Targeting Topoisomerase II Activity in NSCLC with 9-Aminoacridine Derivatives

DAVID M. FERGUSON[2,3], BLAKE A. JACOBSON[1], JOE JAY-DIXON[1], MANISH R. PATEL[1], ROBERT A. KRATZKE and AHMAD RAZA[1]

Departments of [1]Medicine, [2]Medicinal Chemistry and [3]Pharmacology, University of Minnesota, Minneapolis, MN, U.S.A.

Abstract. *Background: Etoposide and other type-II human topoisomerase (TOPOII) poisons are widely used for the treatment of many different cancer types, including non-small cell lung cancer (NSCLC). However, there is a risk for the development of therapy-related secondary leukemia following treatment with these TOPOII poisons. Five to seven years is the typical latency period for the development of secondary leukemia. One of the strategies to overcome this issue is to develop agents that do not act as poisons but still effectively inhibit topoisomerase activity. This has led to the development of acridine-based agents, which are catalytic TOPOII inhibitors, that do not generate DNA strand breaks that can lead to secondary malignancies in in vitro tests. Materials and Methods: In this study, we showd antiproliferative activity of a series of acridine-based catalytic inhibitors of TOPOII using four NSCLC cell lines (H460, A549, H2009 and H2030). Cells were treated with four acridine-based compounds for 72 h. Results: The results indicate that these compounds inhibit NSCLC cell proliferation with half-maximal effective concentration (EC_{50}) ranging from 8.15 to 42.09 μM. Combination therapy with cisplatin resulted in increased potency. Poly (ADP-ribose) polymerase cleavage and Guava Nexin assays confirm that the primary mode of cell death was by apoptosis. Conclusion: This current work is part of a series of studies for this panel of acridine-based compounds bearing TOPOII-inhibitory activity against different solid tumor types. The acridine-based agents were found to substantially reduce NSCLC cell viability and induce apoptosis. In addition, the acridine-based compounds sensitized cells to cisplatin as measured by cell viability. The results are consistent with prior work on mesothelioma, small-cell lung cancer and pancreatic cancer with this same panel of 9-aminoacridine derivatives. These findings support further development of this type of catalytic TOPOII inhibitor as a novel agent for NSCLC therapy.*

Correspondence to: Ahmad Raza, MD, Division of Hematology Oncology and Transplant, University of Minnesota, Minneapolis, MN 55455, U.S.A. Tel: +1 6126245620, Fax: +1 6126256919, e-mail: razax019@umn.edu

Key Words: Non-small cell lung cancer, human topoisomerase II, 9-aminoacridine derivatives.

Topoisomerase inhibitors, including etoposide and teniposide, commonly induce DNA re-arrangements comprising the mixed lineage leukemia gene on chromosome 11q23, which is the driving force behind secondary leukemia (1). A replacement for the type-II human topoisomerase (TOPOII) poisons with agents having similar TOPOII-inhibitory activities but lacking the mechanism for gene re-arrangement would likely diminish or possibly eliminate the risk of secondary malignancies.

With this in mind, the role of topoisomerase inhibitors in cancer therapeutics is evolving. They are effective as single agents and can be successfully combined with other agents in the treatment of lung cancer (2). There has been a constant level of interest in these agents, which includes efforts in the development of dual inhibitors of both TOPOI and TOPOII, catalytic inhibitors of TOPOII, and inhibitors specific to the TOPOIIα isoforms. These new strategies are showing promise of incorporation into the clinical setting, where the likelihood of secondary malignancies would diminish.

Humans have seven types of topoisomerases, which consists of two groups, TOPOI and TOPOII (3). Each type of enzyme has a distinct mechanism of action. TOPOI introduces transient breaks in single-stranded DNA, which helps remove torsional tensions, and then immediately seals them again. TOPOII on the other hand introduces nicks in both strands of the DNA helix simultaneously by an ATP-dependent mechanism. Two sub-classes of TOPOII, TOPOIIα and TOPOIIβ, have ATP-dependent strand passage ability, with few differences in function and structure. TOPOIIα has the unique ability to promote DNA strand relaxation (4). TOPOIIβ does not have the ability to do this and is a morphologically and immunologically distinct molecule (5).

