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Genz-644282, a Novel Non-Camptothecin Topoisomerase I Inhibitor for Cancer Treatment

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Abstract

**Purpose:** Genz-644282 [8,9-dimethoxy-5-(2-N-methylaminoethyl)-2,3-methylenedioxy-5H-dibenzo[c,h][1,6]naphthyridin-6-one] has emerged as a promising candidate for antitumor agents. This report describes the bone marrow colony-forming unit, granulocyte macrophage (CFU-GM) and tumor cell CFU activity of topoisomerase I (Top1) inhibitors, such as Genz-644282, topotecan, irinotecan/SN-38, and ARC-111, and examines their activity in several human tumor xenograft models.

**Experimental Design:** Colony-forming assays were conducted with mouse and human bone marrow and eight human tumor cell lines. In addition, 29 human tumor cell lines representing a range of histology and potential resistance mechanisms were assayed for sensitivity to Genz-644282 in a 72-hour exposure assay. The efficacy of Genz-644282 was compared with standard anticancer drugs (i.e., irinotecan, docetaxel, and dacarbazine) in human tumor xenografts of colon cancer, renal cell carcinoma, non–small cell lung cancer, and melanoma.

**Results:** Human bone marrow CFU-GM was more sensitive to the Top1 inhibitors than was mouse bone marrow CFU-GM. The ratio of mouse to human IC₉₀ values was more than 10 for the camptothecins and less than 10 for Genz-644282, which had more potency as a cytotoxic agent toward human tumor cells in culture than the camptothecins in the colony-forming and 72-hour proliferation assays. Genz-644282 has superior or equal antitumor activity in the human tumor xenografts than the standard drug comparators.

**Conclusions:** On the basis of preclinical activity and safety, Genz-644282 was selected for development and is currently undergoing phase 1 clinical trial.

**Introduction**

Topoisomerase 1 (Top1) is an essential enzyme in mammalian cells (1–4). 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 (5–8). Top1 associates preferentially with transcriptionally active genes and is thought to be involved in relaxing supercoils introduced by RNA polymerase during RNA transcription (9, 10).

Top1 produces a single-strand break in DNA, allowing relaxation of DNA for replication. The single-strand break is then resealed, thus restoring the DNA double strands. The Top1 reaction intermediate consists of the enzyme covalently linked to a nicked DNA molecule. This assembly is known as a “cleavable complex” (11, 12). The Top1-targeted drugs, topotecan and irinotecan, and the Top2-targeted drugs, doxorubicin, amsacrine, etoposide, and teniposide, stabilize the covalent enzyme–DNA complex and, thus, prevent religation (13). The cytotoxicity of Top1 inhibitors is due to entrapment of Top1–DNA cleavage complex as opposed to inhibition of Top1 catalytic activity. The Top1–DNA cleavage complex causes DNA damage during DNA replication and transcription. Repair of Top1-mediated DNA damage has been reviewed and reported (2, 14).

The camptothecin analogues topotecan and irinotecan are Food and Drug Administration (FDA)-approved Top1-targeted drugs. *In vivo*, there is a chemical equilibrium between the lactone form and the E ring–opened form of the camptothecins. The E ring–opened carboxylate form has less than 10% of the potency of the lactone as a Top1 inhibitor and is inactive in cell culture, perhaps, due to inability to cross the cell membrane (15). Both topotecan and irinotecan are substrates for P-glycoprotein (P-gp) efflux pumps and for the pump known as the breast cancer resistance protein (BCRP; refs. 16, 17).

