Camptothecins are broad-spectrum anticancer drugs that specifically target DNA topoisomerase I (Topo I). The formation of a cleavable drug–Topo I–DNA complex results in lethal double-strand DNA breakage and cell death. However, de novo or acquired clinical resistance to camptothecins is common. Studies of the camptothecin analog irinotecan suggest the following general mechanisms of resistance: (i) variable levels of the enzymes involved in the conversion of irinotecan; (ii) reduced cellular accumulation from active drug efflux; (iii) reduced levels of Topo I expression; (iv) alterations in the structure of Topo I from different mutations; (v) alterations in the cellular response to camptothecin–Topo I–DNA complex formation, which involves proteasome degradation of Topo I and/or enhanced DNA repair; and (vi) activation of the transcription factor nuclear factor kappa B by DNA damage and subsequent suppression of apoptosis. Multiple approaches using pharmacological and biological modulation to circumvent the above mechanisms of resistance have been incorporated into ongoing clinical trials and are expected to enhance the antitumor activity of irinotecan and reduce its systemic toxicity.

**Key words:** apoptosis, irinotecan, modulation, nuclear factor kappa B, proteasome, resistance

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Pharmacology

Understanding the pharmacology of irinotecan provides insights into the basis of irinotecan toxicity and tumor resistance [10]. Irinotecan is a unique semisynthetic camptothecin, characterized by the presence of a bulky piperidino side-chain at the C-10 position [11]. This side-chain can be cleaved enzymatically by carboxylesterase to 7-ethyl-10-hydroxycamptothecin (SN-38), which is 1000-fold more potent than irinotecan [12]. Both irinotecan and SN-38 are in equilibrium with their active lactone and inactive carboxylate forms, an equilibrium that is pH- and protein-dependent [13, 14]. The metabolism and mechanism of action of irinotecan are summarized in Figure 1.

Carboxylesterase activity is found in serum, liver, intestine and other sites [15]. Genetic variability of carboxylesterase expression and/or activity is suspected, as variations of SN-38 levels are observed from individual to individual for a given dose of irinotecan [16]. After conversion from irinotecan by carboxylesterase, SN-38 is deactivated through conjugation by uridine diphosphate glucuronosyltransferase isoform 1A1 (UGT 1A1) in the liver to an inactive form, SN-38 glucuronide (SN-38G). This isoenzyme, which is also responsible for the glucuronidation of bilirubin, is mutated in Gilbert’s syndrome [17] and deficient in Crigler-Najjar syndrome type I [18]. As a result, patients with these disorders are at increased risk for severe irinotecan-induced toxicity. The deficiency in Gilbert’s syndrome is due to a homozygous TA insertion in the TATAA promoter of UGT 1A1, leading to a mutated allele [19]. Pretreatment of rats with valproic acid caused 99% inhibition of the formation of SN-38G by inhibiting UGT 1A1 enzyme activity, leading to a 270% increase in the area under the plasma concentration–time curve (AUC) [20]. In contrast, pretreatment with phenobarbital, an inducer of UGT 1A1, caused a 1.7-fold increase in the AUC of SN-38G, and 31 and 59% reduction in the AUC of SN-38 and irinotecan, respectively [20].

The elimination of irinotecan and SN-38 is through biliary excretion, which is dependent on the canalicular multispecific

Figure 1. Depicted are the steps involved in the activation and metabolism of irinotecan, as well as its mechanism of action. Numerals reflect areas in which enhancement (+) or interference (−) with irinotecan action and toxicity have been reported: 1, phenytoin and carbamazepine (−); 2, carboxylesterase (CE) gene transfer (+); 3, genetic profiling (+), phenobarbital and dexamethasone (−), valproic acid (+); 4, antisense canalicular multispecific organic anion transporter (cMOAT) (+), cyclosporin (+); 5, antibiotics (−); 6, intestinal alkalization (−); 7, multidrug resistance-associated protein, P-glycoprotein and breast cancer resistance protein inhibitors (+); 8, topoisomerase I (Topo I) increase (+), Topo I mutation (−); 9, proteasome inhibition (+); 10, gene transfer (IkBα) (+), proteasome inhibition, thalidomide and cyclooxygenase inhibition (+). APC, aminopentanecarboxylic acid; B Gluc, β-glucuronidase; CYP 3A4, cytochrome P-450; ds, double strand; NF-κB, nuclear factor kappa B; NPC, 7-ethyl-10[4-(1-piperidino)-1-amino]carbonyloxycamptothecin; SN-38, 7-ethyl-10-hydroxycamptothecin; SN-38G, SN-38 glucuronide; ss, single strand; SUMO, small ubiquitin-like modifier; UGT, uridine diphosphate glucuronosyltransferase.
organismic anion transporter (cMOAT), a member of the transporters with an ATP cassette [21, 22]. Treatment with cyclosporin A, which decreases biliary flow and inhibits cMOAT, increases the AUC of irinotecan and SN-38 [23]. Subsequently, SN-38G is deconjugated to SN-38 by β-glucuronidase produced by the intestinal bacterial flora, which may account for the late SN-38 double peaks in the plasma, and may be responsible for the delayed intestinal toxicity of irinotecan [24]. Indeed, β-glucuronidase activity is correlated with irinotecan-induced cecal damage in the rat [25]. The toxicity in this model was attenuated by antibiotic administration, which presumably inhibits the intestinal microflora proliferation [25].

