Achieving precision death with cell cycle inhibitors that target DNA replication and repair

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Running Title: Selective Targeting of Checkpoint Kinase Inhibitors

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Abstract:

All cancers are characterized by defects in the systems that ensure strict control of the cell cycle in normal tissues. The consequent excess tissue growth can be countered by drugs that halt cell division and, indeed, the majority of chemotherapeutics developed during the last century work by disrupting processes essential for the cell cycle, particularly DNA synthesis, DNA replication, and chromatid segregation. In certain contexts the efficacy of these classes of drugs can be impressive but, because they indiscriminately block the cell cycle of all actively dividing cells, their side effects severely constrain the dose and duration with which they can be administered, allowing both normal and malignant cells to escape complete growth arrest. Recent progress in understanding how cancers lose control of the cell cycle, coupled with comprehensive genomic profiling of human tumor biopsies, has shown that many cancers have mutations affecting various regulators and checkpoints that impinge on the core cell cycle machinery. These defects introduce unique vulnerabilities that can be exploited by a next generation of drugs that promise improved therapeutic windows in patients whose tumors bear particular genomic aberrations, permitting increased dose intensity and efficacy. These developments, coupled with the success of new drugs targeting cell cycle regulators, have led to a resurgence of interest in cell cycle inhibitors. This review in particular focuses on the newer strategies which may facilitate better therapeutic targeting of drugs that inhibit the various components that safeguard the fidelity of the fundamental processes of DNA replication and repair.

Introduction:

Tissue growth and homeostasis in multicellular organisms necessitates a variety of systems to regulate and coordinate cell division. When these systems fail, unabated and uncoordinated growth can lead to cancer. The core cell cycle machinery required for chromosome duplication, chromosome segregation, and cytokinesis can be distinguished from cell cycle regulators that restrain the core engine via cell cycle...
checkpoints. Many traditional cancer therapies act on the core machinery, either at S phase, preventing nucleotide synthesis and DNA replication (antimetabolites, DNA damaging agents, and radiotherapy) or at mitosis (microtubule inhibitors). Since they act on processes essential for all dividing cells, these classical therapies also affect normal proliferating tissues, and toxicities seemed inevitable for cell cycle inhibitors. Hence, in the 1990s, cancer drug discovery shifted focus from cell cycle targets to mitogenic and oncogenic signaling pathways. Targeting somatic mutations in genes encoding members of these signaling pathways offered a better therapeutic window in the cancers where they were defective. This shift in focus was vindicated by success of oncogene targeting drugs such as trastuzumab, imatinib, erlotinib, vemurafenib, and crizotinib (1).

During this same period, Next-Generation Sequencing ushered in efforts to comprehensively catalog all genomic aberrations that can drive cancer growth (2). Interestingly, these studies identified key cell cycle checkpoint genes that are commonly mutated in cancer. In particular, checkpoints at three stages are targeted by mutations in established cancer genes: (i) G1-phase checkpoints including the restriction point, (ii) S-phase checkpoints including DNA damage and origin firing checkpoints, and (iii) M-phase, particularly the spindle assembly checkpoint.

Recent years have seen the introduction of inhibitors that specifically target kinases that facilitate DNA replication and repair such as inhibitors of ataxia telangiectasia mutated kinase (ATM), ataxia telangiectasia and Rad3-related kinase (ATR), WEE1, checkpoint kinase 1 (CHK1), and checkpoint kinase 2 (CHK2), collectively referred to herein as checkpoint kinase inhibitors. Because their mechanisms of action inhibit crucial components of DNA damage checkpoints, the initial interest in this class of inhibitors was their use in combination with standard cytotoxic (genotoxic) therapies as chemopotentiators. However, preclinical investigations led to a next generation strategy that relies on the identification of underlying genetic or expression alterations that can potentially enhance the susceptibility of cancer cells to checkpoint kinase inhibitor monotherapy or to combination therapy with targeted drugs that do not induce generalized DNA damage. Consequently, this article reviews the underlying genetic drivers in tumors that facilitate better selective targeting of these agents with a focus on checkpoint kinase inhibitors that are currently in development (Table 1).

