Preclinical Development

CK2 Inhibitor CX-4945 Suppresses DNA Repair Response Triggered by DNA-Targeted Anticancer Drugs and Augments Efficacy: Mechanistic Rationale for Drug Combination Therapy

Adam Siddiqui-Jain1, Joshua Bliesath1, Diwata Macalino1, Mayuko Omori1, Nanni Huser1, Nicole Streiner1, Caroline B. Ho1, Kenna Anderes1, Chris Proffitt1, Sean E. O’Brien1, John K. C. Lim1, Daniel D. Von Hoff2, David M. Ryckman1, William G. Rice1, and Denis Drygin1

Abstract

Drug combination therapies are commonly used for the treatment of cancers to increase therapeutic efficacy, reduce toxicity, and decrease the incidence of drug resistance. Although drug combination therapies were originally devised primarily by empirical methods, the increased understanding of drug mechanisms and the pathways they modulate provides a unique opportunity to design combinations that are based on mechanistic rationale. We have identified protein kinase CK2 as a promising therapeutic target for combination therapy, because CK2 regulates not just one but many oncogenic pathways and processes that play important roles in drug resistance, including DNA repair, epidermal growth factor receptor signaling, PI3K/AKT/mTOR signaling, Hsp90 machinery activity, hypoxia, and interleukin-6 expression. In this article, we show that CX-4945, a clinical stage selective small molecule inhibitor of CK2, blocks the DNA repair response induced by gemcitabine and cisplatin and synergizes with these agents in models of ovarian cancer. Mechanistic studies show that the enhanced activity is a result of inactivation of XRCC1 and MDC1, two mediator/adaptor proteins that are essential for DNA repair and that require phosphorylation by CK2 for their function. These data position CK2 as a valid pharmacologic target for intelligent drug combinations and support the evaluation of CX-4945 in combination with gemcitabine and platinum-based chemotherapeutics in the clinical setting.

Introduction

DNA-targeted chemotherapeutics represent fundamental components of modern cancer therapy and are currently prescribed for multiple indications. These agents target the DNA of tumor cells and can activate one or more DNA repair response mechanisms, potentially leading to the development of drug resistance (1). An emerging strategy to boost the effectiveness of these drugs is to combine them with inhibitors of corresponding DNA repair response mechanisms (1, 2). Methods currently subject to clinical investigation include inhibitors of the checkpoint regulators CHK1 and CHK2, inhibitors of the direct repair enzyme MGMT and inhibitors of PARP, a key mediator of base excision repair (1). Because different classes of DNA-targeted anticancer drugs can trigger distinct DNA repair response pathways, it would be advantageous to identify and inhibit a target protein to disrupt multiple DNA repair response pathways simultaneously. Protein kinase CK2, a constitutively active serine/threonine kinase that is widely overexpressed in human cancers (3, 4), has recently emerged as a key participant in DNA repair response, being essential for the surveillance and repair of both single and double strand breaks (6). Among the best characterized of the CK2-dependent DNA repair response substrates...
are the mediator/adaptor proteins XRCC1 and MDC1, which are essential components of the single strand break (SSB) and double strand break (DSB) repair machinery, respectively (Fig. 1).

XRCC1 is a key mediator of SSB repair, which includes both the base excision repair and nucleotide excision repair (NER) mechanisms (12). It exists in a tight complex with DNA ligase IIIα, which serves to religate broken DNA single strands following the processing of damaged bases/nucleotides. XRCC1 is continuously phosphorylated by CK2 (13), an event that is required for its interaction with 2 proteins, aprataxin and polynucleotide kinase (PNK), which participate in DNA end-processing before ligation (Fig. 1). In addition, phosphorylation of XRCC1 by CK2 may also be required to maintain stability of the XRCC1–ligase IIIα complex itself (5, 14, 15).

MDC1 is a key mediator of homologous recombination (HR) DSB repair and is the principle binding partner of γ-H2AX that is anchored to DNA at sites of DSBs (16–18). Once bound to γ-H2AX, MDC1 recruits a key multiprotein complex "MRN" that is required for DSB repair signaling. This interaction is dependent on the phosphorylation of MDC1 at multiple acidophilic sites by CK2 (16–18). The functional consequences of MRN complex binding to MDC1 include activation of both the S-phase and G2-M checkpoints following the treatment of cells with ionizing radiation (19–21) and amplification of ATM signaling (Fig. 1; ref. 22). In addition, the phosphorylation of MDC1 by CK2 also promotes binding of aprataxin, implicating MDC1/CK2 in direct HR repair (6).

