

Development of Chemotherapy with Cell-Cycle Inhibitors for Adult and Pediatric Cancer Therapy

Christopher C. Mills¹, EA. Kolb², and Valerie B. Sampson²



Abstract

Preclinical and clinical development of agents that inhibit cell-cycle progression have brought an understanding of the feasibility of targeting various cell-cycle regulators in patients with cancer. Small molecule inhibitors targeting key proteins that participate in cell-cycle progression including the cyclin-dependent kinases and checkpoint kinases induce cell-cycle arrest and apoptosis in neoplastic cells. Early phase I studies demonstrate targeted inhibitors can be administered safely in adult and pediatric cancer patients, but these agents generally show limited clinical benefits as single agents. In this review,

we discuss biological mechanisms that support dual combination strategies of cell-cycle inhibition with chemotherapeutic agents that are anticipated to achieve rationally targeted therapies for cancer patients. The rationale for evaluating these combination strategies is that DNA damage renders tumors highly responsive to irreversible cell-cycle arrest therapy. This approach is predicted to generate less intensive therapies and to maximize the efficacy of individual agents against solid tumors and hematologic malignancies. *Cancer Res*; 78(2); 320–5. ©2018 AACR.

Introduction

The normal cell cycle consists of complex pathways that regulate the duplication of all molecules and organelles and their separation into two identical daughter cells. This progresses through four phases: G₁ (gap), S (synthesis), G₂ (gap), and M (mitosis) and is coordinated by cell-cycle regulators that drive chromosome duplication, chromosome segregation, and cytokinesis. The accuracy of this process is controlled by cyclin-dependent kinases (CDK) and regulatory cyclins as well as checkpoint proteins that delay cell-cycle progression to detect errors and preserve genomic integrity. Although the replication machinery is highly conserved in higher eukaryotes, defects in these pathways can arise as a result of somatic mutations in genes encoding key regulatory proteins (1). This, along with defects in checkpoints, can lead to adaptations that result in cancer cell growth. Cell-cycle inhibitors target components that control DNA replication, or coordinate the DNA damage response (DDR) signaling networks, and the mitotic spindle. Inhibition of these processes induces cell-cycle arrest and cell death. Current clinical development demonstrates the safety and tolerability of many single agents in adult and pediatric patients, but with limited efficacy, along with various side effects. Combination strategies of agents that induce cell-cycle arrest with cytotoxic therapies demonstrate greater anti-tumor effects in patients. This article reviews the rationale for dual combination treatments that support the idea that the activity of agents is mechanistically coupled to achieve greater efficacy. This

approach is predicted to develop better regimens to improve survival and reduce toxicities in cancer patients.

Cell-Cycle Progression

Human cells enter the cell cycle at G₁ through various mitogenic stimuli. One such mechanism involves signaling through the Ras/Raf/MAPK pathway that increases cyclin D expression (2). The CDKs are a family of multifunctional enzymes that bind regulatory cyclins and modulate several protein substrates involved in the cell cycle. The retinoblastoma tumor suppressor gene product (Rb) is phosphorylated by cyclin D/CDK4/6 and cyclin E/CDK2, to govern the G₁–S transition (2). The single replication of DNA during the cell cycle is ensured by loading of origin recognizing complexes onto the DNA at replication origins during G₁ phase (3). Initiation and termination of DNA replication occurs during the S-phase in which 23 pairs of chromosomes are duplicated. The central DNA polymerase Pol δ, together with Pol ε and Pol α/primase, synthesize the daughter DNA strands at the eukaryotic replication fork (4). Pol δ and accessory proteins including proliferating cell nuclear antigen (PCNA) edit or proofread nascent DNA strands to monitor the correct incorporation of nucleotides.

The significance of the intra-S-phase checkpoint is the defense against mutagenic DNA synthesis in replicating cells upon genotoxic challenge. When DNA polymerases encounter errors including mutations and lesions, checkpoint regulation of S-phase progression via inhibition of DNA synthesis, occurs. This triggers ataxia telangiectasia and Rad3-related (ATR) activation (5) to prevent extension of mismatched primers or the introduction of mutations and loss of genomic stability. Eukaryotic cells typically begin mitotic events, such as chromosome condensation, when DNA replication is complete. During mitosis, the spindle assembly checkpoint restricts the onset of anaphase to facilitate the attachment of kinetochores to spindle microtubules and the tension setting during metaphase, to ensure proper segregation of chromosomes (6). This is followed by nuclear division and cytokinesis. Together, these checkpoints ensure the coordinated

¹University of Delaware, Newark, Delaware. ²Nemours Center for Cancer and Blood Disorders, Nemours/Alfred I. duPont Hospital for Children, Wilmington, Delaware.