ATM, ATR, WEE1, CHK1, and CHK2 are crucial components of DNA damage response (DDR) signaling networks responsible for contributing either to the detection and repair of damage or for coordinating DNA replication (3-6). These DDR checkpoint kinases function collectively to maintain genomic integrity by providing cells time to repair any DNA damage prior to replication or mitosis, and to initiate an apoptotic response if the damage is beyond repair. ATR and ATM act as sensors of single-strand and double-strand breaks (SSB and DSBs), respectively, which activate CHK1 and CHK2, by phosphorylation (Figure 1). CHK1 and CHK2 suppress cell cycle progression through down-regulation of the CDC25A and CDC25C phosphatases, which promote progression by removing inhibitory phosphates on CDK2 and CDK1, respectively (7). WEE1 also prevents cell cycle progression via inhibitory phosphorylation of CDK1 and CDK2 (8-11). The ATR-CHK1 response is also initiated in response to single-strand DNA generated by replication fork stalling (12,13).

**Strategies for Clinical Development: Past and Present**
The initial preclinical studies evaluating these inhibitors clearly demonstrated that the chemical inhibition of checkpoint kinases enhances the activity of cytotoxic therapies that damage DNA through diverse mechanisms. Good reviews have been published recently describing this biology and therefore it will not be summarized here (7,10,12-16). As a result of these data, clinical trials with early CHK1 inhibitors focused on the chemopotentiation of cytotoxic drugs. Although Phase 1 trials demonstrated proof-of-concept that CHK1 inhibitors could be safely combined with chemotherapy (17-25), Phase 2 studies failed to meet their primary endpoints (26,27). Early CHK1 inhibitors were not successful for a variety of reasons including pharmacokinetic properties, unacceptable toxicities, and business considerations.

However, newer inhibitors, which are more diverse and include not only CHK1 inhibitors, but also WEE1, ATR, and ATM inhibitors, continue to test this hypothesis with a variety of genotoxic agents and as monotherapies (Table 1). Although no Phase 2 studies have been published with checkpoint inhibitors as monotherapy, evidence of efficacy has been observed in Phase 1. A Phase 1 evaluation of AZD1775 as a monotherapy enrolled 24 patients, 9 of which had a BRCA1/2 mutation. Two patients with a BRCA1 mutation [squamous cell cancer (SCC) of the base of the tongue and papillary serous ovarian cancer] had partial responses (28). Prexasertib, a CHK1 inhibitor, demonstrated objective responses in patients with SCC of the anus and head and neck cancer in a Phase 1 study with multiple expansion cohorts (29). These preliminary results suggest that the newer inhibitors may be more successful than initial CHK1 inhibitors, which were only developed as chemopotentiators.

In addition, these data suggest the optimal use of these inhibitors may require the identification of contexts where tumor-specific vulnerabilities are leveraged to achieve selective cytotoxicity in cancer cells. TP53 is a critical mediator of the DDR which is activated through phosphorylation by CHK2 and ATM, and participates in a parallel pathway to ATR, CHK1, and WEE1 (Figure 1) (7). For this reason, cancer cells that have lost either TP53 or ATM may show greater reliance on ATR, CHK1, and WEE1 for efficient repair and hence greater sensitivity to treatments that inhibit these kinases (30-35). This hypothesis has been tested in a Phase 2 trial where patients with TP53-mutated ovarian cancer who were refractory or resistant (<3 months) to first-line therapy received AZD1775 in combination with carboplatin. The combination demonstrated manageable toxicity and in 21 evaluable patients, the objective response rate was 43%. Median progression-free and overall survival times were 5.3 months and 12.6 months, respectively. In addition to TP53 mutations, patients with an objective response had alterations in BRCA1, KRAS, MYC, or CCNE (Cyclin E) (36). Similarly, in a randomized Phase 2 trial in platinum-sensitive, TP53 mutant ovarian cancer, the WEE1 inhibitor AZD1775 combined with paclitaxel and carboplatin met the primary and secondary endpoints and significantly prolonged progression-free survival compared to the combination of paclitaxel and carboplatin (37). In a Phase 1 multi-arm combination study assessing AZD1775 with either gemcitabine, cisplatin, or carboplatin, 176 patients were evaluable for response. Responses were observed in patients with ovarian cancer (n=7), melanoma (n=3), breast cancer (n=2), head and neck cancer (n=3), colorectal cancer (n=1), and SCC of the skin (n=1). Patients with TP53 mutations (4/19, 23%) had a partial response compared to 4/33 (12%) of patients with TP53 wild-type tumors. (38)
Ongoing studies with AZD1775 continue to evaluate this hypothesis in TP53-mutated gastric cancer (with paclitaxel, NCT02448329), TP53 or KRAS mutated solid tumors (with olaparib, NCT02576444), and TP53 mutated (with either MYC amplification or CDKN2A mutation) relapsed small cell lung cancer (SCLC) (monotherapy, NCT02688907). Similarly, the ATR inhibitor AZD6738 has an ongoing clinical study (NCT02264678) in ATM low/deficient NSCLC (with carboplatin) or gastric cancer (with olaparib). These studies will further characterize the impact of TP53 mutations and ATM deficiency not only in the context of cytotoxic chemotherapy, but also with targeted agents and monotherapy.

