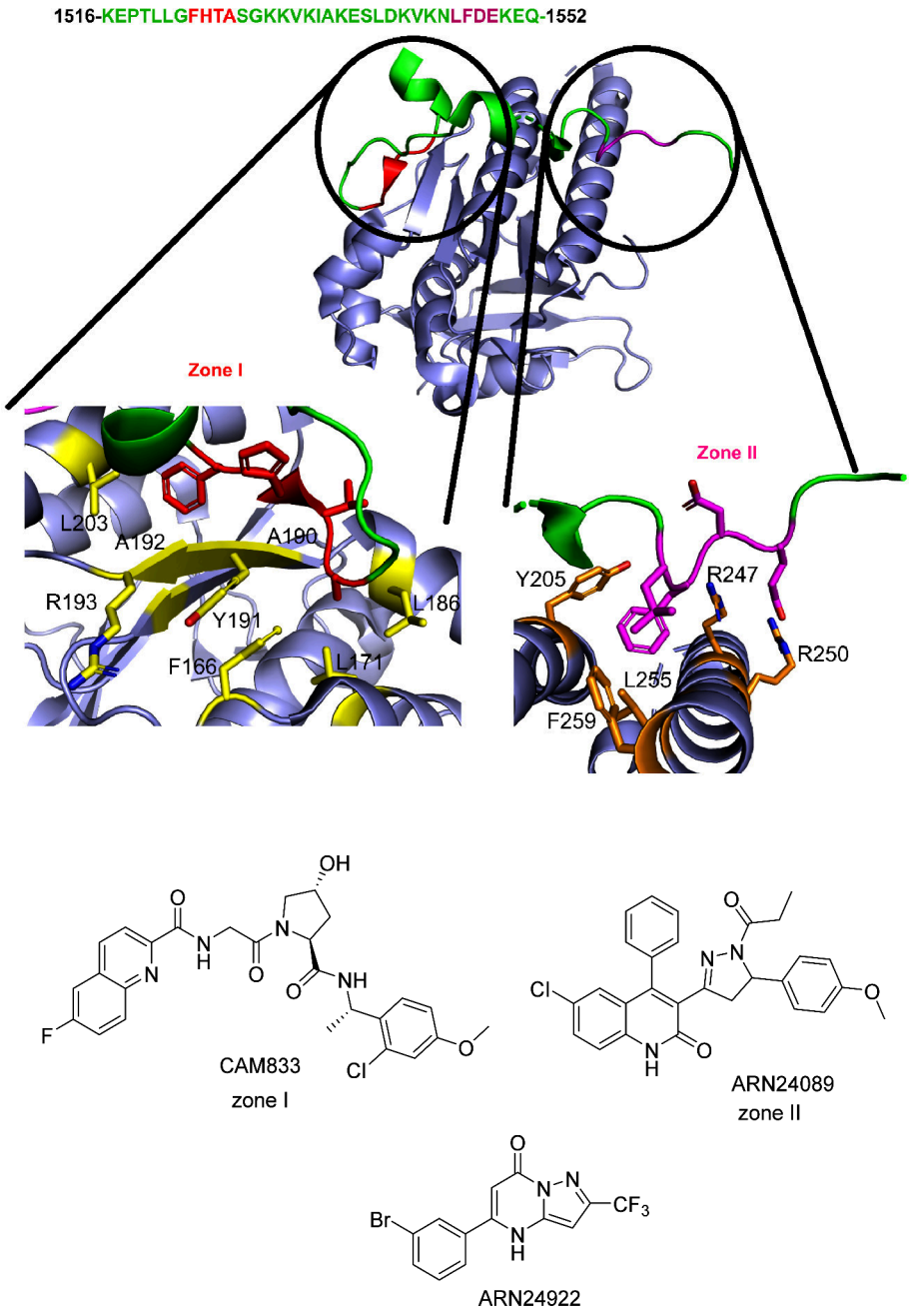

Figure 9. Crystal structure of the RAD51-BRC4 complex (PDB entry 1N0W) (top). In red is highlighted zone I, while in purple zone II (prepared using PyMol). Inhibitors of the BRCA2-RAD51 interaction reported in the text (bottom).

Several compounds that modulate RAD51 activity *in vitro* have been identified, most of which modulate its binding to ssDNA, and have been summarized in recent reviews.[75,84] However, the translation of RAD51 inhibitors (RAD51i) to the clinic has been slow, with no clinical RAD51i in clinical trials to date.[75] The mechanism of action of the only purported RAD51i, CYT-0851, entering clinical trial (NCT03997968), has recently been disclosed and attributed to the inhibition of the monocarboxylate transporter (MCT), therefore ruling it out as a RAD51i.[85]

Recently, our group proposed a new anticancer paradigm dubbed "fully small molecule-induced SL". This paradigm is based on the hypothesis that it could be possible to mimic the effect of BRCA2 germline mutations by using small organic molecules disrupting RAD51-BRCA protein-protein interaction. This could potentially expand the population of patients eligible for treatment with olaparib and other PARPi, especially in otherwise difficult to treat pancreatic cancers.[86−90] BRCA2 interacts with RAD51 through eight homologous sequences known as BRC repeats. In particular, the BRC4 peptide interacts with RAD51 through two different domains: the FXXA domain, critical also for RAD51 multimerization, and the LFDE domain, also known as zone I and zone II, respectively (Figure 9). The X-ray crystallographic structure of the BRC4 repeat in complex with the catalytic domain of RAD51 has been reported (PDB entry 1N0W).[91−93] On the basis of this structure, to obtain BRCA2-RAD51 disruptors, our group conducted virtual screening campaigns based on high-throughput docking upon both pockets followed by SAR optimization.[87−90] The most promising results were observed with a zone II binding dihydroquinoline pyrazoline derivative

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Table 3. Preclinical Evaluation of BRCA2-RAD51 Interaction Inhibitors

inhibitor	target	*in vitro* preclinical data	*in cellulo* preclinical data	*in/ex vivo* efficacy	ref
ARN24089	BRCA2-RAD51	EC_{50} (ELISA) = 19 μM; RAD51 K_d MST = 11 μM	HR inhibition EC_{50} = 18 μM (BxPC-3) synergism (CI[a] < 0.3 at 20 μM) with olaparib in BRCA2 wild-type cells (BxPC-3) over BRCA2 mutation CAPAN-1	NA	88
ARN24922	BRCA2-RAD51	EC_{50} (ELISA) = 28 μM; RAD51 K_d MST = 12 μM (wt) and 55 μM (monomer)	HR inhibition of 80% (HEK-293 m-Clover Lamin A assay at 50 μM), synergism with talazoparib in BxPC-3, AsPC-1, and HPAC cells and a PT291 organoid (all pancreatic tumors)	NA	95
CAM833	BRCA2-RAD51	RAD51 K_d ITC = 366 nM and K_d FP = 355 nM	HR inhibition of 50% above 25 μM, synergic with PARP1i AZD2461 in BRCA2 wild-type cells and radiosensitizer	NA	86

[a]CI, combination index.

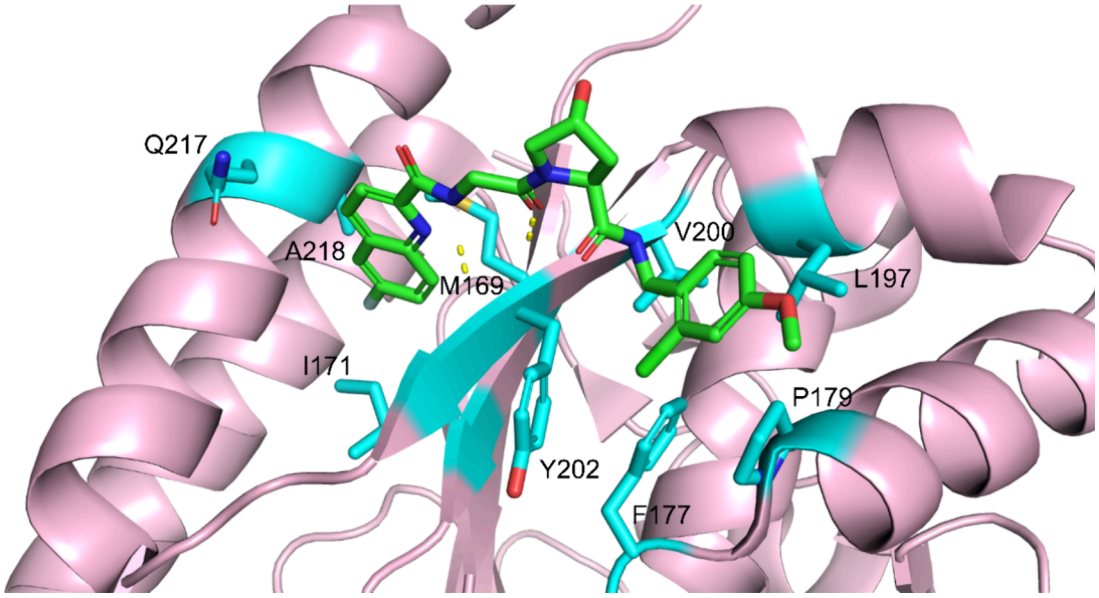

Figure 10. Crystal structure of the RAD51 in complex with CAM833 (PDB entry 6TW9). In pink is the protein, in light blue are highlighted the main interactions, while in green is the compound(prepared using PyMol).

ARN24089 (Figure 9 and Table 3) that disrupts BRCA2-RAD51 protein-protein interaction, thus mimicking the effects of BRCA2 impairment. The compound inhibits HR activity and synergizes with olaparib to trigger SL in pancreatic BRCA2-proficient cancer cells (BxPC-3).[88] RAD51 possesses an intrinsic tendency to form oligomeric structures, which makes it challenging to conduct biochemical, biophysical, and drug discovery investigations. The recent isolation of a fully human monomeric RAD51[94] has allowed us to validate ARN24992, a BRCA2-RAD51 inhibitor, which resulted from a ^{19}F nuclear magnetic resonance fragment screening performed on human wild-type RAD51, followed by medicinal chemistry optimization (Figure 9 and Table 3).[95] ARN24922 showed HR inhibition in BxPC-3 cells. Remarkably, the combination of ARN24922 with PARPi talazoparib showed efficacy also in three-dimensional (3D) pancreatic cancer models, specifically PT291 organoids in which the co-administration of both compounds caused a 50% reduction of cell viability in comparison with that of the single talazoparib treatment.[95] Likewise, Scott and colleagues developed CAM833 (Figures 9 and 10), an inhibitor of BRCA2-RAD51 interaction binding zone I. This discovery was made through a structure-led fragment-based approach utilizing a humanized RadA-RAD51 construct developed in house (HumRadA22F).[86] The crystal structure shows that CAM833 binds to the RAD51 oligomerization pocket and consequently reduces RAD51 foci and filaments, preventing DNA repair through HR. Ultimately, CAM833 increases cytotoxicity induced by IR and synergizes with PARP1i (Table 3).[86] Despite promising preclinical results, the therapeutic potential of BRCA2-RAD51 interaction inhibitors and their combination with PARPi in the clinic has been stymied due to the mechanism-related toxicity in normal tissues that can result from the systemic inhibition of both RAD51 and PARP. Nevertheless, cancer cells depend more strongly on DNA repair mechanisms than do healthy cells, due to their genetic instability. Consequently, the combination of PARPi and BRCA-RAD51 inhibitors may affect healthy cells far less than cancer cells, offering a chance for selectivity. Indeed, this specific chemically induced SL can be leveraged in different scenarios: (i) to treat patients with acquired resistance to PARPi, mostly due to the restoration of the mutated BRCA2 gene or RAD51 overexpression, (ii) to treat tumors with mutations that have a SL relationship with BRCA impairment. Moreover, beyond the specific PARP-BRCA SL pair, this approach forms the basis for future combination strategies targeting SL pairs involved in DDR.

