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Next generation topoisomerase I inhibitors: Rationale and biomarker strategies

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ABSTRACT

Topoisomerase I (TopoI), an essential enzyme, produces a DNA single strand break allowing DNA relaxation for replication. The enzymatic mechanism involves sequential transesterifications. The breakage and closure reactions generate phosphodiester bonds and similar free energies, so the reaction is freely reversible. The TopoI reaction intermediate consists of enzyme covalently linked to DNA dubbed a 'cleavable complex'. Covalently bound TopoI–DNA complexes can be recovered. Camptothecin analogs, topotecan and irinotecan, are approved TopoI-targeted drugs. Both have limitations due to the equilibrium between the camptothecin lactone and ring-opened forms. Several strategies are being explored to develop improved TopoI inhibitors. Homocamptothecins, in which the metabolically labile camptothecin lactone is replaced with a more stable seven-membered β-hydroxylactone, are potent anticancer agents. Gimatecan is a seven-position modified lipophilic camptothecin developed to provide rapid uptake and accumulation in cells and a stable TopoI–DNA–drug ternary complex. Diflomotecan, a homocamptothecin, and gimatecan are in Phase II clinical trial. Among non-camptothecins, edotecarin, an indolocarbazole that results in DNA C/T-G cleavage compared with T-G/A for camptothecins, is in Phase II clinical trial. Indenoisoquinolines were identified as TopoI inhibitors by the NCI 60-cell line COMPARE analysis. Co-crystal structures of two indenoisoquinolines with TopoI–DNA elucidated the structure of the ternary complex. Indenoisoquinolines are in preclinical development. Dibenzonaphthyridinone TopoI inhibitors have undergone extensive structure–activity examination. ARC-111 was selected for in-depth preclinical study. Biomarkers are under investigation to predict clinical efficacy from preclinical models, to allow determination of drug targeting in vivo and to aid selection of patients most likely to benefit from TopoI inhibitor therapy. γ-H2AX formation may be a useful pharmacodynamic marker. A gene signature developed for topotecan sensitivity/resistance may have value in patient identification. Convergence of these efforts should result in clinically effective second generation TopoI inhibitors.

Topoisomerase I (TopoI) is an essential enzyme in mammalian cells [1,2]. TopoI knockout mice die very early in embryogenesis. TopoI produces a single strand break in DNA allowing relaxation of the DNA for replication of the DNA. The single strand break is then religated, thus restoring the DNA double strands. Interestingly, no energy cofactor is required to carry out this reaction suggesting that hydrolysis was not involved in the mechanism of the DNA cleavage; otherwise religation would require a coupled reaction to balance the unfavorable free energy of dehydration in an aqueous medium [3]. It was therefore proposed that the enzymatic mechanism involves two sequential transesterification reactions. In the cleavage

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reaction, the active site tyrosine (Tyr 732 in human TopoI) acts as a nucleophile. The phenolic oxygen attacks a DNA phosphodiester bond, forming an intermediate in which the 3′ end of the broken strand is covalently attached by an O^4-phosphodiester bond to the TopoI tyrosine [3,4]. The religation step consists of a transesterification involving nucleophilic attack by the hydroxyl oxygen at the 5′ end of the broken strand. Both the breakage and closure reactions generate phosphodiester bonds and the free energies of hydrolysis are similar. Therefore the equilibrium constant is near unity and the reaction is freely reversible. However, the equilibrium has been shown to favor religation [3]. Repair of TopoI-mediated DNA damage has been reviewed [5].

DNA topoisomerase enzymes regulate the topological state of DNA, as a result, they control the cellular processes that involve DNA [6]. Topoisomerase activity is crucial for initiation and elongation during DNA synthesis, for the proper separation of sister chromatids during mitosis, for RNA transcription and for illegitimate recombination events [7–16]. DNA topoisomerase I associates preferentially with transcriptionally active genes and is thought to be involved in relaxing supercoils introduced by RNA polymerase during RNA transcription [17–20].

The TopoI-targeted drugs topotecan and irinotecan and the TopoII-targeted drugs doxorubicin, amsacrine, etoposide and teniposide, stabilize the covalent topoisomerase–DNA complex, thereby preventing religation [21–23]. The TopoI reaction intermediate consists of the enzyme covalently linked to a nicked DNA molecule. This assembly is known as a ''cleavable complex''. If cells expressing TopoI are exposed to the detergent sodium dodecyl sulfate (SDS), they undergo protein denaturation and quenching of the topoisomerization reaction [24–26]. Any cleavable complexes present are trapped as such, because the enzymatic machinery necessary to catalyze religation is no longer functional. Covalently bound TopoI–DNA complexes can then be recovered intact and purified. This technique has been used to determine the preferred sites of enzymatic cleavage.