The emerging clinical data with TP53 mutations are one example of potential synthetic lethality (SL), where functional alterations in one pathway lead to enhanced sensitivity to inhibition of another pathway. This is the strategy guiding the use of PARP inhibitors as therapies for BRCA1- or BRCA2-deficient breast or ovarian cancers (39-42). Leveraging SL for checkpoint kinase inhibitors requires knowledge about functional alterations in cancer cells that make them more vulnerable to the inhibition of these kinases than normal cells. Although TP53 mutations are the most well characterized example of SL with the checkpoint inhibitors, the concept of replication stress (RS) is emerging as a potential SL mechanism (43,44). RS can arise in any situation that leads to inappropriate replication origin licensing or firing (45). This results in stalled replication forks and DNA breaks if the stalled forks are not adequately resolved (46-48). In cancer cells, oncogene-driven events can contribute to higher degrees of RS by promoting growth to a point that strains the replicative capacity of the cell (45,49). As a result, inhibitors of checkpoint kinases may induce SL with tumors that have high RS since all of these kinases contribute to sensing and reducing RS (Figure 2). In particular, WEE1 and CHK1 play complimentary roles in restricting the initiation of replication origins by inhibiting CDK2, which when activated, promotes replication (6,50,51). Thus, treatment with an inhibitor of CHK1 or WEE1 augments ongoing RS by effectively neutralizing one of the mechanisms available for suppressing replication origin firing, resulting in more stalling and DNA breaks. Since both CHK1 and WEE1 are important signaling components in the response to DNA damage, damage that results from the additional RS is potentially exacerbated because it cannot be repaired effectively. Likewise, ATR plays an important role as a sensor for RS since it is recruited to regions of ssDNA that become exposed by replication fork stalling. Subsequently ATR is responsible for activating CHK1, which in turn suppresses replication as indicated above (15). An ongoing challenge is to identify tumors that have reached near-critical levels of RS and are likely the most susceptible to treatment with checkpoint kinase inhibitors.

Validation of therapeutic strategies to exploit RS has recently emerged from several studies (52-63). For example, a SL relationship was described between oncogene-induced RS by MYC activation and ATR loss in lymphomas (49). Specifically in the context of ATR-deficient cells, MYC expression induced higher RS and apoptosis and contributed to greater sensitivity to ATR and CHK1 inhibitors. Analogous observations were derived from studies in neuroblastoma models wherein sensitivity to CHK1 inhibition was correlated with MYCN expression (52). Studies in other model systems for hematologic malignancies such as MYC-driven diffuse large B-cell lymphoma, cyclin D1-driven mantle cell lymphomas, T-cell acute lymphoblastic leukemia, and acute myeloid leukemia suggest that oncogene-induced RS in these cancers may make them particularly vulnerable to treatment with checkpoint kinase inhibitors (53-55,58,61,63). The hypothesis that MYC may drive RS and increase sensitivity to checkpoint kinase inhibitors is being
tested in several ongoing clinical trials, including a biomarker-directed study with AZD1775 and durvalumab in muscle invasive bladder cancer. To be eligible patients must have mutations in CDKN2A (p16) or RB1 genes and/or amplification of CCNE1, MYC, MYCL, or MYCN genes, all of which presumably contribute to heightened RS (Figure 2). Other agents are focusing on SCLC, a tumor associated with RS and MYC amplifications: AZD1775 (NCT02593019) and prexasertib (NCT02735980) are being assessed as monotherapy, while VX970 is being evaluated with topotecan (NCT02487095).

