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Molecular Cancer Therapeutics

Combined CDKN1A/TP53 Mutation in Bladder Cancer Is a Therapeutic Target

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Abstract

Invasive bladder cancer has high morbidity and nearly uniform mortality when metastatic, with no therapeutic improvement in many years. Although chemotherapy combined with Chk1 inhibition has been investigated in several cancer types in which *TP53* mutation is seen, this combination treatment approach has not been studied in bladder cancer. Recently, cancer genome sequencing efforts have identified CDKN1A (p21) mutations at 14% frequency in invasive bladder cancer, co-occurring half the time with TP53 mutations. We hypothesized that combined CDKN1A–TP53 loss would make bladder cancer sensitive to combined treatment with gemcitabine and Chk1 inhibitor. Here, we show that TP53–CDKN1A double-mutant bladder cancer cell lines, 647V and RT-112, have a remarkable increase in p-Chk1 levels and G_2–M arrest in response to gemcitabine treatment, with a heightened sensitivity to combination treatment with gemcitabine and either Chk1 inhibitor PF477736 or AZD7762, in comparison with other bladder cancer cell lines (either TP53 or p21 deficient). In addition, CDKN1A restoration in p21-deficient bladder cancer cells significantly reduced their sensitivity to combined treatment by protecting them from DNA damage and apoptosis. Furthermore, xenograft studies using RT-112 showed a significant synergistic effect of combined gemcitabine–PF477736 treatment on tumor growth. Our findings suggest that TP53/CDKN1A double-mutant bladder cancer cells have a unique dependence on Chk1 activity for the G_2–M cell-cycle checkpoint in response to chemotherapy-induced DNA damage. This combination or others involving genotoxic agents and Chk kinase inhibitors is a promising therapeutic approach for bladder cancer with these mutations. *Mol Cancer Ther; 14(1); 1–9. ©2014 AACR.*

Introduction

Cell-cycle checkpoints are regulatory pathways that control the order and timing of cell-cycle progression, and ensure that critical events, for example, DNA replication, are completed with high fidelity before cell division (1–3). Checkpoints respond to DNA damage by arresting the cell cycle to provide time for repair and by induction of transcription of genes that are necessary for repair. When the genome is damaged by irradiation, UV treatment or chemotherapeutic agents, both single-stranded DNA and double-strand breaks (DSB) occur. ATR serine/threonine kinase (ATR) is activated in response to single-stranded DNA, and phosphorylates and activates checkpoint kinase 1 (Chk1). DSBs lead to recruitment of the MRN (Mre11–Rad50–Nbs1) complex, including the ATM serine/threonine kinase. ATM phosphorylates H2AX on Ser139, and leads to recruitment of the Chk1 kinase, which is also phosphorylated by ATM. Activated Chk1 phosphorylates the CDC25A phosphatase, leading to phosphorylation of the CDK2–cyclin complex, resulting in cell-cycle arrest. Both ATM and Chk1 phosphorylate p53, leading to its stabilization and a transcriptional response, enhancing cell-cycle arrest until the DNA damage is repaired (4–7).

Hence, p53-deficient tumor cells rely on Chk1 to arrest cell-cycle progression in the S and G_2–M phases (8). When the S and G_2–M checkpoints are abrogated by inhibition of Chk1, p53-deficient cancer cells undergo mitotic catastrophe and apoptosis (9–14). Several preclinical studies have demonstrated that Chk1 inhibitors potentiate the effects of DNA-damaging agents, such as chemotherapy in p53-deficient cancer cells, and several Chk1 inhibitors are being tested in clinical trials (15–18).

Multiple cell-cycle checkpoint genes are subject to mutation/deletion or amplification in invasive bladder cancer, including *TP53*, *CDKN2A* (encoding $p19^{ARF}$ and $p16^{INK4A}$), *MDM2*, *CCND1*, and *CCND2* (19–23). Mutations in *CDKN1A* (encoding p21, also known as CIP1) have been seen very rarely overall in cancer (http://cancergenome.broadinstitute.org), but have recently been identified in invasive bladder cancer at 14% frequency (19). We hypothesized that *CDKN1A*-mutant and double *TP53*/*CDKN1A*-mutant bladder cancers would be uniquely sensitive to Chk1 inhibition, in combination with DNA-damaging chemotherapy, by abolishing the normal cell-cycle checkpoint response. We examined this hypothesis using bladder cancer cell lines both *in vitro* and *in vivo* in mouse xenograft tumors.

Materials and Methods

DNA analysis methods

Genomic DNA from bladder cancer cell lines was extracted using the Blood and Tissue Extraction Kit (Qiagen). Exons of

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Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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CDKN1A were amplified by PCR, and subjected to Sanger sequencing.

Cell culture, viral infection, and cell viability assays

Thirty bladder cancer cell lines were cultured in DMEM containing 10% FCS and 2 mmol/L glutamine under a humidified atmosphere of 5% CO_2 at 37°C, as described previously (Supplementary Table S1; 24). All cell lines were subject to microsatellite fingerprinting in 2012, which confirmed that they were unique (24).

CDKN1A lentiviral particles, with CDKN1A expression under a tetracycline inducible suCMV promoter (cat# LVP140) and tetracycline regulator (TetR) lentiviral particles (cat# LVP017-Puro) were purchased from GenTarget Inc. The two lentiviruses were added to 647V, RT-112, and 97-1 bladder cancer cell lines, and stably infected cell lines selected by combined puromycin (5 μg/mL) and blasticidin (5 μg/mL) treatment. p21 expression was induced by treatment with 100 ng/mL doxycycline for 24 hours. For cell viability assays, 3,000 cells were plated in sterile 96-well plates and cultured overnight. Compounds were then added in serial dilutions. Cellular viability was determined after 48 hours by the CellTiter-Glo Luminescent Cell Viability Assay (Promega). Plates were measured on a THERMO max microplate reader.

