

Development of Cell Cycle Active Drugs for the Treatment of Gastrointestinal Cancers: A New Approach to Cancer Therapy

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ABSTRACT

The cell cycle represents a series of tightly integrated events that allow the cell to grow and proliferate. An essential part of the cell cycle machinery is the cyclin-dependent kinases (CDKs). When activated, the CDKs provide a means for the cell to move from one phase of the cell cycle to the next (G1 to S or G2 to M). The cell cycle serves to protect the cell from genotoxic stress. In the setting of DNA damage, the CDKs are inhibited and the cell undergoes cell-cycle arrest. This provides the cell the opportunity to repair its own damaged DNA before it resumes cell proliferation. If a cell continues to cycle with its damaged DNA intact, the apoptotic machinery is triggered and the cell will undergo apoptosis. In essence, cell cycle arrest at these critical checkpoints represents a survival mechanism, which provides the tumor cell the opportunity to escape the effects of lethal DNA damage induced by chemotherapy. Over the past several years, a series of new targeted agents has been developed that promote apoptosis of DNA damaged tumor cells either during cell cycle arrest or following premature cell cycle checkpoint exit, such that tumor cells re-enter the cell cycle before DNA repair is complete. An understanding of the cell cycle and its relationship to p53 are critical for the successful clinical development of these agents for the treatment of patients with gastrointestinal cancers.

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THE CELL CYCLE: AN OVERVIEW

The cell cycle is a critical regulator of the processes of cell proliferation and growth, as well as of cell division, following DNA damage. It is the mechanism by which cells reproduce, and is typically divided into four phases. The periods associated with DNA synthesis (S phase) and mitosis (M phase) are separated by gaps of varying length called G1 and G2 (Fig 1). Progression of a cell through the cell cycle is promoted by a number of cyclin dependent kinases (CDKs) which, when complexed with specific regulatory proteins called cyclins, drive the cell forward through the cell cycle. At least 9 structurally related CDKs (CDK1 through CDK9) have been identified, though not all have clearly defined cell cycle regulatory roles. A consider-

able number of cyclins have been identified to date (cyclin A through cyclin T). CDKs/cyclin complexes themselves become activated by phosphorylation at specific sites on the CDKs by cdk7/cyclin H, also referred to as CDK-activating kinase (CAK).¹ Cyclin D isoforms (cyclin D1 through D3) interact with CDK 2, 4, and 6 and drive a cell's progression through G1. The association of cyclin E with CDK2 is active at the G1/ S transition and directs entry into S-phase. S-phase progression is also directed by the cyclin A/CDK2 complex, and the complex of cyclin B with CDK1 (also known as cdc2) is important for the cell cycle entry from G2 to M.

There exist corresponding cell cycle inhibitory proteins (cyclin-dependent kinase inhibitors [CDKIs]) that serve as negative regulators of the cell cycle and

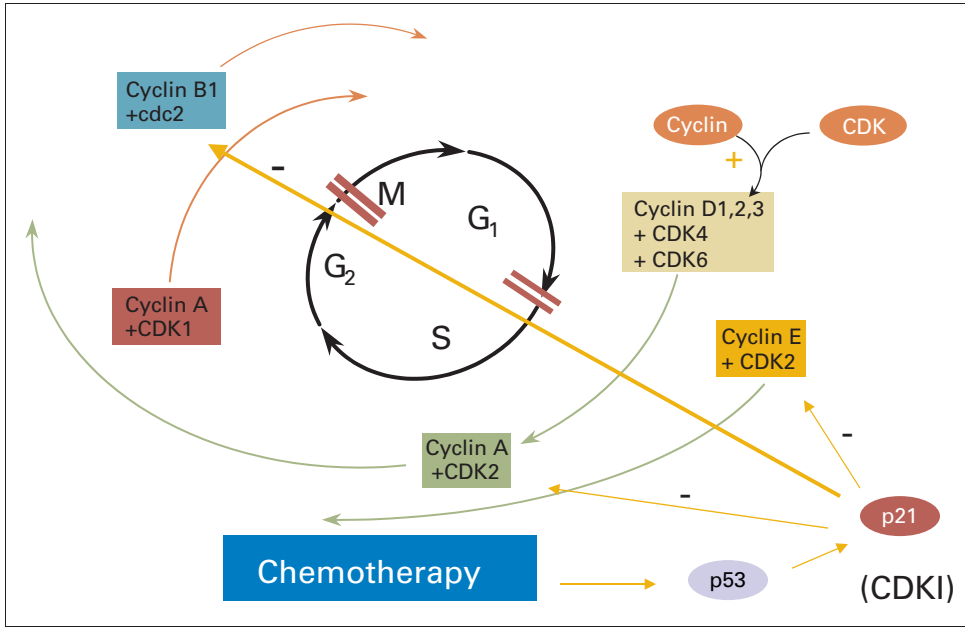


Fig 1. The cell cycle and the response to DNA damage. DNA damage (induced either by chemotherapy or by radiotherapy) activates p53, which acts as a transcription factor for the induction of p21. As a cyclin-dependent kinase inhibitor (CDKI), p21 then binds to and inhibits the activity of the cyclin E/CDK2, cyclin A/CDK2, and the cyclin B1/cdc2 complexes. This causes arrest in both the G1 and G2 phases of the cell cycle.

stop the cell from proceeding to the next phase of the cell cycle. The INK4 (for inhibitor of cdk4) class of CDKI's, notably p16^{lnk4a}, p15^{lnk4b}, p18^{lnk4c}, and p19^{lnk4d}, bind and inhibit cyclin D-associated kinases (CDK2, -4, and -6). The KIP (kinase inhibitor protein) group of CDKI, p21^{waf1}, p27^{kip1}, and p57^{kip2}, negatively regulate cyclin E/CDK2 and cyclin A/CDK2 complexes.²