Another opportunity for SL may be inhibition of ATR in the context of ATM deficiency. In preclinical models of hematologic malignancies, cells that lack ATM (e.g. due to 11q deletions) or have defects in TP53 were particularly vulnerable to the ATR inhibitor AZD6738. Treatment with AZD6738 increased replication initiation and fork stalling, resulting in RS and DNA damage which likely contributed to selective cytotoxicity in cells with TP53 and/or ATM defects (64). In parallel, in a Phase 1 trial with the ATR inhibitor VX970 and carboplatin, a patient with colorectal cancer and ATM loss achieved a complete response (65). This hypothesis is being further tested in a trial of AZD6738 in patients with relapsed/refractory B cell malignancies with prospectively identified 11q-deleted or ATM-deficient relapsed/refractory CLL (NCT01955668).

In addition to neuroblastomas as mentioned above, other pediatric cancers, particularly sarcomas, may represent another opportunity for exploiting oncogenic drivers of RS as demonstrated by recent studies in models for Ewing’s sarcoma (ES) wherein the oncogenic drivers were fusion proteins unique to this tumor (EWS/FLI1 or EWS/ERG) (59). In these studies, ES cell lines were more sensitive to inhibition of ATR compared to sarcoma cell lines which lacked the EWS fusions, and ATR inhibitor monotherapy resulted in near-complete to complete growth inhibition of human ES xenografts. Related studies with the CHK1 inhibitor prexasertib (56) showed that monotherapy resulted in complete regressions in xenograft models for desmoplastic small round cell tumor, which also expresses an oncogenic EWS fusion protein known as EWS-WT1, or alveolar rhabdomyosarcoma, a malignancy driven by another unique oncogenic fusion protein (66,67). These nonclinical studies with prexasertib have led to an interest in exploring prexasertib in pediatric solid tumors (NCT02808650). Additionally, WEE1 was identified as a target in medulloblastoma, the most common pediatric malignant brain tumor (68). AZD1175 induced DNA damage and suppressed the growth of medulloblastoma cells both in vitro and in vivo (68), potentially by exploiting MYC-induced RS (69). AZD1775 has also demonstrated in vitro activity in rhabdomyosarcoma as monotherapy and in combination with conventional therapies (70) and improves the efficacy of radiation in mouse models of pediatric high-grade glioma (71). A Phase 1 study of AZD1775 and radiation in children with diffuse intrinsic pontine gliomas is ongoing (NCT01922076). AZD1775 is also being assessed in the European Proof-of-Concept Therapeutic Stratification Trial of Molecular Anomalies in Relapsed or Refractory Tumors (ESMART) Trial. In this basket study, molecular profiling of pediatric tumors is used to direct patients to individual treatment arms, including a combination of AZD1775 and carboplatin (NCT02813135).

Strategies exploiting RS also include combining inhibitors targeting different checkpoint kinases. Accordingly, RNA interference (RNAi) studies exploring SL with the CHK1 inhibitor PF-00477736 identified WEE1 as the most significant hit (72). Additionally, synergistic growth inhibition was observed with PF-00477736 and AZD1775 in cell lines and in a xenograft model for ovarian cancer (72). Synergy
was also observed with AZD1775 and the CHK1 inhibitor MK-8776 (73). Analogous studies showed that treatment of cancer cells in culture with the CHK1 inhibitor AZD7762 and the ATR inhibitor VE-821 elevated RS leading to replication catastrophe and death by apoptosis. The combination was associated with a significant inhibition of tumor growth and an increase in overall survival in mice bearing human NCI-H460 NSCLC xenografts when compared to the monotherapy treatments (74).

In addition to RS, deficiencies in homologous recombination repair (HRR) may also contribute to greater sensitivity to checkpoint kinase inhibition since homologous recombination deficiencies (HRDs) reduce the efficiency of repair of DSBs that result from RS generated by these inhibitors (Figure 2). Deficiencies in the Fanconi anemia (FA) genes (FANC), like defects in BRCA1/2, also disrupt HRR (75), and studies with the CHK1 inhibitor Gö6976 showed that FA-deficient cell lines were highly sensitive to Gö6976 and to interfering-RNA (RNAi) silencing of CHK1 mRNA expression when compared to isogenic lines that were FA-proficient (76). An RNAi based screen which targeted 230 DNA repair genes identified many FANC genes as SL with Gö6976 treatment, including FANCA, FANCC, FANCD1, FANCD2, FANCE, FANCF, and FANCG.