Scramble nontargeting siRNA control (D-001810-0X) and ON-TARGETplus CDKN1A siRNA (L-003471-00-0005) were purchased from Dharmacon Inc. Transfection was performed according to the manufacturer's protocol using Lipofectamine 2000. For cell viability assays using siRNA, TCCSUP cells were treated with siRNA, then seeded in 96-well plates 24 hours later, and then treated with drugs 48 hours later. Cell viability was assessed by CellTiter-Glo 72 hours after the start of the experiment. Similarly, treated TCCSUP cells were lysed 72 hours after transfection for immunoblot analysis.

Reagents

Gemcitabine, AZD7762, and PF477736 were purchased from Sigma. Cisplatin was purchased from Tocris Bioscience (Cat# 2251). Propidium iodide (PI) staining solution was purchased from BD Biosciences. Antibodies against CDKN1A, TP53, PARP-1, P-γH2Ax, p-Chk1-317, and p-Chk-345 were purchased from Cell Signaling Technology. β-Actin antibody (Santa Cruz Biotechnology) was used as protein loading control. DMEM was obtained from Cellgro and supplements were from Invitrogen.

Western blotting

Immunoblotting was carried out using standard techniques. Briefly, cells were lysed in ice-cold RIPA lysis buffer and protein concentrations determined. Aliquots (50 μg) of protein were denatured in Laemmli loading buffer and separated on precast 4% to 10% NuPAGE Novex 4% to 12% Bis-Tris Protein Gels (Novex-Invitrogen). Proteins were transferred to polyvinylidene difluoride membranes, which were blocked and probed with primary, and then detected using appropriate horseradish peroxidase–labeled secondary antibodies. Proteins were visualized using enhanced chemiluminescence (Pierce; Thermo Fisher Scientific) on Hyperfilm (GE Healthcare).

Cell-cycle analysis

For cell-cycle analysis, cells were seeded in a 10-cm dish at 60% confluency. After 1 hour exposure to gemcitabine, the cells were incubated with fresh DMEM containing 10% FBS with/without 500 nmol/L PF477736 for an additional 23 hours. Then the cells were washed with PBS and fixed by 70% ethanol for 12 hours. The next day, these cells were treated with RNaseA (Sigma Aldrich) and stained with 10 ng/mL PI. Cell-cycle status was determined using a FACSCaliber flow cytometer (Becton Dickinson) and analyzed using FlowJo7.6.5 software.

Analysis of *in vivo* xenograft mice

All animal procedures were performed in accordance with the NIH Guide on the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Children's Hospital Boston. To generate bladder tumor xenografts, 5×10^6 RT-112 bladder cancer cells were injected into the flanks of CB17/SCID mice. Tumor nodules were monitored until they reached 9 to 11 mm^3. Gemcitabine and PF477736 were administered at 50 and 15 mg/kg, respectively, by i.p. injection, as described previously (25). These drugs were given to randomly chosen mice when tumor nodules crossed the threshold size. Tumors were measured with calipers in two dimensions and volume calculated using the equation volume $= \pi/6 \times$ length $\times$ width^2. The comparisons between groups at each time point were made using a Student t test for unpaired samples. The tests were two-sided and a change with a P value of <0.05 was considered statistically significant.

Results

CDKN1A is frequently mutated in human bladder cancer and involved in chemotherapy-induced DNA damage response

In The Cancer Genome Atlas (TCGA) dataset, *CDKN1A* is mutated in 18 of 131 (14%) bladder cancers and nearly all are frame-shift mutations, including indels and nonsense mutations (Fig. 1A; ref. 19). To examine this further, we studied a collection of 30 bladder cancer cell lines and identified mutations in *CDKN1A* in 3 of 30 (10%; Fig. 1A). In the TCGA bladder cancer dataset, TP53 mutations were also common, seen in about half of cancers (19). Eight of the 18 *CDKN1A* mutations reported in the TCGA analysis occurred in cancers that also had *TP53* mutations, whereas 10 occurred in cancers without *TP53* mutation (Fig. 1B).

Among 15 bladder cancer cell lines assessed by immunoblot analysis, we observed that 11 of 15 expressed p21 to some extent, whereas four lacked expression completely, including three lines with defined mutations in *CDKN1A*, and another cell line, 97-1, previously reported not to express p21 (Fig. 1C; ref. 20). One of the four p21 null cell lines grew very slowly and was not studied further. The other three, 647V, RT-112, and 97-1, showed no change in expression of p21 in response to treatment with gemcitabine, a nucleoside analogue that blocks DNA replication and also inhibits ribonucleotide reductase, in contrast with a set of control cell lines (Fig. 1D; Supplementary Table S1). All six of the lines showed increased expression of p-Chk1-S345 in response to gemcitabine treatment, as expected due to blocked DNA replication with activation of ATM/ATR. Notably, higher levels of p-Chk1-S345 were observed in 647V and RT-112, cell lines with concurrent mutation in *TP53* (Supplementary Table S1), suggesting that p53/p21 dual-mutated cells are more dependent on Chk1 mediated cell-cycle checkpoint in response to chemotherapeutic drug.