Corresponding Author: Valerie B. Sampson, Nemours/Alfred I. duPont Hospital for Children, 1701 Rockland Road, Wilmington, DE 19803. Phone: 302-651-4832; Fax: 301-651-4827; E-mail: vsampson@nemours.org

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regulation of cell-cycle progression and maintain genetic fidelity between daughter cells.

Current advances in the development of phase-specific cell-cycle drugs that selectively inhibit the activity of ataxia-telangiectasia mutated kinase (ATM), ATR, Wee1, CDKs, checkpoint kinases (CHK), aurora kinases, and polo-like kinases (PLK) for cancer therapy have been comprehensively addressed in recent reviews (7-9). This review focuses on preclinical and clinical combination studies of chemotherapy with selected cell-cycle modulators for the treatment of cancer.

DNA Damaging Effects of Chemotherapy

Standard chemotherapy regimens are used to treat many types of solid tumors and hematologic malignancies. Cytotoxic agents

induce DNA damage in cells and activate the intra-S-phase checkpoint to inhibit DNA synthesis (10), leading to apoptosis or cellular senescence. Chemotherapeutics have diverse actions and cause different forms of DNA damage. Alkylating agents (e.g., temozolomide, dacarbazine cyclophosphamide, ifosfamide) add an alkyl group to DNA binding proteins to prevent the linking of strands in the formation of the DNA double helix. This causes DNA strand breaks, the production of reactive oxygen species and inactivation of DNA polymerases (11). Platinum-based agents (e.g., cisplatin, carboplatin, oxaliplatin) form DNA intrastand and interstrand crosslinks and DNA protein crosslinks that restrict DNA repair (12). Doxorubicin intercalates DNA, and disrupts the DNA repairing function of topoisomerase-II (13), preventing the DNA double helix from being resealed and thereby stopping the process of replication. Nucleoside analogues [e.g.,