TopoI inhibitors exhibit S-phase cytotoxicity and G2–M cell cycle arrest. A replication fork collision between an advancing replication fork and the inhibitor-trapped TopoI cleavable complex triggering replication fork arrest and breakage to generate a DNA double-strand break and a covalent TopoI–DNA complex, has been proposed to explain the S-phase cytotoxicity [27,28]. This collision is responsible for the G2–M arrest and activation of DNA damage signals including nuclear factor kB activation, p53 up-regulation, replication protein A phosphorylation, Chk1 phosphorylation and ATM/ATR activation [29,30]. Elevated TopoI levels in tumors are a factor in the antitumor activity of TopoI inhibitors [31,32]. In the presence of inhibitors, TopoI is down regulated and targeted to the ubiquitin/proteasome pathway [33–35]. Camptothecin–TopoI–DNA cleavable complexes are rapidly conjugated with SUMO, a ubiquitin-like protein, by UBC9, perhaps as a repair response [36,37].

Camptothecin analogs, topotecan and irinotecan, are the only TopoI-targeted anticancer drugs. Despite clinical success, there are several problems with camptothecin-derived anticancer agents. A major limitation is from the chemical equilibrium between camptothecin lactone form and the E ring-opened form. The E ring-opened carboxylate form has less than 10% the potency of the lactone form as a TopoI inhibitor and is inactive in cell culture, perhaps due to inability to cross the cell membrane [38,39]. Camptothecin analogs suffer from another drawback which further limits antitumor efficacy [5,40–44]. Although these drugs can freely enter cells via passive diffusion across cell membranes, their intracellular concentration is greatly reduced by efflux pumps in a wide variety of tissues. Multi-drug resistance (MDR) results from drug efflux by the well characterized P-glycoprotein (P-gp) [40]. Both topotecan and irinotecan are substrates for P-gp. Additionally, all camptothecins are substrates for the pump known as breast cancer resistant protein (BCRP) [41–44].

1. Homocamptothecins

Modifying the metabolically liable camptothecin lactone ring from a six-membered α-hydroxylactone to a seven-membered β-hydroxylactone ring led to the development of the homocamptothecin family of compounds exemplified by diflomotecan (Fig. 1) [45–51]. Modification of the crucial E-ring by insertion of a methylene group between the keto-group and the hydroxyl-substituent, conserved or enhanced TopoI enzyme inhibition. The homocamptothecins are potently cytotoxic and are active antitumor agents in human tumor xenografts. In contrast to camptothecins, homocamptothecins undergo slow lactone ring opening in plasma. After 3 h in human plasma the homocamptothecin BN80927 was more than 90% intact while 80% of SN38, the active species from irinotecan, was ring-opened. The half-life for ring opening of BN80927 was 21 h and for SN38 was less than 30 min [45]. The homocamptothecins, like the camptothecins, are substrates for cellular efflux pumps such as ABCG2 (Table 1) [47,48]. Cell lines that are resistant to camptothecins by overexpression of multi-drug resistance efflux pumps or mutations in TopoI are also resistant to homocamptothecins [51].

The difluoro homocamptothecin derivative BN80915 (diflomotecan) is one of the most potent topoisomerase inhibitors as measured by number of DNA-strand breaks and cytotoxicity in cell-based assays [50]. In preclinical safety studies, the dose-limiting toxicity of diflomotecan was myelosupression [46]. A Phase I clinical study with diflomotecan was conducted to determine the maximum tolerated dose when diflomotecan was administered as a 20 min intravenous infusion once every three weeks; and to evaluate the pharmacokinetics and plasma concentration versus neutropenic effect relationships (Table 1). The patients in the Phase I trial had a variety of solid tumors. The main toxicological side-effects were hematological especially severe neutropenia. The recommended dose was established to be 4 mg/m^2 for this route and schedule of administration. The time course of the neutropenia could be described by a PK/PD model using the neutrophil cell counts. Diflomotecan is currently in Phase II clinical trial.