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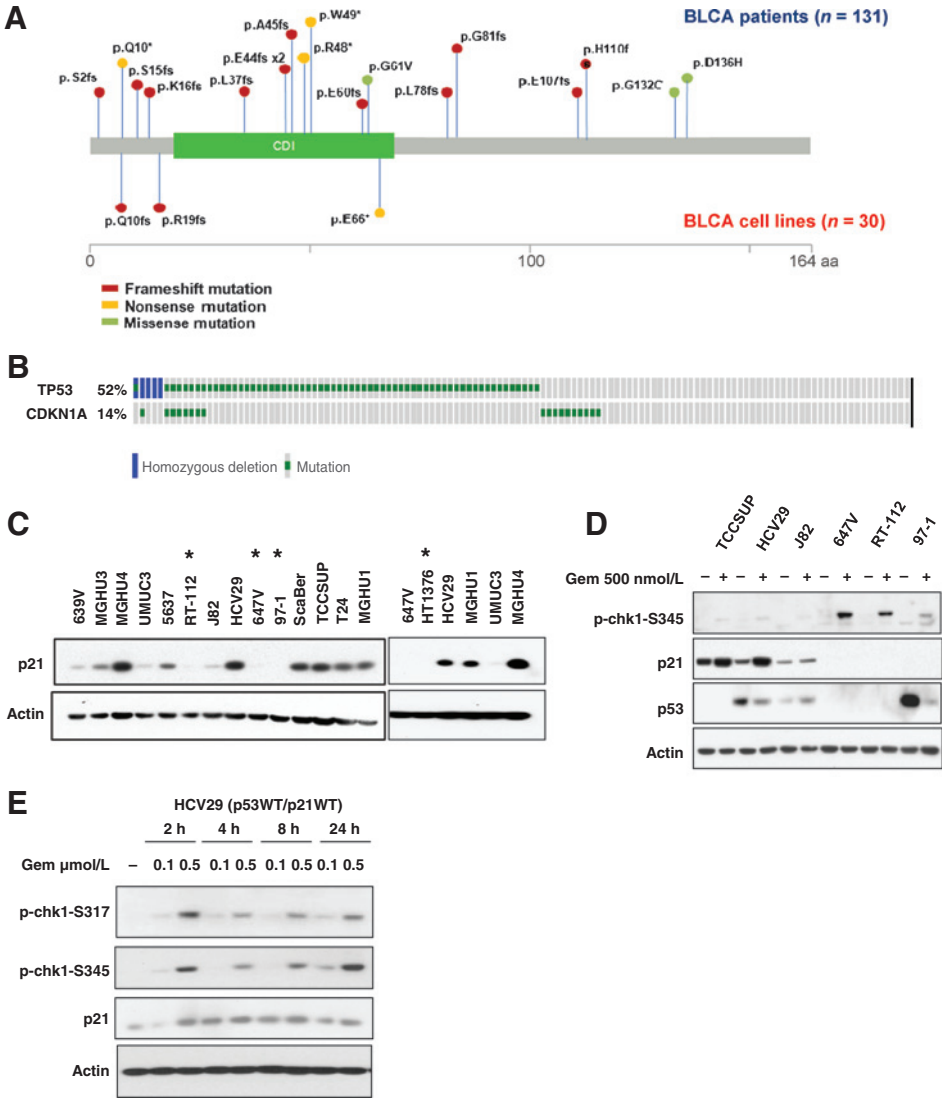

Figure 1.
CDKN1A mutations in bladder cancer. A, diagram of the structure of the CDKN1A encoded protein p21 with mutations identified by the TCGA in bladder cancer (19) indicated above. Red circles, indel frame-shift mutations; yellow circles, nonsense mutations; and green circles, missense mutations. Mutations identified in bladder cancer cell lines are shown below. B, comutation plot for *TP53* and *CDKN1A* mutations in the TCGA bladder cancer dataset (from http://www.cbioportal.org; ref. 19). It can be seen that eight (44%) *CDKN1A* mutations occur in cancers with *TP53* mutations, and 10 (56%) occur in cancers without TP53 mutations. C, p21 expression in 14 bladder cancer cell lines assessed by immunoblotting. Actin was used as loading control; *, p21-mutated or -deficient cell lines. Note that some cell lines are loaded twice, as controls. D, activation of Chk1 by phosphorylation at S345, and induction of p21 by treatment with gemcitabine at 500 nmol/L for 12 hours. Note that cell lines TCCSUP and HCV29 are wild-type for each of *TP53/CDKN1A*; J82 is *TP53* mutant and *CDKN1A* wild-type; 97-1 is *TP53* wild-type and p21-deficient; whereas 647V and RT-112 are mutant for each of *TP53/CDKN1A*. E, p21 induction and pChk1-S345 and pChk1-S317 levels are increased by gemcitabine treatment in the *TP53/CDKN1A* wild-type cell line HCV29.

Next, we examined expression of p21 in TP53^{wt}/CDKN1A^{wt} cell lines in greater detail in response to gemcitabine. We found that expression of p21 was induced in a dose- and time-dependent manner, and could be seen as early as 2 hours after treatment with gemcitabine (Fig. 1E), consistent with p21 involvement in the early response to DNA damage. Hence, this suggested that loss of p21 might lead to dysregulation of the p53-mediated DNA damage pathway.

