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Preclinical Results of Camptothecin-Polymer Conjugate (IT-101) in Multiple Human Lymphoma Xenograft Models

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Abstract

Purpose—Camptothecin (CPT) has potent broad spectrum antitumor activity by inhibiting type I DNA topoisomerase (DNA topo I). It has not been used clinically because it is water-insoluble and highly toxic. As a result, irinotecan (CPT-11), a water-soluble analogue of CPT, has been developed and used as salvage chemotherapy in patients with relapsed/refractory lymphoma, but with only modest activity. Recently, we have developed a cyclodextrin-based polymer conjugates of 20-(S)-CPT (IT-101). In this study, we evaluated the preclinical antilymphoma efficacy of IT-101 as compared to CPT-11.

Experimental Design—We determined an *in vitro* cytotoxicity of IT-101, CPT-11 and their metabolites against multiple human lymphoma cell lines. In human lymphoma xenografts, the pharmacokinetics, inhibitions of tumor DNA topo I catalytic activity, and antilymphoma activities of these compounds were evaluated.

Results—IT-101 and CPT had very high *in vitro* cytotoxicity against all lymphoma cell lines tested. As compared to CPT-11 and SN-38, IT-101 and CPT had longer release kinetics and significantly inhibit higher tumor DNA topo I catalytic activities. Furthermore, IT-101 showed significantly prolonged the survival of animals bearing s.c. and disseminated human xenografts when compared to CPT-11 at its maximum tolerated dose in mouse.

Conclusions—The promising present results provide the basis for a phase I clinical trial in patients with relapsed/refractory lymphoma.

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Introduction

Although great advances have been made in the treatment of malignant lymphoma, more than half of the patients with aggressive non-Hodgkin lymphoma (NHL) and a vast majority of patients with indolent lymphoma have resistant diseases or relapse after the initial treatment and eventually require salvage chemotherapy. In general, patients with Burkitt lymphoma, anaplastic large T-cell lymphoma (ALTC), and advanced-stage Hodgkin lymphoma (HL) who receives first-line combination chemotherapies can achieve 5-year overall survival rate in 65–90%, 37–93%, and 66–82% of patients, respectively (1–5). However, only a small number of these patients can achieve long-term disease-free survival (DFS) after high-dose therapy and hematopoietic stem cell rescue. The limitation of this approach is that not all patients respond to widely used salvage therapies including EPOCH (6), ESHAP (7), and MINE-ESHAP (8). Therefore, a novel agent for the salvage setting in these patients is needed. The development of salvage regimens are based on the combination of non-cross resistant agents from the first-line chemotherapy regimens. The DNA topoisomerase I (Topo I) inhibitors have been explored as candidates for salvage therapy in patients with relapsed/refractory NHL due to an increase of DNA Topo I activity in lymphoma cells. 20(S)-Camptothecin (CPT) is a plant alkaloid present in fruit, bark, and wood of the *Camptotheca acuminata*. CPT has a board spectrum of antitumor activity which mediates through interaction with the nuclear enzyme Topo I and prevents it from resealing the DNA break, resulting in a double strain DNA break and cell death (9–12). Moreover, it is a poor substrate for P-glycoprotein, a class of drug efflux pumps that is upregulated in many multi-drug resistant (MDR) cancer cells. However, the clinical use of CPT has been precluded by its significant treatment-related toxicity (TRT) and low antitumor efficacy (13,14). Irinotecan (CPT-11), an analogue of CPT, has been used alone or in combination with other cytotoxic agents as salvage regimens for patients with relapsed/refractory NHL (15–18). In spite of the high response demonstrated in the phase II study of CPT-11 against a board range of solid tumors, it usually has not been employed in the treatment of malignant lymphoma. This is mainly because of its common TRT including grade 3/4 leukopenia and grade 3/4 diarrhea caused by the recommended dosing schedule of this agent (16–19). Although prolonged intravenous (i.v.) infusion of CPT-11 has been reported to enhance antitumor activity (20, 21), a disadvantage of this delivery method observed in xenograft models and early clinical trials was again a high incidence of TRT including diarrhea, nausea/vomiting, neutropenia, anemia, and pulmonary toxicity (22–25). IT-101, a nanoparticulate conjugate of 20(S)-camptothecin and a β -cyclodextrin-based polymer, has recently been developed to improve biodistribution to tumor tissue, minimize TRT, and increase antitumor activity (26, 27). In a preclinical study, IT-101 has been able to demonstrate antitumor activity against a broad range of solid tumors (28). Therefore, we have performed this study to determine the preclinical efficacy of this novel compound in human lymphoma xenografts.