Following the discovery of CX-4945, a first-in-class clinical stage inhibitor of CK2 (23, 24), we sought to investigate whether the pharmacologic targeting of CK2-dependent DNA repair response functions could potentiate the ability of DNA-targeted chemotherapeutic agents to kill tumor cells. For this purpose we selected cisplatin (or carboplatin) and gemcitabine in the context of ovarian cancer, in which both drugs are used in combination chemotherapy (25). The primary mechanism involved in the repair of DNA–platinum adducts is NER, in which XRR1/ligase IIIα complex plays a prominent role in religating the broken DNA strand (Fig. 1; refs. 12, 26). During DNA replication, unrepaired platinum adducts can stall the replication fork, triggering ATR-mediated repair. Likewise, the triphosphorylated form of gemcitabine is incorporated into DNA during DNA replication, also causing replication forks to stall. Failure to repair either cisplatin- or gemcitabine-induced stalled replication forks leads to replication fork collapse (DSBs), triggering the accumulation of CK2-phosphorylated MDC1 (bound to γ-H2AX), amplified ATM signaling, and repair by HR (Fig. 1; refs. 27–30). Thus, inhibition of CK2 could potentially synergize with cisplatin by disrupting XRC1-dependent NER (SSB repair) and with cisplatin and gemcitabine by disrupting MDC1-mediated HR repair (DSB repair).

Materials and Methods

Materials

CX-4945 [5-(3-chlorophenylamino)benzo[c][2,6]naphthyridine-8-carboxylic acid] was synthesized by Cylene Pharmaceuticals (>99% pure by high-performance liquid chromatography assay). Cisplatin, gemcitabine, and carboplatin were purchased from Sigma-Aldrich (Fig. 2).

Cell culture

A2780 and SKOV-3 human ovarian carcinoma cell lines were purchased from American Tissue Culture Collection (ATCC) and used within 6 months with weekly monitoring for growth rates and morphology consistency. ATCC...

Figure 1. Reported roles of CK2 in DNA repair mechanisms relevant to cisplatin and gemcitabine.
carries out authentication testing of the cell lines using DNA profiling and cytogenetic analysis. Cell lines were cultured according to the suppliers’ recommendations.

**Immunoprecipitation**
Untreated or CX-4945–treated (10 μmol/L, 24 hours) cells were washed twice with PBS and lysed in 1X radio-immunoprecipitation assay Buffer supplemented with phenylmethylsulfonylfluoride and Protease Inhibitor Set 1 (EMD Chemicals). Samples were sonicated on ice and centrifuged at 14,000 × g for 10 minutes at 4°C. Protein was quantitated using the Bradford protein assay. Ten micrograms of anti-MDC1 antibody (Bethyl Laboratories) was added to the cell lysate (2 mg) and 100 μL of 20% protein A suspension. The immunoprecipitation reactions were rotated overnight at 4°C. The samples were centrifuged and the resulting pellets were washed 3 times with 500 μL cold-cell lysis buffer. Samples were analyzed by Western blot.

**Comet assay**
SKOV-3 cells (1 × 10⁵ cells) were combined with molten LMAgarose (Trevigen) at a ratio of 1:10 (v/v) and were immediately pipetted onto CometSlide (Trevigen). Slides were incubated at 4°C in the dark for 10 minutes, then immersed in prechilled Lysis Buffer and incubated at 4°C for 30 minutes. Slides were immersed in Alkaline Unwinding Solution, pH > 13 (200 mmol/L NaOH, 1 mmol/L EDTA) for 20 minutes at room temperature in the dark. Electrophoresis was done at 21 V for 30 minutes using Alkaline Electrophoresis solution (200 mmol/L NaOH, 1 mmol/L EDTA). The slides were washed twice in water for 5 minutes and once in 70% EtOH for 5 minutes, then dried overnight and visualized by microscopy. Under these conditions the formation of “comet tail” is indicative of SSDs, DBSs, and/or active excision repair of DNA cross-links.

**Cell-cycle analysis**
Cell cycle was analyzed by flow cytometry as described elsewhere (23). Data were analyzed with FlowJo software (TreeStar, Inc.).