2.6. RAD52. Human RAD52 is involved in various DNA repair pathways, including HR, single-strand annealing (SSA), and RNA-dependent DNA repair.[96] Moreover, RAD52 is involved in solving stalled replication forks.[96] Human RAD52 appears to be nonessential for the viability of normal cells[97,98]

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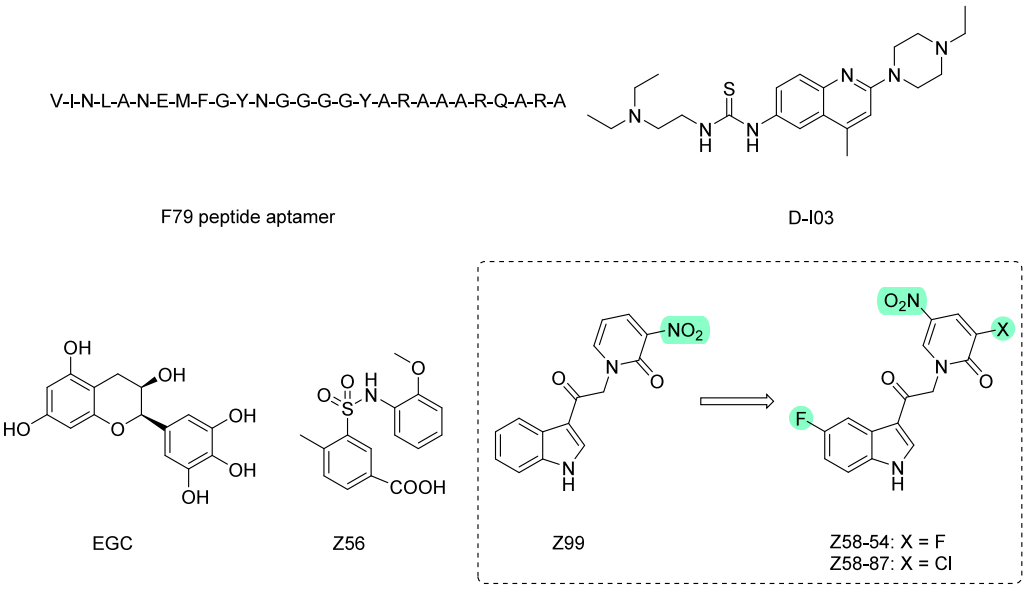

Figure 11. RAD52i discussed in the text. Key structural features are highlighted in green.

but becomes essential under cancer conditions when other DSB repair-related proteins are mutated.[99,100] Therefore, RAD52 has emerged as a novel target to aid chemically induced SL therapies, avoiding resistance and severe side effects. Inhibiting RAD52 induces SL in many cancer cells with defective DNA repair-related proteins, such as BRCA1/2, PALB2, XAB3, and RAD51 paralogues.[101−103]

Researchers have devoted considerable effort to identifying and characterizing RAD52 inhibitors (RAD52i), but none have reached clinical development.[96] Notably, targeting the RAD52 structure represents a remarkable challenge because of the organization of RAD52 into an undecameric protein ring that also results in multivalent interactions with ssDNA.[96,104] Another important challenge is the development of inhibitors selective for RAD52 over other ssDNA binding proteins to avoid secondary target binding toxicity.[105]

A few examples of small molecule RAD52i and their SL applications are reported below. In 2013, Cramer-Morales and colleagues[106] developed peptide aptamer F79 (Figure 11) as a RAD52i. F79 exerts SL in BRCA2 and/or HR-mutated tumor cells. F79 selectively killed BRCA-deficient leukemia cells with a low risk toward normal cells and showed SL also in breast, pancreatic, and ovarian cancer cells.[106] Moreover, F79 showed encouraging results *in vivo* (SCID mice with BCR-ABL1 positive leukemia).[106,107] Despite these promising results, there have been no further reports on the subsequent development of this peptide aptamer (Table 4).

In 2016, Huang et al.[108] identified a new series of RAD52 small molecule inhibitors through an HTS with a fluorescence quenching assay. Among them, D-I03 (Figure 11) was the most potent compound in biochemical and cellular assays.[108] Later, Sullivan-Reed and colleagues reported that D-I03 was effective in slowing the growth of BRCA1-deficient xenograft tumors in mice.[107] Despite these promising *in vitro* and *in vivo* studies, the thiourea scaffold of D-I03 had several drawbacks, including poor solubility and metabolic and chemical instability.[109]

In 2016, Hengel and colleagues[110] reported the development of a novel FRET-HTS assay that led to the identification of epigallocatechin (EGC) (Figure 11), a natural compound that competes with ssDNA to bind to RAD52, but not to RPA. EGC dysregulated DSB repair and caused a reduction in cell viability in BRCA1/2-depleted and MUS81-depleted cells.[110] Once more, despite promising *in vitro* biological results, EGC and its analogues were characterized by poor ADME properties, largely due to low gut absorption, which was found to be further reduced when accompanied by food. Additionally, the very high hydrophilicity of EGC analogues created homogeneity problems in lipid formulations.[111,112] Very recently, Bhat and co-workers[113] tried to overcome ADME challenges associated with EGC to produce new RAD52i.[114] Pharmacophore information about the RAD52 complexation with EGC allowed for scaffold hopping into a drug-like synthetically accessible space. Six distinct chemical scaffolds that occupy the same physical space of RAD52 as EGC were identified. The six compounds were all efficient RAD52i, in particular against the RPA-RAD52-ssDNA complex (IC_{50} ∼ 8−96 μM). Among the selected inhibitors, Z56 and Z99 (Figure 11) were especially attractive as they showed enhanced activity in cell viability assays. Z56 was selective for RAD52 over RPA *in vitro* and selectively killed BRCA mutant cells. In contrast, Z99 inhibited both proteins *in vitro* and displayed toxicity toward BRCA-complemented cells. Z99 showed excellent drug-likeness and was selected for scaffold expansion, resulting in potent and selective RAD52i Z58-54 and Z58-87 (Figure 11) with IC_{50} values in the low micromolar range against RAD52 that were able to discriminate between BRCA1/2 mutant and BRCA-proficient cells (Table 4). Because of the selectivity for RAD52 over RPA of both Z56 and derivatives from Z99 expansion, additional rounds of chemical exploration may be a promising strategy for achieving more potent and selective RAD52i.[113]

2.7. Nuclear Kinases Associated with Genomic Stability. Nuclear kinases play a pivotal role in orchestrating signaling pathways in response to DNA damage. These specialized enzymes act as key regulators by phosphorylating target proteins involved in DNA repair, cell cycle arrest, and apoptosis. Upon sensing DNA lesions, these kinases are activated and initiate a cascade of events that ensure genomic stability and cell survival. Key players include the ATR/

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Table 4. RAD52i Discussed in the Text and Their Preclinical Evaluation

inhibitor	target	tumor type	*in vitro* preclinical data	*in cellulo* preclinical data	*in vivo* efficacy	clinical trial	refs
F79 aptamer	RAD52	BRCA1/2 mutation tumors	RAD52-ssDNA (EMSA)	BRCA1/2 with or without cell survival: BRCA1/2$^-$ cells over BRCA1/2$^+$ and combination with PARPi	PARPi and F79 (2.5 mg/kg/day) synergic against BRCA1-deficient primary AML xenograft in NSG mice	NA	106, 107
D-I03	RAD52	BRCA1/2 mutation tumors	RAD52 K_d (SPR) = 25.8 μM	BRCA1/2 with or without cell survival: IC_{50} = 14.5 μM for BRCA1/2$^-$ cells over BRCA1/2$^+$ and combination with PARPi	PARPi (talazoparib) and D-I03 (50 mg/kg/day) synergic against BRCA1-deficient solid tumor growth in nude mice	NA	107, 108
Z56 and Z99 derivatives	RAD52	BRCA1/2 mutation tumors	RAD52-ssDNA (FRET, IC_{50} = 2–8 μM)	BRCA1/2 with or without cell survival: selective toxicity for BRCA1/2$^-$ cells over BRCA1/2$^+$	NA	NA	113

CHK1/WEE1 axis, the ATM/CHK2/p53 axis, PKMYT1, and DNA-PK (Figure 12).[37,81] PLK1 and Aurora kinase A (AURKA) are also involved in damage checkpoint regulation (Figure 12).[37]

Despite the development of various inhibitors, the toxicity of CHK1/2 inhibitors, stemming from a lack of selectivity, has prevented their clinical application and further drug development.[37] However, the improving understanding of SL interactions presents an opportunity to repurpose existing inhibitors tailored to specific tumor profiles, thereby optimizing their clinical evaluation. In this regard, it is worth mentioning the AURKA inhibitor alisertib, the most advanced AURKA selective (>200-fold more selective than AURKB) inhibitor in clinical studies. This molecule found new applications after Takeda's announcement to terminate its phase 3 trial in relapsed or refractory peripheral T-cell lymphoma due to a lack of efficacy. The drug is now being investigated in phase 1/2 clinical trials in Rb-deficient head and neck squamous cell cancer in combination with pembrolizumab (NCT04555837) and in combination with osimertinib in metastatic EGFR mutant lung cancer (NCT04085315). These trials leverage AURKA SL interactions with tumor suppressor deficiencies,[115] even though balancing risk and benefit in patients remains difficult.

In this context, we focus on the extensively studied ATR, ATM, and DNA-PK kinases, along with emerging players like WEE1, PKMYT1, and others such as PLK1, which are attracting attention because of their roles in controlling DNA damage checkpoints.

2.7.1. Trinity of DDR: ATM, ATR, and DNA-PK. ATM, ATR, and DNA-PK are three closely related kinases of the phosphoinositide 3-kinase-related kinase (PIKK) family.[81,116,117] These kinases orchestrate DDR at the very early stages, acting as sensors that recognize DSBs and activate the signaling cascade by interacting with many different downstream players (Figure 12).[81,116−118] These kinases have been covered extensively by the literature in various reviews.[117,119−121] This work aims to give a brief overview of their function to support the discussion about medicinal chemistry strategies, which has led to recent clinical progress. With regard to detailed clinical trial data, we refer readers to the excellent recent publication by Groelly et al.[119]

2.7.1.1. ATR. ATR is activated by a wider range of genotoxic stresses, expanding from DSBs. Rapidly dividing cells undergo high levels of replication stress and tend to be highly dependent on the ATR pathway.[122,123] ATR activation takes place in RPA-coated ssDNA through the interaction with the ATR interacting protein (ATRIP) and other factors (i.e., TopBP1) (Figure 12).[37] One crucial function of ATR is to induce G2/M arrest by phosphorylating the downstream kinase CHK1 to provide sufficient time for DNA repair (Figure 12).[37] This process is covered in greater detail by Neizer-Ashun et al.[124] Briefly, CHK1 induces cell cycle arrest by phosphorylating a series of CDC25 phosphatases targeting them for degradation or sequestration, causing a signaling cascade, which results in cell cycle arrest at the intra-S and G2/M checkpoints (Figure 12).

ATR inhibition is lethal with the ATM pathway. Indeed, ATR inhibitors (ATRi) cause the increase in the level of formation of DSBs, which in turn augments the reliance of cells on ATM and its associated proteins activity. This relationship explains why the inhibition of ATR through small molecules seems to be synthetically lethal in cells with *ATM* or *p53*

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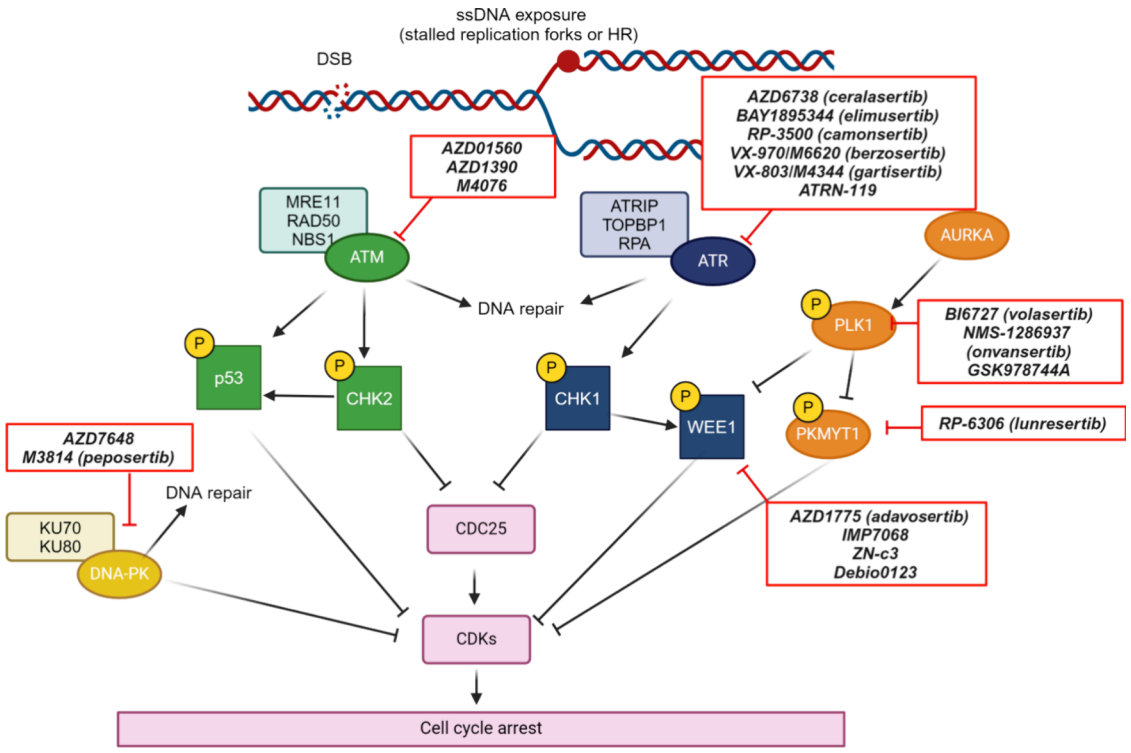

Figure 12. Schematic representation of nuclear kinases associated with DDR and their associated inhibitors discussed in the text (created with BioRender.com).