Materials and Methods

DNA construct

The bifunctional *ffLuc:Zeocin* fusion gene which coexpresses the firefly luciferase (*ffLuc*) and zeocin (*Zeo*) resistance genes was cloned into pcDNA3.1⁺ (Invitrogen, Carlsbad, CA) to generating plasmid *ffLuc:Zeocin*-pcDNA3.1⁺ as previously described (29).

Cell culture and genetic modification

Daudi cell (human Burkitt lymphoma line) was obtained from American Type Culture Collection (Manassas, VA). Karpas 299 cell (human anaplastic large T cell lymphoma line) was obtained from Deutsche Sammlung von Mikroorganism und Zellkulturen GmbH

(Graunschweig, Germany). L540 cell (human Hodgkin lymphoma line) was kindly provided by Dr. Andreas Engert (University Clinic of Cologne, Cologne, Germany). These cell lines were maintained in sterile culture media (CM) as previously described (29). 8×10^6 Daudi and Karpas cells were genetically modified with 10 μg of *ffLuc:Zeocin-pcDNA3.1⁺* linearized DNA plasmid as previously described (29). Beginning on the third day after electrotransfer, zeocin (InvivoGen, San Diego, CA) was added to the culture at a concentration of 0.1–0.4 mg/mL to maintain stable transfection.

In vitro cytotoxicity of IT-101 against human lymphoma cell lines

The toxicities of CPT, IT-101, SN-38 (active metabolite of CPT-11), and CPT-11 were determined in Daudi, Karpas 299, and L540 cells after 72 hours of incubation in RPMI 1640 supplemented with 10% heat-inactivated FBS (Daudi cell and Karpas cell) or 20% FBS (L540 cell) using the MTS assay.

Human lymphoma xenograft models

Mouse care and experimental procedures were carried out in accordance with the Research Animal Care Committee (RACC) of Beckman Research Institute at City of Hope. We first established three localized s.c. models in six- to eight-week-old female severe combined immunodeficiency (SCID/NCr, BALB/C background) mice (National Cancer Institute, Frederick, MD). These animals were injected with 0.2 mL of 1:1 mixture of tumor cell suspension in 1% human serum albumin in Hank's balanced salt solution (HBSS; Mediatech) and Matrigel (BD Biosciences, San Jose, CA) into their right flanks. The cell dose of Daudi cell, Karpas 299 cell, and L540 cell was 5×10^6 , 3×10^6 , and 5×10^6 cells, respectively. To establish two disseminated models, six- to eight-week-old female nonobese diabetic severe combined immunodeficiency (NOD.scid/NCr) mice (National Cancer Institute) were injected with 0.2 mL of 1:1 lymphoma cells stably expressing *ffLuc* activity in a suspension of 1x Dulbecco's phosphate-buffered saline solution (DPBS; Mediatech) and 1% heat-inactivated FBS via lateral tail vein. The cell dose of Daudi cell and Karpas 299 cell was 7.5×10^6 and 5×10^6 cells, respectively.

Plasma and tumor concentrations of IT-101, CPT, CPT-11, and SN-38

Localized s.c. human xenograft-bearing animals with tumor volumes (TVs) reaching approximately 500–800 mm³ were randomly divided into two treatment groups of 20 animals each; group 1: CPT-11 (100 mg/kg, intraperitoneal [i.p.], single dose) and group 2: IT-101 (10 mg/kg, i.v., single dose). Then tumor specimens and plasma from five mice in each treatment group were collected at four time points (before dosing, 2, 24, and 48 hours after dosing) to measure plasma and tumor concentrations of the compounds and their active metabolites. Measurements of plasma and tumor concentrations of IT-101 and CPT were carried out for SCID mice treated with IT-101, whereas those of CPT-11 and SN-38 were carried out for mice treated with CPT-11. The method of these measurements has been previously described (26).

Tumor type I DNA topoisomerase (DNA Topo I) catalytic activity inhibited by IT-101 and CPT-11

At each time point along with the measurements of plasma and tumor concentrations of IT-101, CPT-11, and their metabolites, the inhibition of tumor DNA Topo I catalytic activity after administration of either IT-101 or CPT-11 were evaluated. The catalytic activity of DNA Topo I was determined by measuring the relaxation of supercoiled (form I) plasmid substrate DNA using the Topo I assay kit (TopoGEN, Port Orange, FL) essentially according to the method of Liu and Miller (30). First, preparation of crude extracts from tumor tissues was performed as previously described (31). Second, nuclear extraction was