A second major metabolite of irinotecan is aminopentane-carboxylic acid (APC), produced by oxidation of the terminal piperidine ring by the cytochrome P-450 CYP3A4 enzyme [26]. APC does not hydrolyze to SN-38 and is a poor inhibitor of the Topo I–DNA cleavable complex.

Mechanism of action
Irinotecan interacts with cellular Topo I–DNA complexes and has S-phase-specific cytotoxicity [27]. Topoisomerases reduce DNA twisting and supercoiling that occur in selected regions of DNA as a result of essential cellular processes such as transcription, replication and repair recombination. They cleave and reseal the phosphodiester backbone of DNA, and form a covalent enzyme–DNA linkage, which allows the passage of another single- or double-stranded DNA through the nicked DNA. Topo I binds to single-strand DNA breaks, and the reversible Topo I–irinotecan–DNA cleavable complex is not lethal to the cells by itself. However, upon their collisions with the advancing replication forks, the formation of a double-strand DNA break occurs, leading to irreversible arrest of the replication fork and cell death [27]. The collision of the irinotecan–Topo I complex with the replication fork also results in G2 arrest/delay by signaling the presence of DNA damage to an S-phase checkpoint mechanism [28]. At higher concentrations of irinotecan, non-S-phase cells can also be killed. The mechanism of non-S-phase cell killing appears to be related to transcriptionally mediated DNA damage, and through the mechanism of apoptosis [29].

Mechanisms of irinotecan resistance
Pharmacokinetics and metabolism of irinotecan
Irinotecan is subject to extensive metabolic conversion by various enzymatic systems in the body. The conversion of irinotecan to the more active form SN-38 requires carboxylesterase. A clear relationship between carboxylesterase level and the chemosensitivity of human small-cell and non-small-cell lung cancer cell lines has been demonstrated in vitro, where irinotecan resistance was encountered in cell lines with low carboxylesterase expression [30]. Furthermore, as SN-38 is inactivated to SN-38G by glucuronidation by UGT 1A1, overexpression of UGT at the protein and mRNA levels was found to account for SN-38 resistance in a human lung cancer cell line [31].

Irinotecan’s complex metabolism makes this agent a target of potential interactions with other medications. The most compelling example of interference with irinotecan pharmacokinetics comes from studies in patients with central nervous system tumors [32]. Irinotecan’s clearance in these patients was approximately two-fold higher than reported in previous trials. The observation that the overwhelming majority of these patients were receiving enzyme-inducing antiepileptic drugs (phenytoin, carbamazepine and phenobarbital) led to the suspicion that induction of hepatic cytochrome P-450 enzymes, including CYP3A4, resulted in increased conversion of irinotecan to its APC inactive metabolite. In addition, phenobarbital and dexamethasone are known to induce glucoronyl transferase, enhancing the conversion of SN-38 to SN-38G [20]. Conversely, another anticonvulsant, valproic acid, inhibits SN-38 conjugation, decreasing therefore irinotecan clearance [20]. A phase I study in patients with malignant glioma receiving irinotecan and concurrent enzyme-inducing antiepileptic drugs demonstrated that doses of irinotecan that are two-fold higher than the usually recommended doses can be administered safely to these patients [33].