2. Gimatecan

Gimatecan (ST1481) is a seven-position modified lipophilic camptothecin derivative that was developed to provide rapid

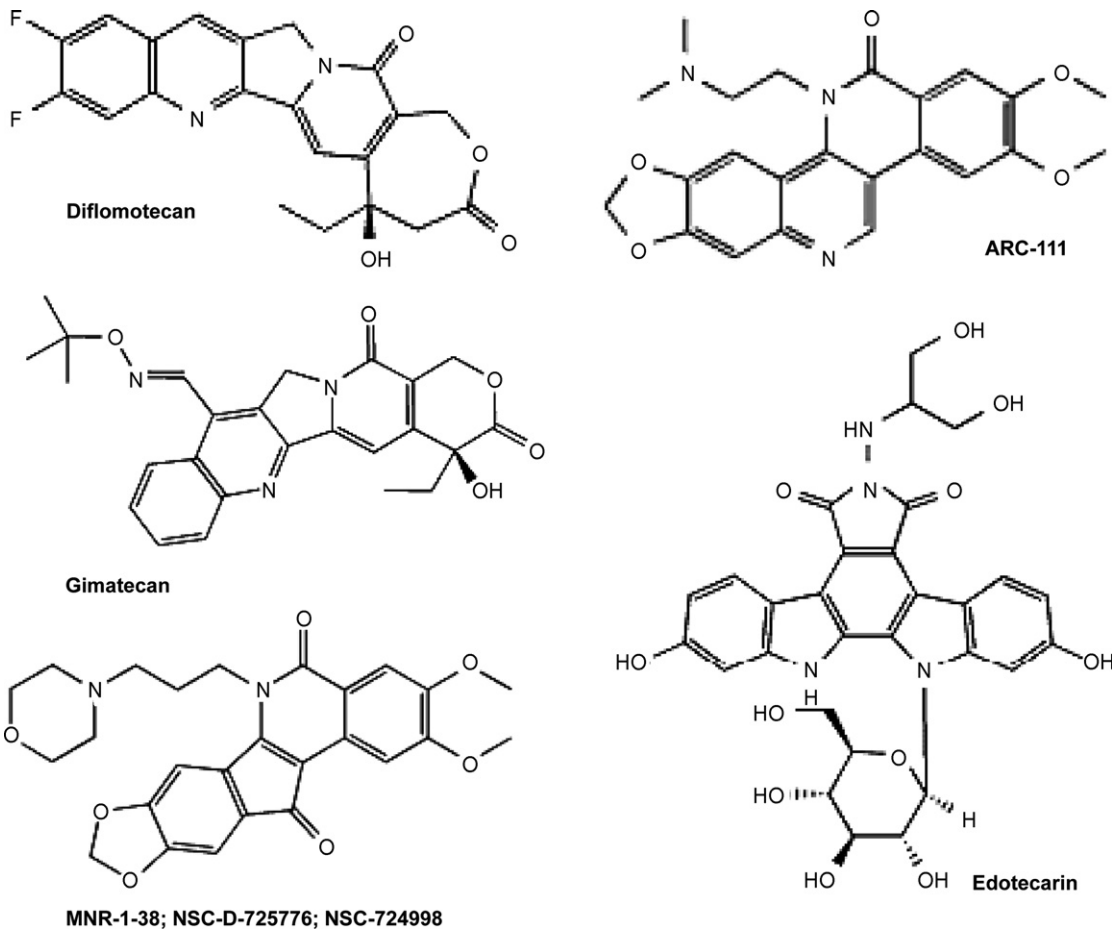

Fig. 1 – Chemical structures of topoisomerase I inhibitors.

uptake and enhanced accumulation in cells and to prolong and stabilize the TopoI–DNA–drug ternary complex (cleavage complex) compared with conventional camptothecins (Fig. 1) [52–56]. Specifically, gimatecan is 7-t-butoxyiminomethyl-camptothecin. Gimatecan is very efficient in forming stable ternary complexes with DNA and TopoI resulting in a higher number of DNA strand breaks than toptotecan and SN38 under the same conditions [52,53]. Unlike topotecan and SN38, gimatecan is not a substrate for the breast cancer resistance protein (BCRP), an efflux pump, or for the multi-drug resistance P-glycoprotein efflux pump (Table 1) [55,56]. Gimatecan has routinely been administered orally in pre-clinical in vivo studies; however, the formulation used in the in vivo studies contained 10% DMSO. Gimatecan administered daily orally at doses between 0.25 and 4 mg/kg was a highly effective antitumor agent in a wide variety of human tumor xenografts grown subcutaneously, intracranially or intraperitoneally [54,55].