performed using cellLytic nuclear extraction kit (Sigma-Aldrich, St. Louis, MO). Then tumor DNA Topo I catalytic activity was determined following the instructions accompanying the Topo I assay kit. Briefly, the reaction mixtures used consists of supercoiled (form I) plasmid substrate DNA (0.5 µg), tumor nuclear extract (0.5 µg total protein) and the assay buffer. Supercoiled (form I) plasmid DNA (0.5 µg) and relaxed DNA (0.5 µg) provided by the Topo I assay kit were used as the control markers. The reaction mixtures were incubated at 37 °C for 30 minutes, and terminated by adding 5 µL stop buffer/gel loading buffer. Samples were loaded onto a 1% agarose gel submersed in 1x TAE buffer (50x TAE buffer: 242 g Tris base, 57.1 mL glacial acetic acid, and 100 mL 0.5 M EDTA) and electrophoresed overnight at room temperature. The gel was stained with 0.2 µg/mL ethidium bromide for 20 minutes at room temperature, destained in water for 20 minutes and imaged under UV light. The background of supercoiled DNA band was subtracted, and the density of the supercoiled DNA band was quantified using FluorChem AlphaEaseFC2 software (Alpha Innotech, San Leandro, CA). The percentage of DNA Topo I catalytic activity inhibited by either IT-101 or CPT-11 was equal to the density of supercoiled DNA band from treated tumor divided by the density of supercoiled DNA band from untreated control and timed by 100.

Tumor burden monitoring

In the s.c. model, detection of tumor growth by serial physical measurements was initiated two to seven days after tumor implant and repeated at least twice a week until the average TV was approximately 100–200 mm³ at which time therapy was initiated. This was repeated at least once a week until the end of the study. The TV was calculated as previously described (28). In disseminated model, *in vivo* biophotonic imaging (see below) was initiated approximately seven days after tumor injection.

Biophotonic imaging

The *in vivo* ffLuc-derived bioluminescent imaging (BLI) signal was evaluated using an IVIS 100 imaging system (Xenogen, Alameda, CA) at 18 minutes after a single intraperitoneal (i.p.) injection of dissolved D-Luciferin (Xenogen) at a dose of 50 mg/kg (0.1 mL of a 10 mg/mL solution per 20-g mouse). Photons were quantified using the Living Image version 2.5 software (Xenogen). Background bioluminescence signal was defined as < 10⁶ p/s/cm²/sr based on the average ffLuc-derived BLI of normal control mice.

Determination of treatment efficacy

The treatment result for each animal may be pathological complete tumor response (pCTR), complete tumor response (CTR), or partial tumor response (PTR). In a CTR, the TV is < 13.5 mm³ for two consecutive measurements in localized s.c. model, whereas the BLI is < 10⁶ p/s/cm²/sr for two consecutive measurements in the disseminated model. A pCTR is defined as CTR combined with evidence of nonviable tumor on histopathological study. In a PTR, the TV is < 50% of its pretreatment volume for two consecutive measurements and the TV ≥ 13.5 mm³ for one or more of these two measurements, whereas the BLI is < 50% of its pretreatment signal and the BLI signal > 10⁶ p/s/cm²/sr for one or both of these two measurements. In accordance with the institutional RACC, the predetermined tumor end point is defined as follows: (1) the TV > 2,000 mm³ and/or ulcerated tumor in localized s.c. model; and (2) the BLI signal > 10¹⁰ p/s/cm²/sr and/or weight loss > 20% with evidence of progressive disease and/or hind limb paralysis and/or death from tumor progression in disseminated model. Percent animal survival is defined by the percentage of animals that did not die of the tumor or TRT or reach the predetermined tumor end point during the course of the study. At the end of the study, all survived animals were euthanized with CO₂. In order to detect some residual tumors by histopathology study, a whole thickness of skin around the tumor injection site with or without gross tumor was harvested from animals with s.c.

xenografts; whereas a brain, a heart, a spleen, a liver, ovaries, kidneys, lungs, femurs, and spines were harvested from animals with disseminated xenografts.

Treatment schedule and tumor burden monitoring

For reference purposes, CPT-11 was used as the internal positive control in the current study as previously described (28). Mice with established human lymphoma xenografts were allocated into five different treatment groups of nine animals each, as follows: group 1 (untreated control); group 2 (CPT-11 at 100 mg/kg, i.p., qwk $\times$ 3); group 3 (IT-101 at 5 mg/kg, i.v., qwk $\times$ 3); group 4 (IT-101 at 10 mg/kg, i.v., qwk $\times$ 3); and group 5 (single dose of IT-101 at 15 mg/kg, i.v.).

Tumor burdens were monitored at least once weekly toward the predetermined endpoints or for 126 days postadministration in the localized s.c. models and for 90 days postadministration in the disseminated models.