Catalytic inhibitors have the ability to block the catalytic cycle of TOPOII prior to formation of the cleavable complex, preventing double-stranded DNA breaks and the formation of mutagenic linear DNA fragments. When proliferating cells are treated with TOPOII inhibitors of this type they stall in the G_1-S transition of the cell cycle (6). The cell responds by activating repair and recombination pathways. As a result, the programmed cell death pathways are activated (7). Multiple catalytic inhibitors of TOPOII have been in development and have shown promise in pre-clinical settings. Few of the agents were tested in phase I clinical trials and were either found to be too toxic or did not have clinical activity against variety of solid tumors (2).

Non-small cell lung cancer (NSCLC) is a heterogeneous group of diseases, which is not considered curable at advanced stages. Molecular characterization of this disease helps guide treatment at advanced stages, however, about 80% of NSCLCs do not harbor one of the driver mutations, which leaves chemotherapy as the only treatment option in these cases (8). Among approved chemotherapeutic agents for NSCLC, human TOPO inhibitors have an established role and are part of standard-of-care in this setting. Etoposide, which is a TOPOII poison, was tested in a phase III trial in combination with cisplatin and was approved for this indication (9). Often high-dose etoposide (>2.0 g/m^2) chemotherapy regimens are required for treatment of some tumors. It has been reported that at this high-dose level, the risk of secondary leukemia increases 336-fold compared to low-dose etoposide levels (10). Irinotecan, which is a TOPOI inhibitor, was also tested in a phase III trial in combination with cisplatin and is currently approved for the treatment of advanced NSCLC (11).

Aminoacridines are well-known TOPO-directed agents and one derivative, amsacrine, proved to be a potent TOPOII poison and demonstrated activity against acute myeloid leukemia (12). The aminoacridine core structure has been further exploited in the development of selective agents that display catalytic inhibitory activity against TOPOII (6, 13). One series in particular has been shown to be active across a wide range of cancer cells, both *in vitro* and *in vivo* (13-15). This panel of agents has been found to inhibit the catalytic cycle of TOPOII prior to formation of linear DNA fragments. These compounds were recently tested in mesothelioma cell lines and small-cell lung cancer cell lines and were shown to induce cell death *via* apoptosis for mesothelioma or *via* apoptosis and autophagy for small-cell lung cancer (16, 17).

Materials and Methods

Cell lines and cell culture. NSCLC cell lines H460, A549, H2009 and H2030 (American Tissue Culture Collection, Manassas, Virginia, USA) were cultured in RPMI-1640 containing L-glutamine (Gibco, Invitrogen, Waltham, Massachusetts, USA) supplemented with 10% calf serum (Biofluids, Rockville, Maryland, USA) and 1%, antibiotic-antimycotic (penicillin, streptomycin and amphotericin B (Life Technologies, Waltham, Massachusetts, USA). Cells were maintained at 37˚C in a humidified atmosphere in 5% CO_2.

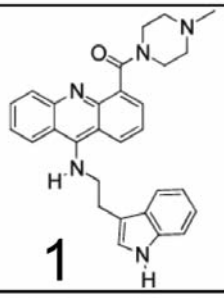
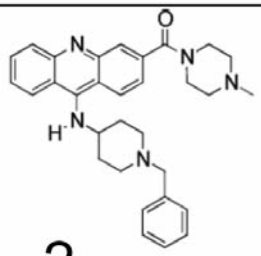
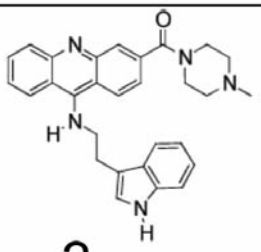
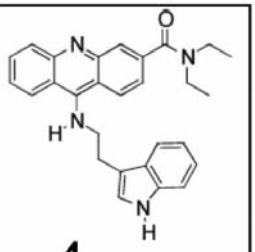
Cell proliferation assays. Cells were seeded as triplicate sets into 96-well plates with 5,000 cells in each well. Following overnight incubation cells were treated with the panel of four acridine-based compounds specified in Table I for 72 h. Control cells were treated with an equal concentration of vehicle (dimethyl sulfoxide) in each experiment. Cell viability was determined employing Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Gaithersburg, MD, USA) of CCK-8 mixture (10 μl) was added to each well, plates were incubated for 2 h at 37˚C, then the absorbance was measured at 450 nm. Cell viability values were normalized to those of untreated cells. The half maximal effective concentration (EC_{50}) of the four compounds for the NSCLC cell lines was determined by using GraphPad Prism 5.0 (La Jolla, California, USA).

Enhanced cytotoxicity assays. Cell viability was measured as above for H460 and A549 cell lines with the inclusion of cisplatin. After overnight incubation, cells were left untreated or treated with compounds 2 or 3 alone or each simultaneously with cisplatin. After an incubation period of 72 h, cell viability was determined using Cell Counting Kit-8.