Cell Cycle Arrest: The p53/p21 Axis

The failure of many chemotherapeutic agents reflects an inability of these drugs to induce apoptosis. The cell cycle and apoptosis, though, are intimately related, as evidenced by the central role of p53, both in cell cycle arrest and in the induction of apoptosis. In terms of cell cycle arrest, the activation of p53 serves to guard genomic fidelity. DNA damage (induced either by chemotherapy or by radiotherapy) activates p53, which acts as a transcription factor for the induction of p21. As a CDKI, p21 then binds to and inhibits the activity of the cyclin E/CDK2, cyclin A/CDK2, and the cyclin B1/cdc2 complexes.² This causes arrest in both the G1 and G2 phases of the cell cycle (Fig 1).^{3,4} G1 arrest is due primarily to the induction of p21, whereas the arrest in G2 is due to the induction of both p21 and 14-3-3σ.^{5,6} Thus, the growth arrest that is mediated by p53 and p21 axis, in the setting of DNA damage, allows the cell to undergo cell cycle arrest and DNA repair. This prevents damaged DNA from being propagated throughout the various stages of the cell cycle to cell division. The cell cycle thus ensures that when cell division does occur, the two resulting cells carry the proper amount of DNA and that the DNA itself is intact without mutations or damage. Otherwise, a proliferating cell with damaged DNA will be recognized by the apoptotic machinery

and apoptosis will be induced. Thus, following genotoxic stress, the cell cycle provides all cells a mechanism for DNA repair and cell survival. From an oncology point of view, this effect will work to the benefit of providing healthy cells a means to escape the toxic effects of chemotherapy. However, these same mechanisms also provide the tumor cell the same protection and will severely limit the effectiveness of chemotherapy.

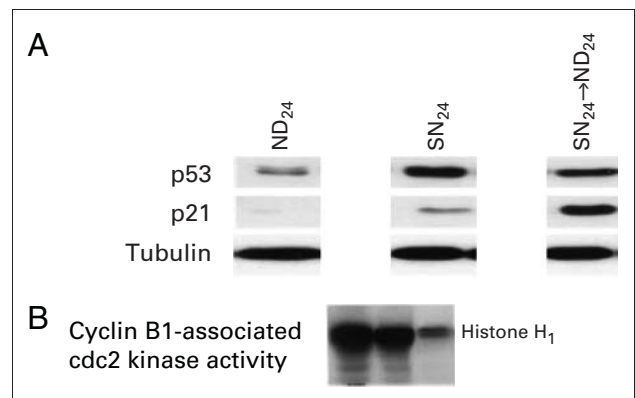


Fig 2. p53 and p21 are induced after SN-38 treatment of HCT116 cells that have normal p53 (HCTp53^{wt}) with inhibition of cyclin B1/cdc2 kinase activity. (A) Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and Western blotting of p53 and p21. Asynchronous cells were either untreated (ND₂₄), treated with SN-38 for 24 hour (SN₂₄), or treated with SN-38 for 24 hour followed by drug free media (SN₂₄ → ND₂₄). Fifty micrograms of protein was resolved on 15% SDS-PAGE gels and transferred to polyvinylidene fluoride membranes. The membranes were probed with mouse monoclonal p53 or p21 antibodies. Tubulin is shown to confirm equal loading of the protein. The results indicate a time dependent increase in p53 and p21 protein expression after treatment with SN-38. (B) Cyclin B1/cdc2 kinase activity. Two hundred micrograms of protein from these cells was immunoprecipitated with anti-cyclin B1 and the precipitates were assayed for kinase activity using histone H1 as substrate. Cyclin B1/cdc2 kinase activity is inhibited after SN-38 followed by no drug at the time of maximal p21 induction (lane 3).

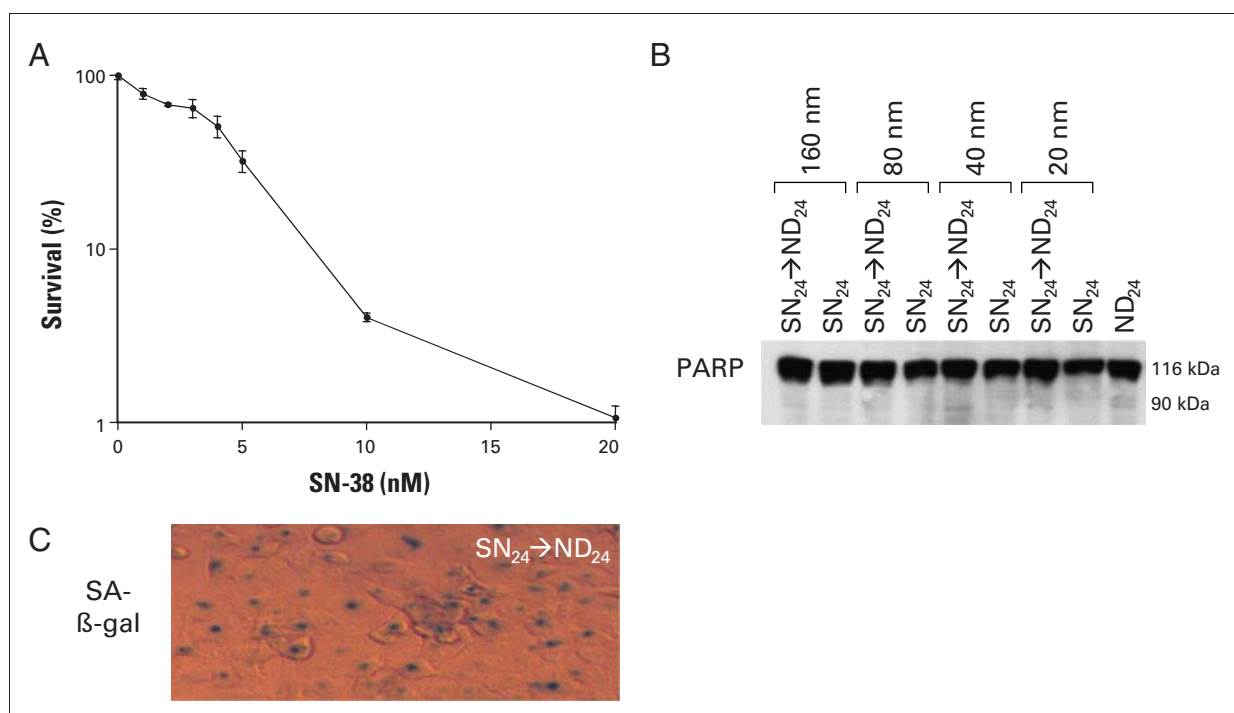


Fig 3. Effect of SN-38 on colony formation, poly-(ADP ribose) polymerase (PARP) cleavage and cellular senescence. HCT116 cells that have normal p53 (HCTp53^{wt}) were treated with increasing concentration of SN-38. (A) Colony formation. Colony formation is inhibited 100% with 20 nmol/L of SN-38. (B) Sodium dodecyl sulfate (SDS)-PAGE and Western blotting for PARP. SN-38 and SN-38 for 24 hour followed by drug free media (SN₂₄ → ND₂₄) at concentrations up to 160 nmol/L induces no PARP cleavage. (C) Cellular senescence. SN-38 induces single cells (no colonies) that stain positive for senescence associated β galactosidase (SA-β-gal).