Decreasing the intracellular level of irinotecan by active efflux
Multidrug resistance, a major obstacle encountered in cancer chemotherapy, is characterized by overexpression of ATP-binding cassette (ABC) transmembrane transporters such as P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP), which actively transport chemotherapy agents out of the cell [34]. P-gp accepts amphipathic cationic or neutral compounds as substrate, whereas MRP acts as a glutathione S-conjugate (GS-X) export pump. Several ABC proteins have been shown to be able to efflux camptothecins. Using membrane vesicles from human cell lines overexpressing P-gp and MRP, it was found that both P-gp and MRP are involved in the active efflux of SN-38 and irinotecan [35]. The involvement of MRP was further shown in MRP-overexpressing transfectants, where the ATP-dependent efflux of irinotecan and SN-38 was inhibited by its specific inhibitor PAK-104P [36]. Another ATP-dependent transporter, cMOAT (also known as MRP2), which shares 49% amino acid identity with MRP1, also exhibits GS-X pump activity [37]. cMOAT is predominantly located in hepatic cells, where it is involved in the excretion of irinotecan to the bile. Transfection of cMOAT antisense cDNA into a human hepatic cancer cell line results in a reduction in cMOAT protein and increased sensitivity to camptothecin derivatives [38].

In addition, the breast cancer resistance protein (BCRP), another member of the ABC family of drug transporters, also mediates resistance to camptothecins [39]. The affinities to
BCRP are as follows in descending order: SN-38, topotecan, 9-aminocamptothecin (9-AC), irinotecan. Administration of a BCRP inhibitor, GF120918, reverses the resistance almost completely at 100 nM, further confirming the involvement of BCRP in irinotecan efflux [40]. Overexpression of BCRP has been described in ovarian, breast, colon and gastric cancer cell lines [40], implying their chemoresistance. In summary, the above results indicate that the P-gp and MRP family of transporters play important roles in the efflux and active excretion of irinotecan and can be targets for pharmacological modulation.

Quantitative Topo I level

As Topo I is the cellular target of irinotecan, it is conceivable that the cellular level of Topo I would be proportional to irinotecan cytotoxic effects. This notion is supported by experimental evidence from yeast systems and mammalian cell lines [41, 42]. In irinotecan-resistant cell lines rendered resistant by stepwise, continuous treatment with irinotecan, the total activity of Topo I was shown to be reduced compared with the irinotecan-sensitive parental cell line [43]. Theoretically, the expression of Topo I in tumor specimens may serve as a predictor for sensitivity to irinotecan chemotherapy. Increased levels of Topo I are demonstrated in colon cancer and prostate cancer, but not in renal cancer [43, 44]. Topo I levels are higher in colorectal tumors compared with normal colon mucosa, suggesting a favorable therapeutic index [43]. Pathological specimens from breast cancer and seminoma examined for Topo I expression using immunohistochemical staining showed great variability, with increased expression observed in only 41% of the breast and 30% of the seminoma specimens tested, respectively [45, 46]. Although no consistent association has been described between pretreatment tumors and Topo I expression and antitumor response to irinotecan, this question has not been properly addressed in prospectively designed clinical trials. In addition, tumor cells may escape irinotecan cytotoxic effects by reducing the level of Topo I expression. Therefore, dynamic studies, evaluating the behavior of Topo I in cancer patients during a treatment period are warranted.

Multiple mechanisms contribute to the regulation of Topo I level in the cell. In tumors demonstrating increased levels of enzyme expression, increased levels of mRNA were also observed [44]. This would indicate either increased transcription or increased mRNA stability. On the other hand, specimens with reduced expression were found to have a nonproductive rearrangement of the Topo I genome, leading to decreased transcription and thus reduced enzymatic production of Topo I [47]. The rearranged allele in these specimens is hypermethylated, resulting in transcriptional silencing.

Topo I mutations

Certain cell lines resistant to camptothecin have been found to harbor mutations in Topo I [48, 49]. Mutations have been found in the area that may alter DNA cleavage or Topo I–DNA–irinotecan interactions, so that camptothecin can no longer enter the complex [50]. Mutations immediately flanking the catalytic tyrosine may affect the catalytic activity of the enzyme, altering its ability to relax supercoiled DNA [51]. Recently, the crystal structure of Topo I–DNA covalent complexes became available, which will allow structural mapping of these mutations and may shed light on the functional roles of the mutations [52, 53].