Tolerability

Mice were weighed twice weekly during treatment and then weekly until the end of the study and were observed for some expected TRT, including progressive weight loss, anorexia, and diarrhea. The unacceptable TRT for the maximum tolerated dose (MTD) was defined as previously described (28).

Statistical and graphical analyses

For DNA Topo I catalytic activity, samples were compared by paired t test. The survival curves were constructed using the Kaplan-Meier method. The log-rank test was used to compare the percent animal survival between treatment groups. $P < 0.05$ was considered statistically significant.

Results

In vitro cytotoxicity of Topo I inhibitors against three lymphoma cell lines

Based on the Developmental Therapeutics Program of NCI/NIH and our unpublished data, the IC₅₀'s of CPT, IT-101, SN-38, and CPT-11 for colon, breast, and prostate cancer cell lines were 0.02–0.2 μ M, 0.22–0.38 μ M, 0.001–0.003 μ M, and 13–45 μ M, respectively. Using MTS assay, the IC₅₀'s ranged from 0.01–0.06 μ M and 0.06–0.4 μ M after 72 hours of incubation with CPT and IT-101, respectively, indicating that both CPT and IT-101 had very high *in vitro* cytotoxicity against all lymphoma cell lines tested (Table 1).

Longer release kinetics of IT-101 and CPT as compared with CPT-11 and SN-38

In plasma, the maximum concentration of polymer-bound CPT, free CPT, CPT-11, and SN-38 was at 2 hours posttreatment in all s.c. tumor xenograft animals tested but the concentration of CPT-11 and SN-38 declined rapidly at 24 hours posttreatment (Fig. 1A). In the s.c. tumor xenografts themselves, the maximum concentration of polymer-bound CPT and free CPT was at 48, 24, and 48 hours posttreatment in Daudi tumors, Karpas 299 tumors, and L540 tumors, respectively; whereas the maximum concentration of SN-38 was at 2, 2, and 24 hours posttreatment in Daudi tumors, Karpas 299 tumors, and L540 tumors, respectively (Fig. 1B). These results indicated that IT-101 has longer release kinetics in tumor than that of CPT-11.

Tumor type I DNA Topoisomerase catalytic activity inhibited by IT-101 and CPT-11

Based on the IT-101 kinetic data above, we predicted that IT-101 would be able to inhibit tumor DNA Topo I catalytic activity for longer time than CPT-11. The sensitivity of tumor

cells to either IT-101 or CPT-11 was determined by inhibition of tumor nuclear Topo I catalytic activity using a DNA relaxation assay. At 2, 24, and 48 hours posttreatment, both IT-101 (10 mg/kg, i.v., $\times 1$) and CPT-11 (100 mg/kg, i.p., $\times 1$) could significantly inhibit nuclear DNA Topo I catalytic activity in all type of s.c. lymphoma xenografts tested. However, at 48 hours, IT-101 could inhibit nuclear DNA Topo I catalytic activity significantly better than CPT-11 in s.c. Daudi tumors and s.c. Karpas 299 tumors ($P = 0.007$; 95% CI, 11.2–30.99 and $P = 0.037$; 95% CI, 2.98–48.08, respectively); though there was only a nonsignificant trend in s.c. L540 tumors ($P = 0.07$). These observations are associated with longer release kinetics of IT-101 in tumor and are consistent with the maximum concentration of polymer-bound CPT and free CPT at 48 hours posttreatment as compared to SN-38 at 2 hours posttreatment (Fig. 2).

Tolerability

Recent studies have demonstrated that at the same accumulative dose, IT-101 qwk $\times 3$ can simultaneously maximize antitumor effect and minimize TRT when compared to multiple daily dosing schedules (28). The maximum tolerated dose (MTD) of IT-101 in solid tumor bearing athymic nude mice (no data in SCID and NOD.scid/NCr mice) was more than 16.1 mg/kg qwk $\times 3$ but less than 25 mg/kg qwk $\times 3$. However, based on our internal study of human lymphoma xenografts in athymic nude mice, the MTD of IT-101 was less than 15 mg/kg qwk $\times 2$ so that three weekly doses of IT-101 at 5 mg/kg (CPT equivalents), three weekly doses of IT-101 at 10 mg/kg (CPT equivalents) and a single dose of IT-101 at 15 mg/kg (CPT equivalents) were planned for use in the present study. Except for three treatment-related deaths (TRDs) in IT-101 (15 mg/kg, i.v., $\times 1$) treated SCID mice, all animals tolerated the treatments well. Treatments with three weekly doses of IT-101 at 5 mg/kg and 10 mg/kg were well tolerated in all animals. Thus, the MTD of i.v. IT-101 in tumor bearing SCID mice was between 10 mg/kg qwk $\times 3$ and 15 mg/kg single dose. As a result, we dropped the IT-101 (15 mg/kg, i.v., $\times 1$) arm from the disseminated models using NOD.scid/NCr mice. Therapy with i.p. CPT-11 was generally well tolerated in both strains of animal. Mean body weight loss of SCID mice and NOD.scid/NCr mice was minimal.