The role of the cell cycle in limiting the effectiveness of chemotherapy has been illustrated in human colon cancer cell lines (HCT116) that have been made deficient in the CDKI p21. Cells with a normal p21 (HCTp21+/+), when irradiated with gamma-radiation, undergo a cell cycle growth arrest followed by clonogenic survival; whereas cells lacking p21 (HCTp21-/-), when irradiated with gamma-radiation, do not undergo a cell cycle growth arrest and proceed to apoptosis.⁷ Similar effects have been shown with chemotherapy against HCT116 cells that either have normal p53 (HCTp53^{wt}) or have been made deficient in p53 (HCT^{mut}). For these studies HCTp53^{wt} cells were treated in vitro with 20 nmol/L of SN-38, a physiologic dose of the active metabolite of irinotecan.⁸ As predicted, the induction of DNA damage by SN-38 on the HCTp53^{wt} cells induces p53 protein expression within 24 hours of drug exposure (SN₂₄; Fig 2A). The expression of p53 is maintained for at least another 24 hours, even in the presence of drug-free media (SN₂₄ → ND₂₄). During this same time course, there is induction of p21 protein, which is initially observed by 24 hours (SN₂₄), but protein expression continues to increase for an additional 24 hours in the presence of drug-free media (SN₂₄ → ND₂₄). Tubulin is shown in this Western blot to confirm equal loading of the protein under this treatment conditions.

This induction of p21 should then inhibit cyclin B1 associated cdc2 kinase activity and result in a G2 cell cycle

arrest. The HCTp53^{wt} were again treated with 20 nmol/L SN-38, under these same treatment conditions, and cyclin B1-associated cdc2 kinase was immunoprecipitated. The activity of the cyclin B1/cdc2 kinase complex was then measured by examining the inhibition of phosphorylation of histone H1 substrate in this kinase assay. As shown in Fig 2B, at the time of maximal p21 induction (SN₂₄ → ND₂₄, lane 3) there is significant inhibition of cyclin B1 associated cdc2 kinase activity, resulting in a G2 cell cycle arrest.⁸

Cell Cycle Arrest Versus Cell Death

In assessing drug response in oncology, clonogenic assays are considered among the gold standard to examine a drug's effect. In these assays, a drug's effect is assessed by inhibiting colony formation (eg, a single cell is plated and drug is added such that if the drug is effective it should prevent the dividing cells from forming a cell colony). Treatment of HCTp53^{wt} with increasing concentrations of SN-38 does inhibit colony formation such that with 20 nmol/L of drug there is complete suppression of colony formation (Fig 3A). However, the question is whether these colon cancer cells, which have undergone G2 cell cycle arrest following treatment with 20 nmol/L of SN-38, are alive or dead. Apoptosis is considered the biochemical process of cell death. The molecular cascade of apoptosis is characterized by the early release of

mitochondrial cytochrome C, activation of apoptotic protease activating factor (Apaf-1), activation of caspase 9, and subsequent cleavage of downstream, or effector caspases in a self-amplifying cascade. Effector caspases finally degrade a number of cellular proteins, such as poly-adenosine 5'-diphosphate- ribosyl polymerase (PARP), laminin and β -actin.^{9,10} PARP cleavage can be assessed by Western blot. In the setting of apoptosis, PARP is cleaved from 116 kDa to its 90 kDa cleaved product. As shown in Figure 3B, with SN-38 alone for 24 hours (SN₂₄) or SN-38 followed by drug-free media (SN₂₄ → ND₂₄), there is no PARP cleavage up to SN-38 concentrations of 160 nM.⁸ This would suggest cell survival. An examination of the treated plates in fact indicates the presence of single cells that have managed to survive without forming colonies. These cells have undergone cell cycle arrest and some have entered a phase of cellular senescence as indicated by positive staining for senescence associated β galactosidase (SA- β -gal; Fig 3C).

This data would indicate that the treatment of colon cancer cells with an intact p53 and p21 axis undergo cell cycle arrest following SN-38-induced DNA damage, but they do not undergo apoptosis. This is not limited to topoisomerase I inhibitors, but extends to all chemotherapy or radiotherapy that results in genotoxic stress and damage to the DNA. The results indicate that though the tumor cell may undergo cell cycle arrest, this is not sufficient to result in cell death, an event we believe is critical for cancer cure. Yet, p53 is also an essential inducer of apoptosis by activating the transcription of multiple apoptosis-associated genes.^{11,12} The p53-target genes involved in apoptosis include *Bax*, *Noxa*, *Puma*, *Pidd*, *perp*, *p53AIP1*, *DR5* and *Bid*.¹³⁻²² For example, it has been reported that *Puma* (p53 upregulated modulator of apoptosis) is tightly regulated by p53 and has properties that indicates it mediates p53-associated apoptosis in colon cancer cells. PUMA is a BH3 only domain protein and is exclusively localized to mitochondria, where it interacts with Bcl-2 and Bcl-xL. Through its BH3 domain, it promotes multimerization of Bax and mitochondria-dependent cell death.¹⁵

Flavopiridol and the Shift From Cell Cycle Arrest to Cell Death

Is it then possible to shift this paradigm such that p53 functions as a mediator of apoptosis rather than an inducer of cell cycle arrest, or can we shift a cell from cell cycle arrest to cell death? Flavopiridol is a novel anti-neoplastic agent that originally was noted for its ability to inhibit the activity of a number of protein kinases. Flavopiridol (Fig 4) is now best classified as a CDKI because of its considerable affinity for CDKs and its ability to induce cell cycle arrest in a number of cell lines.^{23,24} It has been shown to bind to and directly inhibit CDK1,