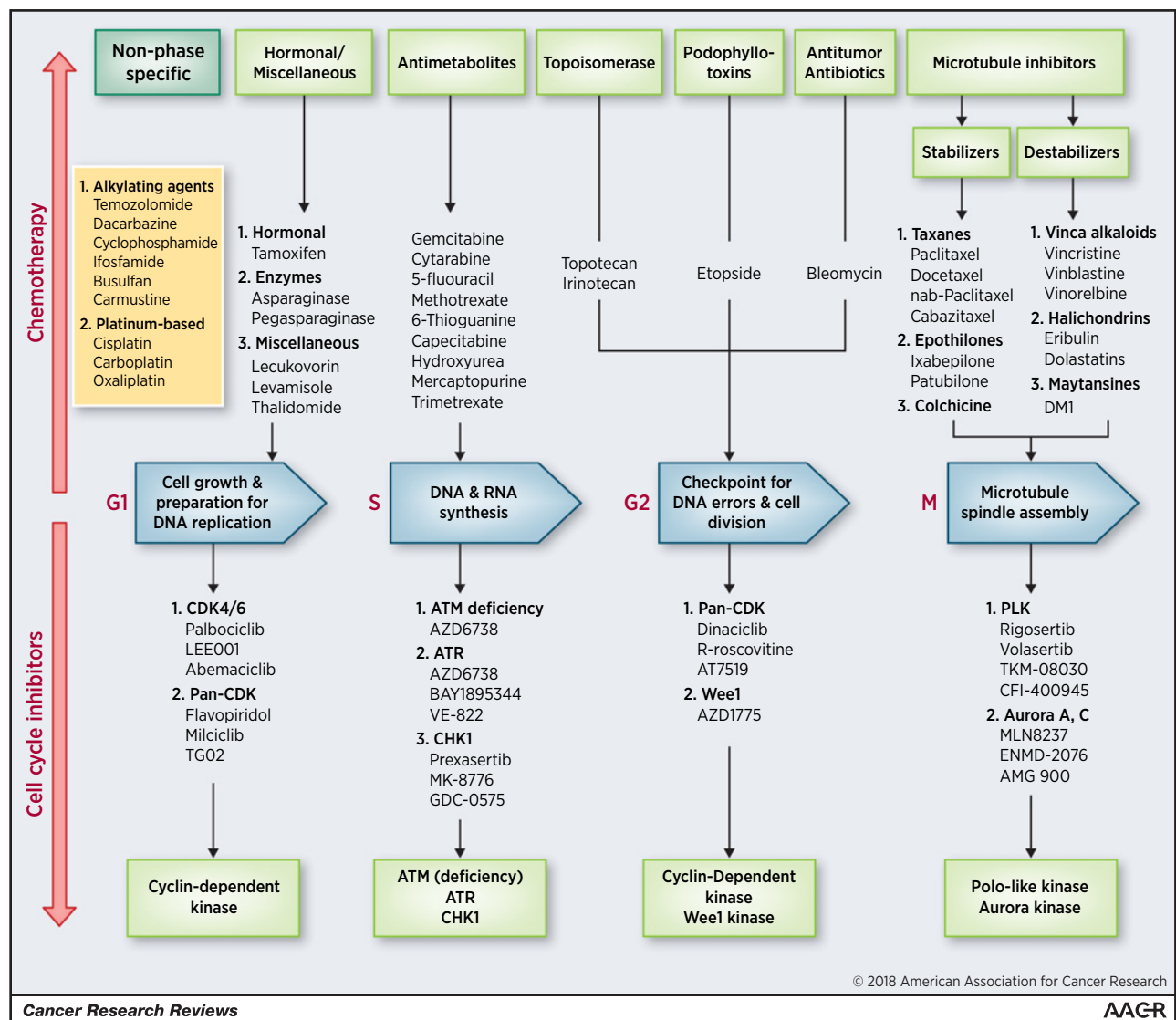


Figure 1.

Therapies that target the cell cycle. Top, the mechanism of action of some cytotoxic chemotherapies (top green boxes) may be cell-cycle independent (e.g., alkylating agents, yellow box) or rely on specific phases in the cell cycle (blue arrows) to induce cell-cycle arrest. Bottom, cell-cycle inhibitors target cell-cycle modulators (bottom green boxes) that act at specific phases to delay cell-cycle progression.

gemcitabine, cytarabine, 5-fluorouracil (5-FU)] are incorporated into DNA strands and act as chain terminators to inhibit DNA polymerase. Anti-metabolites (e.g., methotrexate, 5-FU, mercaptopurine) block essential enzymes necessary for DNA synthesis or also become incorporated into the DNA strand. Microtubule inhibitors (vinca alkaloids and taxane) disrupt microtubule spindle formation during metaphase causing mitotic arrest (Fig. 1).

Although chemotherapy is highly toxic to the cell cycle, cancer cells may adapt a variety of DNA repair mechanisms or rely on faulty checkpoints to reverse or escape chemical damage to DNA. Activation of DNA repair pathways in tumor cells can mediate base excision repair, mismatch repair, or DNA double-strand repair (14). The recruitment of translesion synthesis (TLS) polymerases by ubiquitylated PCNA allows the repair of bulky lesions to compromise many platinum-based-induced lesions (15). Defects at cell-cycle checkpoints may allow some lesions to escape DNA polymerase selectivity and proofreading mechanisms, and become replicated. In addition, some base lesions such as O⁶-methylguanine, which are produced by alkylating agents and reactive oxygen species can introduce mutations during DNA synthesis in replicating cells upon genotoxic stress (16) and in such cases, drugs become ineffective. In another mechanism, the activation of proteins that are necessary for replication initiation following DNA damage (e.g., chromatin licensing and replication factor protein, Cdt1) can lead to DNA re-replication in neoplastic cells (17). This initiates new rounds of replication of chromosome regions in a single cell cycle, resulting in an increase in aneuploid cell populations and gene amplifications. Several studies demonstrate that cells that have been exposed to DNA-damaging agents and topoisomerase blockers exhibit extended G₂ arrest to delay the onset of mitosis, reviewed by ref. 18. This allows more time for DNA repair processes prior to the start of mitosis, which can cause tumor cells to become refractory to chemotherapy. The fate of cancer cells with DNA damage induced by chemicals, radiation, oxidative, and replication stresses may be lethal or may allow cells to acquire mutations and epigenetic modifications. If abnormal cells are not eliminated, they may evolve to a more aggressive and malignant phenotype that is refractory to treatment.