Poly (ADP-ribose) polymerase (PARP) cleavage assessment. A total of 7.5×10^5 cells were seeded on plates (10 cm) and incubated overnight. Cells were then treated with acridine-based compounds (30 μM) or vehicle. Cell lysate was prepared following 48 h of treatment, as follows. Subsequently to washing cells with phophate-buffered saline (PBS), cells were treated with trypsin, centrifuged, washed with PBS, and resuspended in five times the cell volume of TNESV (50 mM Tris-HCl, pH 7.4; 1% NP-40; 2 mM EDTA, pH 8.0; 0.1 M NaCl) lysis buffer supplemented with phosphatase and protease inhibitors. Protein concentration was determined employing the Bradford assays. Eight percent sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was utilized to separate protein samples that were then transferred to hybond-P polyvinylidene fluoride membrane (GE Healthcare, Little Chalfont, United Kingdom). Membranes were probed separately with either mouse anti-β-actin (Sigma-Aldrich, St. Louis, Missouri, USA) at a dilution of 1:20,000 or rabbit anti-poly (ADP-ribose) polymerase (Cell Signaling, Inc. Danvers, Massachusetts) at a dilution of 1:1,000. Membranes were washed three times with tris-buffered saline containing 0.1% Tween-20 and placed in anti-rabbit or anti-mouse horseradish peroxidase-conjugated secondary antibodies at a dilution of 1:2,500 or 1:3,000, respectively, for 1 hour at room temperature with mild shaking. Blots were then washed four times for 5 min and developed using ECL Plus Western blotting system (GE Healthcare, Little Chalfont, United Kingdom) (18). Stimulation of apoptosis was denoted by the presence of two bands at 89 and 116 kDa corresponding to the cleaved and intact protein, respectively.

Annexin V measurement. Phosphatidylserine externalization was measured as a sign of apoptosis employing Guava Nexin Reagent and the Guava EasyCyte flow cytometer (Guava Technologies, Hayward, CA, USA). The Guava Nexin assay utilizes two stains (annexin V and 7-amino actinomycin D) to determine the percentage of apoptotic cells. Cells were collected as above 48 h following compound treatment. Guava Nexin reagent was added to

Table I. *Growth inhibition of four non-small cell lung cancer cell lines using acridine-based compounds 1-4. Values reported are half maximal effective concentration (μM) ±SD, n=3.*

Cell line	Compounds			
	 1	 2	 3	 4
H460	42.07 ± 14.72	17.65 ± 7.02	23.19 ± 5.92	26.88 ± 1.15
A549	15.25 ± 5.04	8.15 ± 1.78	15.56 ± 2.68	12.87 ± 3.31
H2009	20.01 ± 1.14	12.36 ± 3.14	15.28 ± 3.22	19.22 ± 3.33
H2030	33.56 ± 5.22	14.80 ± 1.88	22.17 ± 0.31	26.65 ± 5.68

the diluted (250,000 to 750,000 cells/ml) cells and incubated in the dark for 20 min at room temperature. Samples were examined using flow cytometry according to the manufacturer's recommendations. Findings are stated as the percentage of gated cells positive for annexin V staining. Analysis was performed in triplicate.

Results

NSCLC proliferation is suppressed by acridine-based compounds. Previous research revealed that a panel of acridine-based compounds inhibited proliferation in a variety of cancer types (13-17). To investigate whether these same acridine-based agents would also inhibit proliferation in NSCLC, four NSCLC cell lines were treated with the panel of drugs and cell survival was assessed. All four cell lines (H460, A549, H2009 and H2030) displayed sensitivity to the acridine-based compounds (Figure 1). At low micromolar concentrations, there was a dose-dependent effect on all cell lines. The EC_{50} ranged from 8.15 to 42.09 μM (Table I). The cell line most sensitive to the drugs was A549, while the most resistant was H460. The most potent of the four acridine-based agents was compound 2, with an EC_{50} range of 8.15 (cell line A549) to 17.65 (cell line H460) (Figure 1 and Table I).