In vivo efficacy of IT-101 against localized s.c. human lymphoma xenograft models

In localized s.c. Daudi tumors, therapy was initiated ten days after tumor cell inoculation. Forty-five SCID mice with established localized s.c. tumor were allocated into five different treatment groups of nine animals per group as described earlier. The average TV among all groups was equally distributed. All untreated controls and animals treated with CPT-11 developed progressive tumor growth with an average TV of more than 2,000 mm³ within 38 days and 59 days after tumor injection, respectively. In contrast, IT-101 (5 mg/kg, i.v., qwk $\times 3$) and IT-101 (10 mg/kg, i.v., qwk $\times 3$) could significantly prolong the survival of the animals as compared to those treated with CPT-11 ($P < 0.0001$ and $P < 0.0001$, respectively) (Fig. 3A). Five of nine mice (56%) treated with IT-101 5 mg/kg and seven of nine mice (78%) treated with IT-101 (10 mg/kg, i.v., qwk $\times 3$) survived and were pathologically confirmed disease-free after 126 days posttreatment.

In s.c. Karpas 299 tumors, therapy was initiated four days after tumor cell inoculation. All untreated control mice developed progressive tumor growth with an average TV more than 2,000 mm³ within 25 days after tumor injection. As shown in Fig. 3B, although the significant difference between the groups treated with either IT-101 (5 mg/kg, i.v., qwk $\times 3$) or IT-101 (10 mg/kg, i.v., qwk $\times 3$) was not observed; the animals treated with IT-101 (10 mg/kg, i.v., qwk $\times 3$) had significantly longer survival than those treated with CPT-11 ($P = 0.0072$). At the end of the study, 44% of IT-101 (10 mg/kg, i.v., qwk $\times 3$) treated animals, 33% of IT-101 (5 mg/kg, i.v., qwk $\times 3$) treated animals, and 33% of CPT-11 treated animals achieved a pathologically confirmed disease-free status.

The antilymphoma activity of IT-101 was also evaluated in s.c. L540 tumors. In this model, therapy was initiated 16 days after tumor cell inoculation. Tumors in all untreated control mice grew rapidly with ulceration within 51 days after tumor injection and were then sacrificed. Although a significantly longer survival could be observed in all CPT-11 treated mice as compared with untreated controls ($P = 0.018$), all had progressive tumor growth with ulceration within 79 days after tumor implant. Consistent with the other s.c. xenografts, IT-101 (5 mg/kg, i.v., qwk $\times$ 3) treated animals and IT-101 (10 mg/kg, i.v., qwk $\times$ 3) treated animals had significantly longer survival than those treated with CPT-11 ($P < 0.001$ and $P < 0.0001$, respectively) as shown in Fig. 3C. Seven of nine animals (78%) that received either IT-101 (5 mg/kg, i.v., qwk $\times$ 3) or IT-101 (10 mg/kg, i.v., qwk $\times$ 3) were pathologically confirmed tumor free and survived for at least 126 days after dosing.

In vivo efficacy of IT-101 against disseminated human lymphoma xenograft models

Previous studies have demonstrated that Daudi cells and Karpas 299 cells injected i.v. into mice spread in a pattern comparable with the dissemination of human lymphomas and show preferential localization to the lymph nodes (32, 33).

We further evaluated the antilymphoma efficacy of IT-101 in disseminated Daudi tumors in which the therapy was initiated 11 days after the tumor injection. All untreated controls died of disseminated disease proceeding by progressive weight loss or were sacrificed due to being moribund within 67 days after tumor cell inoculation. In the same xenografts, 55.6% of animals receiving IT-101 (10 mg/kg, i.v., qwk $\times$ 3) and 33.3% of animal receiving IT-101 (5 mg/kg, i.v., qwk $\times$ 3) could achieve pCTR at day 125 posttreatment, whereas none of animals receiving CPT-11 did so (Fig. 4A). In addition, IT-101 (5 mg/kg, i.v., qwk $\times$ 3) and IT-101 (10 mg/kg, i.v., qwk $\times$ 3) could significantly prolong the survival of animals bearing disseminated Daudi⁺ ffLuc tumor when compared to CPT-11 ($P = 0.002$ and $P < 0.0001$, respectively) as shown in Fig. 4B.