**Cell viability assay**
A2780 and SKOV-3 cells were seeded at a density of 750 and 1,000 cells per well, respectively, and treated 24 hours later according to the preaddition or postaddition schedule (Preaddition schedule: 4-hour treatment with CX-4945 followed by 24-hour treatment with cisplatin/gemcitabine. Postaddition schedule: 24-hour treatment with cisplatin/gemcitabine followed 8-hour treatment with CX-4945). Following completion of the treatment schedule, the media was replaced and the cells were cultured at 37°C for the remainder of 96 hours. Cell viability was analyzed by CyQuant assay (Invitrogen).

**In vivo efficacy studies**
Female immunocompromised mice CrTac:Ncr-Foxn1nu (5–7 weeks old) were obtained from Taconic Farms. Animals were maintained under clean room conditions in sterile filter top cages. Animals received sterile rodent chow and water ad libitum. All procedures were conducted in accordance with the Institute for Laboratory Animal Research Guide: The Care and Use of Laboratory Animals. Xenografts were initiated by subcutaneous injection of A2780 cells into the right hind flank region of each mouse. When tumors reached a designated volume of 100 to 150 mm³, animals were randomized and divided into groups of 9 to 10 mice per group. CX-4945 was administered by oral gavage twice daily at 75 or 100 mg/kg as indicated. Cisplatin (5 mg/kg) and carboplatin (75 mg/kg) were administered via intraperitoneal injection once a week. Gemcitabine (60 mg/kg) was administered via intraperitoneal injection according to a once every 3 days × 4 schedule. Tumor volumes and body weights were measured twice weekly. The length and width of the tumor were measured with calipers and the volume calculated using the following formula: tumor volume = (length × width²)/2. Mean percent tumor growth inhibition (TGI) values were calculated on the final day of the study for drug-treated compared with vehicle-treated mice and were calculated as 100 × [1 - (TreatedFinal day − TreatedDay 0)/(ControlFinal day − ControlDay 0)]]. Time-to-endpoint (TTE) is defined as the median time taken for the tumors to reach a median of 2,000 mm³.

**Pharmacodynamic studies**
Mice bearing A2780 tumors (300–400 mm³) were randomized and divided into 3 groups of 5 mice. Following a single dose of gemcitabine, CX-4945 was administered by oral gavage at 3.5 and 15.5 hours. Animals were euthanized 24 hours after administration of gemcitabine; tumors were resected, lysed, and resulting protein extracts were subjected to Western blot analysis.
Results

**CX-4945 enhances the activity of cisplatin and gemcitabine in cell-based antiproliferative experiments**

Gemcitabine and cisplatin are commonly used to treat patients with ovarian cancers (25). Because CK2 is involved in multiple processes that regulate the sensitivity of cancer cells to such DNA-targeted anticancer drugs (10, 13, 16), we asked whether combining CX-4945 with gemcitabine or cisplatin can improve the effectiveness of these chemotherapeutics. As p53 status is known to play an important role in the sensitivity of ovarian cancer cells to DNA-targeted agents (31) and because of the high frequency of p53 mutations in ovarian cancer (32), we used both p53 WT A2780 cells and p53 null SKOV-3 cells for our mechanistic studies. To ensure inhibition of CK2 signaling throughout the experiment, cells were pre-treated with CX-4945 4 hours before addition of either gemcitabine or cisplatin, and the presence of CX-4945 was maintained throughout the treatment period. Under these conditions (Fig. 3A), CX-4945 promoted synergistic antiproliferative effects in both cell lines (antiproliferative activity 8% to 17% greater than Bliss additivity; ref. 33).