Chk1 inhibition sensitizes p53- and p21-deficient bladder cancer cells to gemcitabine

It has been shown previously that p53-deficient cells rely on Chk1 activity for cell-cycle checkpoint arrest in response to DNA damage (21). Thus, Chk1 inhibition has been proposed as a potential therapeutic strategy for p53-deficient cancers, when given concurrently with treatment with conventional chemotherapeutic drugs that induce DNA damage (22). As noted above, we hypothesized that double-mutant p53/p21-deficient bladder cancers might have even greater sensitivity to this therapeutic strategy. To explore this, we examined the effects on cell growth of treatment with varying doses of gemcitabine and the Chk1 inhibitor PF-477736. In a standard cell growth assay using CellTiter-Glo, we found that 500 nmol/L PF-477736 significantly enhanced the reduction in cell growth in response to gemcitabine and reduced the IC_{50} value of all three p21-deficient bladder cancer cell lines (647V, RT-112, and 97-1) by 10- to 100-fold (Fig. 2A and Supplementary Fig. S1). In contrast, there was no significant synergy observed in combined treatment of three p21 wild-type lines (J82, HCV29, and TCCSUP) with this drug combination (Fig. 2A). The doses of gemcitabine used to achieve significant cell growth inhibition in 500 nmol/L PF-477736 were particularly low for the 647V and RT-112 cell lines, which had concurrent loss of TP53 (Figs. 1D and 2A). Furthermore, those two cell lines showed marked sensitivity to concurrent treatment at doses of PF-477736 as low as 50 nmol/L (Fig. 2B). To insure that the effect of PF-477736 was specific to Chk1 inhibition, we also examined the effects of a second Chk1 inhibitor, AZD7762. AZD7762 also showed significant synergy in combination with gemcitabine over a range of doses, with near complete death of

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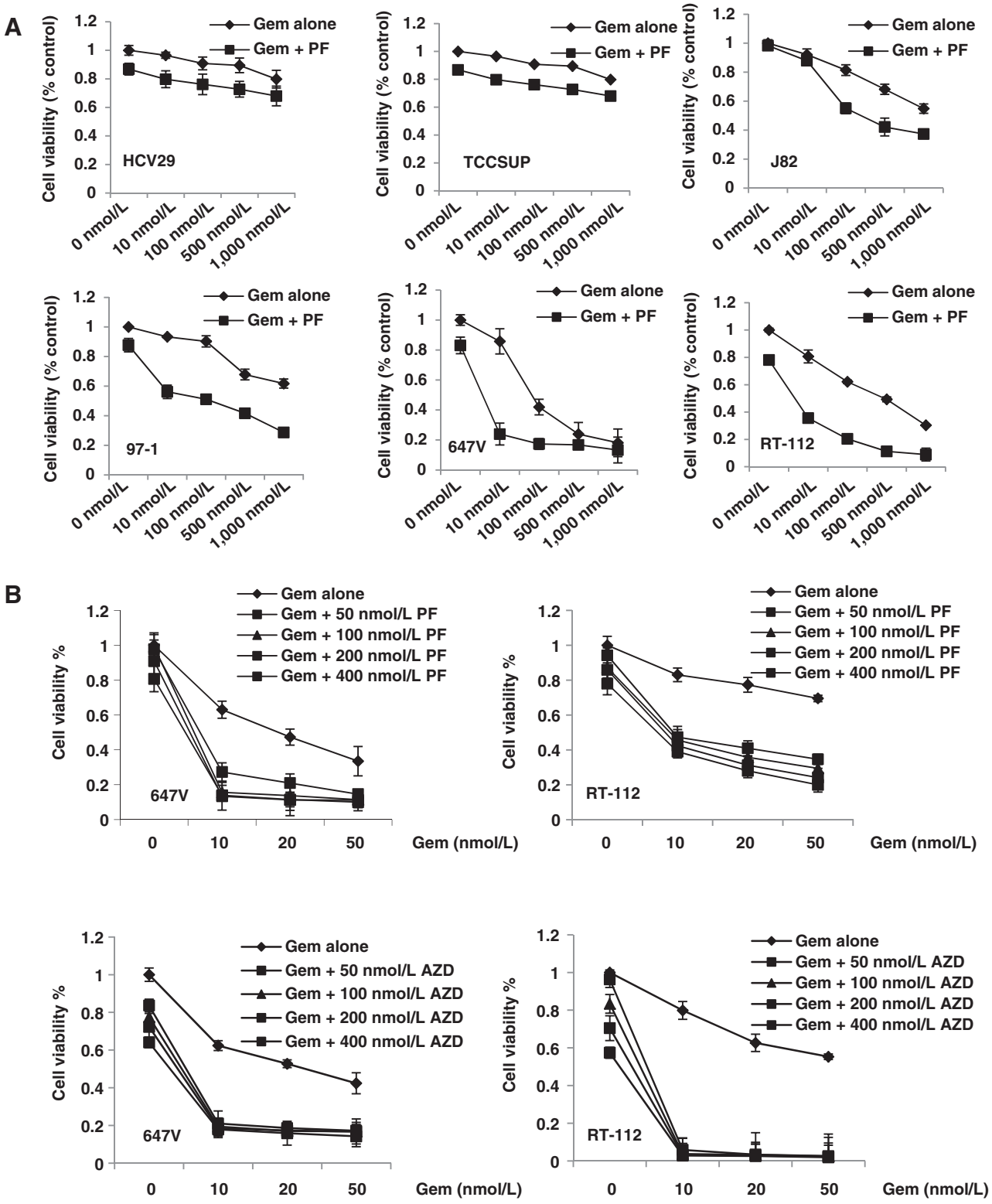

Figure 2.
Synergistic effect of combined Chk1 inhibition and gemcitabine treatment in bladder cancer cell lines. A, cell viability curves are shown for a fixed dose of PF-477736 (PF, 500 nmol/L) and variable dose of gemcitabine (Gem, *x*-axis). Cell counts were assessed by CellTiter-Glo assay. Note that the reduction in cell viability is much higher for the *CDKN1A*-mutant cell lines 647V and RT-112 (both also mutant for *TP53*) and 97-1. B, cell viability curves are shown for various doses of PF-477736 (PF, top) and AZD7762 (AZD, bottom; 0, 50, 100, 200, and 400 nmol/L for each), and variable doses of gemcitabine (Gem, 0–50 nmol/L, *x*-axis). Cell viability was determined after 2 days incubation using CellTiter-Glo.