Acridine-based agents enhance susceptibility of NSCLC cells to cisplatin. In the treatment of NSCLC, platinum agents are used in combination with other currently used TOPO inhibitors such as etoposide and irinotecan. This is based on proven superior clinical efficacy of combination regimens (19). To explore the possibility that acridine-based agents would enhance chemotherapy-induced cell killing, we treated NSCLC cells with cisplatin alone and in combination with acridine-based compounds 2 or 3. Cell viability was reduced for cell lines H460 and A549 treated for 72 h with the combination of acridine-based drugs and cisplatin compared to each agent alone (Figure 2). In each instance, cell viability for the combined treatment was significantly reduced compared to that with each drug alone. Cisplatin-induced cell death was enhanced at the indicated concentrations by 23% (H460) and 21.9% (A549) when comparing cells treated with cisplatin alone to those treated with the addition of acridine-based compound 2 and by 40% (H460) and 31.2% (A549) when evaluating cells treated with cisplatin to those also treated with acridine-based compound 3 (Figure 2). These *in vitro* findings parallel the clinical efficacy of TOPO inhibitors such as etoposide in combination with platinum agents (9).

Apoptosis is induced by acridine-based drugs in NSCLC. DNA topoisomerase inhibition and TOPOIIα depletion in mammalian cells is known to induce apoptosis (20). Previous studies on these compounds confirmed their ability to induce apoptosis of pancreatic cancer cells (14, 21). We explored the possibility that acridine-based agents would lead to apoptotic cell death in NSCLC. Two markers of apoptosis were studied, poly (ADP-ribose) polymerase (PARP) cleavage and annexin V staining. NSCLC cell line H2030 was treated with acridine-based compounds 1 and 2 (30 μM), while H460 was treated

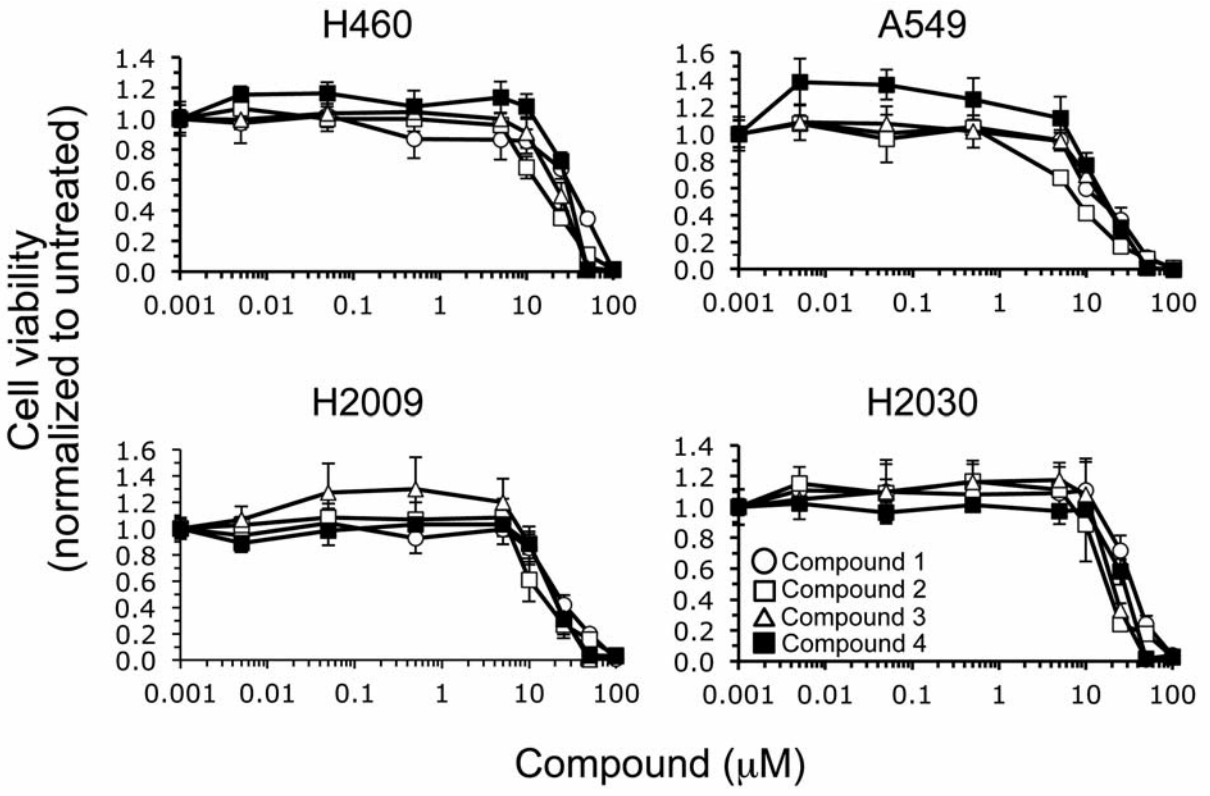


Figure 1. *Acridine-based compounds suppress non-small cell lung cancer (NSCLC) proliferation. NSCLC cell lines H460, A549, H2009 and H2030 were left untreated or treated with compounds at the indicated concentrations for 72 h. Data are expressed as the mean ±SD of three independent determinations of cell number normalized to untreated cells.*