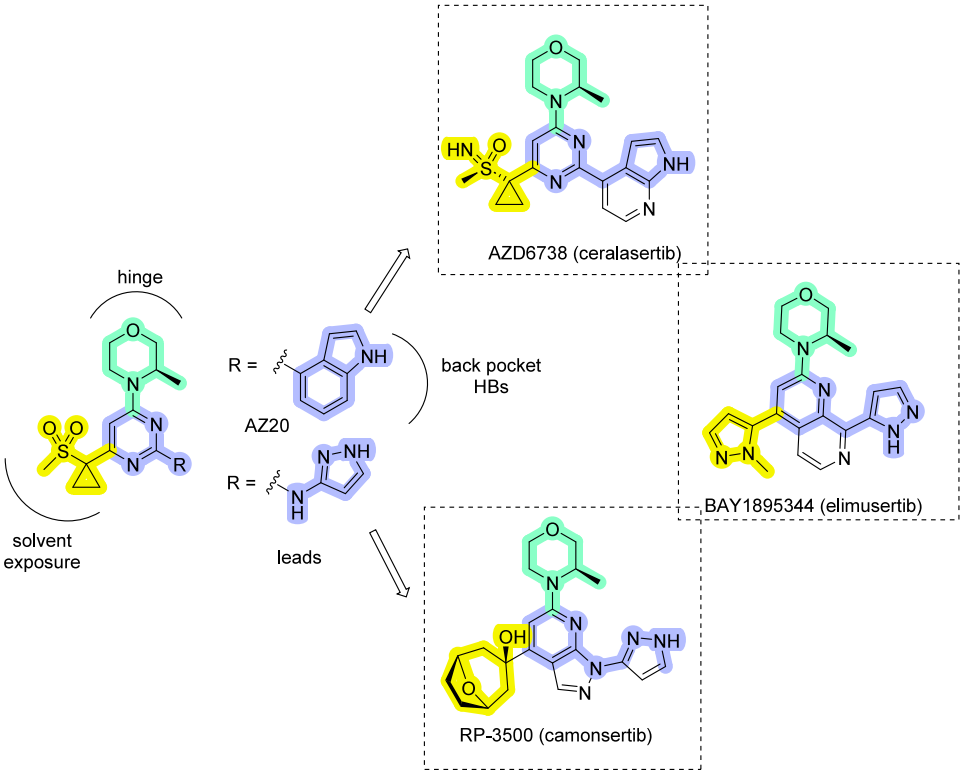

Figure 13. Structures of ATRi discussed in the text. Key and common structural features are highlighted in green, violet, and yellow.

deficiency.[125] In *ATM*- or *p53*-deficient pancreatic cells, ATR inhibition causes the accumulation of DNA damage with the induction of mitosis prior to the completion of DNA repair, causing subsequent mitotic catastrophe.[123] Furthermore, ATRi show synergism when combined with therapeutic agents that increase replication stress such as traditional chemotherapeutics (i.e., DNA topoisomerase I or IR).[126,127] Additionally, the recent combination study of ATR inhibition with immunotherapeutic targeting PDL1 resulted in a successful synergistic antitumor activity in mouse models of castration-resistant

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Table 5. ATRi Inhibitors Discussed in the Text, Their Preclinical Evaluation, and Relevant Clinical Trials[a]

compound	ATR HeLa IC_{50} (μM)	mTOR HeLa selectivity (x-fold)	LoVo[b] antiproliferative IC_{50} (nM)	biomarker (clinical trial)
AZD6738 (ceralasertib)	0.18	6	377	–
BAY1895344 (elimusertib)	0.0023	35	27	DDR gene mutations (NCT04095273[d])
RP-3500 (camonsertib)	0.0009	27	28	*TP53*, *ATM*, *SF3B1*, *XPO1*, or *POT1* gene mutations (NCT05405309)
M6620/VX-970 (berzosertib)	0.074	1.3	NA	DDR gene mutations (NCT04266912)
M4344/VX-803 (gartibsertib)	0.005	27	86	*ARID1A*, *ATRX*, *DAXX*, or *ATM* gene mutations (NCT02278250[d])
ATRN-119	0.004[c]	>2000[c]	NA	DDR gene mutations (NCT04905914)
ART0380	NA	NA	NA	ATM deficiency (NCT04657068)
IMP9064	NA	NA	NA	– (NCT05269316)

[a]NA, not available. Preclinical data for all compounds except ATRN-119 taken from ref 134. [b]Human *MRE11* mutant colorectal cancer cell line frequently used for ATRi evaluation. [c]Data reported for the HCT116 cell line. [d]Study completed.

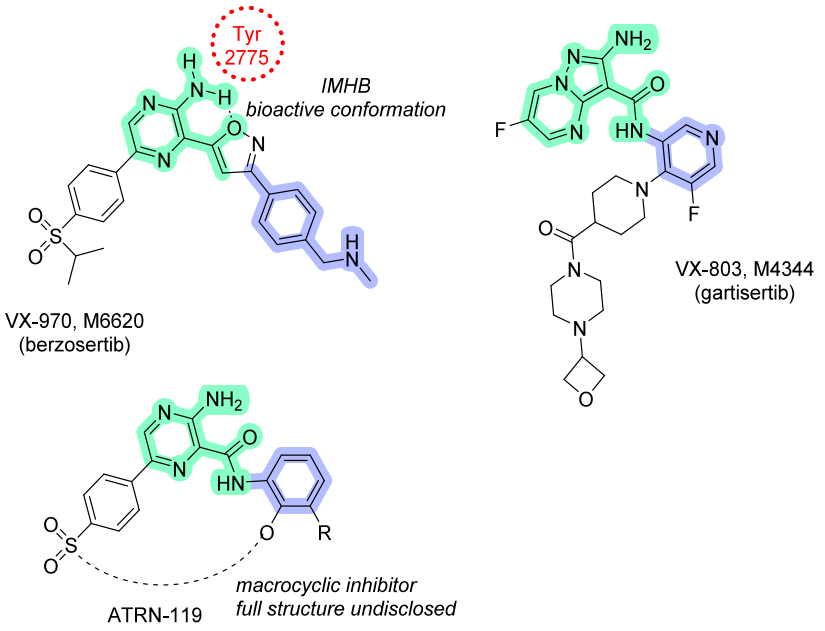

Figure 14. Structures of ATRi discussed in the text. Key structural features are highlighted in green and purple, and the amino acid that is important for the binding mode is represented in the dashed red circle.

prostate cancer.[128] Lastly, the link between ATR inhibition and PARP is well established, which perhaps represents the most promising prospect for ATRi in the clinic. PARP inhibition, as mentioned in this Perspective, has shown remarkable success in the clinic in BRCA1/2-deficient cancers. However, acquired resistance occurs with high frequency where alternative DNA repair pathways are leveraged to bypass BRCA1/2 pathway and PARP inhibition. Remarkably, the use of ATRi has been shown to block this event and resensitize tumor cells to PARP inhibition.[129] Inhibition of ATR has progressed from the highlighted *in vitro*/*in vivo* studies into clinical trials, with a number of ATRi identified. An extensive description of ATRi development and their clinical trials has been thoroughly reported in recent reviews.[37,81,121,130] Herein, we narrow the description to a selection of medicinal chemistry strategies that led some ATRi entering clinical trials.

The structures of ATRi fall into two classes. The first class is the groups of the morpholine hinge binders exemplified by AZ20 (Figure 13). Compounds grouped in this class are ATP-competitive and bind to the hinge region of the protein through their methyl-morpholine substituent. Moreover, the heterocycle attached to the pyridine or pyrimidine central core establishes important HB with the protein backbone (Figure 13). AZD6738 (also known as ceralasertib, developed by AstraZeneca) was developed from AZ20 to improve the aqueous solubility and eliminate CYP3A4 time-dependent inhibition. Despite the moderate inhibition against mTOR, which eventually may hamper its selectivity, AZD6738 has been the first oral ATRi to reach clinical trials (Table 5).[131] Interestingly, AZD6738 has recently showed a clinical therapeutic benefit by underpinning broad immunomodulation that suggests a wider antitumor therapeutic potential.[132] BAY1895344 (elimusertib, Bayer)[133] and RP-3500 (camonsertib, Repare Therapeutics, now developed in collaboration with Roche)[134] are two other oral ATRi in clinical trials as single agents in DDR-deficient tumors (Table 5). The equivalent cell-based potency and selectivity over mTOR of the two compounds (Table 5) translated into a similar preclinical efficacy when administered on a weekly (3 days on/4 off) intermittent schedule both at their respective maximum tolerated doses (MTDs, 50 mg/kg BID for BAY1895344, and 30 mg/kg QD for RP-3500).[134,135] However, when both were

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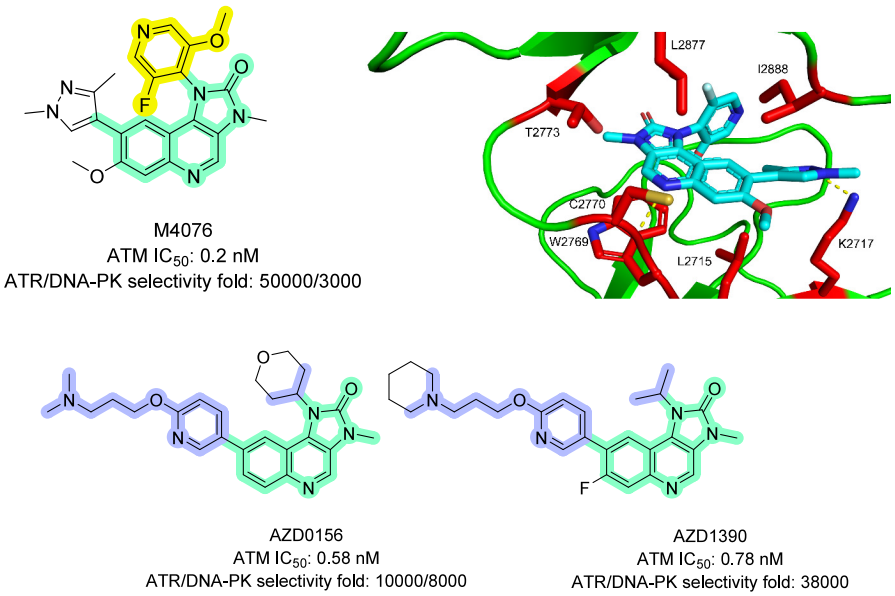

Figure 15. Structures of ATMi discussed in the text. Key and common structural features are highlighted in green, violet, and yellow. Cryo-EM structure of ATM (green, PDB entry 7NI4) in complex with inhibitor M4076 (cyan) (top right). Amino acids that are crucial for the interaction with the inhibitor are colored red, while HBs are represented as dashed yellow lines.

administered on an intermittent dosing schedule in phase I clinical evaluation, the two compounds, with different ADME properties, particularly $t_{1/2}$ (BAY1895344 $t_{1/2}$ = 11.5 h, and RP-3500 $t_{1/2}$ = 5.8 h), led to the induction of a higher rate of anemia with BAY1895344 than with RP-3500.[136,137]

The second class of ATRi encompasses the aminopyrimidines discovered by Vertex (Figure 14). Berzosertib (previously known as M6620 or VX-970, developed by Vertex and Merck) was the first ATRi to enter clinical investigation. Berzosertib was the result of a medicinal chemistry optimization aimed at maintaining an intramolecular hydrogen bond (IMHB) interaction between the hydrogen of the pyrazin-2-amine and the oxygen of the oxazole ring, resulting in a bioactive conformation that avoids the unwanted steric clash between the molecule and Tyr2775 (Figure 14). Furthermore, functionalization of the benzyl ring with a methylamine enabled the exploitation of a negatively charged area of the ATP binding site, resulting in a potent molecule (IC_{50} = 0.074 μM) with favorable physicochemical properties (aqueous solubility of 52 μM) (Figure 14).[138] Despite being under clinical evaluation, both as monotherapy and in combination, berzosertib suffers from limited selectivity toward mTOR (Table 5). For this reason, Vertex and Merck have recently reported gartisertib (M4344 or VX-803), a more potent (IC_{50} = 0.005 μM) and selective aminopyrimidine derivative (Figure 14 and Table 5).[139] In its first in-human phase I study, gartisertib has been tested in patients bearing loss-of-function mutations, specifically, ARID1A, ATRX, DAXX, and ATM genes, known to be synthetically lethal with ATR inhibition. However, the study reported unexpected liver toxicity, which prevented further advancement of gartisertib and led to its recent discontinuation.[140] In any case, the use of loss-of-function mutations as biomarkers offered an efficient patient stratification, along with the opportunity to leverage SL as an anticancer strategy.

ATRN-119 (Aprea Therapeutics) is another recent molecule that belongs to the aminopyrimidine class of ATRi. Preclinical evaluation data for ATRN-119 are limited, but it is described as a potent (IC_{50} = 0.004 μM) and orally bioavailable derivative (Table 5) that could get its high selectivity (>2000-fold for mTOR) from its macrocyclic structure (Figure 14).[141] ATRN-119 is in an ongoing phase 1/2a dose escalation trial in solid tumors with DDR deficiencies (Table 5).