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RT-112 cells in response to low doses of each of gemcitabine and AZD7762 (Fig. 2B).

Chk1 inhibition enhanced gemcitabine-induced DNA damage and apoptosis in p53 and p21^{Cip1} dual-mutated bladder cancer cells due to abrogation of G_2–M cell-cycle checkpoint

Next, to assess the mechanism of cell growth inhibition seen in response to concurrent Chk1 inhibitor–gemcitabine treatment, we examined cell-cycle progression in response to these drugs. Gemcitabine (0.5 μmol/L for 1 hours) caused S phase arrest in p53/p21 wild-type (HCV29), p53-mutant/p21-wild-type (J82) and p53-wild-type/p21-deficient (97-1) bladder cancer cell lines 24 hours after exposure (Fig. 3A, top). However, similar treatment of the p53-mutant/p21-mutant bladder cancer cell lines (647V, RT-112) led to G_2–M phase arrest rather than S phase arrest, suggesting that S phase continued in those cells (Fig. 3A, bottom). PF-477736 alone had minimal to no effects on cell-cycle distribution after treatment with 0.5 μmol/L for 24 hours (data not shown). The addition of 0.5 μmol/l PF-477736 for 23 hours following short-term gemcitabine treatment caused a substantial reduction in the percentage of cells at G_2–M in both 647V cells (32% reduction) and RT-112 cells (36% reduction; Fig. 3B). Similar treatment led to a reduction in S phase cells in the HCV29, 97-1, and J82 cell lines. These observations suggest that Chk1 inhibition can overcome gemcitabine-induced G_2–M phase cell-cycle arrest specifically in p53–p21-deficient bladder cancer cells.

To examine the mechanism of cell death induction by combined treatment in greater detail, we assessed apoptosis in these bladder cancer cells lines by immunoblotting. For these experiments, we used continuous treatment with these drugs for 18 hours. We found that gemcitabine induced p-Chk1-S345 expression in all six bladder cancer cell lines, and this was reduced to near baseline levels in a dose-dependent manner by treatment with PF-477736 (Fig. 3C). Combination treatment led to significantly increased phospho-γH2A.X-S139 expression in all cell lines, consistent with a DNA damage response. However, apoptosis, as assessed by cleavage of PARP, was seen to a major extent only in the double p21–p53-mutant 647V and RT-112 cell lines, and to a lesser extent in the p21- or p53-deficient cell lines (Fig. 3C).

Reexpression of p21 attenuates the effect of Chk1 inhibition on gemcitabine-induced cytotoxicity in p21-deficient bladder cancer cells

To confirm that loss of p21 was the direct cause of enhanced sensitivity to gemcitabine–Chk1 inhibitor treatment, we used lentiviral-based delivery to express doxycycline-inducible p21 in the 97-1, 647V, and RT-112 cell lines. Treatment with 100 ng/mL doxycycline led to a significant increase in p21 expression (Fig. 4A–C), while markedly increasing surviving cell number in response to combination treatment with gemcitabine and 500 nmol/L PF-477736 (Fig. 4A–C). Furthermore, immunoblot assays showed that there was a significant decrease in expression of cleaved PARP-1 and p-γH2A.X-S139 levels in doxycycline-treated, p21-expressing 97-1, 647V and RT-112 cell lines (Fig. 4A–C). In addition, knockdown of p21 in the TCCSUP cell line (p53mut/p21wt) sensitized those cells to treatment with gemcitabine–Chk1 inhibitor combinations, with enhanced PARP1 cleavage and reduced cell viability (Supplementary Fig. S2A and S2B). These results confirm that this sensitivity to combined gemcitabine–Chk1 inhibitor treatment is indeed dependent upon p21 status in bladder cancer cells.

Effectiveness of gemcitabine–Chk1 inhibition in a bladder cancer xenograft model

To examine the potential synergistic effect of Chk1 inhibition with gemcitabine on *in vivo* tumor growth, we used the RT-112 cell line in a xenograft mouse model. Four groups of tumor-bearing CB17/SCID mice were treated with vehicle, gemcitabine, PF-477736, or the combination of both drugs. Mice were treated every 3 days after tumor volume crossed a threshold size. Treatment continued until humane sacrifice was required, or 7 weeks had passed. Both gemcitabine and PF-477736 alone caused a small but significant decrease in tumor growth (Fig. 5A). However, combination treatment led to significantly slower tumor growth than either agent alone with little net tumor growth during the 7 weeks of this therapy (Fig. 5A). Furthermore, tumor weight and size were significantly reduced in the combination therapy mice, compared with controls (Fig. 5B and C). None of the treated mice showed any evidence of toxicity or weight loss (Fig. 5D). These results demonstrate that a combination of a Chk1 inhibitor (PF-477736) with gemcitabine has synergistic effects on inhibiting tumor growth in this xenograft model.