DNA repair: ubiquitin/26S proteasome-mediated degradation of Topo I

Another cellular mechanism of resistance to irinotecan is repair of irinotecan-induced DNA damage; a mechanism which is coupled with RNA transcription [27]. According to this model, the collision between the elongation RNA polymerase complex and the Topo-I cleavable complex (on the template strand) results in transcription arrest and the formation of a Topo I linked single-strand break. This collision triggers degradation of Topo I through an ubiquitin/26S proteasome-dependent system [54–56]. Subsequent to Topo I destruction, repair of the single-strand break can presumably occur. The model is illustrated by studies in breast cell lines, demonstrating that the most sensitive cell line was completely defective in irinotecan-induced Topo I reduction, whereas the least-sensitive line exhibited effective Topo I reduction. Tumor sensitivity to proteasome degradation can therefore serve as an important parameter for determining irinotecan sensitivity/resistance [55]. However, studies on a panel of breast, colon and leukemia cell lines have demonstrated that irinotecan-induced Topo I reduction is defective in most tumor cell lines [27], whereas Topo I reduction in peripheral blood monocellular cells has been observed in patients treated with irinotecan [57]. It remains to be established whether irinotecan-induced Topo I reduction occurs preferentially in normal cells to protect them from irinotecan toxicity, while tumor cells remain sensitive.

In addition to the ubiquitin degradation pathway, a small ubiquitin-like modifier (SUMO-1) can also conjugate Topo I [58]. The role of SUMO–protein conjugation is still unclear, and diverse functions have been proposed. One speculation is that SUMO may target Topo I for relocation to a different cellular compartment, so it cannot participate in the formation of Topo I cleavable complexes [58]. Likewise, a gene that encodes an enzyme that can hydrolyze the bond between Topo I–DNA has been isolated in yeast systems [59]. Enzyme-defective mutants of this gene are hypersensitive to treatments that increase the amount of covalent complexes. Identification of this gene in humans may have implications for the effectiveness of Topo I interactive agents in the clinic.

Nuclear factor kappa B activation

As a result of irinotecan–Topo I–DNA complex formation and its collision with the replication fork, a double-stranded DNA
break ensues. The double-stranded DNA damage is lethal and causes cell-cycle arrest and apoptosis. Recently, multiple experiments also showed that treatment of irinotecan activates nuclear factor kappa B (NF-κB), an ubiquitous transcription factor which controls the transcription of a wild variety of genes involved in inflammation and immunity [60–64]. The activation was dependent on initial nuclear DNA damage, followed by cytoplasmic signaling events [62]. This very important discovery has raised awareness of another mechanism of chemoresistance, as many experiments on the diverse roles of activated NF-κB indicated that NF-κB acts as an antiapoptotic factor, especially in early transforming tumor cells. Activated NF-κB can suppress the apoptotic cascade induced by tumor necrosis factor-alpha (TNF-α), oncogenic Ras, and chemotherapy agents, particularly irinotecan [61–65]. Inhibition of NF-κB activation augments irinotecan-induced apoptosis [63, 64]. In addition, NF-κB knockout mice die embryonically from extensive apoptosis within the liver [66], indicating again that NF-κB may normally be antiapoptotic.

How does NF-κB protect cells from apoptosis? It has been suspected that the antiapoptotic effect of NF-κB involves the regulation and activation of pro-survival genes; however, only a few of them have been identified. Major recent discoveries have revealed that the NF-κB cell-survival pathway cross-talks with the c-Jun N-terminal kinase (JNK) pathway, substantially blunting this pathway [67, 68]. JNKs are part of the evolutionarily conserved mitogen-activated protein kinase family, and are implicated in cell-death pathways stimulated by environmental stresses and TNF-α. Two JNK inhibitory proteins have been discovered. In one model, activation of NF-κB mediates transcriptional increase of a protein called gadd45b/myd118, which then lowers JNK signaling induced by the TNF-α receptor [67]. The other protein (NF-κB-induced X-chromosome-linked inhibitor of apoptosis) is switched on by TNF-α in an NF-κB-dependent manner and also blunts JNK activation [68]. These discoveries undoubtedly shed light on elucidating the downstream events of NF-κB activation, and its role of antiapoptosis and cell survival following TNF-α stimulation and chemotherapy.