Medicinal chemistry campaigns have been hindered by the lack of a crystal structure with an appropriate resolution for drug discovery campaigns,[142] therefore relying on only the ATR homology models built on PI3K-γ as a structural template. Nevertheless, continuing research efforts are focused on finding a highly selective ATRi possessing favorable ADME characteristics to find the optimal clinical dosing schedule. In this regard, ART0380 (Artios) and IMP9064 (IMPACT Therapeutics) are two other recently developed small molecule ATRi in clinical trials but for which preclinical data and structure are undisclosed so far (Table 5).

2.7.1.2. ATM. ATM can be activated by two different mechanisms, either in reaction to afford reactive oxygen species (ROS) or more commonly in response to DSBs. In this latter mechanism, the MRN (MRE11-RAD50-NBS1) complex recruits the ATM homodimer, which in turn monomerizes with the MRN complex, subsequently promoting the interaction between the DNA break and the ATM monomer (Figure 12).[143] The activation of ATM is finely regulated by post-translational modifications, including the acetylation of Lys3016 by acyltransferase TIP60 that in turn promotes Ser1981 autophosphorylation, known as a marker of activated ATM, as it occurs immediately after the formation of DSBs (Figure 12). Activated ATM in turn phosphorylates MDC1 or γH2AX to amplify repair signaling. The ATM/CHK2/p53 pathway controls the G1/S checkpoint in addition to being partially involved with G2/M checkpoint regulation through phosphorylation and degradation of CDC25A, responsible for the removal of the inhibitory phosphorylation of CDKs.[37]

For its role in DDR, ATM has been identified as a potential druggable target, with SL partnerships being observed with PARP, BRCA1, DNA-PK, Polθ, XRCC1, APE1, and the Fanconi anemia pathway.[118] Consistently, success has been

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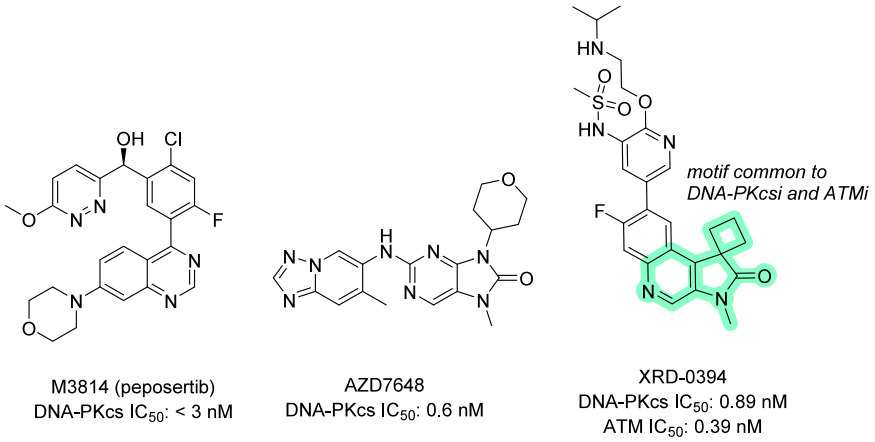

Figure 16. Structures of DNA-PKcsi and of dual DNA-PKcsi/ATMi discussed in the text. Key structural features are highlighted in green.

seen in phase I clinical trials using PARPi (e.g., NCT02975934) and ATRi (e.g., NCT04657068) to treat ATM-deficient tumors. In turn, it follows that tumors defective in one or more of those genes may be candidates for therapy using ATM inhibitors (ATMi). AZD0156 was the first inhibitor developed by AstraZeneca to enter clinical trials.[144] However, the company removed it from its pipeline after severe hematological toxicities in patients treated with a combination of AZD0156 and olaparib (Figure 15).[145] Moreover, AZD0156 turned out to be a substrate for efflux transporters, hindering its eventual development for CNS malignancies. Subsequently, AstraZeneca developed AZD1390, an orally bioavailable ATMi with improved BBB properties in several preclinical models (efflux ratio in MDCK cells of 1.8 at 0.1 μM and $K_{p,uu}$ in cynomolgus monkey brain of 0.33), making it a suitable clinical candidate for CNS malignancies (Figure 14).[146] Currently, M4076 (Merck KGaA) has been the most potent and selective ATMi (ATM IC_{50} = 0.2 nM) so far in clinical trials. It shares an imidazo[5,4-*c*]quinolin-2-one core with AZD0156 and AZD1390 and an analogous substitution pattern (Patent WO2020/193660A1) (Figure 15). The cryo-EM structure of ATM in complex with M4076 revealed structural insights into its mode of action (Figure 15).[147] In fact, the high selectivity of M4076 is attributed to the stacking interactions with W2769 in the hinge region and to the close contact between the 1,3-dihydro-imidazo[4,5-*c*]quinolin-2-one moiety of M4076 and C2770, also located in the hinge region (Figure 15).[147] Cryo-EM technology can support structure-based drug design (SBDD), especially to help with the challenge of ATM mutations, which are common tumor features.[119] In conclusion, despite the fact that the clinical evaluation is proceeding, ATM have proven to be targetable and ATMi appear as a promising treatment option in DDR-mutated cancers.

2.7.1.3. DNA-PKcs. DNA-PK comprises a catalytic subunit (DNA-PKcs) and a regulatory heterodimer known as Ku (composed of Ku70 and Ku80). Upon localization on DSB, Ku70/Ku80 recruits DNA-PKcs to form an active DNA-PK complex (Figure 12).[116] In contrast to the other PIKK members, it plays a key role in NHEJ, a DSB repair mechanism used by cells outside the G2/S phase or when HR is impaired (Figure 12).[116] In addition, DNA-PK has a multifaceted role, including participation in the replication stress response and DNA damage checkpoints.[37]

DNA-PKcs have been reported to be synthetically lethal with many genes in the DDR process. The primary SL partnership is the aforementioned ATM due to the complementary nature of these two genes with inactivation of both genes causing embryonic lethality.[148,149] Furthermore, as simultaneous inhibition of NHEJ and HR tends to cause SL in tumors overly reliant on HR, synthetic lethality can be seen between DNA-PKcs and the main drivers of HR such as BRCA1, BRCA2, CHK2, RAD50, PTIP, PAXAP, and MSH3.[118]

Due to the aforementioned SL relationships, drug discovery efforts have attempted to inhibit DNA-PKcs activity. This has proven to be challenging as selectivity issues have arisen between DNA-PKcs and the other PIKK members due to structural similarities between the various kinases.[120] M3814 (peposertib, Merck KGaA, Patent WO2020/193660A1)[150] and AZD7648[151] (AstraZeneca) are two extensively described DNA-PKcs inhibitors (DNA-PKcsi) with demonstrated selectivity over other PIKKs (Figure 16). Despite both molecules potentiating radiation and chemotherapy both *in vitro* and *in vivo* (remarkably, AZD7648 sensitized ATM-deficient cancer cells to both radiation therapy and PARPi), their clinical translation was not successful.[150,152] Indeed, targeting DNA-PKcs is not without risks. Systemic radiosensitization of healthy cells in addition to tumor cells has led to side effects, which were not counterbalanced with sufficient satisfactory patient responses, ultimately leading to program discontinuation for both molecules.[153]

Among the newest strategies for pursuing DNA-PKcsi is the recent development of XRD-0394, an ATM/DNA-PKcs dual inhibitor that shares the imidazo-2-one core as a common motif between DNA-PKcsi and ATMi and leverages the SL between the two targets, by XRad Therapeutics (Figure 16). This orally available small molecule is described as a potent inhibitor of both proteins, without significant inhibition of structurally related PIKK kinases in human tumor cells, including ATR and mTOR.[154] *In vitro* and *in vivo* efficacy showed marked dose-proportional radiosensitization, with no toxicity in the absence of IR. Additionally, in cells lacking BRCA1/2, XRD-0394 showed synergism in combination with PARPi, therefore potentially expanding their application in the case of resistance.[154] Because of these promising results, XRD-0394 has recently entered a clinical trial for the treatment of solid tumors in combination with palliative radiotherapy (NCT05002140).

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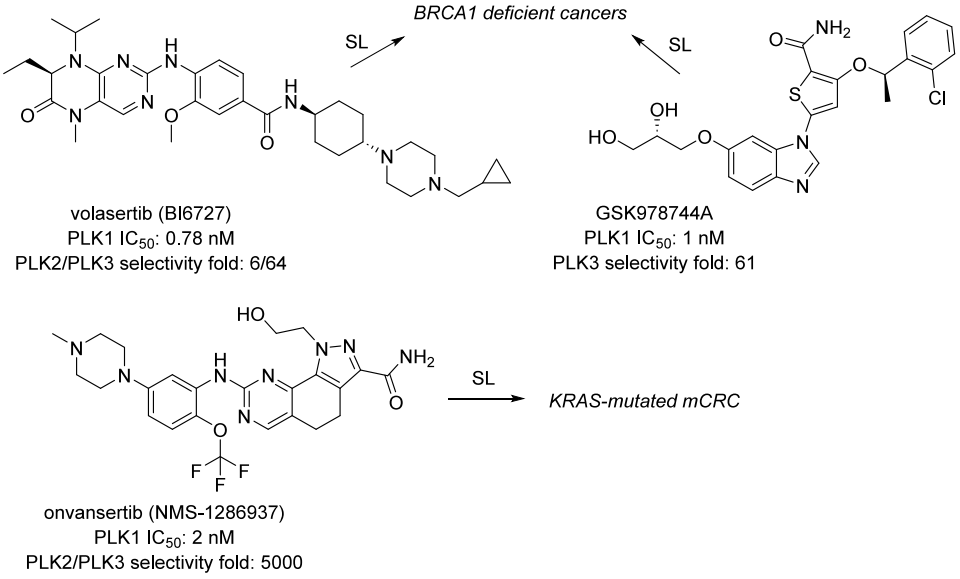

Figure 17. Structures of PLK1i discussed in the text and their applications to achieve SL.

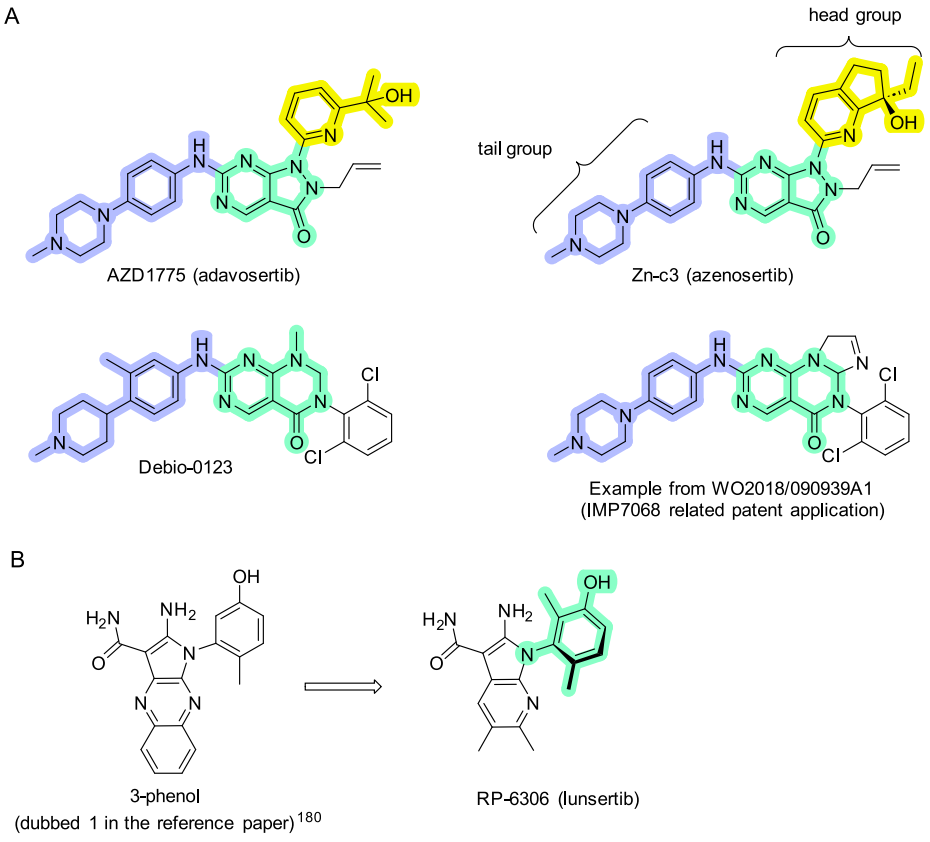

Figure 18. (A) WEE1i discussed in the text. (B) PKMYT1i discussed in the text. Key and common structural features are highlighted in green, violet, and yellow.