In separate experiments using postaddition of CX-4945, we carried out combination studies using a schedule wherein CX-4945 was added 24 hours after treatment with gemcitabine or cisplatin and then maintained in combination for only 8 hours. Under these conditions, the chemotherapeutic agents have sufficient time to cause DNA strand breaks before the addition of CX-4945, whereas the presence of CX-4945 for only 8 hours contributes minimal single-agent antiproliferative activity. Using this

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Synergistic antiproliferative effects of combining CX-4945 with either cisplatin or gemcitabine in A2780 and SKOV-3 cells. The additivity line is equivalent to the theoretical combined antiproliferative effects of 2 drugs A and B [%(A + B) - %B(100) - %A])/100] in which %A and %B are the percentage of cells killed by drug A and B, respectively, at given concentrations. In A2780 cells, 3 μmol/L CX-4945, 300 nmol/L cisplatin (Cis), and 3 nmol/L gemcitabine (Gem) were used. In SKOV-3 cells, 10 μmol/L CX-4945, 1 μmol/L cisplatin (Cis), and 10 nmol/L gemcitabine (Gem) were used. A, CX-4945 preaddition schedule. B, CX-4945 postaddition schedule. C, inhibition of replication recovery for A2780 cells treated with 10 nmol/L gemcitabine and 10 μmol/L DX-4945.
schedule (Fig. 3B), CX-4945 significantly enhanced the antiproliferative effects of gemcitabine and cisplatin (antiproliferative activity 23%–38% higher than Bliss additivity). These data were consistent with an enhancement of antiproliferative activity by CX-4945 as a consequence of inhibiting DNA repair response mechanisms.

Stalled or collapsed replication forks produced by DNA-targeted drugs are known to cause cancer cells to arrest in S-phase (34). Indeed, we showed that SKOV-3 cells and, to a greater extent, A2780 cells can respond to gemcitabine or cisplatin treatment by accumulating in S-phase (data not shown). To determine the effects of CX-4945 on chemotherapeutically induced cell-cycle arrest, we evaluated the effects of gemcitabine alone or the combination of gemcitabine with CX-4945 in A2780 cells. After 28 hours, A2780 cells treated with gemcitabine alone reached the maximal S-phase arrest and then began recovery from S-phase and progressed to G2-M by 36 hours. However, the combination of CX-4945 with gemcitabine delayed replication recovery (Fig. 3C), whereas CX-4945 alone produced G2-M arrest, as previously described (24). These data suggested that by inhibiting DNA repair response in ovarian cancer cells, the combination with CX-4945 inhibits replication recovery and increases cancer cell death induced by DNA-targeted drugs.

CX-4945 decreases XRCC1 and MDC1 phosphorylation and prevents DNA repair response in combination with cisplatin and gemcitabine

To further define the mechanistic processes underlying the synergistic antiproliferative activity, we asked whether CX-4945 alone or in combination with cisplatin or gemcitabine could reduce the phosphorylation of the DNA repair mediator/adaptor proteins XRCC1 and MDC1, thus inhibiting DNA repair response. Treatment of A2780 or SKOV-3 cells with CX-4945 led to a significant

Figure 4. Western blot analysis of XRCC1 and MDC1 from CX-4945 combined with cisplatin (A) or gemcitabine (B) in A2780 or SKOV-3 cells. Cells were treated with gemcitabine (10 nmol/L) or cisplatin (3 μmol/L) for 24 hours followed by CX-4945 (10 μmol/L) and the cells lysed 8 hours after the addition of CX-4945; quantified phospho-XRRC1 levels (normalized to total XRCC1 levels). C, levels of phospho-MDC1 in CX-4945 (10 μmol/L, 24 hours) treated A2780 and SKOV-3 cells by immunoprecipitation.
decrease in the phosphorylation of XRCC1 at multiple CK2-specific sites (Fig. 4A and B). As specific antibodies for the CK2 phosphorylation sites on MDC1 were unavailable, we probed immunoprecipitated MDC1 from A2780 and SKOV-3 cells treated with CX-4945 using an antibody designed to bind to phospho-peptides with the CK2 substrate consensus sequence. Treatment of either cell type with CX-4945 led to significant reductions in MDC1 phosphorylation (Fig. 4C).

To determine whether decreased XRCC1 and MDC1 phosphorylation prevented DNA repair after treatment with gemcitabine or cisplatin, we used the alkaline comet assay to monitor the production of DNA strand breaks (35). At concentrations at which neither CX-4945 nor gemcitabine or cisplatin caused significant comet formation in A2780 cells, the combination of CX-4945 with either gemcitabine or cisplatin produced prominent tails. Such tails are indicative of SSBs, DSBs, and/or active excision repair of DNA cross-links. This shows that CX-4945 prevented DNA repair after gemcitabine or cisplatin treatment (Fig. 5A). Addition of the pan-caspase inhibitor zVAD-FMK did not reduce tail formation, indicating that