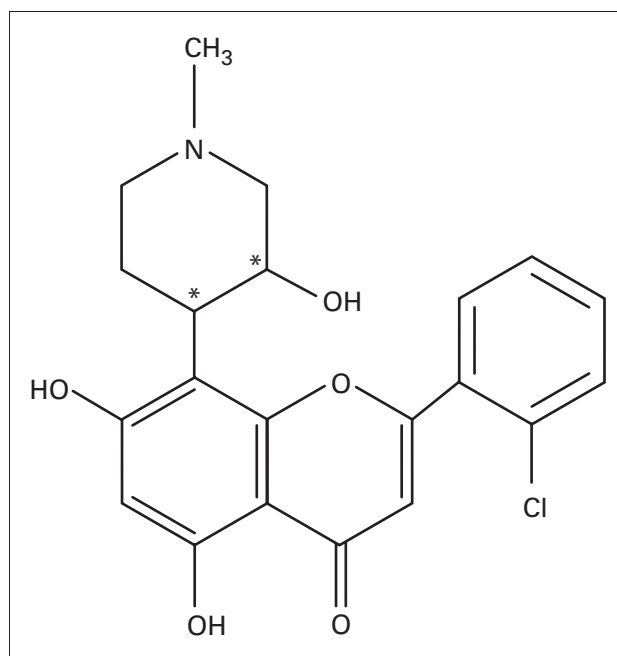


Fig 4. Flavopiridol.

CDK2, CDK4, and CDK6. Flavopiridol administration has been associated with the selective induction of apoptotic cell death, particularly in hematopoietic cell lines.^{25,26} This induction of apoptosis may be mediated by an early activation of the MAPK protein kinase family of proteins (MEK, p38, and JNK), leading to activation of caspases.²⁷ It has also been shown to inhibit anti-apoptotic molecules

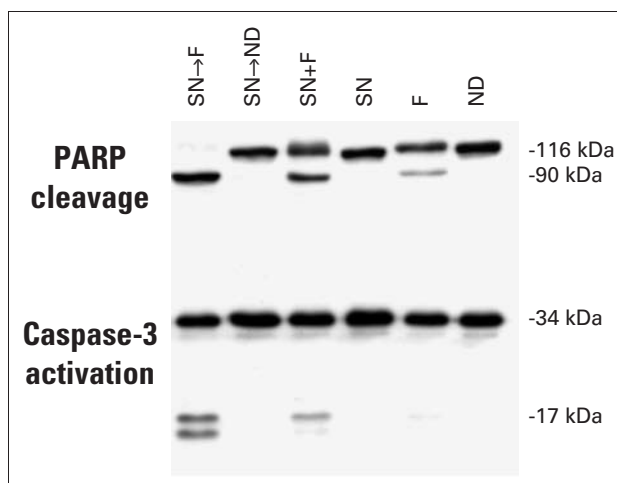


Fig 5. Flavopiridol potentiates SN-38 induced apoptosis: sodium dodecyl sulfate (SDS)-PAGE and Western blotting with poly-(ADP ribose) polymerase (PARP) and caspase-3. HCT116 cells that have normal p53 (HCTp53^{wt}) were treated with 20 nmol/L SN-38 and 150 nmol/L flavopiridol with different schedules and protein was isolated. Fifty micrograms of protein was loaded on 8% or 16% (PARP and caspase-3, respectively) SDS-PAGE gels, transferred to polyvinylidene fluoride membranes and probed with PARP or caspase-3 antibodies. The equal loading of protein was examined by amido black. As shown, the greatest effect is observed with sequential SN-38 followed by flavopiridol (SN→F). SN+F, concomitant therapy; SN→ND, SN-38 followed by drug free media.

including bcl-2,²⁵ XIAP,⁸ p21,⁸ mcl-1,^{28,29} cyclin D1,³⁰ and phospho-survivin.³¹ The suppression of these proteins is, in part, due to flavopiridol's general effect on inhibiting transcription, resulting in a suppression of gene expression.³²

Therefore, in view of flavopiridol's diverse effects on inhibiting anti-apoptotic proteins, is it possible that the addition of flavopiridol to HCTp53^{wt} cells, following the induction of G2 cell cycle by SN-38, will induce apoptosis? As shown in Figure 5, as predicted, treatment with 20 nmol/L of SN-38 alone for 24 hours (SN₂₄) or SN₂₄→ND₂₄, failed to induce PARP cleavage. However, PARP was partially degraded into its 90 kDa cleaved product with concomitant therapy of SN-38 and flavopiridol (SN + F) and completely degraded with sequential therapy of SN-38 followed by flavopiridol (SN→F).⁸ PARP is the substrate for the final common executioner of apoptosis caspase-3.¹⁰ In keeping with these observations, higher activation of caspase-3 (ie, formation of 24, 17 and 12 kDa form) was observed in sequential therapy of SN-38 followed by flavopiridol compared to other treatment schedules (Fig 5). The combination of SN-38 and flavopiridol together for 24 hours ([SN+F]₂₄) also showed significant activation of caspase-3, albeit to a lesser degree, when compared to SN-38 followed by flavopiridol.

The augmentation of irinotecan's antitumor effect was also validated in HCTp53^{wt} xenografts.⁸ HCTp53^{wt} tumor implants were treated with irinotecan, flavopiridol, and irinotecan followed by flavopiridol separated by varying intervals from 4 to 16 hours (Fig 6). In these experiments, the greatest tumor regression (the percent decrease in tumor volume) and cures were observed if the interval between irinotecan and flavopiridol was at least 7 to 16 hours (CPT11-F₇ and CPT11-F₁₆). Two weeks after the end of treatment (day 30), there was a 40% ± 25% regression of the tumor in mice treated with irinotecan alone (CPT-11); whereas in mice treated with irinotecan followed by 7- or 16-hour flavopiridol, the tumor regression was 86% ± 9% or 82% ± 5%, respectively. The difference between the areas under the volume-time curve of irinotecan alone and irinotecan followed by flavopiridol after 7 or 16 hours were statistically significant ($P = .0002$ and $P = .0005$, respectively). The cure rates for irinotecan followed by 7-hour or 16-hour flavopiridol were 30% (3 of 10) or 29% (2 of 7), respectively. This was in contrast to irinotecan alone or irinotecan followed by flavopiridol at 4 hour, where no cures were found.⁸

The conversion from cell cycle arrest to cell death by flavopiridol on SN-38 and irinotecan treated colon cancer cells remains the subject of ongoing laboratory studies.