**Strategies to overcome resistance to irinotecan**

**In vivo transfer of carboxylesterase cDNA to solid tumors**

Irinotecan is activated by carboxylesterase to SN-38 in vivo. Although plasma and liver carboxylesterase presumably produce sufficient SN-38 for cytotoxicity, a lack of such conversion at the tumor site may result in decreased sensitivity to irinotecan. Conversely, selective increase of carboxylesterase levels in tumors may produce tumor-specific activity. Carboxylesterase expression has been detected in some, but not all, squamous cell lung carcinomas and adenocarcinomas at various levels [69, 70]. Thus, conceivably it would be desirable to identify tumors deficient in carboxylesterase and stimulate its expression. A gene-therapy-based approach of transferring the carboxylesterase gene directly to the tumor followed by systemically administered irinotecan has been attempted in cell lines and a nude mouse tumor model with success [71, 72]. This strategy may have potential as a local therapy for solid tumors, such as the treatment of intrabronchial tumors, lung tumors invading into the chest wall and inoperable intrapulmonary tumors. Another very interesting application of this gene therapy-based approach is to purge contaminating tumor cells from their mixture with CD34+ cells while preparing for autologous stem cell transfer. Adenovirus-mediated transfer of carboxylesterase followed by exposure to irinotecan successfully killed neuroblastoma cells mixed with CD34+ cells in vitro, while the cytotoxic effect did not affect the colony formation by CD34 cells [73]. This approach shows potential clinical utility to reduce the tumor burden of peripheral stem cells in autologous transplantation.

**Increased cellular level of Topo I**

In preclinical systems, coadministration of irinotecan and mitomycin C (MMC) had a synergistic effect [74]. It was further demonstrated that MMC increases Topo I catalytic activity as measured by relaxation of supercoiled DNA, although Topo I protein level was not increased [75]. Based on these preclinical data, our group has evaluated the combination of MMC and irinotecan (given 24 h after MMC) in patients with refractory solid malignancies [76]. Topo I gene expression was measured in pre-MMC and post-MMC peripheral mononuclear specimens by RT-PCR studies and quantified with a competitive, reaction-specific internal standard and analysis by capillary electrophoresis with laser-induced fluorescence [77]. This combination chemotherapy showed promising activity in heavily pretreated gastric, esophageal, breast and non-small-cell lung cancers. Interestingly, patients experiencing a major response to treatment (complete and partial responses, n = 5) had a 46-fold induction of Topo I at 3 h after MMC compared with baseline, and a 312-fold induction at the end of the irinotecan infusion (P = 0.00021, ANOVA) [77]. In contrast, non-responding patients (stable disease or tumor progression, n = 29) did not experience significant Topo I induction (P = 0.64). Although these results are of interest, it is not certain if the dynamics of Topo I following MMC in peripheral mononuclear cells adequately reflect intratumoral events. Ongoing phase II tumor-specific studies with the combination, in which tumor tissues are sampled pre- and post-treatment for Topo I and carboxylesterase gene expression, will help clarify this issue.

Studies on another Topo I-interactive agent, topotecan, noted the occurrence of depleted cellular levels of Topo I in relation to prolonged infusion of topotecan (for 21 days). Topo I levels decreased progressively in a weekly fashion and returned to baseline levels 1 week after stopping the infusion [78, 79]. This association indicates that prolonged infusions
may have advantages because of their ability to target maximum amounts of free Topo I to form DNA–drug–Topo I complexes. This regimen was used in a phase II trial as second-line therapy in patients with previously treated ovarian cancers [79]. The regimen was well tolerated, and produced a response rate of 35%, which is at the upper level for topotecan therapy in this group of patients. Laboratory data suggested that the depletion of free Topo I was partly due to the formation of cleavable complexes [78]. However, this experiment cannot distinguish from two other reported possibilities of free Topo I depletion: (i) translocation of Topo I from the nucleus to cytoplasm in the presence of low doses of topotecan [80], and (ii) the transcription-triggered proteasome degradation of Topo I from the Topo I–DNA complex as described above [54–56]. Randomized studies are required to elucidate the therapeutic advantage and true mechanism in this administration schedule.