2.7.2. PLK1. Polo-like kinase 1 (PLK1) is a serine/threonine nuclear kinase with different functions in controlling genome stability. Its primary role regards the progression of mitosis, maturation of the centrosome, and spindle assembly. Moreover, recent evidence suggests it is involved in DNA damage and the replication stress response (Figure 12).[155] The primary physiological function of PLK1 that has been extensively studied is its involvement in the G2/M checkpoint. Following activation by AURKA, PLK1 facilitates inhibitory phosphorylation on WEE1 and PKMYT1, leading to their degradation and subsequent activation of CDK1, inducing cell cycle arrest (Figure 12).[37] PLK1 overexpression in cancers is often correlated with poor prognosis, identifying PLK1 as a promising drug discovery target. PLK1 consists of two domains, a kinase domain (KD) and a polo-box domain (PBD), which provide druggable targets for the design of PLK1 inhibitors (PLK1i). As summarized in recent reviews, considerable effort has been spent on the development of new

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PLK1i, with at least 10 PLK1i reaching clinical trials.[155,156] However, the promising results of the preclinical studies did not translate into clinical efficacy because of PLK1i limited effects, resistance, and hematological toxicities often arising from the lack of selectivity mostly toward PLK2/PLK3.[157] Worth reporting is the FDA designation in 2013 of breakthrough therapy to volasertib [BI6727 (Figure 17)], an ATP-competitive dihydropteridinone PLK1i (IC_{50} = 0.78 nM, and PLK2/PLK3 selectivity fold = 6/64) developed by Boehringer Ingelheim for the treatment of patients with acute myeloid leukemia (AML). Although early results of volasertib in AML patients seemed promising, the reported clinical efficacy following a phase 3 trial of volasertib, monotherapy or in combination with chemotherapy, failed to reproduce positive results, leading to the discontinuation of its development.[158] In this context, the search for PLK1 SL pair partners could therefore be an efficacious strategy, although it is still in its infancy. Promising SL interactions were reported between PLK1 and *BRCA1*,[159] *TP53*-mutated tumors,[160] *CCND1*-driven,[161] or *KRAS* mutation,[162] suggesting possible clinical application of PLK1i in specific subgroups of patients. Indeed, in 2020, the FDA assigned the fast-track designation to onvansertib [NMS-1286937 (Figure 17)], another potent and more selective ATP-competitive PLK1i (IC_{50} = 3 nM, PLK2/PLK3 selectivity fold = 5000)[163] for patients with *KRAS*-mutated metastatic colorectal cancer (mCRC) (NCT03829410). Recent preclinical research suggests revisiting previously established PLK1i and evaluating their potential in exploiting SL in specific mutated tumors. This approach has gained traction following findings such as the application of volasertib and GSK978744A, a thiophene-based PLK1i developed by GlaxoSmithKline (with an IC_{50} of 1 nM and a PLK3 selectivity fold of 61),[164] in BRCA1-deficient cancer models. This exploration holds promise for potentially effective therapeutic interventions targeting specific genetic vulnerabilities in cancer cells.[159]

2.7.3. WEE1/PKMYT1. Due to p53 mutations, many human cancer cells suffer from a deficient regulation of the G1/S checkpoint and thus rely heavily on G2/M checkpoint regulation.[165] Among the many kinases responsible for orchestrating cellular responses, WEE1 is involved in cell cycle progression (Figure 12). WEE1 functions as a key suppressor of the G2/M checkpoint through phosphorylation of CDK1 and CDK2, causing G2/M arrest and allowing time for DNA repair (Figure 12). Therefore, inhibition of WEE1 leads to premature entry into mitosis and subsequent mitosis catastrophe.[166] WEE1 is strongly expressed in many cancers, and therefore, its inhibition has emerged as an appealing strategy in cancer therapies, especially in SL strategies in p53 mutation cancers and in combination with other DNA-damaging agents or kinase inhibitors (e.g., ATRi).[14,165]

Several different WEE1 inhibitors (WEE1i) have so far been reported in the literature, bearing different scaffolds and developed through various medicinal chemistry approaches.[167,168]

The WEE1i adavosertib [AZD1775 (Figure 18A)], developed by AstraZeneca, has recently been discontinued due to a poor toxicity profile (Table 6).[166] Indeed, the correlation between the activity of AZD1775 and p53 status seemed to be controversial, as it exhibited cytotoxic effects in sarcoma cells regardless of their p53 status.[169] One possible explanation is that AZD1775 was discovered to target multiple kinases and exhibits comparable potency against both WEE1

Table 6. WEE1 and PKMYT1 Inhibitors Discussed in the Text and Their Preclinical/Clinical Evaluation

inhibitor	target	SL gene/protein pair	*in vitro* preclinical data	*in cellulo* preclinical data	*in vivo* efficacy	clinical trial	ref
adavosertib (AZD1775)	WEE1	DDR deficiencies/p53 mutation tumors	WEE1 IC_{50} = 3.9 nM; 13 other kinases ≥90% inhibited at 1 μM	A427 IC_{50} = 78 nM	A427 CDX model, TGI[a] at 80 mg/kg as a single agent	discontinued	174
IMP7068	WEE1	DDR deficiencies/p53 mutation tumors	WEE1/PLK1 selectivity (>435-fold)	ND	ND	NCT04768868	171
ZN-c3	WEE1	DDR deficiencies/p53 mutation tumors	WEE1 IC_{50} = 3.8 nM; five other kinases ≥90% inhibited at 1 μM	A427 IC_{50} = 75 nM	A427 CDX model, TGI at 80 mg/kg as a single agent	NCT04158336	174
Debio0123	WEE1	DDR deficiencies/p53 mutation tumors	WEE1 IC_{50} = 0.8 nM	ND	ND	NCT03968653	182
lunsertib/ RP-6306	PKMYT1	*CCNE1* amplification	PKMYT1 nanoBRET IC_{50} = 2 nM (2050-fold against WEE1)	PKMYT1 cell assay IC_{50} = 0.014 μM	OVCAR3 (TGI 60%, 15−300 ppm/BID)	NCT05147350, NCT05147272, and NCT04855656	180

[a]TGI, tumor growth inhibition.

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and PLK1, suggesting a more intricate mechanism of action than a simple dual SL pair.[170]

IMP7068 (Impact Therapeutics) is another WEE1i that has recently entered clinical trials, specifically in tumors with p53 mutations. The structure of IMP7068 has not been disclosed, but a company-related patent application (WO2018/090939A1) describes a compound belonging to a class of 2,3-dihydropyrimido[4,5-*d*]pyrimidin-4(1*H*)-ones as AZD1775 (Figure 18A). IMP7068 possesses a superior WEE1/PLK1 selectivity (>435-fold) compared to that of AZD1775 with a favorable PK profile in preclinical species (Table 6).[171]

Recently, there has been significant interest in a novel SL approach that exploits vulnerabilities in cancer cells reliant upon *CCNE1* amplification.[172] The *CCNE1* gene encodes cyclin E1, which forms complexes with CDK2 and facilitates cell progression from the G1 phase to the S phase, thereby promoting DNA replication. Increased levels of cyclin E have been observed across various cancers and are correlated with unfavorable outcomes. This overexpression of cyclin E induces DNA replicative stress and triggers DNA repair responses, rendering cells more susceptible to the WEE1 inhibitor AZD1755. Consequently, cyclin E overexpression has emerged as a valuable prognostic biomarker for AZD1775 therapy in tumors exhibiting high cyclin E levels.[173]

The recently reported azenosertib [ZN-c3 (Figure 17A)], developed by Zentalis Pharmaceuticals, shares with AZD1775 both the 1,2-dihydro-3*H*-pyrazolo[3,4-*d*]pyrimidin-3-one central core and the (4-methylpiperazin-1-yl)phenylamino tail group. ZN-c3 showed higher selectivity for WEE1 over other kinases, with efficacy similar to that of the parent AZD1775 *in vivo*. Interestingly, the chiral ethyl moiety in the bicyclic headgroup of ZN-c3 provided an improvement in WEE1 potency, maintaining excellent ADME and PK properties.[174] Its promising preclinical results and selectivity profile allowed ZN-c3 to enter the clinical evaluation with a continuous dosing regimen and achieving promising clinical efficacy in a phase 2 trial (Table 6). Notably, ZN-c3 was shown to be more sensitive in cyclin E1 overexpression in ovarian cancer cells *in vitro* and *in vivo*.[175]

CCNE1 has been exploited to investigate SL with other protein kinases involved in cell cycle control.[176] In particular, the WEE1-like kinase PKMYT1 was identified through a CRISPR-Cas9 screen as a SL partner with cyclin E in cancers overexpressing *CCNE1*.[177] This is particularly relevant also because it was found that a mechanism of acquired AZD1775 resistance is mediated by the upregulation of PKMYT1 kinase.[178] This mechanism of resistance relies on PKMYT1's intrinsic biological role as a negative regulator of CDK1, allowing cells to avoid mitotic catastrophe (Figure 12).[166,179] To address the need of selective inhibitors that would allow for investigation of the pharmacological role of PKMYT1, very recently Repare Therapeutics conducted a focused screen of known kinase inhibitors using a fluorescence polarization-based competitive displacement assay. Among the inhibitors identified as hits, the 3-phenol compound [dubbed **1** in the reference paper (Figure 18B)] appears to be an excellent lead structure, particularly considering the 50−60-fold selectivity observed over the highly homologous enzyme WEE1.[180] SBDD supported by multiple cocrystal structures enabled the optimization of keys properties, including PKMYT1 cell-based potency and kinase activity. The medicinal chemistry campaign took a fortunate turn with the introduction of a dimethylphenol substituent into the azaindole core. The resulting molecules, existing as specific atropoisomers, could be separated and assessed as single enantiomers, one of which with increased potency on PKMYT1. Parallel optimization of ADME properties led to identification of the orally available lunresertib [RP-6306 (Figure 18B)]. RP-6306 showed nanomolar cell-based potency against PKMYT1, excellent selectivity toward a panel of kinases, including WEE1, favorable PK, and ultimately efficacy in an ovarian *CCNE1*-amplified xenograft model (Table 6).[180] The first-in-class clinical candidate RP-6306 reported by Repare Therapeutics is currently being evaluated in phase 1 clinical trials for the treatment of various solid tumors alone or in combination therapies with FOLFIRI (NCT05147350), gemcitabine (NCT05147272), and the ATR selective inhibitor RP-3500 (NCT04855656).[180]

Most recently, Repare Therapeutics has partnered with Debiopharm to explore SL combination of PKMYT1 and WEE1 in cancer. The collaboration aims to explore the synergism between RP-6306 and Debio0123, a potent and brain penetrant WEE1i (Figure 18A and Table 6),[181,182] in a new arm of Repare's ongoing clinical trial NCT04855656.

Overall, the data suggest that WEE1/PKMYT1 inhibition is suitable for SL strategies in cancer therapy. The broad spectrum of activities exerted by the two kinds of kinases across the cell cycle makes them good candidates for a diverse array of therapeutic combinations. However, preclinical and clinical studies should be conducted to identify specific molecular applications in which WEE1/PKMYT1 inhibitors may be recommended.[179]

3. SL BEYOND DDR

While >70% of oncology trials in SL focus on genes within DDR pathways, there are emerging opportunities in SL associated with pathways outside DDR (non-DDR).[183] Particularly of note is the significant presence of non-DDR studies in preclinical research, indicating a growing interest in SL beyond DDR.[183]

Distinguishing between SL pairs inside or outside the DDR context can sometimes be challenging because one gene may be associated with DDR while the other is not. Examples falling into this category have been discussed in the previous section, typically involving the use of specific DDR protein inhibitors in mutated tumors (such as *KRAS*, *MYC*, *RB-1*, or *ARID1A*) or exploiting tumor deficiencies (through gene loss of function or protein inhibition) to afford a HRD phenotype, thereby enhancing sensitivity to PARPi. Notably in this regard, PARPi talazoparib has been shown to be effective in polybromo-associated BAF complex (*PBRM1*)-deficient cancer cells[184] or in combination with BET inhibitors (epigenetic factor).[185]

Nevertheless, there are many examples of SL pairs in which neither gene is a DDR gene, with a reassuring trend of increased application in clinical trials over the past decade.[183] Herein, we describe two exclusive non-DDR SL interaction examples, one between *MTAP* deletion and PRMT5 or MAT2A and the other between SMARCA4 and SMARCA2. Both of these stand out as extensively studied and well-defined SL pairs in terms of medicinal chemistry campaigns, with neither gene belonging to the DDR category.