We also evaluated the efficacy of IT-101 in disseminated Karpas 299⁺ ffLuc tumor in which the therapy was initiated eight days after tumor cell injection. All untreated controls died of disseminated disease with severe weight loss or were sacrificed due to being moribund or reaching the predetermined endpoint of ffLuc activity $> 10^{10}$ p/s/cm²/sr within 29 days after tumor cell inoculation (Fig. 5A). Although all of the animals receiving therapy succumbed from disseminated disease by day 83 after tumor cell injection, IT-101 (10 mg/kg, i.v., qwk $\times$ 3) treated animals had significantly longer survival than that of IT-101 (5 mg/kg, i.v., qwk $\times$ 3) treated animals and CPT-11 treated animals ($P = 0.0009$ and $P = 0.0049$, respectively) as shown in Fig. 5B.

Discussion

In order to mitigate the disadvantages of CPT, i.e. high toxicity and low clinical therapeutic efficacy, we recently attached it to a hydrophilic β -cyclodextrin-based polymer known as IT-101 (26). IT-101 maintains CPT in its active lactone form. This novel compound is too large to pass through normal vessel walls and renal clearance is inhibited. Therefore, the compound can achieve a long plasma half-life, permits a large amount of the compound to reach the tumor site through an abnormally leaky tumor vasculature, and accumulates in tumor tissue due to a lack of effective lymphatic drainage (34–36). Based on our previous study and unpublished data, pharmacokinetics and pharmacodynamics of IT-101 at various dose levels similar to the ones explored here (0.9 – 9 mg/kg in rat, equivalent to 1.8 to 18 mg/kg scaled allometrically to mouse) were previously studied in rats (26). Area under the curve (AUC) and maximum plasma concentration ($c_{\max}$) for both polymer conjugated and unconjugated CPT scaled linearly with dose and were similar when normalized for the dose administered. Mean terminal half-life of conjugated CPT was 18 hours and independent of

dose. These results indicate a linear dose-plasma exposure correlation for both conjugated and unconjugated CPT after administration of IT-101. In animal models, not only is the mean plasma elimination half-life ($T_{1/2}$) for IT-101 (17–19 hours) significantly longer than that of CPT (1.3 hours), but much higher average 24-hour total CPT levels are achieved in tumors after treatment with IT-101 compared to those treated with CPT alone (26). Since CPT is an S-phase-specific drug, an optimal level of DNA Topo I inhibition is needed in which the tumors are exposed to the compound for a prolonged period of time. In addition, the lower level of freely circulating CPT may reduce the TRT (26).

Our current data clearly demonstrate the advantage of this nanoparticle technology, namely that IT-101 can provide superior antilymphoma activity than that of CPT-11 against xenografts of human lymphoma. *In vitro*, both IT-101 and CPT-11 caused marked inhibition of growth of three distinct human lymphoma cell lines. Because the *in vitro* conditions lacked bio-distribution and drug delivery variables, the observed cytotoxicity of either IT-101 or CPT-11 was most likely due to its direct antiproliferative effect against these lymphoma cell lines tested. Since previous study has shown that the sensitivity of tumor cells to DNA Topo I-targeted cytotoxic agents is related to the level of DNA Topo I catalytic activity in the nucleus, we have chosen the three distinct s.c. lymphoma xenografts which exert a high level of DNA Topo I catalytic activity as the representatives in our study (11). Our short term *in vivo* studies showed that IT-101 was able to significantly inhibit DNA Topo I catalytic activity at 48 hours postadministration in Daudi tumors and Karpas 299 tumors as compared to CPT-11. Such an effect results from the prolonged release kinetics of CPT from IT-101 in tumor tissue, leading to higher degree of cytotoxicity as compared to CPT-11. This was consistent with our observation of higher levels of both IT-101 prodrug and free CPT observed in tumors at 24 and 48 hours postadministration compared to CPT-11 and its active metabolite SN-38. Moreover, the long-term therapeutic efficacy of IT-101 was clearly superior to CPT-11. In localized s.c. xenografts, upon discontinuation of the IT-101 treatment, most of animals attained pCTR at the end of the study, in agreement with the disseminated Daudi tumors treated with IT-101 (10 mg/kg, i.v., qwk $\times$ 3). Among treatment groups, IT-101 (10 mg/kg, i.v., qwk $\times$ 3) gave the best results in terms of pCTR and survival benefit in both s.c. and disseminated xenografts.