Sequential treatment with Topo I and Topo II inhibitors

Cell lines selected for resistance to camptothecin often demonstrate collateral sensitivity to Topo II poisons and increases in Topo II enzyme activity or content [48]. Reciprocal changes in Topo I activity have been observed in cell lines resistant to Topo II poisons [81]. This phenomenon was also observed in cancer patients. Several patients demonstrated decreased levels of Topo I accompanied by increased levels of Topo II when treated with the Topo I inhibitor 9-AC [82]. In colon cancer cell lines, sequential exposure to camptothecin and etoposide leads to additive cytotoxicity [83]. Sequential treatment of athymic mouse bearing human colon tumor with topotecan followed by etoposide also demonstrated increased tumor sensitivity to etoposide [84]. However, results from a recent phase I translational study of sequential administration of the Topo I and II inhibitors, topotecan and etoposide, did not fully support the above proposed mechanism. By assaying Topo I and II levels in the tumor specimens after treatment, the increase in Topo II was only clearly demonstrated in one out of six patients tested [85]. Thus, the rationale of sequential administration of Topo II and Topo I requires further investigation.

Inhibition of the activation of NF-κB to enhance irinotecan-induced apoptosis

Irinotecan exposure frequently results in increased production of TNF-α, interleukin-1 (IL-1), phorbol esters and lipopolysaccharides, as well as in activation of the transcription factor NF-κB [86]. Activation of NF-κB leads to inhibition of apoptosis. NF-κB is a heterodimer consisting of two proteins, p65 and p50. In unstimulated cells, NF-κB is located in the cytoplasm and is bound to IκBα and IκBβ, which prevents it from entering the nuclei. The external stimuli modulate signal transduction pathways leading to IκB phosphorylation, causing its rapid degradation by proteasomes. The release of NF-κB from IκB results in the passage of NF-κB into the nucleus, where it binds to specific sequences in the promoter regions of target genes [64]. As has recently been shown, the NF-κB complex negatively cross-talk to the JNK cascade, reducing the TNF-α-mediated JNK activation [67, 68].

A number of strategies have been employed to inhibit the activation of NF-κB. The first approach is the molecular gene-therapy approach of introducing a dominant negative variant of IκBζ via adenovirus vector to tumor cells [63]. This form of IκBζ is a ‘super-repressor’, which is resistant to phosphorylation by IκB kinase, and presumably remains bound to NF-κB, thus sequestering NF-κB in the cytoplasm. This approach has been tested in multiple tumor cell lines and mouse tumor models (fibrosarcoma, colorectal cancer) by different groups. It consistently demonstrated increased cell death or tumor regression in the presence of irinotecan and increased induction of apoptosis [63, 87]. Inhibition of NF-κB using the ‘super-repressor IκBζ’ was found to sensitize non-small-cell lung cancer cells to gemcitabine-induced apoptosis [88]. However, using the same approach, enhanced chemosensitivity was not confirmed in similar studies done by two other groups [89, 90]. This discrepancy may represent the intricate cellular protein interaction and control of apoptosis. NF-κB has been connected with multiple aspects of oncogenesis, including promotion of cell proliferation, cell migration, angiogenesis, metastasis and blockage of cell differentiation and apoptosis [64]. In many late-stage tumor cell lines and other experimental tumor systems, NF-κB appears to be proapoptotic [64].

As the use of gene therapy to deliver NF-κB inhibitors is relevant to certain cancers, but is limited when considering widely disseminated metastases, an alternative approach is to use pharmacological inhibitors of proteasome function. The inhibition of proteolytic function effectively blocks degradation of cellular proteins that have undergone ubiquitination, such as IκB [91]. MG-132, an inhibitor of proteasomes, has been shown to significantly inhibit NF-κB activation induced by irinotecan [90]. However, in that experimental system, apoptosis was decreased, again raising the question of the role of NF-κB to be pro- or antiapoptotic in that tumor line. PS-341, a potent boronic acid dipeptide that is highly selective for proteasome inhibition, was also tried, in order to inhibit proteasome function in human colon cancer cell lines [92]. Pretreatment of cancer cells with PS-341 can effectively block the activation of NF-κB that is induced by exposure to SN-38/irinotecan. NF-κB inhibition using PS-341 also augmented the sensitivity of tumor cells to chemotherapy in tumor xenograft models. The increased antitumor effect was attributable in part to regulation of apoptosis, with markedly increased levels of apoptosis compared with single-agent treatment alone [92]. In pancreatic xenografts, the combination of PS-341 and irinotecan results in 89% inhibition of tumor growth and increased cellular apoptosis [93]. While using PS-341 appears to be very promising, it is important to keep in mind that the increased antitumor effect may in part be attrib-
uted to the prolonged binding of irinotecan to Topo I, rendered by the inhibition of degradation of bound Topo I–irinotecan complex, in the presence of proteasome inhibition [54, 55]. In addition, proteasome inhibition may also stabilize a wide range of key cell-cycle regulatory proteins, although it has been shown to be at least independent of p53 [93].