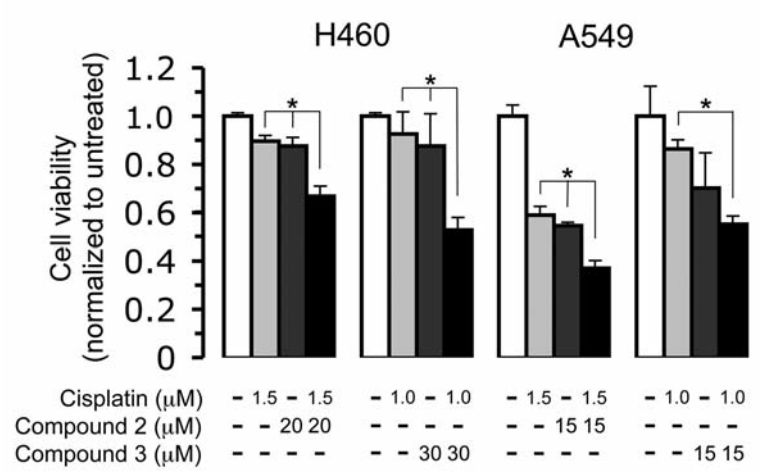


Figure 2. *Susceptibility of non-small cell lung cancer (NSCLC) cells to cisplatin is enhanced by acridine-based compounds. NSCLC cell lines treated with compounds 2 or 3 were treated with the indicated concentrations of cisplatin. Viable cells were counted after 72 h. Vehicle dimethyl sulfoxide (DMSO) was present and equal in all treatments. Data are the mean±SD of three independent determinations of cell number normalized to untreated cells. Averages of combination treatment were compared to either agent alone by Student's t-test. *Significantly different at p<0.02.*

with compounds 3 and 4 (30 μM) or both cell lines were left untreated for 48 h and lysates prepared. Western blot analysis revealed that treatment of H2030 cells with compounds 1 and 2 and of H460 cells with compounds 3 and 4 led to increased poly (ADP-ribose) polymerase cleavage, signifying apoptosis compared to untreated cells (Figure 3). A consequence of apoptosis that can be measured by annexin V staining is the externalization of phosphatidylserine. NSCLC cell lines A549

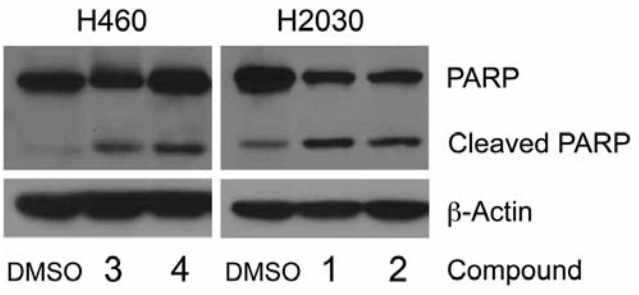


Figure 3. *Apoptosis is induced in non-small cell lung cancer (NSCLC) by acridine-based compounds. H460 cells treated with compounds 3 and 4 and H2030 cells were treated with compounds 1 and 2 at the concentration of 30 μM for 48 h. Proteins from lysates were immunoblotted with anti-poly (ADP-ribose) polymerase. β-Actin served as a loading control. Poly (ADP-ribose) polymerase cleavage, an indicator of apoptosis, was signified by the presence of an 89-kDa fragment.*