3.1. MTAP and PRMT5 or MAT2A. The chromosome 9p21 (chr9p21) locus contains the *CDKN2A* gene, a gene encoding critical tumor suppressors and homozygously deleted in ∼15% of all human cancers.[186] Due to the proximity of the

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genes, *CDKN2A* deletion is generally associated with the homozygous deletion of the methylthioadenosine phosphorylase (*MTAP*) gene, which encodes a critical enzyme in the methionine and adenine salvage pathway.[186] MTAP metabolizes 5′-methylthioadenosine (MTA), a byproduct of polyamine synthesis;[186] therefore, deletion of *MTAP* results in the accumulation of MTA and the blockade of the methionine salvage pathway (Figure 19). The deletion of these passenger

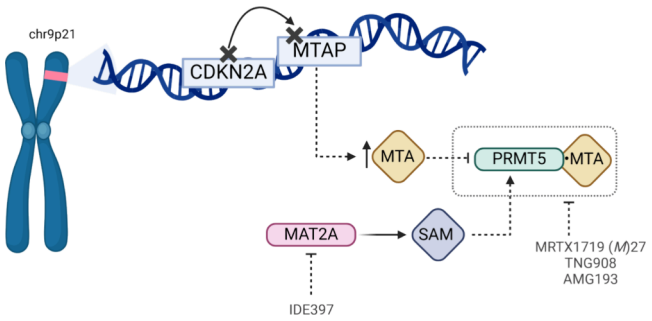

Figure 19. Schematic representation of the SL relationship between *MTAP* depletion and the inhibition of PRMT5, either directly or indirectly via MAT2A inhibition (created with BioRender.com).

genes creates therapeutically tractable cancer vulnerabilities, exploiting what is termed "collateral SL".[187] So far, research around MTAP SL partners has identified, through RNAi screenings, two metabolic enzymes, the protein arginine methyltransferase (PRMT5) and the methionine adenosyl transferase II α (MAT2A) as SL targets with *MTAP* deletion (Figure 19).[186,188,189] PRMT5 belongs to the family of protein arginine methyltransferases (PRMTs) and acts by transferring a methyl group from the *S*-adenosyl-L-methionine (SAM) to coordinate multiple regulatory programs, including cell growth.[190] The role of PRMT5 has been documented in the pathogenesis of different diseases, including cancer.[190] The accumulation of MTA in *MTAP*-deficient cells exerts an inhibitory effect on PRMT5, creating a vulnerability that can disrupt cell viability. Indeed, PRMT5 inhibitors (PRMT5i) leverage this dysregulated metabolic state and in turn induce apoptosis in cancer cells. The reader is advised to refer to the cited reviews for a more thorough summary of other PRMT5i.[191,192] The essential role of PRMT5 for cell survival, as demonstrated by knockout mouse models, explains the issue of toxicity commonly observed with PRMT5i in clinical or preclinical studies. To counteract this toxicity, inhibitors, named MTA-competitive inhibitors, that preferentially bind the target only in the presence of MTA have been designed, to reduce MTA-independent inhibition.[191]

Following this research hypothesis, Mirati Therapeutics recently reported MRTX1719 (*M*)-27, a small molecule that stabilizes the interaction between PRMT5 and MTA. The development of MRTX1719 (*M*)-27 is an example of fragment-based lead discovery (FBLD). An initial fragment screening with SPR identified fragment F1, which underwent fragment growth via SBDD guided by the crystal structure of F1 in complex with PRMT5·MTA, giving MRTX1719 (Figure 20).[193] Subsequently, MRTX1719 (*M*)-27 was identified as the active and stable atropoisomer (Figure 20).[193] MRTX1719 (*M*)-27 is a potent and selective binder of the PRMT5·MTA complex and selectively inhibits PRMT5 in *MTAP*-deleted cells at low nanomolar concentrations, compared to those of *MTAP* wild-type cells.[193] Importantly, MRTX1719 (*M*)-27 avoids the hematopoietic toxicity issues of previous PRMT5i.[193] Given the promising preclinical *in vivo* efficacy results, in which MRTX1719 (*M*)-27 elicited strong antitumor activity across a panel of *MTAP*-del cell line-derived xenograft (CDX) and PDX models, phase 1/2 trials of this drug are proceeding in a subset of cancers, including lung and mesothelioma (Table 7).[193]

Similarly, phase 1/2 clinical trials are currently ongoing for TNG908, a MTA-cooperative PRMT5i developed by Tango Therapeutics (Figure 20).[194] TNG908 is a brain penetrant small molecule, and its oral administration drives dose-dependent, selective activity against *MTAP*-null tumors in xenograft models, including glioblastoma (Table 7).[195]

Recently, Amgen Inc. has been proceeding with phase 1/2 dose escalation studies of AMG193, a second-generation MTA-cooperative PRMT5i, to treat advanced *MTAP*-null solid tumors with an improved toxicity profile (Figure 20 and Table 7).[196] AMG193's structure, currently undisclosed, has been

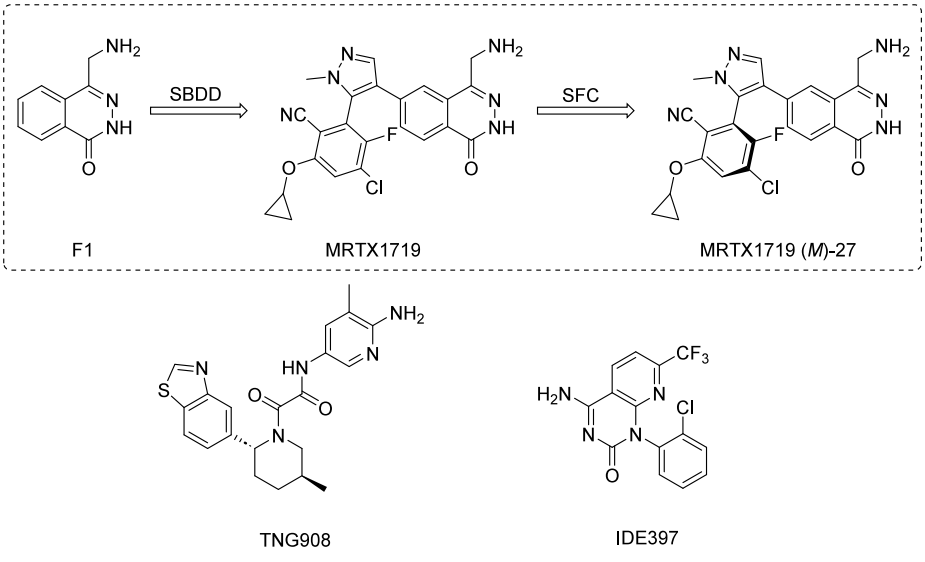

Figure 20. Scheme of the development of MRTX1719 (*M*)-27 and structures of TNG908 and IDE397.

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Table 7. PRMT5/MTA and MAT2A Inhibitors Discussed in the Text and Their Preclinical Evaluation

inhibitor	target	SL gene/protein pair	*in vitro* preclinical data	*in cellulo* preclinical data	*in vivo* efficacy	clinical trial	ref
MRTX1719	PRMT5/MTA	*MTAP*-deleted tumors	K_d (SPR) = 0.14 pM	*MTAP* deletion IC_{50} = 12 nM vs *MTAP*-wt IC_{50} = 890 nM	*MTAP* deletion CDX and PDX models, 86% TGI[a] at 50 mg/kg QD	NCT05245500	193
TNG908	PRMT5/MTA	*MTAP*-deleted tumors (included glioblastoma)	K_d = 0.3 nM	*MTAP* deletion GI_{50} = 0.10 μM vs *MTAP* wild-type selectivity = 15	*MTAP* deletion CDX models: 100% TGI at 60 mg/kg BID	NCT05275478	194
AMG193	PRMT5/MTA	*MTAP*-deleted tumors	ND	ND	ND	NCT05975073 and NCT05094336	196
IDE397	MAT2A	*MTAP*-deleted tumors	ND	ND	efficacy at 5−10 mg/kg in PDX *MTAP* deletion models	NCT05975073 and NCT04794699	198

[a]TGI, tumor growth inhibition.

indicated in the company patent (Patent WO2021/163344A1).

The PRMT5 methyl source, SAM, is produced by the enzyme MAT2A (Figure 19). In parallel, another strategy for the inhibition of PRMT5 has been the inhibition of MAT2A, the enzyme responsible for producing SAM, which is the main methyl source of PRMT5. Indeed, the inhibition of MAT2A results in the reduction of SAM and in turn the starving of PRMT5 of its substrate. With this aim, Ideaya has advanced this approach, developing MAT2A inhibitors (MAT2Ai), such as IDE397 that prevents the production of SAM (Figure 20 and Table 7). However, SAM is a common substrate used broadly by multiple methyltransferases. Thus, there are concerns about the potential off-tissue effects of these drugs. Nevertheless, IDE397 has been reported as the best-in-class MAT2Ai, with a tolerability profile greater than those of existing nontargeted treatments. For this reason, IDE397 is now in phase 1/2 clinical trials, as a monotherapy and in combination with classical chemotherapy in advanced solid tumors harboring *MTAP* deletion (Table 7).[197,198] Promisingly, MAT2Ai could represent an additional class of drugs with which to exploit the paradigm of SL in *MTAP*-deleted cancers. This is even more important if we consider that most SL pairs studied in the clinic so far involve DDR players. If successful, those recent examples would make SL significantly leap forward in anticancer therapy.

3.2. SMARCA2 and SMARCA4. The SWI/SNF chromatin remodeling complexes are highly conserved ATP-dependent multiprotein epigenetic regulators that allow bonding of transcription factors through opening of a tightly packed chromatin structure in the nucleus.[199] The SWI/SNF complex contains two mutually exclusive catalytic ATPase paralog subunits: SMARCA2 (also known as BRM) and SMARCA4 (BRG1).[199] The other subunits are defined by the specific biological role required, resulting in many variations in the final composition.[199] Mutations in SWI/SNF complexes have been linked to cancer growth and resistance; in particular, cancer cells with a loss of SMARCA4 expression via mutation are highly dependent on SMARCA2 for survival, characterized as an SL interaction.[200,201]

These findings have inspired extensive efforts to develop inhibitors targeting the ATPase activity of SMARCA2.[202] As a result, Novartis previously disclosed an optimized small molecule [compound dubbed **14** in the reference paper (Figure 21A)] that demonstrated *in vivo* antitumor efficacy in SMARCA4-deficient cancers. The compound is described as an allosteric inhibitor of both SMARCA2 (IC_{50} < 0.005 μM) and SMARCA4 (IC_{50} < 0.005 μM), binding to a pocket near the ATP site (Glu852). Despite the rather specific *trans* conformation guaranteed by the sulfur−oxygen distance required for activity, the lack of selectivity against the two paralogs hampered its development, resulting in dose-limiting tolerability issues when administered *in vivo*.[203] Nevertheless, another enzymatic inhibitor of both SMARCA4 and SMARCA2, FHD-286 (Figure 21A), has reached the clinic, possibly with the intent of recapitulating the corresponding genetic SL interaction, despite potential toxicity issues.[204] The small molecule developed by Foghorn Therapeutics is described as highly potent and orally available and is now in a phase 1 clinical trial in combination with decitabine or cytarabine in patients with relapsed and/or refractory AML (NCT04891757). Despite the binding mode of FHD-286 not being disclosed, it shares with compound **14** the sulfur−

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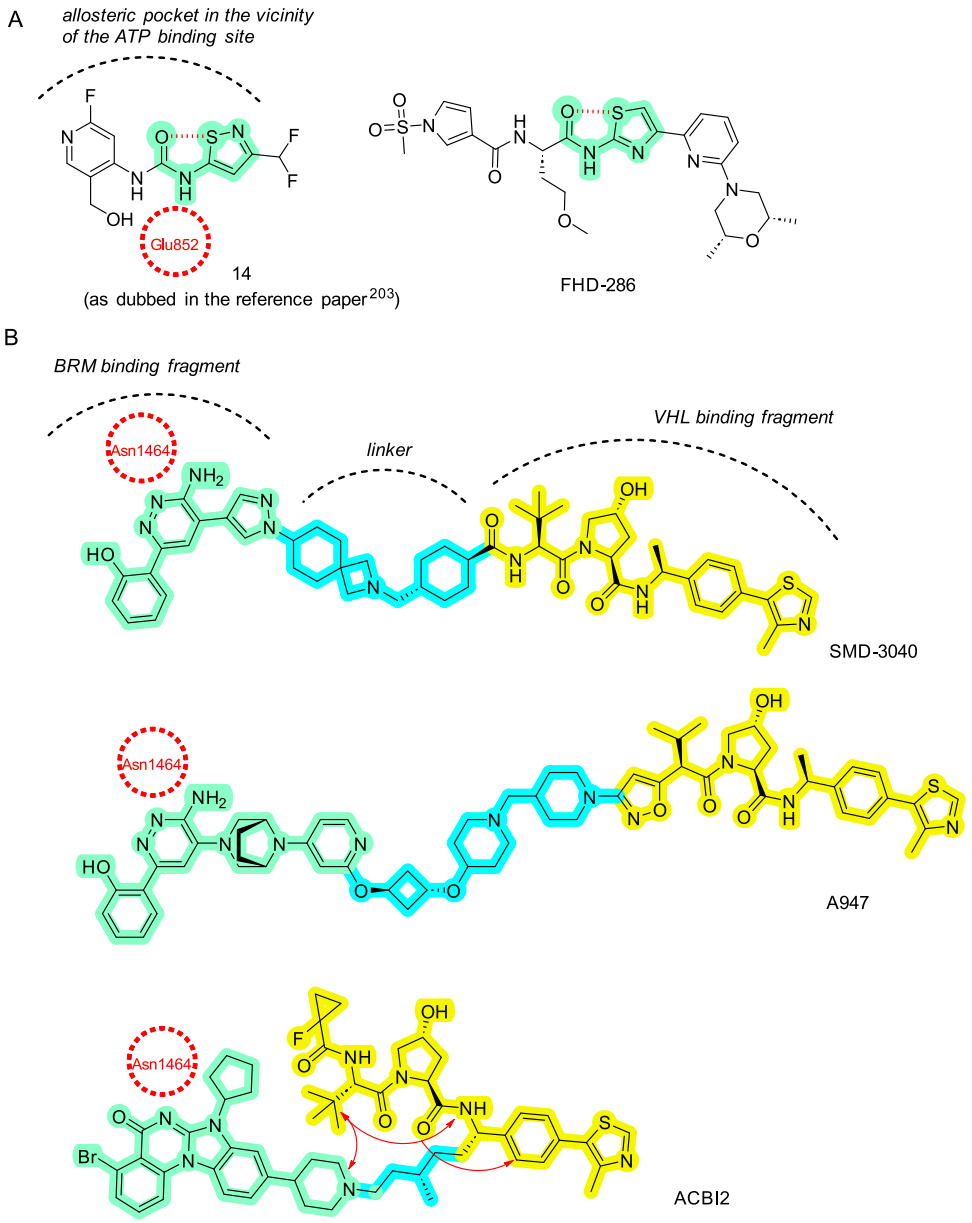

Figure 21. (A) Structures of SMARCA2/4 small molecules inhibitors discussed in the text. (B) Structures of SMARCA2 PROTACs discussed in the text. Selected long-range nuclear Overhauser enhancements in two-dimensional NOESY spectra that guaranteed a specific more compact arrangement for ACBI2 are highlighted as red arrows. Key structural features are highlighted in green, blue, and yellow. Amino acids important for the binding mode are represented via the dashed red circles.

carbonyl motifs, hinting at a similar binding behavior (Figure 21A).