Discussion

In the present study, we have shown that Chk1 inhibition can sensitize bladder cancer cells to gemcitabine through abrogating DNA damage–induced G_2–M cell-cycle arrest. Importantly, we demonstrated that p53/p21 dual-mutant bladder cancer cells undergo massive apoptotic cell death in response to combined Chk1 inhibition and gemcitabine treatment (Fig. 3C), and that this effect contrasts with what is seen in other bladder cancer cell lines without dual p53–p21 mutation. Our reexpression experiments demonstrate that this response is critically dependent on lack of p21 expression, as reexpression rescues the sensitivity to dual treatment (Fig. 4). In addition, our knockdown experiments (Supplementary Fig. S2) demonstrate that CDKN1A knockdown leads to enhanced sensitivity to this combination therapy. Finally, our mouse xenograft experiments demonstrate that combined treatment is effective in causing a marked reduction of the p53/p21 dual-mutant bladder cancer cell line growth *in vivo* in the absence of toxicity (Fig. 5).

Although bladder cancer cell lines with loss of either p53 or p21 alone showed some sensitivity to gemcitabine–Chk1 inhibitor treatment, this was much less than what was seen in the dual-mutant cell lines. This sensitivity correlated with a higher level of phosphorylation and activation of Chk1 kinase (p-Chk1-S345, Fig. 1D) in response to gemcitabine in the dual p53/p21-mutant bladder cancer cell lines (647V and RT-112) compared with either p53- or p21-deficient cells (TCCSUP, J82, and 97-1). Cell-cycle analysis also showed that G_2–M phase arrest occurred only in p53/p21 dual-mutated tumor cells upon gemcitabine treatment, suggesting that loss of either p53 or p21 function alone is not sufficient to disrupt the G_1–S cell-cycle checkpoint in bladder cancer cells.

It has been recently reported that knockdown of p21 can enhance the cytotoxic response to a genotoxic agent combined

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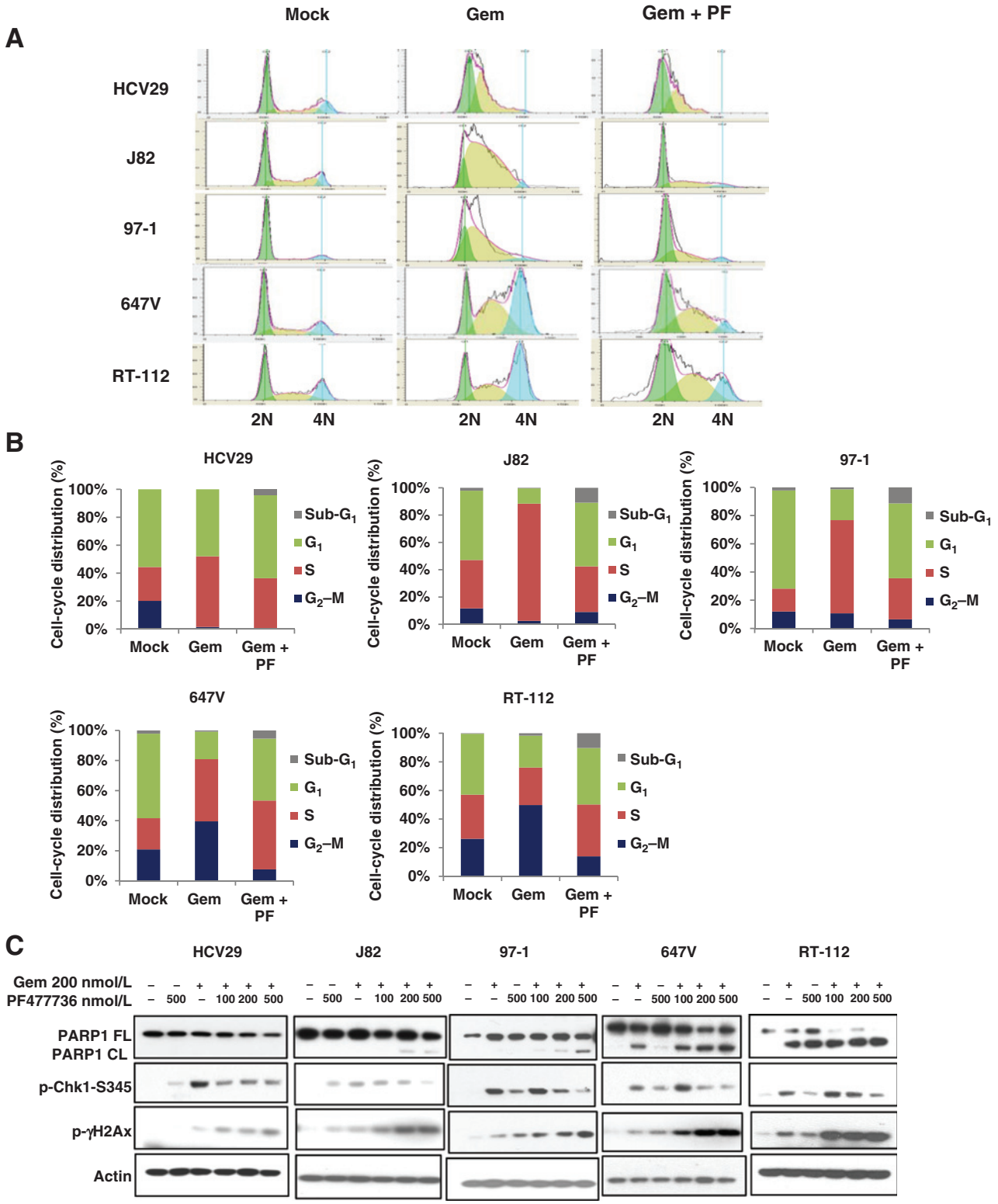