A similar approach using siRNA to target 240 DNA replication and repair genes explored SL with ATR inhibitors (57). These studies uncovered that loss of expression of ATR pathway genes had the strongest association for SL including losses of ATR itself, ATRIP, RPA, and CHK1. Since the ATR pathway is crucial for responding to RS, the observations from this study as well as others provide support regarding the therapeutic benefits that may be derived from the combined inhibition of targets within this pathway to elevate RS further (62,72-74). In addition, the results confirm the benefit that may be attained through monotherapy by selective targeting of tumors with preexisting mutations in this pathway. Of note, these studies demonstrated that loss of excision repair cross-complementation group 1 protein (ERCC1), which functions in the repair of bulky DNA adducts, DSBs, and interstrand crosslinks (77), was a significant SL interaction with ATR and CHK1 inhibition. Similar SL interactions were observed between ATR inhibition and loss of another repair gene known as XRCC1 which plays a role base excision repair (78) and with ATM deficiency (34).

These studies suggest that therapeutic strategies aimed at disrupting HRR may facilitate SL with checkpoint kinase inhibitors. In this regard, CHK1 in addition to its role in limiting RS is also required for HRR by facilitating the essential recruitment of RAD51 to sites of repair (79). Thus, CHK1 inhibition would be expected to promote further deficiencies in HRR in addition to exacerbating RS. In support of this concept, augmented growth inhibition by the PARP inhibitor, olaparib, was observed upon the RNAi silencing of genes encoding CHK1, CHK2, ATR, ATM, and RPA1 as well as genes encoding proteins that contribute to HRR such as RAD51, NBS1, FANCA, FANCD2, and FANC (40). Additionally, studies in breast carcinoma cell lines showed that the combination of PARP inhibitors (olaparib, veliparib, NU1025, or rucaparib) with CHK1 inhibitors (UCN-01, AZD7762, or LY2603618) increased DNA damage and cytotoxicity as compared to the single-agent treatments (80). More recent studies have shown in BRCA1/2 mutated high-grade serous ovarian cancer (HGSOC) models that combination therapy with olaparib and either MK8776 (CHK1) or AZD6738 (ATR) acted synergistically to decrease survival and colony formation in vitro and inhibit tumor xenograft growth in vivo (81). Clinical trials evaluating PARP inhibition with AZD1775 (NCT02511795), AZD0156 (NCT02588105), VX970 (NCT02723864), or AZD6738
(NCT02264678) are ongoing. In addition, a study with AZD1775 and olaparib (OLAPCO) is a molecularly directed trial that requires mutations in TP53 or KRAS (NCT02576444).

As outlined above, the optimal context for a checkpoint kinase inhibitor might be tumors that have both high RS and HRD. Of notable interest are ovarian cancers, particularly HGSOCs, since approximately half harbor mutations in genes that modulate HRR, including mutations that impact BRCA1 and BRCA2 (39,82). Additionally, nearly all HGSOCs have mutations in TP53 (83). Mutations in ATM and ATR have been observed in 2% of HGSOCs, and 5% have mutations in genes of the FA DNA repair pathway (39). Mutually exclusive with mutations in BRCA1/2, CCNE1 is amplified in 15-20% of HGSOCs (82,84). RB1 loss is also observed in about 15% of these cancers (83). Since cyclin E is required for activation of CDK2, its overexpression induces RS and DNA damage that activates HRR and may increase sensitivity to single-agent CHK1, ATR1, and/or WEE1 inhibition (85). Likewise loss of RB1 can contribute to RS by promoting the progression of cells into S-phase. Therefore, HGSOCs present a provocative context for therapy with the checkpoint kinase inhibitors by providing an opportunity to leverage the dual presence of higher RS and HRD (28,86). Accordingly, AZD1775, prexasertib, LY2880070, VX970, VX803, and AZD0156 are being assessed in a variety of subsets of ovarian cancer (Table 1), and it may be notable that AZD1775 has demonstrated clinical activity in both platinum sensitive and platinum resistant ovarian cancer (36,37).