An alternative strategy for targeting SMARCA2 in the treatment of SMARCA4 mutant cancers involves leveraging small molecules that target the SMARCA2 bromodomain region (Asn1464) as a handle for their development into PROTACs, as demonstrated by the growth inhibition of SMARCA2-dependent (SMARCA4 mutant) cancer cells after intracellular knockdown of SMARCA2.[205] This approach capitalizes on the unique advantages of PROTACs over traditional small molecule inhibitors, allowing for selective degradation of a specific protein while sparing its closely related homologues[206] and therefore circumventing the selectivity challenges encountered with inhibitors that target the ATPase activity of the protein.

In 2019, Farnaby et al. introduced ACBI1, the first PROTAC degrader of SWI/SNF complex subunits, SMARCA2 and SMARCA4,[207] swiftly followed by ACBI2 (Figure 21B), a notable achievement as an oral VHL-based PROTAC, a domain typically associated with CRBN-recruiting bifunctional degraders.[208] A factor contributing to the success of this molecule is the more compact arrangement of the branched linker, which afforded a smalle 3D polar surface area (Figure 21B).[208] ACBI2 displayed selective degradation of SMARCA2 over SMARCA4 in *ex vivo* and *in vivo* assays, though it achieved tumor stasis only *in vivo* despite efficient SMARCA2 degradation (for SMARCA2, DC_{50} = 1 nM and D_{max} = 81%; for SMARCA4, DC_{50} = 32 nM and D_{max} = 67%).[208] Subsequent reports unveiled A947 (for SMARCA2, DC_{50} = 0.039 nM and D_{max} = 96%; for SMARCA4, DC_{50} = 1.1 nM and D_{max} = 92%)[209] and SMD-3040 (for SMARCA2, DC_{50} = 12

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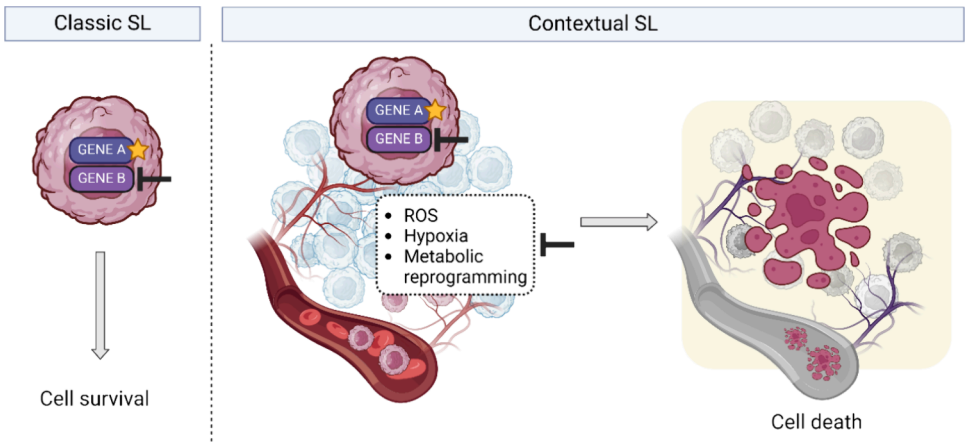

Figure 22. Schematic comparison of classic and contextual SL (created with BioRender.com).

nM and D_{max} = 91%; for SMARCA4, DC_{50} > 1000 nM and D_{max} = 44%),[210] SMARCA2 selective VHL-based PROTAC degraders with notable selectivity for SMARCA2 over SMARCA4, showing stronger efficacy in SMARCA4 mutant cancer cell lines and xenograft models than in SMARCA4 wild-type cells. An interesting expansion of the VHL-SAR for SMD-3040 introduced the morpholine group onto the VHL-methyl benzyl core, resulting in SMD-3236, which features improved DMPK properties and further increased degradation selectivity against SMARCA4 (full structure undisclosed; DC_{50} = 0.5 nM, and D_{max} = 96%; for SMARCA4, DC_{50} > 1000 nM and D_{max} = 41%), thus representing a promising candidate for further advanced preclinical studies possibly overcoming DMPK issues often associated with SMARCA2 degraders.[211]

While potential differences in cellular adaptation as a cause for the often observed tumor stasis rather than regression in *in vivo* models have been acknowledged, more potent PROTACs (D_{max} > 95%)[212] or rational drug combinations are being explored as a means of potentiating the activity of PROTACs against tumor cells. In this regard, MCL1 protein is being evaluated as a synergistic partner for SMARCA2 degradation across various SMARCA4 mutated models, underscoring broader applicability beyond specific cellular contexts.[209]

Nevertheless, the therapeutic rationale of SMARCA2 degraders in SMARCA4 mutant tumors suggests a promising clinical future, as demonstrated by the VHL-based PROTAC undisclosed by Prelude Therapeutics (PRT3789) that recently entered a phase 1 clinical trial (NCT05639751).[213]

4. EXPANDING THE DEFINITION OF SL TO CONTEXTUAL SL

Contextual SL is a concept that has recently been added to the classic genetically based SL. In fact, the effects of classic SL can be weaker or unattainable due to particular tumor conditions, such as the heterogeneity of tumor cells, their metabolic state, and the tumor microenvironment, which ultimately contribute to the onset of resistance to therapy.[214] In this context, contextual SL represents a strategy that aims to target both genetic vulnerabilities and nongenetic abnormal conditions to achieve a more decisive anticancer effect (Figure 22).

Tumor tissue promotes neoangiogenesis to grow new vessels for nutrient demand, in particular glucose to sustain the abnormally hyperactivated glycolysis.[13] However, tumor tissue is not efficiently vascularized, and this creates hypoxic regions. Generally, hypoxia correlates with cancer aggressiveness and resistance to chemotherapy. Interestingly, cancer hypoxia connects to SL as it is linked to DDR deficiency; thus, as they are likely HR-deficient, hypoxic tumor cells can be sensitive to PARPi.[215] As a proof of concept, Chan et al.[215] observed that repair-defective hypoxic cancer cells experience SL with PARPi, in a RKO (human colon carcinoma cell line) xenograft model, which encourages further investigation of hypoxia as an inducer of the HR-defective phenotype.[215]

Moreover, the hyperactivity of the tumor hypoxia-induced pH regulator carbonic anhydrase IX (CAIX) is seen to block the lethal mechanism of ferroptosis by maintaining an alkaline intracellular pH. Taking into account this evidence, Chafe et al.[216] performed a SL CRISPR screen in hypoxic TNBC cells and discovered that CAIX-NFS1/xCT axis are genetic SL pairs that when dysregulated enhance ferroptosis and limit tumor growth.[216] CAIX is a recognized anticancer target and, with CAIX inhibitors, is advancing in the clinic (e.g., SLC-0111).[217] Therefore, this SL strategy could be implemented as a tool for the treatment of hypoxic cancer cells.

Cancer cells generally face complex metabolic reprogramming.[218] This makes them more dependent on a variety of pathways and metabolites, opening up contextual SL applications that could be considered in addition to genetic SL. For instance, in KRas-driven cancer cells, the deprivation of glutamine is SL in combination with the ATP-citrate lyase (ACLY) inhibitor, SB-204990, as reported by Hatipoglu et al.[219] This SL exploits the addiction of KRas-driven cancer cells to glutamine due to their metabolic reprogramming. Moreover, the same authors proved that the combination of SB-204990 with an inhibitor of transaminase, aminooxyacetate, exerted an even stronger SL effect on KRas mutated cells, opening up the application of this relationship as a therapeutic combination.[219]

Increased levels of ROS are a characteristic feature of cancer.[218] While increased ROS concentrations are crucial for cancer development and progression, levels surpassing a cytotoxic threshold lead to cancer cell death.[220] As anticipated above, through metabolic reprogramming, cancer cells can adapt also to high ROS concentrations through mechanisms such as antioxidant production and often rely on tumor-specific dependencies like antioxidant glutathione (GSH) to neutralize ROS.[220] Consequently, restricting GSH availability can selectively induce tumor cell death and contribute to the development of targeted treatment strategies. For example, Ogiwara et al.[221] demonstrated how cancer cells lacking *ARID1A* are particularly sensitive to inhibition of antioxidant

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GSH and the key enzyme in GSH synthesis, glutamate-cysteine ligase synthase catalytic subunit (GCLC). Inhibiting GCLC significantly reduces GSH levels in *ARID1A*-deficient cancer cells, thereby enhancing apoptotic cell death triggered by excess ROS. The susceptibility of glutathione metabolism in *ARID1A*-deficient cancers can thus be considered in the context of genetic SL pairs, as previously discussed in this Perspective.

The examples described above provide a mere hint of this expanding field; a detailed overview of the state of the art is outside the scope of this Perspective. We therefore suggest the recent review by Ge et al.[222] for a more comprehensive analysis. Nevertheless, it is worth emphasizing how careful consideration of tumor heterogeneity could overcome some of the difficulties that SL strategies have had regarding translation from cellular to *in vivo* models and, ultimately, patients.[223] This reflection calls for the attention of the medicinal chemistry community, providing new potential strategies for drug development.

5. FINAL THOUGHTS AND FUTURE PERSPECTIVES

SL therapies have paved the way to personalized therapies, with the hope of treating specific types of cancers with a very rational approach. In this context, PARPi and their use in BRCA1/2 mutation tumors led to a true revolution in anticancer therapy. Nevertheless, the wide use of PARPi in clinics has highlighted PARPi limitations. In particular, the acquired resistance to PARPi represents the most frequent event that threatens the effectiveness of the treatment and its prolonged use. In this context, aid can come from the identification of genes and proteins involved in DDR, to be either predictive biomarkers or SL molecular targets. This helps in predicting the effectiveness of prescribed therapy and establishing patient eligibility. Furthermore, it provides a fresh source of targets for medicinal chemistry campaigns in drug discovery, in particular to investigate the druggability of said targets and lead to the development of newer DDR-targeting agents as monotherapy or in combination therapies. In addition to the examples previously described in this Perspective, worth mentioning is the chemoproteomic approach employed by Vividion Therapeutics. Importantly, it yielded VVD-214, an allosteric covalent inhibitor of the Werner (WRN) DNA helicase with an important SL application in tumors with microsatellite instability (MSI), a common hallmark of cancers with DNA mismatch repair deficiency.[224] Beyond direct DDR inhibition, SL paradigm is also effective through the inhibition of DDR adjacent targets, such as epigenetic regulators and transduction signaling mediators, i.e., tyrosine kinase receptors, achieving an HRD-like status that can confer sensitivity to PARPi. Targeting those indirect players of DDR increases the scope for medicinal chemists and expands the use of SL to more varied cohorts of oncological patients. Knockout screens to catch new SL pairs have focused so far on two-way interactions, silencing one gene across a specific cancer cell line. Newly developed double-knockout CRISPR-Cas9 screening approaches are under development, opening the way to paralogue knockout to provide proteins with redundant activity.[225] Some promising SL interactions between paralogue pairs have been reported, including some tumor suppressors such as the described *SMARCA4*, but also *ARID1A* and *CREBBP*.[226] In some cases, structural similarity can be exploited to achieve a paralogue pair knockout in genetically altered cancer cells. An example is the paralog SL interaction of VRK1 nuclear kinase in glioblastoma deficient of VRK2 reported by Tango Therapeutics,[227] supporting the VRK1 case as a promising drug discovery target using recently developed pyridine-based inhibitors.[228] In other cases, paralogue inhibition discrimination is essential for avoiding toxicities and achieving selectivity. In this regard, the use of PROTACs can afford a selectivity better than that of small molecule inhibitors. As described above for SMARCA2 degraders, the same approach is being applied to other targets, among which the effort of Beactica Therapeutics should be mentioned. They developed YAP/TEAD protein−protein interface degraders, where one of the four TEAD paralogs (TEAD1) is proven to be SL in certain altered genetic settings.[229]

RNAs are increasingly garnering interest from the medicinal chemistry field as a renewed source of clinically relevant targets. Upon determination of multiple roles in the regulation of pathological processes, including cancer, the identification of RNAs will offer the opportunity to leverage SL paradigm using RNA targeting agents, including small molecules and RNA-based therapeutics. For example, an option may be the modulation of the spliceosome to induce SL in splicing factor-altered cancers. Indeed, the dysregulation of the splicing of pre-mRNAs is involved in the development of different malignancies, and frequent somatic mutations are found in components of the spliceosome in myeloid malignancies.[230] For instance, mutations in *SF3B1*, a spliceosome component of mRNA of DDR genes, induce a BRCAness-like phenotype that confers SL to DNA-damaging agents and PARPi. Whether drugs in development that target the SF3B complex can achieve this with minimal toxicity remains to be seen.[231] We therefore expect that the number of SL strategies focused on RNA will increase in the future.