Thalidomide and cyclooxygenase-2 inhibitors

The teratogenic sedative and antinausea drug thalidomide has come back to the clinic based on its anti-inflammatory and anti-oncogenic properties. It is an inhibitor of TNF-α by enhancing the degradation of TNF-α mRNA [94]. Thalidomide has been recently demonstrated to block NF-κB activation through a mechanism that involves the inhibition of activity of the IκB kinase [95]. Thalidomide could inhibit the activation of NF-κB induced by stimulation with TNF-α and IL-1β, and could inhibit the transcription of many endogenous downstream genes controlled by NF-κB [95]. This study suggests the inhibition of NF-κB as the mediator for the anti-inflammatory and anti-oncogenic properties of thalidomide; in addition, it also provides a rationale for the use of thalidomide in combination with irinotecan to modulate irinotecan-induced chemoresistance. A clinical trial with the combination of irinotecan and thalidomide in 5-FU-refractory metastatic colorectal cancer is ongoing at the University of Arkansas. Patients receive 350 mg/m² irinotecan every 3 weeks and 400 mg thalidomide daily. Preliminary results are encouraging, including one complete response and three partial responses among 14 evaluable patients. Interestingly, grade 3–4 diarrhea, a toxicity frequently associated with single-agent irinotecan, has rarely been observed [96].

Aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenases (COXs) to prevent prostaglandin synthesis. They also play important roles in the chemoprevention of colon cancer. COX-1 is constitutively expressed in many types of tissue, while COX-2 expression is induced by cytokines and growth factors, and is increased in 90% of sporadic colon cancers and 40% of colonic adenomas, but is not elevated in the normal colonic epithelium [95]. Both the non-specific COX inhibitor sulindac and the COX-2 inhibitor celecoxib have been shown to cause regression of polyps in patients with familial adenomatous polyposis [97, 98]. The mechanism of chemoprevention by COX-2 inhibitors may involve an increase in apoptosis [97]. In addition, NSAIDs also act through COX-independent mechanisms by inhibiting the NF-κB pathway [99]. The NSAIDs specifically bind to IκB kinase b, inhibiting its kinase activity. This inhibition results in prevention of IκB degradation and nuclear translocation of NF-κB. The above studies provide evidence that NSAIDS, or more specifically COX-2 inhibitors, are potential agents that may inhibit the activation of NF-κB.

In order to evaluate if the target of thalidomide and COX-2 inhibitors mediating favorable interactions with irinotecan is NF-κB, we are conducting a phase I clinical trial in patients with solid malignancies in which the combination of irinotecan with thalidomide and subsequently the triple drug combination irinotecan/thalidomide/celecoxib are evaluated. The principal end point is demonstration of inhibition of NF-κB nuclear translocation in peripheral mononuclear cells. Preliminary results in 10 patients receiving irinotecan 125 mg/m² on days 1 and 8 every 3 weeks in combination with thalidomide 400 mg daily confirms the attenuated toxicity profile previously reported with the irinotecan/thalidomide combination and no effect of thalidomide on the pharmacokinetics of irinotecan.

Pharmacological modulations to reduce irinotecan toxicity

Pharmacological modulation to reduce irinotecan toxicity has attracted many investigations. Myelosuppression and diarrhea are the two major toxicities associated with irinotecan treatment. It is believed that toxicity correlates with levels of SN-38 in the plasma and bowel. Systemic SN-38 levels depend on the expression and activity of carboxylesterases in serum and liver, which convert irinotecan into SN-38 [15], and on hepatic glucuronosyltransferase (UGT 1A1) activity, which converts SN-38 into its glucuronidated and inactive form SN-38G [18]. Although genetic variability in carboxylesterase expression is suspected, and wide variability on the ability of carboxylesterases to metabolize irinotecan has been documented among its different isoenzymes [100, 101], no genetic disorders of deficiency of this enzyme have been characterized. UGT 1A1, however, is mutated in Gilbert’s syndrome and deficient in Crigler–Najjar type 1 syndrome, resulting in increased risk for irinotecan-induced severe toxicity [17, 18]. A clinical trial has been planned at the University of Chicago to test the predictive value of hepatic UGT 1A1 genotyping for irinotecan pharmacokinetics and toxicity [19]. In addition, pharmacological modulation with cyclosporin A, an inhibitor of the active irinotecan/SN-38 transporter cMOAT on hepatic cells [23], and phenobarbital, an inducer of UGT 1A1 [20] has been tested in cancer patients in a phase I clinical trial [102]. Cyclosporin A significantly inhibited the clearance of irinotecan while phenobarbital induced glucuronidation of SN-38, both consistent with preclinical findings.