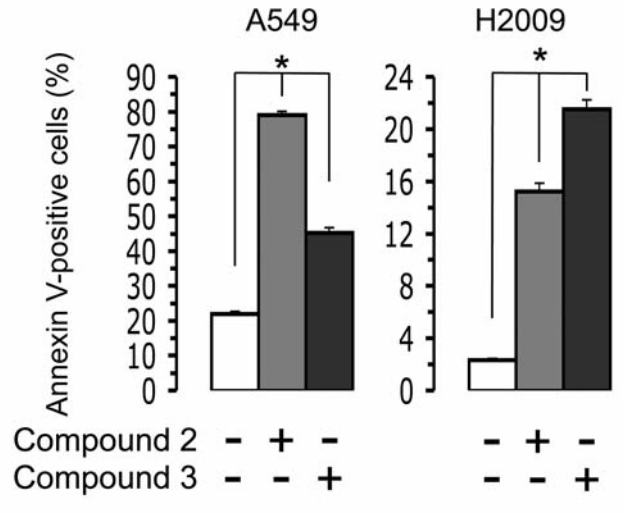


Figure 4. *Acridine-based compounds stimulate apoptosis of non-small cell lung cancer (NSCLC) cells. Graphic depiction of flow cytometric analysis of A549 and H2009 cell lines treated with compound 2 and 3 (30 μM) or vehicle dimethyl sulfoxide (DMSO) for 48 h. Annexin V staining is elevated in compound treated NSCLC cells. Results are expressed as the mean±SD of three independent determinations. *Significantly different at p<0.0003 between treated and untreated cells.*

and H2009 were treated with acridine-based compounds 2 or 3 or were left untreated, and cells were then subjected to annexin V measurement. Annexin V-positive cells increased from 21.9 to 79% and 21.9 to 45.2% for A549 cells and from 2.3 to 15.2% and 2.3 to 21.5% for H2009 cells following treatment with acridine-based compounds 2 and 3, respectively (Figure 4). Overall these results demonstrate that acridine-based agents are capable of inducing apoptotic cell death in NSCLC cells.

Discussion

The present investigation demonstrates that treatment with each compound of a panel of four acridine-based agents suppresses NSCLC proliferation and induces apoptosis. Importantly, two of the acridine-based drugs were shown to potentiate NSCLC cell killing when combined with cisplatin. These data suggest that these 9-aminoacridine compounds, upon further development, could potentially be an effective and less toxic therapy for patients with NSCLC.

Multiple new strategies to block the DNA replication cycle of proliferating cells are revitalizing the role of TOPO inhibitors in the treatment of cancer. TOPO poisons, such as etoposide and doxorubicin continue to be frontline agents in the war on cancer. The utility of these compounds, however, is often limited due to toxicity, stemming in part from their mechanism of action that is prone to produce linear DNA fragments (through trapping of the cleavable complex) that cause secondary leukemia. Catalytic inhibitors of TOPO, on the other hand, can function through a variety of mechanisms that disrupt the catalytic activity of the enzyme to induce cell-cycle arrest and apoptosis without producing secondary malignancies (5). The drug dexrazoxane, for example, belongs to this class of inhibitor and is a member of *bis*dioxopiperazine family of agents. These compounds bind to the *N*-terminal domain of TOPOII homodimer and lock the enzyme in the closed clamp form, preventing the release of the DNA (and hydrolysis of the second ATP molecule) (20, 22). In contrast to *bis*dioxopiperazines that function late in the catalytic cycle after ligation has occurred, the compounds examined in this study have been shown to block DNA from binding to the enzyme early in the cycle (2).

Structural studies have indicated that the primary mechanism of action of these compounds is through DNA intercalation, either through duplex or quadraplex association prior to enzyme recognition (2). These agents have been further shown to inhibit TOPOII before it breaks the phosphodiester backbone of DNA, thereby avoiding the generation of linear DNA fragments (2). Such a mechanism may have significant advantages in the design of safer treatments that diminish side-effects compared to poisons or other catalytic inhibitors that function late in the catalytic cycle. In fact, pre-clinical studies employing acridine-based compounds 1-4 in a mouse model of glioma have been performed and produced promising results in both pharmacokinetic measurements and survival (15). Similar results were shown both *in vitro* and *in vivo* using a pancreatic cancer model (21). In another investigation, these same substituted 9-aminoacridine derivatives have shown success *in vitro* as anticancer agents in small cell lung cancer and malignant mesothelioma (16, 23).

Lately, emerging concepts of dual inhibitors of TOPOI and TOPOII, catalytic inhibitors of TOPOII, and inhibitors specific to the TOPOIIα isoforms indicate a great potential for expanding treatment options. A compound with dual activity against TOPOI and TOPOII has the theoretical advantage of increasing activity and potential advantage of overcoming drug resistance. Compounds such as quinone derivatives and homocamptothecins have demonstrated dual inhibitory activity in preclinical studies (2). The Croton lechleri derivative alkaloid tapsine has shown activity against both TOPOI and TOPOII and proved effective against cells overexpressing drug efflux transporters (24). Many other compounds which include tafluposide, batracylin, and phenazine derivatives have shown promise and were tested in phase I and II clinical trials (25). Further research into multi-drug resistance protein pumps and P-glycoprotein and breast cancer resistance protein transporters will be needed to ascertain the relationship of dual inhibition of human TOPO and drug resistance mechanisms.