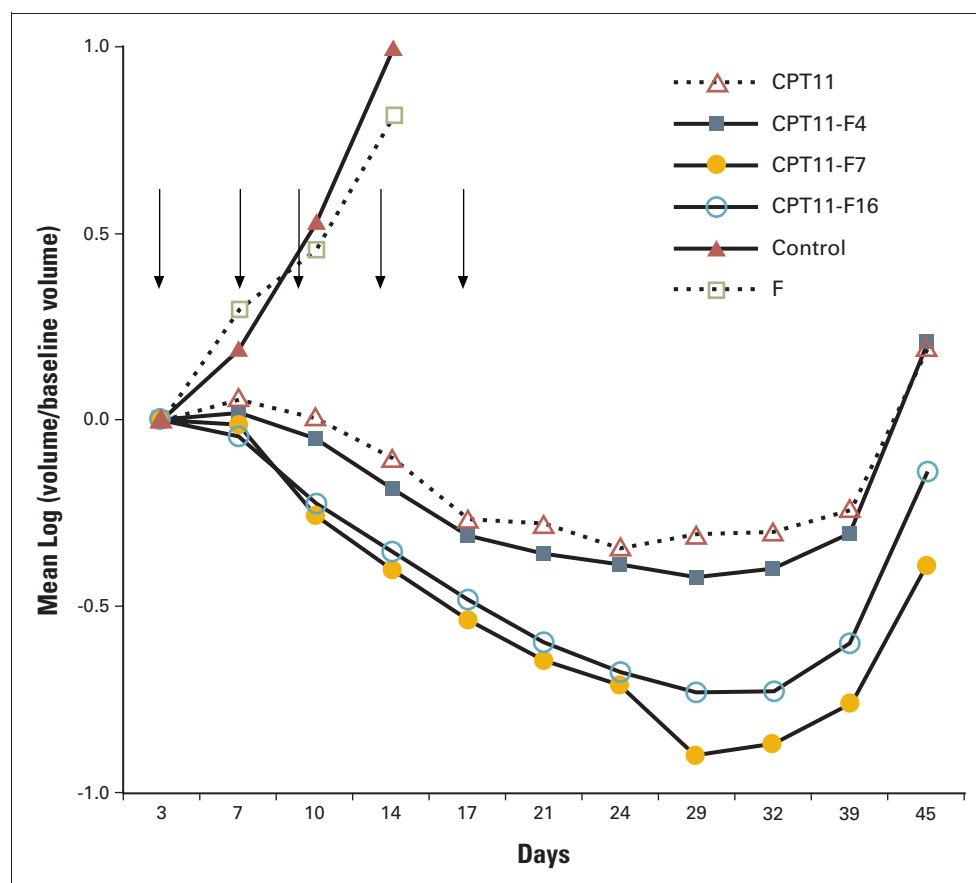


Fig 6. The effect of CPT11 and flavopiridol on growth of established xenografts of HCT116 cells that have normal p53 (HCTp53^{wt}) in nude mice. Mice were treated with CPT11 and flavopiridol alone or in combination as CPT11 followed 4, 7 and 16 hour later by flavopiridol. In these experiments, the greatest tumor regression (the percent decrease in tumor volume) and cures were observed if the interval between irinotecan and flavopiridol was at least 7 to 16 hour (CPT11F7 and CPT11F16). CPT11, irinotecan alone; CPT-F4, CPT11 followed by flavopiridol separated by an interval of 4 hours; CPT-F7, CPT11 followed by flavopiridol separated by an interval of 7 hours; CPT11-F16, CPT11 followed by flavopiridol separated by an interval of 16 hours; F, flavopiridol alone. Arrows indicate days and frequency of therapy.

However, there are recent studies indicating that with the sequential therapy of SN → F in the HCTp53^{wt} cells there is inactivation by flavopiridol of small molecules that inhibit apoptosis (XIAPs) and there is induction of molecules such as PUMA that promote apoptosis.^{8,33} In addition, the combination therapy induces phosphorylation of p53 at sites that have been associated with the induction apoptosis.³³ It has also been shown that flavopiridol suppresses the induction of p21 by SN-38.⁸ P21 has dual functions. First, it functions as a CDKI. Second it inhibits apoptosis by binding to and inhibiting the activity of caspase 3. The proapoptotic effect of flavopiridol on G2 arrested cells is highly dependent on cells with a functional p53. In fact, HCT116 colon cancer cells, which are deficient in p53 (HCTp53^{mut}), are resistant to the effects of SN-38 with flavopiridol.³³ Thus, by modulating the expression of a wide range of molecular events associated with apoptosis, in tumor cells that are wild type for p53, flavopiridol is able to convert an arrested cell to a dying cell.

On the basis of these preclinical observations, a hypothesis can be generated indicating that patients who are wild type for p53 would have the most benefit from the combination of sequential CPT-11 followed by flavopiridol, whereas patients with mutant p53, in fact, would

Table 1. Response According to p53 Status

Patient No.	p53	p21 Change	Response	Duration (months)
1	WT	↑	POD	2.3
2	WT	NA	SD	16.5
3	Mut	No Δ	POD	2.7
4	Mut	NA	POD	2.7
5	Mut	No Δ	POD	2.7
6	WT	No Δ	SD	8.3
7	WT	NA	SD	9.3
8	WT	No Δ	SD	7.1
9	WT	No Δ or ↓	PR	7.6

NOTE: Boldfaced type indicates all patients with mutant p53.
Abbreviations: WT, wild type; POD, disease progression; NA, not assessable; SD, stable disease; mut, mutated; PR, partial response.

be resistant to the combination therapy and show no clinical benefit. This hypothesis has been tested in the phase I clinical trial of weekly sequential CPT-11 followed by flavopiridol, administered 4 weeks out of 6.³⁴ At the expanded maximum tolerated dose, serial biopsies were obtained on patients before treatment and then 24 to 48 hours following week 2 of therapy. Tumors were evaluated for staining by immunohistochemistry for p53 and p21.³⁴ The results are summarized in Table 1. We observed that patients who demonstrated disease control indeed were