As our understanding of SL interactions and the underlying mechanisms of the checkpoint kinase inhibitors grows, the optimal context may shift from a focus on a particular histology such as ovarian cancer, to the genetic attributes of the tumor, regardless of histology. This approach is already being implemented in multiple clinical trials. In a prexasertib basket trial (NCT02873975), patients whose tumors show alterations consistent with RS (MYC amplification, CCNE1 amplification, Rb loss, or FBXW7 mutation) or HRD (BRCA1, BRCA2, PALB2, RAD51C, RAD51D, ATR, ATM, CHK2, or the FA pathway genes) are eligible. Similarly, AZD1775 is being assessed in the Molecular Profiling-Based Targeted Therapy (MPACT) trial which assigns patients with solid tumors to a treatment regimen based on their mutation/amplification category. One of these arms assesses AZD1775 in combination with carboplatin (NCT01827384). One obvious goal from these types of studies, besides improved efficacy, is to identify tumor-specific biomarkers which can be used to predict response to monotherapy or combination therapy with one or more checkpoint kinase inhibitors. Preclinical studies indicate that alterations inducing RS or HRD contribute to greater sensitivity to these inhibitors. However, the crucial challenge from a clinical perspective is going beyond just identifying which of these alterations indicate the presence of RS or HRD to identifying situations where tumors are at near-unstainable levels of RS or HRD and therefore are most susceptible to interventions that further augment RS or compromise HRR.

The identification of expression or genetic alterations in a small subset of stand-alone biomarkers for predicting response is certainly desirable and may be achievable in certain cancers, but for other cancers, additional methodologies may be required to adequately measure the extent to which RS and HRD are near-catastrophic. However, these challenges should not dampen our enthusiasm for the ongoing clinical trials with the various checkpoint kinase inhibitors since some of these trials may provide patient data that will allow us to validate and refine our biomarker hypotheses. The preclinical studies have provided a solid foundation, leading to the concept of leveraging RS and HRD to achieve SL
in human tumors. The clinical data will allow us to return to the bench to explore additional concepts that will further enhance the therapeutic benefit of the checkpoint kinase inhibitors.

**Figure Legends:**

**Figure 1:** ATR, ATM, CHK1, CHK2, and WEE1 inhibit cell cycle progression into S-phase and mitosis following DNA damage. This regulation occurs ultimately through the control of the cyclin-dependent kinases (CDKs) that facilitate entry into the S and M phases of the cell cycle, namely CDK2 and CDK1, respectively. The activity of CDK1 and CDK2 are controlled by phosphorylation as well as by the partnership of these kinases with their regulatory cyclin subunits whereby activation of CDK2 requires association with cyclin E and CDK1 activation requires association with either cyclins A or B. Phosphorylation of either CDK1 or CDK2 is regulated in part by the opposing actions of the WEE1 kinase, and the CDC25A/CDC25C phosphatases. ATR and ATM are sensors of single-strand breaks (SSBs) and double-strand breaks (DSBs), respectively, which activate CHK1 and CHK2, respectively, by phosphorylation. The inhibition of cell cycle progression by activated (phosphorylated) CHK1 and CHK2 results from the inhibition of CDK1 and CDK2 which is mediated by preventing the removal of inhibitory phosphates placed on CDK1 and CDK2 by WEE1. CHK1 and CHK2 prevent the removal of these phosphates by suppressing the CDC25A and CDC25C phosphatases through phosphorylation whereby this phosphorylation facilitates proteolytic degradation of CDC25A and the sequestration of CDC25C by 14-3-3. p53 (TP53) is activated upon DNA damage by phosphorylation by ATM and CHK2 and serves as an additional pathway to inhibit cell cycle progression by promoting transcription of p21 which in turn inhibits the kinase activity of the cyclinE-CDK2 complex.