Drugs that target TOPOIIα can potentially eliminate the risk of secondary malignancies. Many TOPOIIα-specific poisons and catalytic inhibitors are under development. Poisons such as etoposide and doxorubicin have potent activity against the TOPOIIα isoform, while mitoxantrone is specific to the β isoform (2). A TOPOIIα inhibitor NK314 has shown preclinical activity against NSCLC (26). The α isoform has also been studied in depth in human epidermal growth factor receptor 2 (*HER2*)-amplified breast cancer. The gene for TOPOIIα is located in close proximity to *HER2* oncogene and copy number aberrations are found in *HER2*-amplified breast cancer (2). However, gene overexpression or amplification in this case did not increase susceptibility to *HER2*-directed treatment and its role as predictive biomarker remains unclear (27).

The data reported herein extend the potential utility of these compounds to treating NSCLC and further validates the catalytic cycle of TOPOII as a universal target in drug discovery and development. The journey of development of catalytic TOPOII inhibitors has been challenging. Multiple agents have been tested in clinical trials with varying results and so far, no catalytic inhibitor has been approved for clinical use. However, continued research and effort is helping us understand these challenges, which will lead to the creation of more potent and less toxic catalytic inhibitors of human TOPO enzyme.

Grant Support

Funding from the John Skoglund Lung Cancer Research Endowment supported this work.

Acknowledgements

The Authors would like to thank Ashley McDonald for her excellent technical assistance.

References

1 Ezoe S: Secondary leukemia associated with the anti-cancer agent, etoposide, a topoisomerase II inhibitor. Int J Environ Res Public Health *9*: 2444-2453, 2012.

2 Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, Milhem M, Demidov LV, Hassel JC, Rutkowski P, Mohr P, Dummer R, Trefzer U, Larkin JM, Utikal J, Dreno B, Nyakas M, Middleton MR, Becker JC, Casey M, Sherman LJ, Wu FS, Ouellet D, Martin AM, Patel K, Schadendorf D and Group MS: Improved survival with MEK inhibition in BRAF-mutated melanoma. N Engl J Med *367*: 107-114, 2012.

3 Leppard JB and Champoux JJ: Human DNA topoisomerase I: relaxation, roles, and damage control. Chromosoma *114*: 75-85, 2005.

4 Vologodskii A: Theoretical models of DNA topology simplification by type IIA DNA topoisomerases. Nucleic Acids Res *37*: 3125-3133, 2009.

5 Austin CA and Marsh KL: Eukaryotic DNA topoisomerase II beta. Bioessays *20*: 215-226, 1998.

6 Makhey D, Yu C, Liu A, Liu LF and LaVoie EJ: Substituted benz[a]acridines and benz[c]acridines as mammalian topoisomerase poisons. Bioorg Med Chem *8*: 1171-1182, 2000.

7 Bailly C: Contemporary challenges in the design of topoisomerase II inhibitors for cancer chemotherapy. Chem Rev *112*: 3611-3640, 2012.

8 Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, Jenkins RB, Kwiatkowski DJ, Saldivar JS, Squire J, Thunnissen E, Ladanyi M, College of American Pathologists International Association for the Study of Lung C and Association for Molecular P: Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Mol Diagn *15*: 415-453, 2013.

9 Belani CP, Lee JS, Socinski MA, Robert F, Waterhouse D, Rowland K, Ansari R, Lilenbaum R and Natale RB: Randomized phase III trial comparing cisplatin-etoposide to carboplatin-paclitaxel in advanced or metastatic non-small cell lung cancer. Ann Oncol *16*: 1069-1075, 2005.

10 Pedersen-Bjergaard J, Daugaard G, Hansen SW, Philip P, Larsen SO and Rorth M: Increased risk of myelodysplasia and leukaemia after etoposide, cisplatin, and bleomycin for germ-cell tumours. Lancet *338*: 359-363, 1991.

11 Ohe Y, Ohashi Y, Kubota K, Tamura T, Nakagawa K, Negoro S, Nishiwaki Y, Saijo N, Ariyoshi Y, and Fukuoka M: Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. Ann Oncol *18*: 317-323, 2007.

12 Kessler T, Mohr M, Muller-Tidow C, Krug U, Brunnberg U, Mohr B, Schliemann C, Sauerland C, Serve H, Buchner T, Berdel WE and Mesters RM: Amsacrine containing induction therapy in elderly AML patients: comparison to standard induction regimens in a matched-pair analysis. Leuk Res *32*: 491-494, 2008.