Gimatecan showed good tolerability in Phase I clinical trial (Table 1). The absorption, metabolism and excretion of [^{14}C]gimatecan was studied in patients after oral administration. Gimatecan had uncomplicated metabolism with rapid absorption (T_{max} was 1 h (range 0.5–3 h)) and a long elimination phase (mean $t_{1/2}$ = 91 h) with clearance of 0.6 L/h [57].

Table 1 – Chemical and clinical characteristics of topoisomerase I inhibitors of varied structure are listed

Compound	Chemical class	Multi-drug resistance substrate	Clinical trial status	Dose limiting toxicity
Topotecan	Camptothecin	Yes	Approved ovarian ca small cell lung ca	Neutropenia
Irinotecan	Camptothecin	Yes	Approved colorectal ca	Diarrhea, neutropenia
Diflomotecan	Homocamptothecin	No	Phase II	Neutropenia
Gimatecan	Camptothecin	No	Phase II	Thrombocytopenia
Edotecarin	Indolocarbazole	Yes	Phase II	Neutropenia, granulocytopenia
NSC-725776/724998	Indenoisoquinoline	?	Preclinical	?
ARC-111	Dibenzonaphthyridinone	No	Preclinical	?

Fecal excretion was the main elimination pathway. Gimatecan has undergone Phase II clinical study in advanced epithelial ovarian, fallopian tube and peritoneal cancers, advanced breast cancer, malignant glioma and metastatic colorectal cancer [58–61]. These studies indicate that gimatecan is active as a single agent with bone marrow suppression being the main dose limiting toxicity.

3. Edotecarin

Among the novel non-camptothecin topoisomerase I inhibitors, the indolocarbazoles are the most advanced [62,63]. The two most prominent of these compounds are NB-506 and edotecarin (PHA-782615; J-107088) (Fig. 1). Edotecarin is a derivative of NB-506 that as a TopoI inhibitor induces single-strand DNA cleavage more effectively than either NB-506 or camptothecin. Although TopoI-mediated cleavage can be demonstrated with these compounds in vitro and in cell-based assays, other mechanisms contribute to the cytotoxicity of these compounds. Indolocarbazoles are also well-known as kinase inhibitors. Urasaki et al. [64] used three cell lines with known mutations in three different TopoI domains, human prostate carcinoma cells DU-145/RC1 (mutation R364H), Chinese hamster fibroblasts DC3F/C10 (mutation G503S) and human leukemia CEM/C2 cells (mutation N722S). The TopoI enzymes in these cells are resistant to both camptothecin and NB-506. However, the three cell lines which were 2-logs or more resistant to camptothecin, were only 2–10-fold resistant to NB-506 cytotoxicity, indicating that another mechanism contributes significantly to the cytotoxicity of this compound. Edotecarin (J-107088) emerged as an interesting novel indolocarbazole differing from campthothecin in selectivity in DNA cleavage at C/T-G compared with T-G/A for camptothecin and formation of very stable, persistent DNA-enzyme-drug cleavage complexes. Like the camptothecins, edotecarin is subject to multi-drug resistance by efflux from cells via ATP-binding cassette transporters such as breast cancer resistance protein (BCRP, MXR, ABCP) (Table 1) [65]. Edotecarin was tested extensively in human tumor xenografts including central nervous system tumors, colon cancer and breast cancer alone and in combination with other anticancer agents [66–68]. In preclinical safety studies, edotecarin was largely eliminated as unchanged parent molecule via biliary excretion.

Edotecarin has undergone several Phase I and Phase II clinical trials (Table 1) [69–72]. Edotecarin has been administered to patients as a 2 h intravenous infusion once every 21 days. The recommended dose for Phase II for patients in Japan and in the United States was 13 mg/m^2 intravenously every 3 weeks. The dose limiting toxicity was hematologic toxicity especially neutropenia, leukopenia and anemia. The pharmacokinetics of edotecarin was fully characterized [73]. Steady-state plasma concentrations were rapidly achieved during infusion followed by a bi-exponential decline with a very steep initial phase and a relatively shallow terminal phase (terminal $t_{1/2}$ = 20–25 h). Edotecarin has a moderate plasma clearance (45–53 L/h/m^2) and a large volume of distribution (650–850-L/m^2) [73]. As in the preclinical studies, biliary excretion was the major route of elimination. Edotecatrin has undergone Phase II clinical trials in irinotecan-naive metastatic colorectal cancer and breast cancer and Phase III clinical trial in glioblastoma multiforme [71].