Beyond the genetic-based principle, SL can evolve into a more versatile paradigm ("contextual SL") to tackle tumor-induced conditions and environment, often poorly addressed by the classical combination strategies based on SL pairs. Indeed, cancer complexity has been widely embraced in the past decade, as it significantly undermines the efficacy of current therapeutic strategies.

In this regard, an exciting field that is emerging is the phenomenon of "metabolic SL", defined as an SL relationship not only for targeting gain-of-function and loss-of-function mutations but also for metabolic vulnerabilities.[232] Another definition of "metabolic SL" is the one given by Li et al.[214] that define it as the SL dependency of organelles (e.g., mitochondria) and genes/proteins involved in maintaining the metabolic state of the cell. Caution needs to be used when describing these relationships as SL, because they often fall into the definition of cancer dependencies rather than genetic SL. Nevertheless, metabolic reprogramming is an emerging hallmark of cancer cells, and large-scale metabolic networks can fruitfully open the way to novel combinatorial strategies, expanding the concept of SL.[233,234]

Finally, the growing collection of genomic data from oncological patients, coupled with artificial intelligence and machine learning algorithms, will enable the identification of potential SL targets, within or outside DDR. Together with the advent of groundbreaking medicinal chemistry approaches, the toolbox of SL therapeutics is set to undergo a great expansion. For this reason, we hope that this work will provide inspiration for the medicinal chemistry community to capitalize on those

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innovative approaches and accelerate the development of drug-like compounds to exploit anticancer SL.

AUTHOR INFORMATION

Corresponding Author

Marinella Roberti − *Department of Pharmacy and Biotechnology, University of Bologna, 40126 Bologna, Italy;* orcid.org/0000-0001-9807-2886; Email: marinella.roberti@unibo.it

Authors

Viola Previtali − *Computational & Chemical Biology, Istituto Italiano di Tecnologia, 16163 Genova, Italy*

Greta Bagnolini − *Department of Pharmacy and Biotechnology, University of Bologna, 40126 Bologna, Italy*

Andrea Ciamarone − *Computational & Chemical Biology, Istituto Italiano di Tecnologia, 16163 Genova, Italy; Department of Pharmacy and Biotechnology, University of Bologna, 40126 Bologna, Italy*

Giovanni Ferrandi − *Computational & Chemical Biology, Istituto Italiano di Tecnologia, 16163 Genova, Italy; Department of Pharmacy and Biotechnology, University of Bologna, 40126 Bologna, Italy;* orcid.org/0009-0004-1656-2961

Francesco Rinaldi − *Computational & Chemical Biology, Istituto Italiano di Tecnologia, 16163 Genova, Italy*

Samuel Harry Myers − *Computational & Chemical Biology, Istituto Italiano di Tecnologia, 16163 Genova, Italy*

Andrea Cavalli − *Computational & Chemical Biology, Istituto Italiano di Tecnologia, 16163 Genova, Italy; Department of Pharmacy and Biotechnology, University of Bologna, 40126 Bologna, Italy;* Present Address: CECAM, EPFL, Avenue Forel 3, CH-1015 Lausanne, Switzerland

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.4c00113

Author Contributions

[▽]V.P. and G.B. contributed equally to this work.

Notes

The authors declare no competing financial interest.

Biographies

Viola Previtali completed her doctoral studies in medicinal chemistry at Trinity College Dublin (TCD, Ireland) under the guidance of Prof. Isabel Rozas. After completing her postdoctoral research in medicinal chemistry and chemical biology with Prof. Mads H. Clausen's group at the Technical University of Denmark (DTU, Denmark), she joined the research group of Prof. Cavalli at the Italian Institute of Technology (IIT) in 2019. Currently, her research is focused on designing and developing drugs for synthetic lethality strategies in precision oncology.

Greta Bagnolini received her Ph.D. in biocomputational, biotechnological, pharmaceutical, and pharmacological science at the University of Bologna (Italy) in affiliation with the Italian Institute of Technology (IIT) in 2021. After postdoctoral studies at Duke University (USA) working on RNA targeting, she joined the University of Bologna as a postdoc in medicinal chemistry in 2023. Among the different projects on which she works, the design and synthesis of protein-protein inhibitors for synthetic lethality and RNA targeting by small molecules are the center of her research activity.

Andrea Ciamarone received his M.S. in organic chemistry from the University of Bologna (Italy) in 2021. In the same year, he joined the Italian Institute of Technology (IIT) as Ph.D. student in biotechnological, biocomputational, pharmaceutical, and pharmacological sciences. His research is mainly focused on the design and synthesis of small molecules for cancer treatment.

Giovanni Ferrandi is a Ph.D. student in biotechnological, biocomputational, pharmaceutical, and pharmacological sciences from the University of Bologna in collaboration with the Italian Institute of Technology (IIT). He received his M.S. in 2021 in organic synthesis and bio-organic chemistry from the University of Bologna with a thesis on the synthesis and biological evaluation of organometallic complexes with potential anticancer activity at the University of Brussels ULB. In 2021, he started his Ph.D. working in drug discovery projects mainly focused on the design and synthesis of small molecules to chemically induce synthetic lethality in cancer cells.

Francesco Rinaldi studied Pharmacy at the University of Bologna (Unibo, Italy), carrying out his M.S. work at ETH Zurich (Switzerland). In 2023, he obtained his Ph.D. in biotechnological, biocomputational, pharmaceutical, and pharmacological sciences at Unibo, after developing his three-year work at the Italian Institute of Technology (IIT), where he is currently acting as a postdoc. Since 2021, his work has been supported by the Italian Association for Cancer Research (AIRC), thanks to a three-year fellowship.

Samuel H. Myers received his Ph.D. in medicinal chemistry from the University of Edinburgh (U.K.) in 2016. After postdoctoral work in medicinal chemistry and chemical biology at University College London (UCL, U.K.), in 2018 he joined the Italian Institute of Technology, Genova (Italy), where he worked as a postdoc in medicinal chemistry for 5 years. He has since moved into industry having joined Lo.Li Pharma srl, Roma (Italy), in 2023.

Marinella Roberti is Professor of Medicinal Chemistry at the University of Bologna. Her research field regards the design and the synthesis of biologically active small molecules that can be either innovative lead candidates for drug discovery or valuable chemical tools for studying molecular pathways. The therapeutic field of main interest is cancer. Within the different strategies applied for the design of new molecular entities, the development of protein-protein interaction inhibitors to chemically induce synthetic lethality is the center of her most recent research activities. The scientific research activity is documented by >100 publications in peer-reviewed journals.

Andrea Cavalli is a professor at the University of Bologna and the director of CECAM/EPFL. He was the Director of Computational Sciences and the Vice-Scientific Director of IIT. He combines computational chemistry with drug discovery. He designed algorithms and protocols to accelerate the discovery of new lead candidates. He pioneered molecular dynamics and related methods to (multitarget) drug discovery, creating algorithms for dynamic docking, allosteric modulation, residence time prediction, free energy estimation, etc. He has also led oncogenomics programs with clinics and hospitals that aim to find actionable drug genes through next-generation sequencing, bioinformatics, and machine learning. New targets from omics analyses support new drug discovery programs focusing on synthetic lethality. He has published >300 scientific papers and delivered >150 lectures.

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■ ABBREVIATIONS USED

ACLY, ATP-citrate lyase; APE1, apurinic/apyrimidinic endonuclease; ARID1A, AT-rich interactive domain-containing protein 1A; ATM, ataxia telangiectasia mutated; ATMi, ATM inhibitor; ATR, ataxia telangiectasia and Rad3-related protein; ATRi, ATR inhibitor; ATRIP, ATR-interacting protein; AURKA, Aurora kinase A; BET, bromodomain and extra-terminal domain; BRCA1/2, breast cancer type 1 susceptibility protein; BRD, bromodomain; CCND1, cyclin D1; CDC25A, cell division cycle 25 A; CDKN2A, cyclin-dependent kinase inhibitor 2A; CDX, cell line-derived xenograft; cGAS, cyclic GMP-AMP synthase; DDR, DNA damage response; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; DNA-PKcsi, DNA-PKcs inhibitor; DSB, double-strand break; DUB, deubiquitinase; EGC, epigallocatechin; EMA, European Medicine Agency; FA, Fanconi anemia; FANCD2, Fanconi anemia complementation group 2D; FBLD, fragment-based lead discovery; FLT3-ITD, Fms-like tyrosine receptor kinase 3; GBM, glioblastoma; gBRCAm, germline BRCA mutation; HB, hydrogen bond; HelD, helicase-like domain; HR, homologous recombination; HRD, homologous recombination-deficient; ICL, interstrand cross-links; IMHB, intramolecular hydrogen bond; IR, ionizing radiation; ITD, internal tandem duplication; KD, kinase domain; KRAS, Kirsten rat sarcoma; MAT2A, methionine adenosyltransferase 2A; MAT2Ai, MAT2A inhibitor; MCL1, myeloid cell leukemia-1; MCT, monocarboxylate transporter; MDC1, mediator of DNA damage checkpoint 1; MEK, mitogen-activated protein kinase kinase; METTL16, m6A methyltransferase; miRNA, microRNA; MRN, MRE11-RAD50-NBS1; MSH3, MutS homologue 3; MTD, maximum tolerated dose; MTA, methylthioadenosine; MTAP, methylthioadenosine phosphorylase; mTOR, mammalian target of rapamycin; NFS1, iron−sulfur cluster enzyme; NHEJ, non-homologous end joining; PALB2, partner and localizer of BRCA2; PARG, poly(ADP-ribose) glycohydrolase; PARGi, PARG inhibitors; PARP, poly(ADP-ribose) polymerase; PARPi, poly(ADP-ribose) polymerase inhibitor; PBD, polo-box domain; PBRM1, polybromo-associated BAF complex; PCNA, proliferating cell nuclear antigen; PDX, patient-derived xenograft; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; PIKK, phosphoinositide 3-kinase-related; PLK1i, PLK1 inhibitor; PKMYT1, membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase; PolD, polymerase domain; Polθ, polymerase θ; Polθi, Polθ inhibitor; PRMTs, protein arginine methyltransferases; PRMT5, protein arginine methyltransferase 5; PRMT5i, PRMT5 inhibitor; PROTAC, proteolysis targeting chimera; qHTS, quantitative high-throughput screening; RAD52i, RAD52 inhibitor; Raf, rapidly accelerated fibrosarcoma; RB-1, retinoblastoma protein 1; RPA, replication protein A; SAM, *S*-adenosyl-L-methionine; SF3B, splicing factor 3B; SDL, synthetic dosage lethality; SFC, supercritical fluid chromatography; shRNA, short RNA; siRNA, silencing RNA; SL, synthetic lethality; SMARCA2/4, SWI/SNF-Related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 2/4; SSA, single-strand annealing; SSB, single-stranded break; ssDNA, single-strand DNA; STING, stimulator of interferon genes; TBNC, triple-negative breast cancer; TEAD, transcriptional enhanced associated domain; TLS, translesion synthesis; TMEJ, Polθ-mediated end joining; TMZ, temozolomide; UAF1, USP1-associated factor 1; USP1, ubiquitin-specific protease 1; USP1i, USP1 inhibitor; VHL, Von Hippel−Lindau; VRK, Vaccinia-related kinase; WEE1i, WEE1 inhibitor; xCT, cystine-glutamate antiporter; XRCC1, X-ray repair cross-complementing protein 1; YAP, yes-associated protein 1

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