Figure 3.
Cell-cycle kinetics and apoptosis of bladder cancer cell lines treated with gemcitabine and Chk1 inhibitor. A, effects of no treatment, gemcitabine alone (200 nmol/L × 1 hour), and gemcitabine followed by PF-477736 (500 nmol/L × 23 hours) on cell-cycle distribution of HCV29, J82, 97-1, 647V, and RT-112 cell lines measured 23 hours following cytotoxic treatment. Cell-cycle distribution was assessed by PI-DNA staining and FACS assay. B, cell-cycle distribution in gemcitabine-treated bladder cancer cells (as in A). Histograms, for cell-cycle distribution, as assessed by PI-DNA staining and FACS assay. C, bladder cancer cell lines were exposed to gemcitabine or PF477736, as well as the combination of these two agents for 18 hours. Immunoblot analysis was used to examine levels of PARP1, p-Chk1-345, p-γH2A.X-S139, and β-actin. Note major increase in cleaved PARP1 (PARP1-CL) and p-γH2A.X-S139 in the 647V and RT-112 cell lines. PARP1-FL is full-length PARP1.

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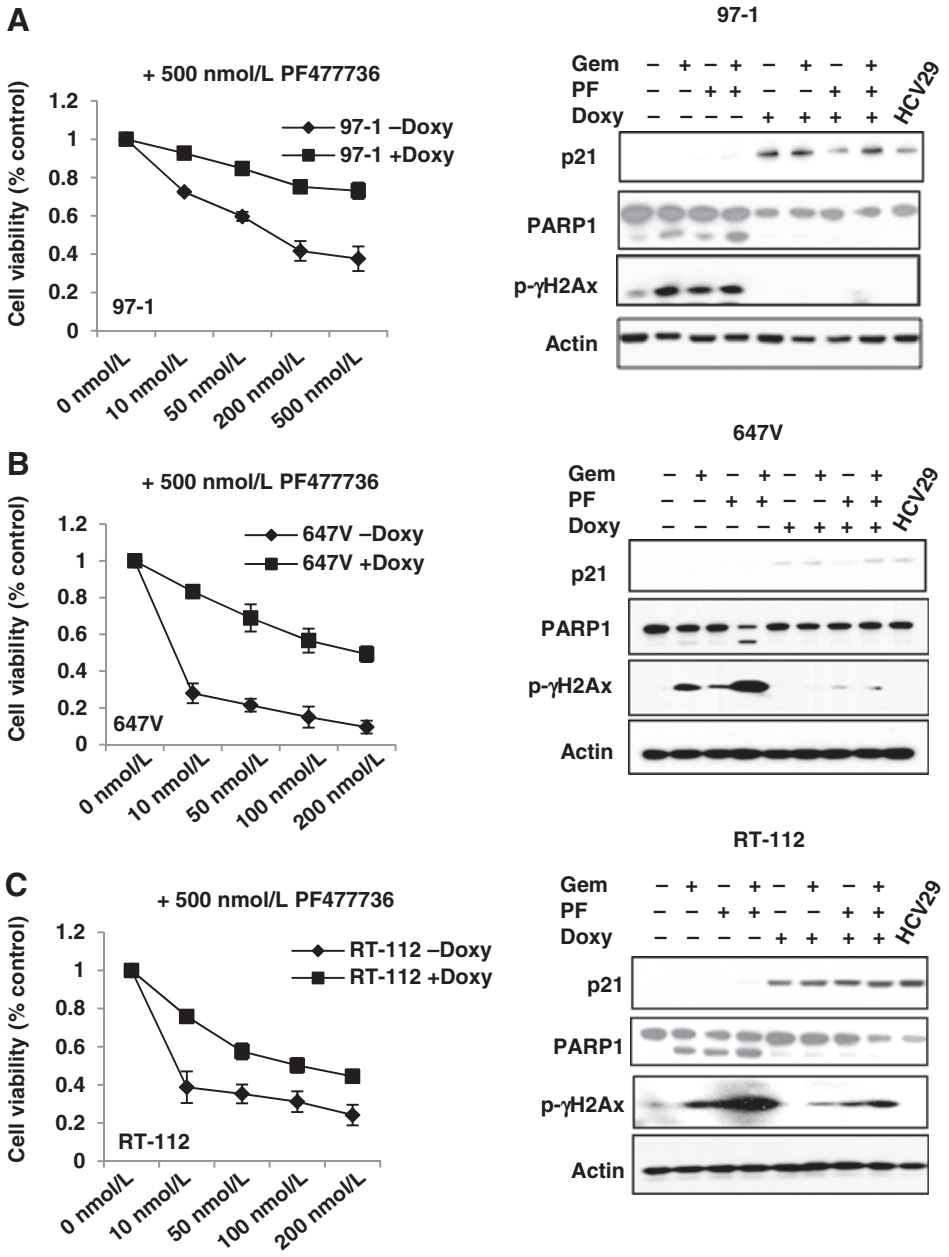

Figure 4.
p21 expression is critical to the response to combination treatment in p21-deficient bladder cancer cell lines. A–C, left, cell viability curves for 97-1, 647V, and RT-112 cell lines expressing doxycycline (Doxy)-inducible p21. Cell number was assessed by CellTiter-Glo after treatment with or without doxycycline to induce $p21^{Cip1}$ expression, and treatment with 500 nmol/L PF-477736 and variable doses of gemcitabine. Right, immunoblot analysis of these treated cells. Note the induction of p21 and reduction in cleaved PARP-1 and p-γH2A.X expression in cells exposed to doxycycline.

with Chk1 inhibition in TP53-mutant colonic epithelial cells (26). However, it is notable that TP53/CDKN1A double-mutant cancers are seen at appreciable frequency only in bladder cancer, at a frequency of 7%. Nonetheless, our findings (Supplementary Fig. S2) as well as the previous publication (26) suggest that methods that inhibit CDKN1A function in tandem with nucleoside analogue treatment and Chk1 inhibition might be effective for a variety of malignancies in which TP53 function is lost. However, one can anticipate that this combination approach might lead to much greater toxicity as all cells would be exposed to effects of inhibition of CDKN1A function.