4. Indenoisoquinolines

The indenoquinoline NSC314622 was identified as a potential TopoI inhibitor by COMPARE analysis from 48 h cytotoxicity screening of the NCI 60-cell line panel (Fig. 1) [62]. Further investigation showed that NSC314622 produces TopoI-mediated DNA strand breaks at the same site as camptothecin and at additional sites. NSC314622 also inhibits TopoI-mediated supercoiled DNA relaxation but not by intercalation into DNA. Exposure of MCF-7 breast cancer cells to NSC314622 for 1 h resulted in formation of protein-associated breaks in genomic DNA, reflecting formation of stabilized DNA–TopoI cleavable complexes. These findings initiated development of a series of more than 400 compounds to elucidate the structure–activity relationship for indenoisoquinoline TopoI inhibitors [1,74–79]. The indenoquinolines have several different characteristics from camptothecins (Table 1). They are chemically stable and do not contain the labile hydroxylactone E-ring characteristic of camptothecins. Indenoisoquinolines target different DNA sequences in formation of DNA–TopoI cleavable complexes than camptothecins and the complexes formed are much more stable than those formed by camptothecins. Co-crystal structures of camptothecin and two indenoisoquinolines in ternary complexes with TopoI and DNA show that the small molecules interact with the DNA in hydrophobic stacking interactions and with TopoI by a network of hydrogen bonds revealing a common interfacial inhibitory paradigm [1,74]. Various regions of the indenoisoquinoline molecule have been optimized for TopoI inhibitory activity including examination of nitrogen heterocycles on the lactam ring, nitration in the isoquinoline ring, length of the lactam side-chain and substituents in the 9-position [76–79]. The potential of bisindenoisoquinolines has also been explored [75,80].

Indenoisoquinoline derivatives, NSC-725776 and NSC-724998, have been selected for clinical development [1,81]. Pilot single-dose safety studies were carried out in beagle dogs [81]. When administered as a 15-min intravenous infusion, the maximum tolerated dose for NSC-725776 was 3 mg/kg and for NSC-724998 was 7.5 mg/kg. An assay for gamma-H2AX has been developed and validated as a pharmacodynamic biomarker for potential use in early clinical trials of the indenoisoquinoline derivatives [82].

5. Dibenzonaphthyridinones

Nitidine and fagaronine are benzo[c]phenanthridine alkaloids with good antitumor potency [83]. Both compounds are active as TopoI inhibitors [84,85]. Synthetic compounds of this chemical class such as ARC-111 are as potent as camptothecin in stimulating TopoI-mediated DNA cleavage using purified human TopoI and are more potent than irinotecan in many human tumor xenograft efficacy models (Fig. 1) [86–88]. An extensive structure–activity relationship was conducted around the dibenzo[*c*,*h*][1,6]naphthyridin-6-one family of

compounds that elucidated structural features associated with potent TopoI-targeting activity and suitable pharmaceutical properties [89]. Enhanced TopoI-targeting, cytotoxic potency, and robust antitumor activity were associated with: (1) methoxy substitutents at both the 2- and 3-positions of the A-ring, (2) a 8,9-methylenedioxy moiety with in the D-ring and (3) hetero-atom substitution adjacent to the benzo ring that incorporates the methylenedioxy substituent. These compounds are not substrates for MDR1 or BCRP efflux (Table 1). The compound 8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-2,3-methylenedioxy-5H-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (ARC-111, topovale) was selected for in-depth study in cell-based and efficacy models [88–90]. The cytotoxicity of ARC-111 was assessed in seven human tumor cell lines of varied histology and resistance mechanisms by MTT assay or colony formation. Compared with topotecan and SN38, ARC-111 was a more potent cytotoxic agent and was highly effective in cells expressing the efflux pumps. ARC-111 was an active antitumor agent in SCID mice bearingseveral standard human tumor xenografts. ARC-111 was as active as irinotecan in the HCT-8 colon carcinoma and as active as topotecan or irinotecan in the SKNEP anaplastic Wilm's xenograft [88]. ARC-111 and several congeners were also very effective antitumor agents in animals bearing the SJ-BT45 medulloblastoma [89]. The efficacy of ARC-111 was compared with irinotecan in human HCT116 colon cancer xenografts. Irinotecan was administered at 60 mg/kg/day, iv, Q4Dx3, and ARC-111 was administered at 2 mg/kg/day, iv, QODx3x2wk. Tumor growth delays were 17 days for both irinotecan and ARC-111. A similar study was performed with the human HT29 colon cancer xenograft, and the tumor growth delays were 10 days for irinotecan and 9 days for ARC-111. ARC-111 was compared with docetaxel in human NCI-H460 non-small cell lung carcinoma xenografts. Docetaxel (20 mg/kg/day, iv, QODx3) and ARC-111 administration each resulted in a tumor growth delay of 21 days. ARC-111 was compared with gemcitabine in human MiaPaCa2 pancreatic cancer xenograft. Gemcitabine (90 mg/kg/day, iv, Q3Dx4) administration resulted a tumor growth delay of 7 days as did ARC-111 [90].