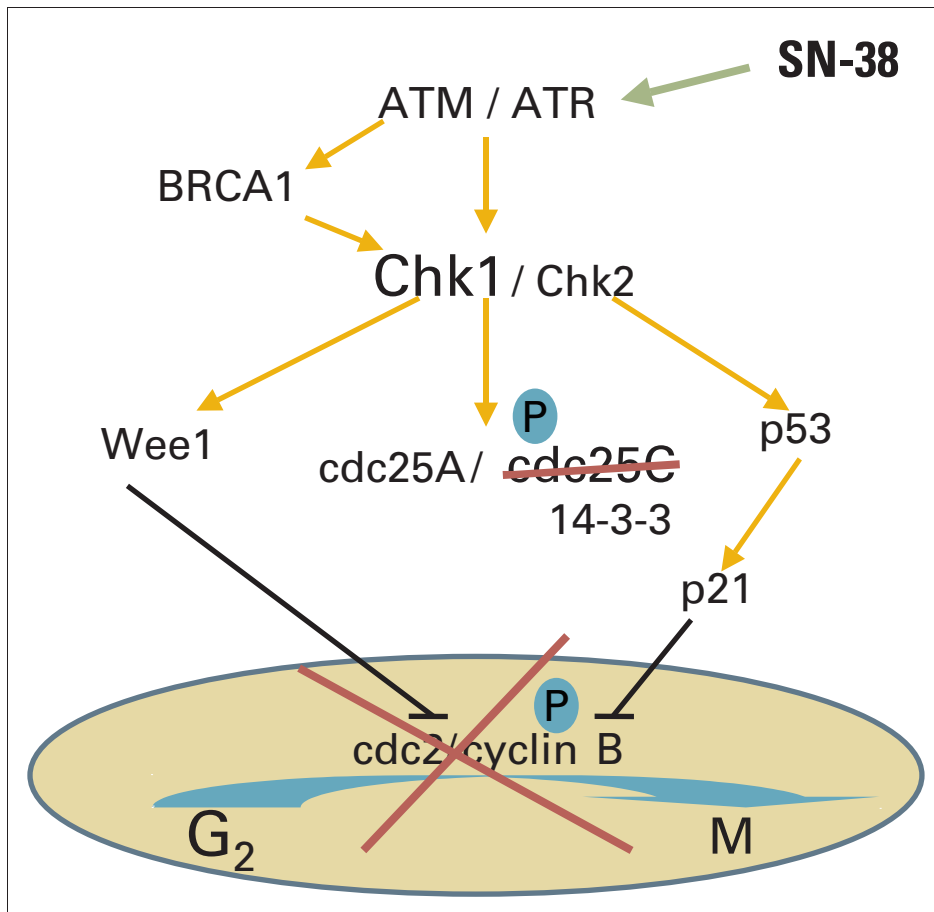


Fig 7. DNA damage induced by SN-38 activates Chk1. In response to DNA damage, Chk1 is activated, which results in the phosphorylation of the phosphatase Cdc25c on serine 216. The 14-3-3 protein binds to the phosphorylated form of Cdc25C. As cyclinB1/cdc2 kinase complex must be dephosphorylated to allow cells to exit G2 into M, this complex prevents this phosphatase from entering the nucleus, where it is required to dephosphorylate cdc2. As a result, cells remain arrested in G2 and can not enter the M phase of the cell cycle. ATM/ATR, ataxia-telangiectasia mutated/ataxia-telangiectasia and Rad3 related.

p53 wild type and had a low p21 level that did not increase or, in fact, decreased with therapy. Patients with disease progression had an increase in p21 or were mutant for p53. Though preliminary, these data support the preclinical model that sensitivity to the combination is related to the p53-p21 DNA damage/repair axis. This is not the first study to suggest that suppression of p21 may be predictive of benefit to treatment in gastrointestinal cancers. In 66 patients undergoing preoperative chemotherapy and radiotherapy, expression of *p53*, *BAX*, *p21*, *Ki-67*, and *hMSH2* were investigated by immunohistochemistry in pre- and post-treatment tumor samples. Patients with a decrease in p21 expression following radiochemotherapy had better disease-free survival ($P = .03$). The dynamic induction of p21 was associated with a worse treatment outcome following neoadjuvant radiochemotherapy and tumor resection.³⁵

Chk1 and the G2 Checkpoint

Flavopiridol is an example of a new targeted drug that can convert a cell from cell cycle arrest to cell death. However, this depends on a cell expressing wild type p53 with an intact p53 and p21 axis. Yet, approximately 50% of all gastrointestinal cancers carry a mutation in p53 and cells with mutant p53 still undergo G2 cell cycle arrest when treated with DNA-damaging agents such as CPT-11. Thus, an alternative approach to cancer therapy needs to be considered. An examination of the cell cycle provides insights into other means to convert a cell with mutant p53 from cell cycle arrest to cell death. As shown in Figure 7, the G2 checkpoint is regulated by p53, as well as by other kinases and phosphatases. The predominant kinase is called Chk1, which is involved in the regulation of the Cdc25C and 14-3-3 proteins.^{36,37} In response to DNA damage, Chk1 is activated, which results in the phosphorylation of the phosphatase Cdc25c on serine 216. The 14-3-3 protein binds to the phosphorylated form of Cdc25C. Because the cyclinB1/cdc2 kinase complex must be dephosphorylated to allow cells to exit G2 into M, this complex prevents this phosphatase from entering the nucleus, where it is required to dephosphorylate the cyclin B1/cdc2 kinase complex. As a result, cells remain arrested in G2 and can not enter the M phase of the cell cycle. Thus, even in cells with mutant and a nonfunctional p53, DNA damage will induce a G2 cell cycle arrest by preventing the dephosphorylation and subsequent activation of cyclinB1/cdc2 kinase.