Figure 5. A, comet assay showing increased DNA strand breaks resulting from the combination of CX-4945 (10 μmol/L) with gemcitabine (100 nmol/L) or cisplatin (10 μmol/L), with and without ZVAD (100 μmol/L). Western blot analysis of γ-H2AX and CHK1/2 from CX-4945 combined with cisplatin (B) or gemcitabine (C) in A2780 or SKOV-3 cells. Cells were treated with gemcitabine (10 nmol/L) or cisplatin (3 μmol/L) for 24 hours followed by CX-4945 (10 μmol/L) and the cells lyzed 8 hours after the addition of CX-4945; quantified phospho-CHK1/2 levels normalized to total CHK levels. Quantified γ-H2AX levels are shown in Supplementary Fig S4.
the observed DNA strand breaks were not secondary to the induction of apoptosis. To confirm these findings, we monitored changes in the levels of γ-H2AX, a widely established marker of DNA strand breaks (36). Combining CX-4945 with either gemcitabine or cisplatin in either A2780 or SKOV-3 cells increased levels of γ-H2AX compared with either agent used alone, confirming that levels of DNA strand breaks were increased in the cancer cells (Fig. 5B and C and Supplementary Fig. S1).

Mechanistic data with CX-4945 presented thus far indicated that CK2, XRCC1, and MDC1, all act in a coordinated and essential fashion to facilitate the DNA repair response that is triggered by cisplatin or gemcitabine treatment. This contention is corroborated by studies in which we used short interfering RNA to knockdown CK2 (both the α and α’ isoforms simultaneously), XRCC1 or MDC1 in SKOV-3 cells. Knockdown of XRCC1, MDC1, or simultaneous knockdown of CK2α/α’ significantly increased the levels of γ-H2AX produced by either cisplatin or gemcitabine treatment alone (Supplementary Fig. S2). These data confirmed the relevance of CK2, XRCC1, and MDC1 in mediating gemcitabine or cisplatin-induced DNA repair response and highlight the utility of CK2 as a drug target to prevent the DNA repair response triggered by such chemotherapeutic agents.

The presence of DNA strand breaks would be expected to trigger the activation of ATR and ATM, 2 kinases that play prominent roles in DNA repair response signaling, which in turn phosphorylate CHK1 and CHK2, respectively, halting cell-cycle progression to allow DNA repair.
CX-4945 Enhances Activity of Gemcitabine and Cisplatin

Figure 7. A2780 ovarian cancer xenograft efficacy studies. Mean tumor volumes, median times to endpoint, and changes in body weights are presented. A, CX-4945 was dosed 75 mg/kg orally twice daily for 21 days; cisplatin was dosed 5 mg/kg i.p. once every 7 days for 21 days. B, CX-4945 was dosed 75 mg/kg orally twice daily for 21 days; carboplatin was dosed 75 mg/kg i.p. once every 7 days for 21 days. C, CX-4945 was dosed 100 mg/kg orally twice daily for 21 days; gemcitabine was dosed 60 mg/kg i.p. once every 3 days for 10 days (days 1, 4, 7, and 10). D, CX-4945 was dosed 100 mg/kg orally twice daily (days 2, 5, 8, and 11); gemcitabine was dosed 30 mg/kg i.p. once every 3 days (days 1, 4, 7, and 10). Combination treatments used combined single-agent schedules. E, analysis of cleaved PARP levels in A2780 tumor cells subjected to CX-4945 and gemcitabine alone or in combination.
to progress (1). In all combinations tested, addition of CX-4945 increased phosphorylation of CHK2 when compared with the cytotoxic agent used alone (Fig. 5B and C). The effects on CHK1 phosphorylation were mixed, particularly in A2780 cells, where significant reductions of total CHK1 levels were also observed. Taken together, our data showed that inhibition of CK2 by CX-4945 prevents activation of MDC1 and XRCC1 proteins and consequently suppresses the ability of cancer cells to repair DNA strand breaks caused by treatment with gemcitabine or cisplatin.