13 Goodell JR, Madhok AA, Hiasa H and Ferguson DM: Synthesis and evaluation of acridine- and acridone-based anti-herpes agents with topoisomerase activity. Bioorg Med Chem *14*: 5467-5480, 2006.

14 Goodell JR, Ougolkov AV, Hiasa H, Kaur H, Remmel R, Billadeau DD and Ferguson DM: Acridine-based agents with topoisomerase II activity inhibit pancreatic cancer cell proliferation and induce apoptosis. J Med Chem *51*: 179-182, 2008.

15 Teitelbaum AM, Gallardo JL, Bedi J, Giri R, Benoit AR, Olin MR, Morizio KM, Ohlfest JR, Remmel RP and Ferguson DM: 9-Amino acridine pharmacokinetics, brain distribution, and *in vitro*/*in vivo* efficacy against malignant glioma. Cancer Chemother Pharmacol *69*: 1519-1527, 2012.

16 Raza A, Jacobson BA, Benoit A, Patel MR, Jay-Dixon J, Hiasa H, Ferguson DM and Kratzke RA: Novel acridine-based agents with topoisomerase II inhibitor activity suppress mesothelioma cell proliferation and induce apoptosis. Invest New Drugs *30*: 1443-1448, 2012.

17 Ryan E, Blake AJ, Benoit A, David MF and Robert AK: Efficacy of substituted 9-aminoacridine derivatives in small cell lung cancer. Invest New Drugs *31*: 285-292, 2013.

18 Goodell LS, Pierce MB, Bravo CM and Ferris AM: Parental perceptions of overweight during early childhood. Qual Health Res *18*: 1548-1555, 2008.

19 Delbaldo C, Michiels S, Syz N, Soria JC, Le Chevalier T and Pignon JP: Benefits of adding a drug to a single-agent or a 2-agent chemotherapy regimen in advanced non-small-cell lung cancer: a meta-analysis. JAMA *292*: 470-484, 2004.

20 Akimitsu N, Kamura K, Tone S, Sakaguchi A, Kikuchi A, Hamamoto H and Sekimizu K: Induction of apoptosis by depletion of DNA topoisomerase IIalpha in mammalian cells. Biochem Biophys Res Commun *307*: 301-307, 2003.

21 Oppegard LM, Ougolkov AV, Luchini DN, Schoon RA, Goodell JR, Kaur H, Billadeau DD, Ferguson DM and Hiasa H: Novel acridine-based compounds that exhibit an anti-pancreatic cancer activity are catalytic inhibitors of human topoisomerase II. Eur J Pharmacol *602*: 223-229, 2009.

22 Galvez-Peralta M, Hackbarth JS, Flatten KS, Kaufmann SH, Hiasa H, Xing C and Ferguson DM: On the role of topoisomerase I in mediating the cytotoxicity of 9-aminoacridine-based anticancer agents. Bioorg Med Chem Lett *19*: 4459-4462, 2009.

23 Etchison RJ, Benoit B, Ferguson D and Kratzke R: Efficacy of substituted 9-aminoacridine derivatives in small cell lung cancer. Invest New Drugs 2012.

24 Fayad W, Fryknas M, Brnjic S, Olofsson MH, Larsson R and Linder S: Identification of a novel topoisomerase inhibitor effective in cells overexpressing drug efflux transporters. PLoS One *4*: e7238, 2009.

25 Salerno S, Da Settimo F, Taliani S, Simorini F, La Motta C, Fornaciari G and Marini AM: Recent advances in the development of dual topoisomerase I and II inhibitors as anticancer drugs. Curr Med Chem *17*: 4270-4290, 2010.

26 Toyoda E, Kagaya S, Cowell IG, Kurosawa A, Kamoshita K, Nishikawa K, Iiizumi S, Koyama H, Austin CA and Adachi N: NK314, a topoisomerase II inhibitor that specifically targets the alpha isoform. J Biol Chem *283*: 23711-23720, 2008.

27 Jarvinen TA, Tanner M, Rantanen V, Barlund M, Borg A, Grenman S and Isola J: Amplification and deletion of topoisomerase IIalpha associate with ErbB-2 amplification and affect sensitivity to topoisomerase II inhibitor doxorubicin in breast cancer. Am J Pathol *156*: 839-847, 2000.

Received May 26, 2015
Revised July 13, 2015
Accepted July 15, 2015