Targeting Ck1 for Cancer Therapy: UCN-01 and Beyond

The general interpretation is that p53 deficient cells rely more on the G2 checkpoint for survival. However, since these cells lack a functional p53/p21 axis, following DNA damage they are dependent on activation of the Chk1 pathway for G2 cell cycle arrest and DNA repair

to avoid cell death. Therefore, it has been hypothesized that in the setting of co-existent DNA damage by chemotherapy or radiation, pharmacologic disruption of the Chk1-dependent pathway may selectively sensitize cells with mutant p53 to exit G2 before DNA repair can be completed and force the cell to undergo apoptosis. UCN-01 (7-hydroxystaurosporine) is a staurosporine analogue isolated from the culture broth of *Streptomyces* species, and was first reported to be a selective inhibitor of protein kinase C.³⁸ However, UCN-01 has also been shown to abrogate the G2 checkpoint by inhibiting the Chk1 and Chk2 kinase (Fig 8).^{36,37,39} This then results in the inhibition of phosphorylation of Cdc25C, which prevents its binding to 14-3-3.^{36,37} Cdc25c can then enter the nucleus where it dephosphorylates and activates the cyclin B/Cdc2 kinase complex, allowing cells to exit G2 and progress towards mitosis. Laboratory studies now indicate that sequential treatment with SN-38 followed by UCN-01 markedly enhances the induction of apoptosis in cells with mutant p53, but in only a small fraction of cells with intact p53.⁴⁰ This effect is mediated by inhibition of Chk1, which results in the activation of cyclin B1/cdc2 kinase and the marked abrogation of the G2/M checkpoint in p53 mutant cells.⁴⁰ Following the prolonged G2 arrest induced by 24 hours of SN-38 (Fig 9B), UCN-01 forces HCT cells into the M phase of the cell cycle after 8 hours of drug exposure (Fig 9C). Then, starting at approximately 16 hours, the colon cancer cells undergo apoptosis (Fig 9D), micronucleation, and induction of a process called "mitotic catastrophe."^{40,41} This combination of UCN-01 and CPT-11 has also been shown to prolong survival in a preclinical liver metastasis model of human

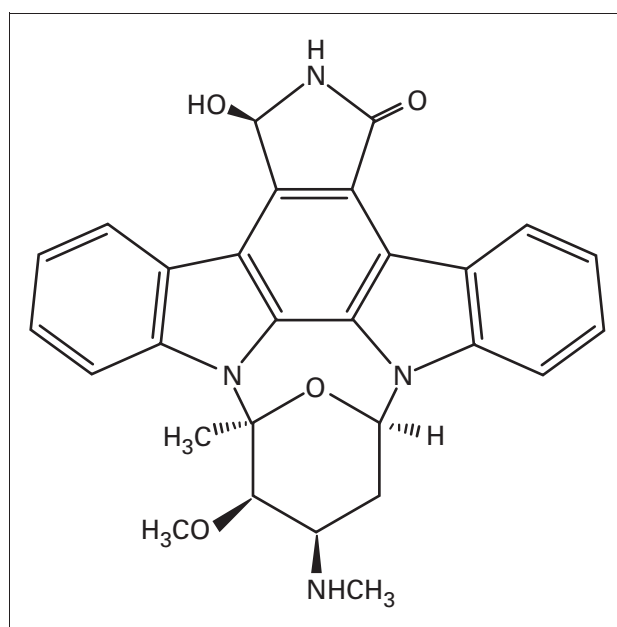


Fig 8. UCN-01.