Combining Conventional Chemotherapy with Cell-Cycle Modulators against Adult and Pediatric Cancer

Several adult cancers are characterized by chromosomal variations and abnormalities (19), which allow genetic or pharmacologic approaches for drug development. In contrast, childhood cancers have fewer and rare gene abnormalities including mutations and fusion genes [e.g., histone mutations for diffuse intrinsic pontine glioma (DIPG) (20), EWS-FLI1 for Ewing sarcoma (21), and PAX-FKHR for alveolar rhabdomyosarcoma (22)]. These are generally not relevant against any approved molecularly targeted agents. Other malignancies, e.g., osteosarcoma contain several known and detectable lesions at either the primary or metastatic tumor but the modulation of these using targeted agents have limited measurable effect on disease regression (23). Preclinical therapeutic studies with cell-cycle inhibitors and selected cytotoxic agents that target adaptive processes for cell proliferation (19) as well as oncogenic signaling that affects the cell-cycle machinery (24, 25) have shown antitumor responses.

There are several strategies that potentially synergize selective inhibition of cell-cycle pathways with DNA-damaging chemotherapy. Distinct DNA lesions that promote nucleotide excision repair and stalled replication forks activate ATM, ATR, and checkpoint kinase 1 and 2 (CHK1 and CHK2) to halt cell-cycle progression at G₁ and prevent cells from replicating the DNA damage (26). Thus, compounds targeting these checkpoint kinases will abrogate G₁-phase arrest leading to the unscheduled entry of cells with damaged chromosomes into mitosis. This can potentially trigger a delay at the spindle assembly checkpoint to promote mitotic catastrophe and cell death. Cancer cells demonstrating a faulty G₁ checkpoint due to loss of p53 (27), pRb (2), or p21 (28) may show greater sensitivity to G₂-M checkpoint inhibition, e.g., through targeting Wee1 kinase and Aurora kinase A and B. This allows cells with damaged DNA to undergo aberrant mitosis, leading to apoptosis (29). Other studies show that DNA re-replication induced by agents that stabilize Cdt1 (30) or chromosome missegregation induced by Aurora B kinase (31) and microtubule inhibitors (25) can lead to the development of aneuploidy. Synergizing these inhibitors with aneuploidy-producing chemotherapeutics may amplify this form of DNA damage to eliminate aneuploid cells. Here, we focus on selected combinations of small molecule inhibitors that target the progression of the cell cycle and conventional chemotherapeutics, which are being investigated in children and adult patients.

Palbociclib with chemotherapeutic agents

Currently, the most promising cell-cycle CDK4/6 inhibitors undergoing clinical investigation are palbociclib, ribociclib, and abemaciclib. Palbociclib and ribociclib are approved for the treatment of hormone receptor-positive and HER2-negative breast cancer in combination with aromatase inhibition (32). Abemaciclib received breakthrough therapy designation status and is also being developed for breast cancer. The primary mechanism of action of these agents is inhibition of phosphorylation of the Rb tumor suppressor, induction of G₁-phase arrest, and delaying DNA replication in tumor cells. Palbociclib shows high sensitivity in Rb-positive human cancer cells, including breast cancer (33) and neuroblastoma (34). Cancer cells with G₁ checkpoint defects (e.g., TP53 mutations), as well as cells that progress to S-phase, will be more susceptible to an S-phase selective drug. Gemcitabine is selectively cytotoxic to cells in S-phase. Synergy between palbociclib and gemcitabine is supported by pharmacokinetic modeling (35) but was antagonistic in human pancreatic cancer cell lines (36). Characterizing the mechanistic coupling of specific regimens that regulate CDK4/6 activity with genotoxic compounds will be critical to maximize cytotoxic effects. McClendon and colleagues (37) also found that palbociclib diminished sensitivity of triple negative breast cancer cells to doxorubicin as both agents act through G₁-phase Rb-mediated cell-cycle control. Together, these studies illustrate that this approach might achieve best cytotoxicity in cancer cells with an intact G₁ checkpoint. In addition, cells arrested at the early G₁ checkpoint can be selectively targeted by cytotoxic agents later in the cell cycle.