SN-38G is largely eliminated by biliary excretion [103]. In the bowel, fecal bacteria-derived β-glucuronidase converts the non-active SN-38G to active SN-38, which can produce direct injury to the mucosa. Since the conversion to SN-38 is believed to be a major factor in the diarrhea produced by irinotecan, inhibition of bowel β-glucuronidase is a potential strategy to reduce irinotecan toxicity. In a small controlled trial, the broad-spectrum antibiotic neomycin was given to patients receiving irinotecan in order to inhibit microflora in the intestines. Amelioration of diarrhea was found in six out of seven patients treated [104]. However, a recent study has
detected up to 30% of irinotecan excreted unchanged in the bile [105]. Thus, the potential exists for direct bowel conversion of irinotecan into SN-38 by the recently identified intestinal carboxylesterases [101]. In the absence of a direct inhibitor of intestinal carboxylesterases, strategies aimed at ameliorating irinotecan-induced diarrhea must consider the contribution of intestinal irinotecan conversion.

The pH dependency of the equilibrium between the active lactone forms and less active carboxylate forms of both SN-38 and irinotecan [13] provides an additional opportunity to use the understanding of irinotecan pharmacology to ameliorate toxicity. Under acidic conditions, the lactone form is favored, but at physiological or higher pH, the lactone form is unstable, and hydrolysis to open the lactone ring into its carboxylate form is favored. In addition, the lactone forms are passively transported in intestinal cells, resulting in an intestinal uptake 10 times greater than the carboxylate forms [106]. Therefore, intestinal alkalization has been proposed as a potential strategy to decrease irinotecan gastrointestinal toxicity [107, 108]. In a phase II trial, oral alkalization utilizing sodium bicarbonate, magnesium oxide, base water and ursodeoxycolic acid combined with ‘controlled’ defecation resulted not only in a decreased incidence of diarrhea compared with a non-randomized control group, but also in decreased myelosuppression, while maintaining anticancer activity [108].

Finally, oral administration of immunomodulators, which induce endogenous IL-15 for protection of intestinal epithelium, and Chinese/Japanese herbal concoctions with an unclear mechanism of action have been used [109, 110]. A need for carefully designed randomized clinical trials will be needed to assess the efficacy (decreased toxicity without affecting anticancer activity) of all the strategies discussed above. In addition, feasibility aspects need to be considered, since efficacy and simplicity will both be needed to guarantee the success of these approaches in clinical practice.

**Future directions**

The intricate pathways of metabolic activation/deactivation of irinotecan make this agent especially suitable for interactions with commonly used medications. Therefore, it is extremely important to be mindful of the potential for increased clearance or increased toxicity that these medications may have when used in combination with irinotecan. However, knowledge of these metabolic pathways can be used to identify modulation strategies aimed at increasing the antitumor activity and reducing the toxicity of this unique agent. Similarly, with the increasing understanding of irinotecan intracellular interactions and the intracellular mechanisms mediating resistance to this agent, the potential to prospectively identify patients with tumors especially susceptible to irinotecan therapy warrants careful attention. Clinical trials of irinotecan should incorporate, whenever feasible, analyses of potential markers of clinical activity and toxicity.

In addition, based on this knowledge, pharmacologically based or biologically based novel therapeutic approaches to modulate irinotecan activity and toxicity have been incorporated into clinical trials. Although none of the novel strategies discussed in this manuscript has yet been validated, modulation of NF-κB activity and Topo I expression and activity appears to hold the most promise for improvement in clinical activity in early clinical trials. Randomized phase II trials evaluating several novel strategies may help to identify the most promising regimens for further study. It is the bias of the authors that these approaches should first be tested in irinotecan-naïve patients and later in irinotecan-refractory patients, in the event that encouraging activity is found on the former. Given the broad spectrum of activity of irinotecan, validation of these approaches in any of these settings might translate into significant advances in clinical practice.

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**References**


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