Muscle-invasive bladder cancer is a difficult and relatively common malignancy, for which there has been no major advance beyond cisplatin-based combination chemotherapy and surgery in the past 30 years (23). There is no widely recognized second-line therapy for treatment of this disease, and no new drugs have been approved for bladder cancer in the past 20 years. Recent genomic profiling of bladder cancer by multiple groups, including TCGA, has led to identification of multiple potential therapeutic targets (19). Based upon our results, we propose that combined TP53/CDKN1A mutation with loss of function of both proteins defines a molecular subset of bladder cancer with particular sensitivity to the combination of a DNA-damaging chemotherapy (gemcitabine used here) and a Chk1 inhibitor. Hence, on the basis of these preclinical data, we encourage translation of these findings to human clinical trials in patients with bladder cancer with combined TP53/CDKN1A mutation.

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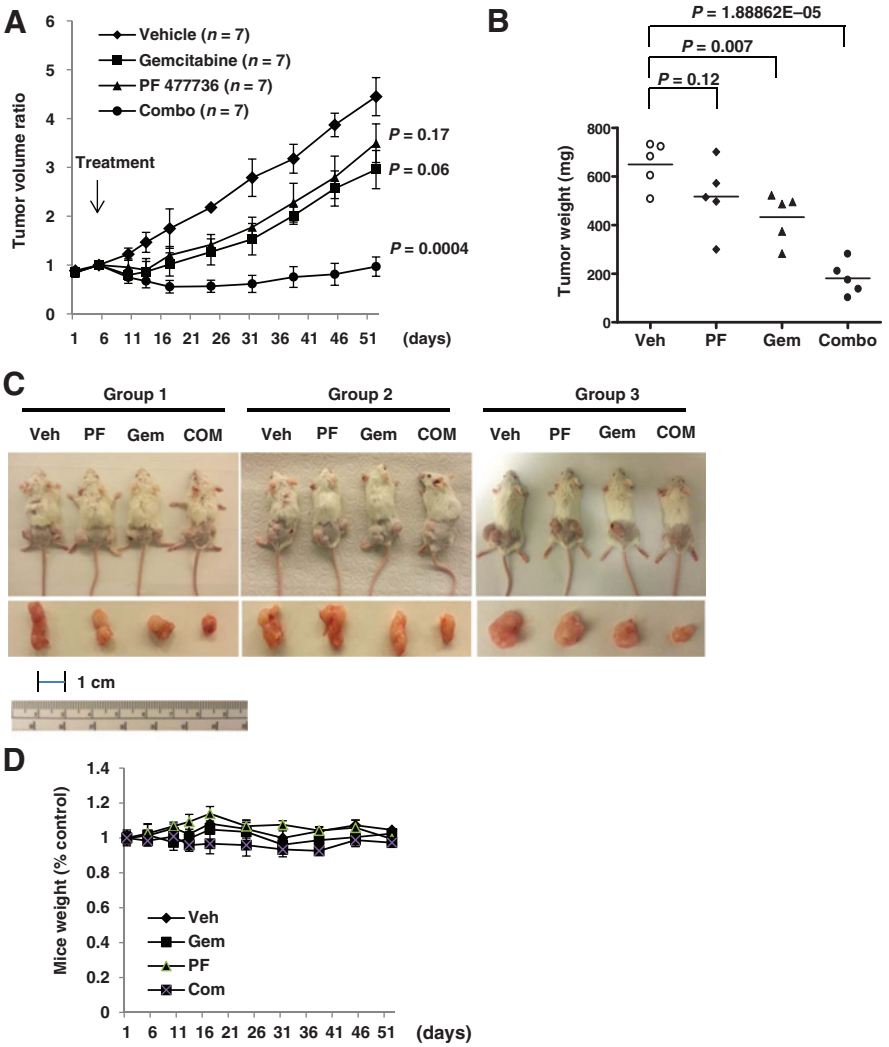

Figure 5.
Chk1 inhibition synergizes with gemcitabine to reduce bladder cancer cell growth in a xenograft model. A, RT-112 (5×10^6) cells were injected into the flanks of CB17/SCID mice. After the subcutaneous tumors reached a size of 10 cm^3, mice were randomized to treatment with vehicle, gemcitabine (50 mg/kg) every 3 days, PF-477736 (15 mg/kg) every 3 days, or both drugs every 3 days at the same dose. Mean ± SD of tumor volume is shown. B and C, tumor weight (B) and tumor images (C) 7 weeks after mice were injected s.c. with the RT-112 cell line, and treated with gemcitabine or PF-477736, or the combination, as in A; mean ± SD. D, weights of mice undergoing treatment with vehicle, gemcitabine, PF-477736, or the combination. Mean ± SD of the normalized weight from the time of treatment initiation is shown.

Disclosure of Potential Conflicts of Interest

D.J. Kwiatkowski is a consultant/advisory board member for Novartis. No potential conflicts of interest were disclosed by the other author.

Authors' Contributions

Conception and design: Y. Liu, D.J. Kwiatkowski
Development of methodology: Y. Liu
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Liu, D.J. Kwiatkowski
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Liu, D.J. Kwiatkowski
Writing, review, and/or revision of the manuscript: Y. Liu, D.J. Kwiatkowski
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D.J. Kwiatkowski
Study supervision: D.J. Kwiatkowski

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