**Figure 2:** The ATR-CHK1 pathway reduces replication stress (RS). The kinase function of CDK2 requires activation by association with cyclin E. When activated by cyclin E, CDK2 facilitates the progression of cells from G1 into S-phase and subsequently promotes replication origin firing. ATR and CHK1 suppress replication origin firing in part by suppressing the activity of CDK2 through regulation of downstream effectors that coordinate the phosphorylation of CDK2. Specifically, the presence of long-stretches of single-stranded DNA, which can occur in situations where the DNA polymerases lag behind the unwinding activity of the helicases, trigger ATR which is recruited by a complex of proteins including replication protein A (RPA), topoisomerase-binding protein 1 (TopBP1), and ATR interacting protein (ATRIP). ATR then activates CHK1 (through phosphorylation) which subsequently through phosphorylation of the CDC25A phosphatase negatively regulates CDK2 by preventing the removal of inhibitory phosphates by this phosphatase. The actions of CDC25A are also opposed by WEE1 which contributes to the inhibition of CDK2 by catalyzing the inhibitory phosphates that are removed by CDC25A. As shown at the bottom half of the figure, RS can result from endogenous or exogenous DNA damage as well as through the action of growth-promoting oncogenes such as MYC which serve to drive progression into S-phase. In addition RS can also arise through other alterations that disrupt control at the G1-S interface such as changes that lead to enhanced expression of cyclin E or loss of inhibition of CDK2. Alterations which may enhance cyclin E expression or CDK2 activity possibly include 1) losses in FBXW7, which facilities proteasomal degradation of cyclin E or 2) alterations which disrupt regulation of the primary restriction point controlling progression from G1 into S including activation of CDK4, CDK6, D-type cyclins, or loss of RB1 which serve to activate E2F thereby promoting the transcription of the
genes that encode cyclin E and CDK2 as well as other gene products which promote DNA replication. Notably as indicated by the red X’s, the inhibition or loss of function of ATR, CHK1, or WEE1 can elevate ongoing RS by effectively removing the control over CDK2. The result of this inhibition or loss is replication fork collapse with eventual DNA double-stranded breaks leading to the activation of DNA damage and repair responses such as homologous recombination repair.
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<td><strong>CRC:</strong> KRAS/BRAF mutations</td>
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<td><strong>NSCLC:</strong> KRAS/BRAF mutations</td>
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<td><strong>Ovarian:</strong> BRCA1/2m</td>
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<td>CRC, ovarian, TNBC</td>
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<td>ATR VX970</td>
<td>II</td>
<td><strong>Monotherapy</strong></td>
<td>HNSCC (HPV-), NSCLC, ovarian, SCLC, urothelial,</td>
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Table 1: Cell Cycle Kinase Inhibitors Currently in Development
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<th>Drug</th>
<th>Phase</th>
<th>Monotherapy</th>
<th>Chemotherapy combinations</th>
<th>Targeted combinations</th>
<th>Biomarker Focused</th>
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<td>carboplatin, Paclitaxel</td>
<td>olaparib</td>
<td>B-cell malignancies, gastric/GEJ, HNSCC, NSCLC</td>
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<td>0156</td>
<td>Monotherapy</td>
<td>irinotecan, olaparib</td>
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Source: www.clinicaltrials.gov

Abbreviations: AML = acute myeloid leukemia, amp = amplification, CRC = colorectal cancer, GEJ = gastro esophageal junction, HPV = human papilloma virus, HNSCC: squamous cell head and neck cancer, m= mutation, MDS = myelodysplastic syndrome, NSCLC: non-small cell lung cancer, SCC = squamous cell cancer SCLC: small-cell lung cancer, TNBC: triple negative breast cancer,
References:


Figure 1:

- **Endogenous or exogenous events**

  **Double-strand break**
  - ATM → P
  - CHK2 → P
  - CDC25A → P
  - CDK2 → P
  - Cyclin E → P
  - p53 → P
  - p21 → P

  **Single-strand break**
  - ATR → P
  - CHK1 → P
  - CDC25C → P
  - CDK1 → P
  - Cyclin A/B → P

- **Proteolysis**
  - CHK2

- **Sequestration**
  - CHK1

- **Cyclin phases**
  - G1 → S → G2 → M

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Figure 2:

- Oncogenes
- Enhanced cyclin E or CDK2 expression/activity
- Loss of regulation of G1 → S progression
- Loss of function of ATR, CHK1, or WEE1

Replication stress → DSBs → Homologous recombination repair
Clinical Cancer Research

Achieving precision death with cell cycle inhibitors that target DNA replication and repair

Aimee Bence Lin, Samuel C. McNeely and Richard P Beckmann

Clin Cancer Res Published OnlineFirst March 22, 2017.

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