**The combination of CX-4945 with cisplatin or gemcitabine results in apoptosis in p53 WT A2780 cells and mitotic catastrophe in p53 null SKOV-3 cells**

It has previously been reported that cisplatin-treated ovarian cancer cells undergo distinct modes of cell death that are dependent on the status of p53 (34). Therefore, we examined the mode of cell death of A2780 and SKOV-3 cells treated with CX-4945 and gemcitabine or cisplatin. First, we analyzed the change in levels of effector caspase-3/7 activity. In p53 WT A2780 cells, the combination of CX-4945 with either cisplatin or gemcitabine resulted in a significant increase in caspase-3/7 activity compared with either agent used alone (Fig. 6A). In p53 null SKOV-3 cells, the same combinations did not produce significant increases in caspase-3/7 activity. Consistent with these observations, in p53 WT A2780 cells a significant increase in cleaved PARP was seen upon combination treatment, confirming elevated apoptosis, whereas in p53 null SKOV-3 cells the levels of cleaved PARP were undetectable (Fig. 6B and Supplementary Fig. S1).

Because p53 null SKOV-3 cells were previously shown to be susceptible to mitotic catastrophe in response to cisplatin treatment (34), we wanted to test whether the combination of CX-4945 with cisplatin would lead to the same outcome. For this purpose we monitored nuclear morphology changes following drug treatment of SKOV-3 cells by 4′,6-diamidino-2-phenylindole (DAPI) staining (Fig. 6C). The nuclear morphology of SKOV-3 cells treated with cisplatin for up to 72 hours was unchanged. Combination treatment of cisplatin with CX-4945 for 72 hours produced enlarged multinucleated cells, a phenotypic response previously characterized as cisplatin-induced mitotic catastrophe (34, 37, 38). Similar results were also seen when CX-4945 was combined with gemcitabine in SKOV-3 cells (Fig. 6C).

**CX-4945 synergizes with cisplatin, carboplatin, and gemcitabine in xenograft models of ovarian cancer**

To determine whether the synergy observed between CX-4945 and cisplatin or gemcitabine in vitro could be translated into increased antitumor efficacy in vivo, we tested these combinations in a xenograft model. For this purpose we selected the A2780 model because it is aggressively tumorigenic (TTE approximately 15 days), and it allowed us to investigate cleaved PARP as a pharmacodynamic biomarker of combination activity in tumors. CX-4945 (dosed intraperitoneally at 5 mg/kg twice daily) or cisplatin (dosed intraperitoneal at 5 mg/kg once every 7 days) had minimal antitumor effects as single agents in this model. However, combining CX-4945 with cisplatin produced robust TGI and extended TTE to 30 days (Fig. 7A). Because cisplatin is known to cause negative effects on the body weights of treated animals, we combined CX-4945 with carboplatin, a cisplatin analog with a reduced side-effect profile that is widely used in the treatment of ovarian cancer. Neither agent was effective when administered alone, whereas the combination resulted in robust TGI and extended TTE to 34 days. The body weight effects for combination were comparable with those produced by single agents (Fig. 7B). In combination with gemcitabine, CX-4945 improved the therapeutic benefit by increasing TGI to 99% and extending TTE to 39 days, while being well tolerated (Fig. 7C).

Our *in vitro* antiproliferative studies suggested that transient exposure to CX-4945 was sufficient to produce synergy with gemcitabine or cisplatin. Therefore, we conducted an additional A2780 xenograft study wherein CX-4945 was dosed only on 4 occasions, each dose given 24 hours after administration of gemcitabine (Fig. 7D). This dosing schedule delivered significantly longer TTE than gemcitabine alone (51 vs. 37 days). Moreover, the enhanced efficacy using this dosing regimen provides *in vivo* support that CX-4945 augments the antitumor effects of chemotherapeutic agents as a consequence of inhibiting DNA repair response mechanisms.

To investigate the potential of cleaved PARP as a pharmacodynamic biomarker, we treated mice implanted with A2780 xenografts to a single dose of gemcitabine (60 mg/kg i.p.), followed by 2 subsequent doses of CX-4945 (75 mg/kg orally) at 3.5 and 15.5 hours after administration of gemcitabine. Twenty four hours following gemcitabine addition, the tumors were resected, lysed, and the resulting protein extracts were analyzed for levels of cleaved PARP using Western hybridization (Fig. 7E). A clear increase in cleaved PARP levels was observed in tumors from mice treated with CX-4945 and gemcitabine in combination compared with either drug used alone, confirming that the antitumor effect of combining the 2 drugs resulted in increased apoptosis in A2780 xenografts *in vivo*.