Palbociclib combined with bortezomib and dexamethasone was assessed in five patients with multiple myeloma (38). Two dose-limiting toxicities were reported [grade 4 thrombocytopenia and grade 3 neutropenia ($n = 1$) and grade ≥ 3 metabolic acidosis ($n = 1$)]. The most common adverse effect was thrombocytopenia, and other adverse effects were grade ≤ 3 , consistent

with known safety profiles of palbociclib and bortezomib. Safety, tolerability, and efficacy of palbociclib in combination with cisplatin or carboplatin will be assessed among patients with advanced/metastatic solid tumors (NCT02897375). Eligible patients for this study include head and neck cancer, pancreatic cancer, breast cancer, lung cancer, sarcoma, ovarian cancer, colorectal cancer, and bladder cancer. Secondary outcomes include antitumor efficacy of the combinations as well as pharmacokinetic profiles of cisplatin and carboplatin. Combinations of palbociclib and either sorafenib, decitabine, or dexamethasone will be evaluated in pediatric patients with hematopoietic/lymphoid cancer, acute myeloid leukemia (AML), and acute lymphoblastic leukemia (ALL; NCT03132454). Palbociclib will be administered at a concentration of 125 mg/kg for cycle 1 on days 1 to 21 with one selected chemotherapeutic agent for cycle 2 on days 1 to 28 to establish the maximum tolerated dose (MTD). Loss of the p16 inhibitory protein (encoded by *INK4A* or *CDKN2A*) is being investigated as a biomarker for combinations of palbociclib and gemcitabine for recurrent or metastatic squamous cell carcinoma of the head and neck (NCT03088059).

AZD1775 with chemotherapeutic agents

AZD1775 is a G₂ checkpoint Wee1 kinase inhibitor that impairs homologous recombination by indirectly inhibiting breast cancer type 2 (BRCA2). AZD1775 is considered to be genotoxic as a result of its mechanism of action and blocks mitotic entry. This agent demonstrates single-agent activity in sarcoma cell lines (39) and is sensitive to SETD2 mutations (the sole methyltransferase for H3K36me₃) in kidney cancer (40, 41). These mutations are also found in pediatric high-grade gliomas (42). *In vitro* studies show AZD1775 sensitizes AML and T-ALL cells to genotoxic chemotherapies including the anti-metabolite cytarabine (43) that inhibits DNA synthesis during the S-phase of the cell cycle. AZD1775 showed strong synergy with gemcitabine in a patient-derived osteosarcoma xenograft mouse model (39).

The phase II study of AZD1775 with carboplatin (non-cell-cycle phase-specific chemotherapy; Fig. 1) in adult patients with TP53-mutated ovarian cancer refractory to first-line therapy (44) and phase I evaluation in combination with gemcitabine, cisplatin, or carboplatin in patients with advanced solid tumors (45) demonstrated appreciable drug safety and tolerability in chemotherapy combinations at doses associated with target inhibition. In the clinical setting, AZD1775 enhanced carboplatin efficacy in TP53-mutated tumors. Common adverse events for combination regimens include fatigue, nausea and vomiting, diarrhea, and hematologic toxicity. Two pediatric phase I trials are underway to study side effects and best dose of combinations of AZD1775 with irinotecan against advanced solid tumors (NCT02095132) and with local radiation therapy in treating newly diagnosed diffuse intrinsic pontine glioma (DIPG; NCT01922076).

Pevonedistat with decitabine

Pevonedistat (MLN4924) is a first-in-class inhibitor of the NEDD8-activating enzyme (NAE; ref. 30). NAE is required for activation of cullin ligase that participates in ubiquitination and degradation of short-lived cell-cycle proteins. MLN4924 causes stabilization of the critical DNA replication licensing factor Cdt1 and re-replication (30). The ability of MLN4924 to increase the aneuploid cell population and induce apoptosis of breast cancer cells represents a unique DNA-targeted biological strategy (46). Because chromosome instability (CIN) and aneuploidy are

tolerated by solid tumors and can arise following chemotherapy, exacerbation of DNA re-replication that drives apoptosis has the potential to eliminate the production of aneuploid cells.