We also have demonstrated that the MTD of IT-101 in SCID/NCr (*BALB/C* background) and NOD.*scid*/NCr mice bearing human lymphoma xenograft was less than that of athymic nude mice (28). This result may be explained by the fact that the SCID/NCr and NOD.*scid*/NCr mice are more immunocompromised as compared to athymic nude mice. Recently, a novel SN-38-incorporating polymeric micelle, NK012, has been developed (37). This compound produced a much higher cytotoxic effect against lung and colon cancer cell lines as compared to CPT-11, mainly due to an enhancement and prolonged distribution of free SN-38 in the tumor tissues. However, SN-38 is cross-resistant with the first-line chemotherapeutic agents commonly used in NHL such as doxorubicin and mitoxantrone (38, 39). Moreover, in a phase II study of CPT-11, a good response (CR + PR) was seen in only 0%, 38%, and 0% of patients with relapsed/refractory Hodgkin's lymphoma, Burkitt lymphoma, and T-cell lymphoma (40). As a result, CPT-11 is not a good candidate in treating relapsed/refractory NHL. Therefore, the positive outcome of this study clearly demonstrates the potential clinical benefit of IT-101 and can help in the design of treatment schedules in phase I clinical trials. IT-101 monotherapy should be tested in patients with relapsed/refractory NHL. Many preclinical and clinical studies have demonstrated that a sequential administration of a DNA Topo I inhibitor followed by a DNA Topo II inhibitor exerts a synergistic antilymphoma effect (17, 41, 42). A recent study indicated that single agent fluoroquinolone can inhibit both DNA Topo I and II activities in eukaryotic cells; and in combination with either CPT or etoposide, it led to a synergistic inhibition of DNA Topo I or II activity, respectively (43). As a result, it would be of value to further test an

antilymphoma effect of IT-101 combined with a DNA Topo II inhibitor or fluoroquinolone. The dose-limiting toxicity of IT-101 is currently being determined in ongoing phase I clinical trial at City of Hope Comprehensive Cancer Center.

In conclusion, we have demonstrated that IT-101 is able to control and inhibit tumor growth both *in vitro* and *in vivo*, and prolong survival of animals bearing multiple distinct human lymphoma xenografts. This preclinical therapeutic efficacy of IT-101 supports its clinical evaluation in patients with NHL and HL.

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Statement of Translational Relevance

Topoisomerase inhibitors have a broad spectrum of activity against human cancers including malignant lymphoma. Although camptothecin (CPT), a type 1 DNA topoisomerase inhibitor, has significant antitumor activity, its water insolubility and toxicity has precluded its clinical use and derivatives, such as irinotecan, have been used clinically with some activity. We have tested the efficacy of IT-101. IT-101 is comprised of a cyclodextrin-containing polymer conjugate of CPT that self assembles into a nanoparticle of ca. 30 nm diameter. The drug was designed to reduce the toxicity of CPT and relies on intracellular chemistry to liberate the drug from the protective polymer once inside the cell. When tested in five different animal models of lymphoma, the drug demonstrated prolongation of survival and the achievement of a complete remission in most of the animals compared to irinotecan, without increased toxicity. As a result, it is our plan to initiate a phase I/II clinical trial in a broad group of patients with relapsed/refractory lymphoma.

A

B

Fig. 1.

(A) Plasma concentration of polymer-bound (■), free CPT (▲), CPT-11 (▼), and SN-38 (◆) as a function of time after a single bolus i.v. injection of IT-101 (10 mg/kg) and a single i.p. injection of CP-11 (100 mg/kg). (B) Tumor concentration of polymer-bound (■), free CPT (▲), CPT-11 (▼), and SN-38 (◆) as a function of time after a single bolus i.v. injection of IT-101 (10 mg/kg) and a single i.p. injection of CPT-11 (100 mg/kg). Points and error bars indicate mean and standard error of mean, respectively.

Fig. 2.

Administration of IT-101 (10 mg/kg, i.v., single dose) can significantly inhibit tumor DNA Topo I catalytic activity in lymphoma xenografts as compared to CPT-11. DNA relaxation assay was done with nuclear extracts containing 0.5 μ g total protein, isolated from s.c. lymphoma xenografts at four time points (before dosing, 2 hours, 24 hours, and 48 hours after dosing), as depicted. The mean percentage of tumor DNA Topo I catalytic activity inhibited by either IT-101 or CPT-11 was calculated from three or more separate experiments. Error bars indicate standard error of mean.

Fig. 3.