**Discussion**

DNA-targeted chemotherapeutics are commonly used as single agents or in combination for the treatment of various types of cancer. Although these drugs are known for their robust initial efficacy, they are often limited by toxicity and inherent or acquired resistance (39). One of the mechanisms driving such resistance is DNA repair response, which limits the ability of DNA-targeted chemotherapeutics to kill cancer cells (1). Several approaches aimed at combining DNA-targeted chemotherapeutics...
with inhibitors of DNA repair response are being investigated in the clinic. The majority of these inhibitors target highly specific DNA repair response pathways; therefore, there is considerable need for DNA repair response inhibitors that target a broader spectrum of DNA repair response and potentially offer combinability with a greater number of anticancer chemotherapies. CK2, with its newly recognized role in the genomic surveillance and repair of both single and double strand breaks and its established overexpression in cancer cells, ideally fulfills these criteria.

We showed that the presence of the CK2 inhibitor CX-4945 strongly enhances the antiproliferative activity of gemcitabine or cisplatin in ovarian cancer cells (Fig. 3A and B). By varying dosing schedules we showed that relatively short exposure of cells already treated with gemcitabine or cisplatin to CX-4945 was sufficient to produce synergy. Cell-cycle analysis established that CX-4945 prevented the replication recovery of cells treated with gemcitabine, indicating that the inhibition of DNA repair response is the primary mechanism behind the observed synergy (Fig. 3C).

We show that inhibiting CK2 with CX-4945 decreased the phosphorylation of XRCC1 and MDC1 at CK2-specific phosphorylation sites (Fig. 3A), which prevented efficient DNA repair response in cisplatin- or gemcitabine-treated ovarian cancer cells, as judged by tail formation in the comet assay and increased H2AX phosphorylation (Fig. 5A–C). The combination treatments also resulted in amplification of DNA repair signaling, as evident from the increased levels of phospho-CHK2. Interestingly, changes in the phosphorylation of CHK1 were cell line dependent, with relative levels of phospho-CHK1 being somewhat higher for the combination in SKOV-3 cells, but lower in A2780 cells, when compared with the effects of single agents (Fig. 5B and C). This can potentially be explained by the reduction of total CHK1 levels produced by both gemcitabine or cisplatin and combination treatments with CX-4945, which may be linked to the previously described caspase-mediated cleavage of CHK1 that can occur during apoptosis (40, 41). It is also possible that CK2 inhibition may directly regulate CHK1 stability in A2780 cells, as CHK1 is a client protein of HSP90 co-chaperone CDC37, which is known to depend on phosphorylation by CK2 for its activity (8).

Using short interfering RNA knockdowns, we showed that CK2ζ/α, MDC1, and XRCC1 proteins were essential for efficient DNA repair response resulting from cisplatin or gemcitabine treatment. Although XRCC1 has been implicated in cisplatin DNA repair response in HepG2 cells (42), the role of XRCC1 in repairing gemcitabine-induced DNA repair response has not previously been described. One possible explanation is that XRCC1 has been shown to bind to RAD51, implicating XRCC1 in HR repair (43). An alternative explanation derives from the emerging role of XRCC1 in the coordination of DNA repair and replication during S-phase, mediated by its interaction with the p58 subunit of DNA Pol α-primase (44). This model would involve XRCC1 in the stabilization and repair of stalled replication forks created by gemcitabine, although the putative role of CK2 in this process remains to be investigated (Fig. 1).

The role of p53 in the cellular and clinical response to chemotherapies reflects the dual nature of p53 as an activator of both DNA repair and apoptosis (45). In the context of ovarian cancer, p53 mutations have been shown to confer resistance to cisplatin in vitro (46) and also correlate with resistance to platinum-based chemotherapy and poor prognosis in the clinic (31). Although in antiproliferative experiments the combination of CX-4945 with gemcitabine or cisplatin was synergistic in both A2780 and SKOV-3 cells, the fate of these cells was found to be different, with p53 WT A2780 cells readily undergoing increased apoptosis, whereas p53 null SKOV-3 cells showed evidence of mitotic catastrophe. These data suggest that even though different cell death pathways are activated, the ability of CX-4945 to augment the antiproliferative activity of gemcitabine or cisplatin seems not to depend on the status of p53, suggesting that such combinations can be used successfully in a broad spectrum of patients with ovarian cancer, a disease characterized by a high frequency of p53 mutation (32).