The investigation of pevonedistat pharmacokinetics and pharmacodynamics in a cohort of 23 adult patients with AML and myelodysplastic syndromes (MDS) achieved two complete responses and two partial responses (47). The MTD for two tested dosing schedules were 59 and 83 mg/m², and hepatotoxicity and multiorgan failure were dose limiting, respectively. A phase I study of MLN4924 and decitabine in combination therapy will be assessed in recurrent childhood AML (NCT03009240). Decitabine acts as a nucleic acid synthesis inhibitor. Patients will receive pevonedistat IV on days 1, 3, and 5 and decitabine on days 1 to 5 and 8 to 12. Treatment repeats will be administered every 28 days up to 24 courses in the absence of disease progression or unexpected toxicity. This dual therapy is anticipated to restrict DNA synthesis as well as induce irreparable aneuploidy to improve AML patient outcome.

Prexasertib with chemotherapy

Prexasertib (LY2606368) is a small molecule inhibitor of checkpoint kinase 1 (CHK1) and is being evaluated in clinical trials against several types of cancer including squamous cell carcinoma, head and neck cancer, KRAS or BRAF-mutated colorectal cancer, or non-small cell lung cancer (NSCLC), AML, and pediatric solid tumors. The ATR-CHK1 and ATM-CHK2 signaling pathways regulate DNA replication and DNA damage response. Inactivation of the ATR-CHK1 pathway sensitizes cancer cells to radiotherapy and chemotherapy (26).

Preclinical investigations with prexasertib demonstrated *in vitro* and *in vivo* sensitivity against neuroblastoma cell lines and xenograft tumors (48) and NSCLC in combination with cisplatin or olaparib (49). Prexasertib is being evaluated in combination with gemcitabine in adults with advanced solid tumors (NCT01341457) as well as combined with EGFR inhibitor cetuximab, or cisplatin (NCT02124148). Clinical studies will focus on solid tumors exhibiting replicative stress [including MYC amplification, *CCNE1* (encodes cyclin E protein) amplification, Rb loss, or a *FBXW7* (F-Box and WD repeat domain containing 7)]. The Children's Oncology Group is conducting a phase I study to assess the safety and tolerability of single-agent prexasertib in pediatric patients with recurrent/refractory solid tumors (NCT02808650). The objective will be to evaluate prexasertib in combination strategies with cytotoxic therapies for future pediatric studies.

Conclusion

Newer generation cell-cycle checkpoint inhibitors are being evaluated for children and adults with cancer. Combination therapy provides promise for improved efficacy of conventional treatment alone. In order for novel therapies that target cell-cycle progression to advance, clinical studies analyzing combination regimens will be required in the drug development path. An important question will be whether agents are additive, synergistic, or antagonistic when combined. Drug interaction studies evaluated in *in vitro* preclinical models can provide insight in the mechanistic coupling of agents and guide the design of minimally toxic and effective therapies.

Preclinical studies could support the development of these inhibitors by identifying somatic mutations, chromosomal

abnormalities, and changes in protein expression patterns in regulatory pathways that predict response. Importantly, members of the Cip/Kip family, p21 and p27 (46) that inhibit cyclin-CDK complexes are indicated to function in dual roles as both tumor suppressors and oncogenes (50, 51). Dysregulation of the PKB/Akt pathway inactivates the tumor-suppressor functions of these proteins but activates oncogenic signaling (51). In addition, the high rate and mutation pattern of TP53 and Rb in many primary solid tumors and tumor heterogeneity can impact the activity of these agents (27). Studies of how agents affect key regulatory pathways should lead to a better understanding of the efficacy of treatments.

Another challenging aspect of these combination therapies is to prevent the development of adaptive mechanisms that reduce treatment efficacy. For some drugs, the mechanism of action may be independent of a specific cell-cycle phase, but for others toxicity may be phase-specific (Fig. 1). In such cases, combinations, dose, and schedule of inhibitors will be important considerations for the selection of agents. This supports the application of multiple approaches based on different tumor char-

acteristics. Finally, emerging studies show cell-cycle inhibitors can render immunotherapies highly effective against various types of cancer (52–54). This reinforces the expectation that combination strategies with cell-cycle inhibitors hold the promise to improve outcomes further in patients with refractory, recurrent and rare diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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