6. Predictive markers and biomarkers

6.1. *Bone marrow CFU-GM*

Bone marrow is a normal tissue that is critically sensitive to many antineoplastic agents. Compounds including TopoI inhibitors kill rapidly dividing bone marrow progenitor cells resulting in acute reversible neutropenia and thrombocytopenia 4–20 days later (Table 1) [91]. Repopulation of the marrow progenitor niche precedes recovery of peripheral cell counts by several days. A goal during preclinical development is to predict whether a new agent will be toxic to the bone marrow, whether the toxicity will be specific to one hematopoetic cell lineage and whether bone marrow progenitor cells will be much more sensitive to the agent than will a variety of human malignant cells.

Bone marrow granulocyte–macrophage-colony forming unit (CFU-GM) assays comparing the sensitivity of bone marrows across species have been useful in predicting the blood levels of agents that might be reached in patients compared with blood levels in preclinical efficacy and safety species. With many cytotoxic agents, the bone marrow of mice is less sensitive than human bone marrow, thus allowing blood levels to be achieved in preclinical efficacy testing that cannot be reached in patients. The bone marrow toxicity of several TopoI inhibitors has been studied (Table 2) [90–93]. While human and canine bone marrow may have similar sensitivity to TopoI inhibitors, murine bone marrow is 4.5- to 27-fold less sensitive to these compounds. The differential sensitivity between murine and human bone marrow progenitor cells to TopoI inhibitors may explain, in part, why curative doses/blood levels of topotecan and 9-amino-camptothecin in mice with human tumor xenografts are not achievable in patients [93]. The corollary is that compounds with smaller or no differential in bone marrow progenitor sensitivity amongst species would likely have a better potential for reaching similar blood levels in patients as in mice, if bone marrow toxicity is dose-limiting in humans, these compounds would be more likely to be successful in reaching therapeutic doses. From these data, camptothecin and ARC-111 would be predicted to be most promising; however, camptothecin suffers from metabolic instability.

Pessina [91] went further to suggest that through use of the ratio of mouse/human CFU-GM IC_{90} values and the maximum tolerated dose of the compound in mice that the maximum tolerated dose of the compound in patients could be predicted and thus the potential for achieving a therapeutic blood level in patients estimated.

6.2. *Pharmacodynamic DNA damage markers*

There is an increasing effort early in the clinical experience with investigational anticancer agents to confirm that the molecule is achieving the desired biological effect [94,95]. The driving forces are to discontinue molecules that for some reason are not 'hitting' the desired therapeutic target (yet may be toxic), to efficiently select a dose of the investigational agent that achieved the desired biological effect and to facilitate the selection of patients who may best benefit from treatment with the new therapeutic agent. Thus, the identification of biomarkers (pharmacodynamic and pharmacogenomic markers) in the tumor and/or normal tissue(s)in the patient is often proceeding in parallel with preclinical development of potential new anticancer therapies. With greater frequency, the early clinical exploration of investigational anticancer agents is being integrated with pharmacodynamic assays [95]. The anticipation is that the time required for anticancer drug development will be shortened through the application of pharmacokinetic and pharmacodynamic measurements, and biomarker determinations in the earliest clinical trials.