Administration of IT-101 (5 mg/kg, i.v., qwk $\times$ 3) and (10 mg/kg, i.v., qwk $\times$ 3) to animals bearing three distinct s.c. human lymphoma xenografts can result in significant better survival as compared to CPT-11. Treatments were initiated at 10, 4, and 16 days after subcutaneous injection of Daudi cells, Karpas 299 cells, and L540 cells, respectively. Treatment arms included untreated control (■), CPT-11 (100 mg/kg, i.p., qwk $\times$ 3) (◇), IT-101 (5 mg/kg, i.v., qwk $\times$ 3) (○), and IT-101 (10 mg/kg, i.v., qwk $\times$ 3) (x). The tumor burden was monitored longitudinally by physical measurements. Using Kaplan-Meier method, the survival curves were plotted for each treatment group and the log-rank test was used to compare the percent animal survival between treatment groups. In Daudi tumors and L540 tumors, a significant difference between the groups treated with either IT-101 (5 mg/kg, i.v., qwk $\times$ 3) or IT-101 (10 mg/kg, i.v., $\times$ 3) was not observed. However, these two treatment groups achieved significantly better survival as compared to the groups treated with CPT-11 ($P < 0.0001$ and $P < 0.0001$, respectively). In Karpas 299 tumors, no significant difference between the groups treated with either IT-101 (5 mg/kg, i.v., qwk $\times$ 3) or IT-101 (10 mg/kg, i.v., qwk $\times$ 3) was observed; but animals treated with IT-101 at (10 mg/kg, i.v., qwk $\times$ 3) had significantly better survival as compared to those treated with CPT-11. ($P = 0.0072$). Points indicate mean survival.

Fig. 4. Administration of IT-101 (5 mg/kg, i.v., qwk $\times$ 3) and IT-101 (10 mg/kg, i.v., qwk $\times$ 3) in disseminated human B-cell lymphoma xenograft-bearing NOD/*scid* mice could result in significant better survival as compared to CPT-11. Treatments were initiated on 11 days after tumor inoculation. The treatment arms included untreated control (■), CPT-11 (100 mg/kg, i.p., qwk $\times$ 3) (◇), IT-101 (5 mg/kg, i.v., qwk $\times$ 3) (○), and IT-101 (10 mg/kg, i.v., qwk $\times$ 3) (x). The tumor burden was monitored longitudinally by quantification of tumor-derived ffLuc-activity. (A) Total photon flux normalized for exposure time and surface area and expressed in units of photons/second/cm²/steradian (p/s/cm²/sr) for individual mice was graphed over time and serial pseudocolor images representing light intensity from Daudi⁺ ffLuc in selected mice were shown. (B) Kaplan-Meier survival curves were plot for each treatment group. The log-rank test was used to compare the percent animal survival between treatment groups. A significant difference between the groups treated with either IT-101 (5 mg/kg, i.v., qwk $\times$ 3) or IT-101 (10 mg/kg, i.v., qwk $\times$ 3) was not observed; but these two treatment groups could significantly establish better survival as compared to the animals treated with CPT-11 ($P = 0.0002$ and $P < 0.0001$, respectively). Points indicate mean survival.

Fig. 5. IT-101 (10 mg/kg, i.v., qwk $\times$ 3) had superior antilymphoma activity as compared to IT-101 (5 mg/kg, i.v., qwk $\times$ 3) and CPT-11 in disseminated human anaplastic large T-cell xenografts. NOD.*scid*/NCr mice were intravenously injected with ffLuc⁺ Karpas 299 cells to establish disseminated human anaplastic large T-cell xenografts and treated with the indicated cytotoxic agent beginning eight days later. Treatment arm included untreated control (■), CPT-11 (100 mg/kg, i.p., qwk $\times$ 3) (◇), IT-101 (5 mg/kg, i.v., qwk $\times$ 3) (o), and IT-101 (10 mg/kg, i.v., qwk $\times$ 3) (x). The tumor burden was monitored longitudinally by quantification of tumor-derived ffLuc-activity. (A) Total photon flux normalized for exposure time and surface area and expressed in units of photons/second/cm²/steradian (p/s/cm²/sr) for individual mice was graphed over time and serial pseudocolor images representing light intensity from Karpas 299⁺ ffLuc in selected mice were shown. (B) Kaplan-Meier survival curves for each treatment group. The log-rank test was used to compare the percent animal survival between treatment groups. IT-101 (10 mg/kg, i.v., qwk $\times$ 3) treated animals had significantly longer survival as compared to those treated with IT-101 (5 mg/kg, i.v., qwk $\times$ 3) and those treated with CPT-11 ($P = 0.0009$, $P = 0.0049$, respectively). Points indicate mean survival.

Table 1

IC50's of CPT, IT-101, SN-38, and CPT-11 in various lymphoma cell lines after a 72-hour incubation period

Lymphoma cell line	CPT (μM)	IT-101 (μM)	SN-38 (μM)	CPT-11 (μM)
Daudi	0.06	0.17	0.003	62
Karpas 299	0.02	0.4	0.008	11
L540	0.01	0.06	0.008	2