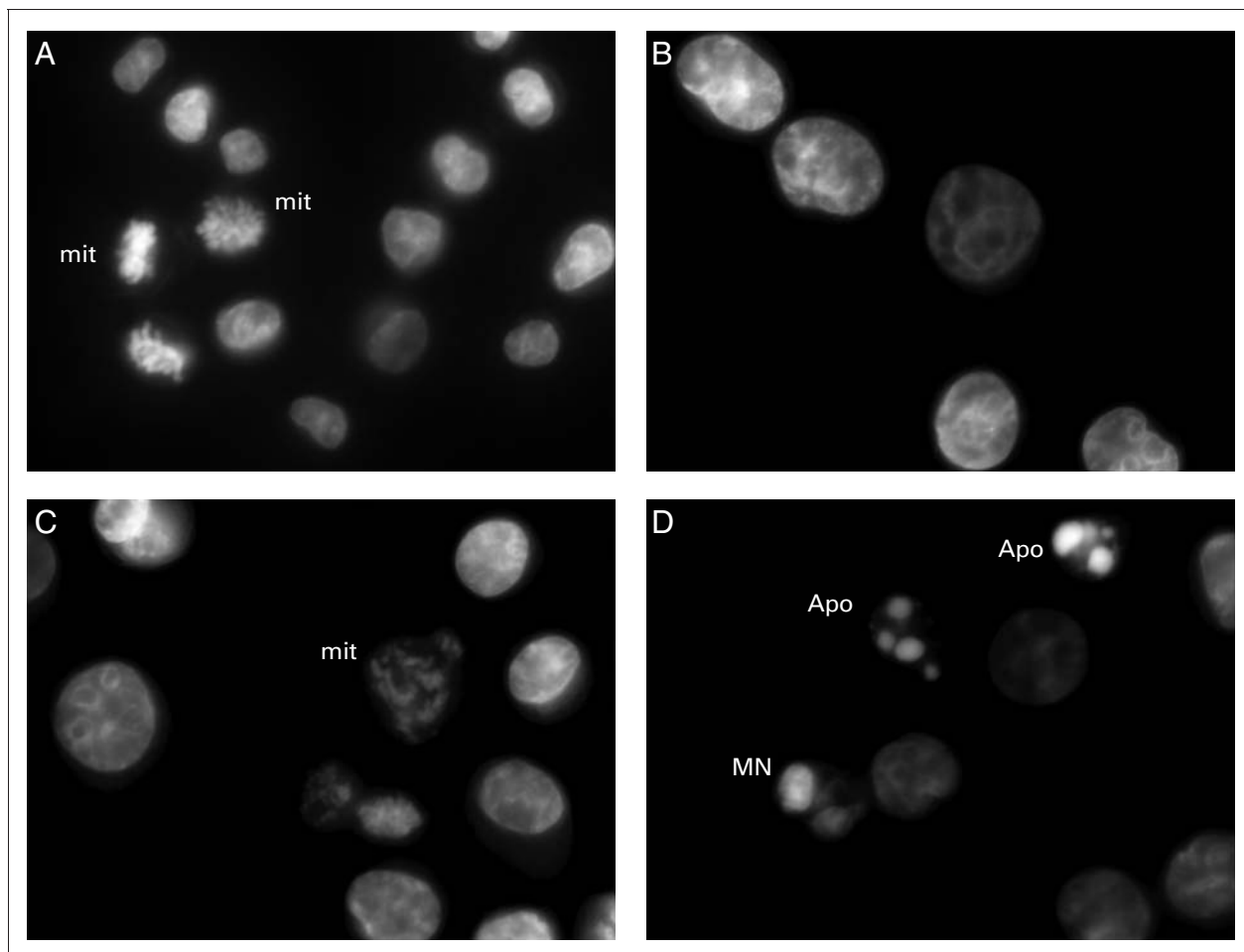


Fig 9. Treatment of HCT116 colon cancer cells that are deficient in p53 (HCTp53^{mut}) with sequential SN-38 and UCN-01. Control cells (A) treated with 20 nmol/L SN-38 for 24 hours undergo a G2 cell cycle arrest. (B) Subsequent treatment of these G2 arrested cells with 200 nmol/L UCN-01 abrogates the G2 checkpoint such that cells enter mitosis after 8 hours of drug exposure (C) with the induction of apoptosis noted after 16 hours of drug exposure (D) mit, mitosis; apo, apoptosis; MN, micronucleation.

colon cancer cells.⁴² In view of these promising results, the combination of sequential CPT-11 and UCN-01 is now being tested in a phase I clinical trial for patients with advanced solid tumors at the Washington University School of Medicine (St. Louis, MO).

The clinical development of UCN-01 has been complicated by UCN-01-induced hyperglycemia. Using the recommended phase II dose of UCN-01 in a phase I study with escalating doses of fluorouracil, Kortmansky et al⁴ have reported that one patient treated with this combination developed grade 4 hyperglycemia requiring hospitalization with a continuous insulin infusion. Furthermore, after amending the eligibility criteria to exclude patients with diabetes mellitus, four additional patients developed grade 3 hyperglycemia.⁴³ The etiology of the hyperglycemia is unclear, although inhibition of AKT and downstream insulin-receptor pathways appears to be the most probable cause. It has been recently shown that at clinically relevant concentrations UCN-01 inhibits glucose transport in the

presence of increasing concentrations of insulin.⁴⁴ These investigators show that UCN-01 inhibits insulin-induced phosphorylation of AKT at Thr308 but not Ser473.

Thus, alternate means of inhibiting Chk1 need to be developed. There has been some recent progress in this area. For example, CHIR124, a new inhibitor of Chk1 that is structurally distinct from UCN-01, has recently been reported to enhance the effects of SN-38 in vitro and CPT-11 in vivo.⁴⁵ Chk1 is also a client protein for HSP90. It has been shown that 17-AAG, a derivative of geldanamycin which intercepts the interactions between HSP90 and its client proteins,⁴⁶ suppresses Chk1 expression.⁴⁷ Therefore, following the induction of G2 cell cycle arrest by SN-38 in HCTp53^{mut} cells, 17-AAG abrogates the G2 checkpoint. This results in an increase in the M phase population and the induction of apoptosis.^{47,48} On the basis of preclinical data, a phase I trial of CPT-11 and 17-AAG will be shortly initiated at Memorial Sloan-Kettering Cancer Center, (New York, NY).

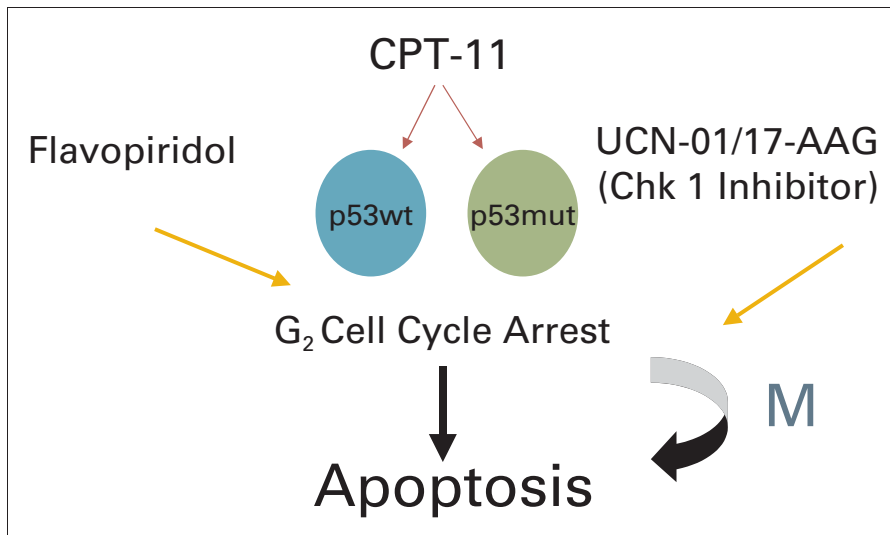


Fig 10. A new model for improving the efficacy of irinotecan (CPT-11) according to p53 status with cell cycle active agents in gastrointestinal cancers.

CONCLUSION

Collectively, these studies provide a new basis for drug development in the treatment of gastrointestinal cancer. An understanding of the cell cycle provides insights into the limitations of conventional chemotherapy. This new approach in cancer therapy is based on the belief that cell cycle arrest induced by the genotoxic stress of chemotherapy is insufficient to induce cell death and yet it is death of the tumor cell that is eventually needed to cure cancer. At the same time the cell cycle does provide opportunities to develop new agents that can convert an arrested cell to a dying cell. For a drug such as flavopiridol, this approach appears to be most effective for cells with wild type p53, such that tumor cells die in the G₂ phase of the cell cycle. For a Chk1 inhibitor such as UCN-01, this approach is

most effective in a cell with mutant p53, which allows the cell to exit G₂ and prematurely enter the M phase of the cell cycle, where it undergoes cell death (Fig 10). Thus, the success of this approach will depend on determining the patient's p53 status and then ultimately selecting the appropriate drug combination based on the drug's established targets and its effects on promoting apoptosis. The clinical trails with these agents are now ongoing but this scientific rationale for drug development does provide new hope for the future treatment of patients with gastrointestinal cancer.

Author's Disclosures of Potential Conflicts of Interest

The author indicated no potential conflicts of interest.

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