TopoI inhibitors are highly targeted molecules that stabilize the DNA single strand break–enzyme complex (TopoI cleavage complex). DNA single strand breaks result in replication fork collapse and the efficient formation of DNA double strand breaks that have been described as replication-mediated double strand breaks [96]. The Mre11·Rad50·Nbs1 (MRN) complex binds DNA double strand breaks to repair DNA and activate checkpoints. Replication-mediated DNA double strand breaks induced by TopoI cleavage complexes interact

Table 2 – The concentrations of topoisomerase I inhibitors (nM) inhibiting bone marrow CFU-GM from human canine and mouse are shown

Compound	Mouse CFU-GM IC_{50} (nM)	Human CFU-GM IC_{50} (nM)	Canine CFU-GM IC_{50} (nM)	Canine CFU-GM IC_{90} (nM)	Mouse CFU-GM IC_{90} (nM)	Human CFU-GM IC_{90} (nM)	Fold difference between mouse and human IC_{90}
Camptothecin	18	1.7	0.5	7.6	42	16	5.5
9-Amino-camptothecin	20	0.6	0.3	7.6	66	6.2	11
Topotecan	128/166	2.8/6.5	1.7	7.6	381/519	39/19	10/27
SN-38	108	10			331	26	13
ARC-111	8	1.9			28	6.2	4.5

The data are for continuous exposure (12–16 days) of the cells to the compounds in a methylcellulose media over a broad concentration range of compound. The values represent 50% (IC_{50}) and 90% (IC_{90}) cell killing [90,93]. Colonies of 30 cells or more were counted.

with MRN which activates the Chk2 checkpoint downstream from ATM (Fig. 2). In addition to activation of the ATM-Chk2 pathways, replication-mediated DNA double strand breaks also activate RPA2 phosphorylation, histone γ-H2AX and BLM phosphorylation. Therefore, both ATM and Chk2 are recruited together with MRN and γ-H2AX and both Mre11 and Nbs1 are likely phosphorylated by ATM [97]. MRN is critically important for the functional activation of ATM-Chk2 and it is known the NBS-, AT- and Chk2-deficient cells are hypersensitive to camptothecin, thus suggesting that MRN and Chk2 may have prognostic value in selecting patients who could most benefit from treatment with a TopoI inhibitor [96].