In vitro studies with mice bearing A2780 xenografts confirmed that the combination of CX-4945 with cisplatin, carboplatin, or gemcitabine increased the antitumor efficacy compared with the efficacy observed with single agents. We also showed that administration of CX-4945 on an intermittent schedule, that is, 24 hours after each dose of gemcitabine could enhance the efficacy of gemcitabine in mice. These findings further illustrate that CX-4945 prevents CK2 from activating DNA repair response mechanisms, thereby preventing replication recovery. Finally, we showed that the increased antitumor activity of the CX-4945/gemcitabine combination correlates with enhanced apoptosis by measuring cleaved PARP as a pharmacodynamic biomarker in xenograft tumors.

CK2 potentially regulates multiple functions within DNA repair response. However, a clearly prevailing mechanism is to facilitate the binding of signaling molecules and DNA end-processing factors to noncatalytically active mediator/adaptor proteins involved in both SSBR and DSBR. Remarkably, mirroring its function with XRC1, phosphorylation by CK2 promotes the binding of both aprataxin and PNK to XRC4, the mediator/adaptor binding partner of DNA ligase IV (7, 15). Because XRC4/Ligase IV complex is an essential component of nonhomologous end-joining repair (NHEJ) of DSBs, the primary repair pathway triggered by ionizing radiation, it is possible that CK2 inhibition may synergize with radiotherapy in addition to the large number of DNA-targeted anticancer drugs that trigger SSBR, HR, and NHEJ (Supplementary Fig. S3).

Along with the DNA repair response, CK2 positively regulates an extensive list of additional cellular processes that are also established effectors of sensitivity to DNA-targeted chemotherapeutics as well as other anticancer
drugs, including PI3K/AKT/mTOR signaling, NF-xB transcription, Hsp90 machinery activity, hypoxia, inhibition of apoptosis, and IL-6 expression (3, 8–11). Indeed, sensitization of pancreatic cancer cells to gemcitabine and head and neck cancer cells to cisplatin by genetic manipulation of CK2 expression has been attributed to resulting suppression of Akt and NF-xB activity, respectively (47, 48). Thus it is possible that in addition to the suppression of DNA repair response, CX-4945 enhances activity of gemcitabine and cisplatin by modulating PI3K/Akt and NF-xB signaling. Our data combined with previous reports showing enhancement of therapeutic activity by suppression of CK2 (e.g., ref. 49) shows that CK2 represents a promising pharmacologic target that can be explored in combination therapy with multiple anticancer therapeutics.

Here we have described how targeting CK2 with CX-4945 can inhibit multiple DNA repair response mechanisms by blocking phosphorylation of XRCC1 and MDC1 and synergize with the DNA-targeted anticancer drugs cisplatin and gemcitabine in ovarian cancer cells. These data provide a strong rationale for combining CX-4945 with gemcitabine and platinum-based chemotherapeutics in clinical trials for ovarian and possibly other cancers.

Disclosure of Potential Conflicts of Interest


Grant Support

This work was supported by Cylene Pharmaceuticals.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 9, 2011; revised December 5, 2011; accepted January 9, 2012; published OnlineFirst January 20, 2012.

References

26. Furuta T, Ueda T, Aune G, Sarasin A, Kraemer KH, Pommier Y. Transcription-coupled nucleotide excision repair as a determinant


CK2 Inhibitor CX-4945 Suppresses DNA Repair Response Triggered by DNA-Targeted Anticancer Drugs and Augments Efficacy: Mechanistic Rationale for Drug Combination Therapy

Adam Siddiqui-Jain, Joshua Bliesath, Diwata Macalino, et al.


Updated version: Access the most recent version of this article at: doi:10.1158/1535-7163.MCT-11-0613

Supplementary Material: Access the most recent supplemental material at: http://mct.aacrjournals.org/content/suppl/2012/01/20/1535-7163.MCT-11-0613.DC1

Cited articles: This article cites 46 articles, 18 of which you can access for free at: http://mct.aacrjournals.org/content/11/4/994.full.html#ref-list-1

Citing articles: This article has been cited by 2 HighWire-hosted articles. Access the articles at: /content/11/4/994.full.html#related-urls

E-mail alerts: Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions: To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions: To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.