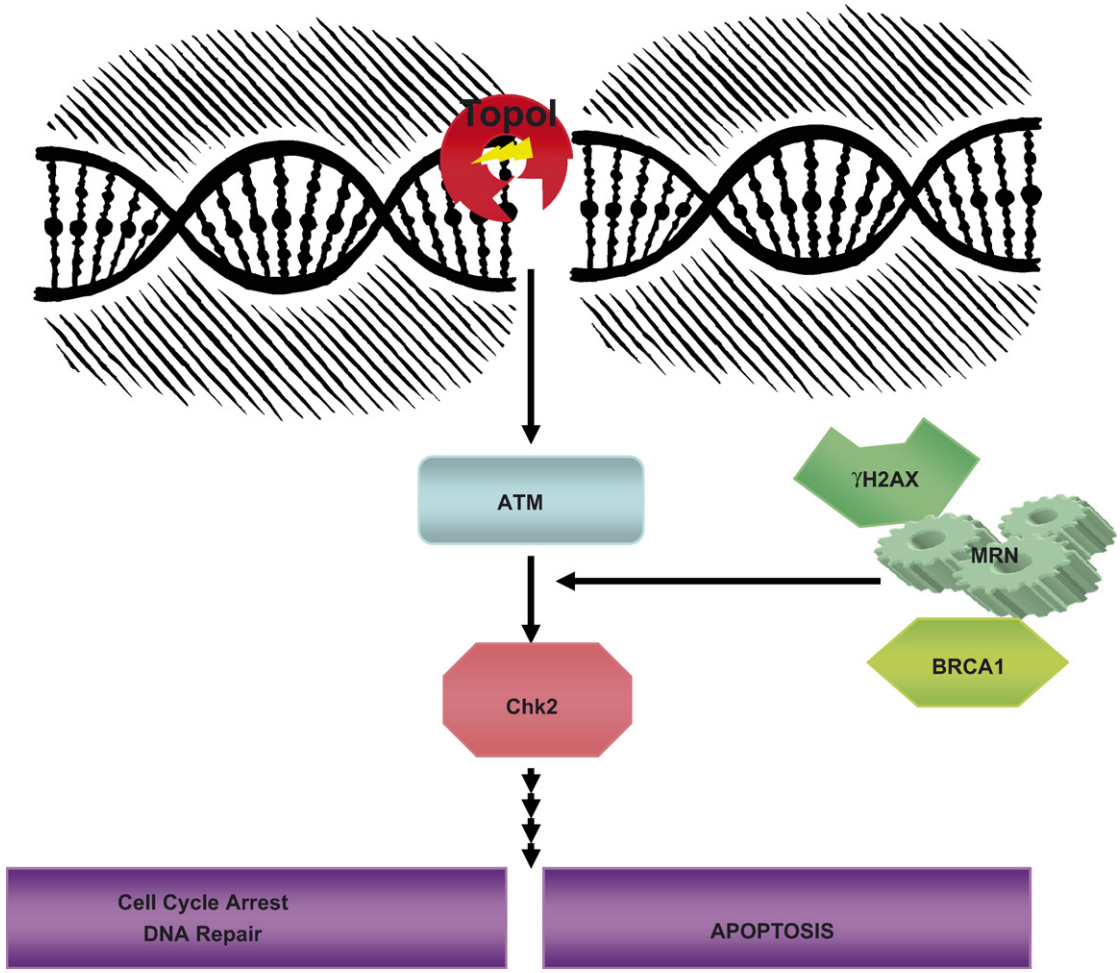

Fig. 2 – Schematic showing pharmacodynamic markers in the molecular pathway involved in cellular responses to TopoI cleavage complexes. TopoI inhibition induced replication-mediated DNA double strand breaks activate ATM and Chk2. Phosphorylated histone H2AX (gamma-H2AX) occurs in response to the DNA double strand breaks. Phosphorylation of Mre11 and Nbs1 two protein in the MRN complex (Mre11·Rad50·Nbs1) is also induced by replication-mediated DNA double strand breaks. Ultimately downstream of Chk2 either the cells are arrested in the cell cycle and DNA repair can occur or the cells proceed to apoptosis.

The phosphorylated histone H2AX (γ-H2AX) produced in response to DNA double-strand breaks can be detected by immunofluorescence and has been correlated directly with DNA damage severity and genomic repair (Fig. 2) [82]. A γ-H2AX immunocytochemical assay has been developed and validated to monitor response to DNA damage in human blood and tumor biopsies. Other markers of DNA damage studied were p53BP1, Chk2 and ATM. Formation of γ-H2AX foci were quantitative with DNA damage dosing with ionizing radiation and were detectable within 2 h of treatment. γ-H2AX formation appears to be a robust pharmacodynamic biomarker with potential for clinical monitoring of DNA damage in PBMC and tumor specimens from early clinical trial patients [82].

6.3. *Gene signatures*

The ready availability of genomic microarray technology for determination of RNA expression levels from varied biological samples has allowed the application of this technology to identification of biomarker gene signatures for diseases and drug response, drug sensitivity and drug resistance [98]. A gene signature consists of a list of genes whose expression is correlated with the biological state of interest. Much of the early work in the application of this biomarker technology to oncology began with the NCI-60 cell line panel for which gene expression patterns have been profiled providing a baseline of cell line molecular characteristics. The NCI-60 gene expression profiles along with the vast database of compound response data for these cell lines facilitated the initial approaches to connecting gene expression patterns with sensitivity or resistance to particular compounds/drugs. Using cell-based drug sensitivity coupled with Affymetrix microarray data, Potti et al. [99] developed gene expression signatures that predict sensitivity to six chemotherapeutic agents including topotecan. The NCI-60 cell line data was used as a starting point and the gene signatures were then tested in an additional cell lines. The gene signature for topotecan sensitivity/resistance included about 150 genes. Data from the analysis of thirteen ovarian cancer cell lines was used to link prediction of chemosensitivity to topotecan with expression of a deregulated oncogenic pathway. Ovarian cancer cell lines that were predicted to be topotecan resistant had a higher likelihood of Src pathway deregulation [99]. The development of gene expression profiles that can predict response to investigational new agents should allow the selection of patients who can best benefit from these treatments in early clinical trials and shorten the time required for clinical trial.

7. Conclusion

TopoI remains a target of active interest in the development new anticancer agents because TopoI inhibitors are clearly active and effective anticancer drugs and because the current TopoI inhibitors are molecules that can be improved upon. There are currently camptothecin and non-camptothecin TopoI inhibitors in preclinical and clinical development. Each of these investigational molecules may have properties that lead to improved therapeutic benefit to patients. There are also very active preclinical efforts based upon protein phosphorylation levels and mRNA levels in tumor and blood samples to define biomarkers that can select patients most likely to benefit from treatment with investigational TopoI inhibitors and to guide clinical investigators toward definition of the lowest effective dose and optimal schedule for administration of these new agents. The convergence of these efforts should result highly clinically effective second generation TopoI inhibitors for the treatment of malignant disease on patients.

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