Bone marrow is critically sensitive to many antineoplastic agents and contributes to toxicity. Top1 inhibitors kill
rapidly dividing bone marrow progenitor cells and tumor cells, resulting in acute reversible neutropenia and thrombocytopenia 4 to 20 days after administration (18). Bone marrow colony-forming unit-granulocyte macrophage (CFU-GM) assays comparing the sensitivity of bone marrow cells across species are useful in predicting the blood levels of an agent that might be achieved with acceptable toxicity in patients (18–21). In general, murine bone marrow has a lower sensitivity to Top1 inhibitors than does human bone marrow. The difference in sensitivity between murine and human bone marrow progenitor cells to Top1 inhibitors may explain, in part, why curative doses/blood levels of topotecan and 9-aminocamptothecin in mice with human tumor xenografts are not achievable in patients (21). A compound with similar bone marrow progenitor sensitivity across species may have better potential of reaching the same blood level in patients as in mice. Pessina and colleagues (18) suggested that, through use of the ratio of mouse/human CFU-GM IC90 values and the mouse maximum tolerated dose, the human maximum tolerated dose of a compound could be predicted and the potential for achieving a therapeutic blood level in patients could be estimated.

Nitidine and fagaronine are benzo[c]phenanthridine alkaloids with good antitumor potency and are active Top1 inhibitors (22, 23). Synthetic compounds of this class are as potent as camptothecin in stimulating Top1-mediated DNA cleavage by using purified human Top1 and are more potent than irinotecan in many human tumor xenografts (24, 25). An extensive structure–activity relationship was conducted around the dibenzo[c,h][1,6]naphthyridin-6-one compound family to identify structural features associated with potent Top1-targeting and suitable pharmaceutical properties (26). Genz-644282 [8,9-dimethoxy-5-(2-N-methylaminoethyl)-2,3-methyleneoxy-5H-dibenzo[c,h][1,6]naphthyridin-6-one] emerged as a promising candidate from the structure–activity relationship. The current report describes the bone marrow CFU-GM and tumor cell CFU activity of Top1 inhibitors, such as Genz-644282, topotecan, irinotecan/SN-38, and ARC-111, and examines their activity in several human tumor xenograft models. Genz-644282 is currently in phase I clinical trial.

**Material and Methods**

**Materials**

The purity and chemical identity of Genz-644282 [8,9-dimethoxy-5-(2-N-methylaminoethyl)-2,3-methyleneoxy-5H-dibenzo[c,h][1,6]naphthyridin-6-one] and ARC-111 [8,9-dimethoxy-5-(2-N,N-dimethylaminoethyl)-2,3-methyleneoxy-5H-dibenzo[c,h][1,6]naphthyridin-6-one] was confirmed (Fig. 1A; ref. 23, 24, 27). Topotecan, irinotecan, and SN-38 were used as purchased from LKT Laboratories. Genz-644282 and ARC-111 were prepared as a 100 μmol/L stock solution in D5W (5% Dextrose, aqueous, Injection USP; B. Braun Medical Inc.). For cell culture, topotecan and SN-38 were prepared as 10 mmol/L stock solutions in dimethyl sulfoxide (DMSO) from Sigma (#D5879) and dilutions for experiments prepared in RPMI/5% FBS.

**Mouse bone marrow**

Male Balb/c mice were purchased from Charles River Laboratories at 6 to 8 weeks of age and used for studies when they were 7 to 10 weeks old. Procedures were carried out according to a protocol approved by the Institutional Animal Care and Use Committee in accordance with the Federal Animal Welfare Act (9 CFR, 1992) and were conducted in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-accredited facility. For sterile bone marrow collection, mouse femoral and tibial shafts were flushed with sterile RPMI 1460/5% FBS with a syringe and blunt 27G needle. The resulting cellular suspension was collected in a 50-mL conical-bottom tube and kept on wet ice. Approximately 25 million cells were obtained from each mouse.

**Human bone marrow**

Viable human bone marrow cells were purchased as frozen vials from AllCells, LLC., via StemCell Technologies (#ABM009). They were prepared with a DNase treatment (#07900; StemCell Technologies) and washed according to a protocol described by the vendor in preparations for culture.

**Bone marrow CFU-GM assay**

For mouse CFU-GM assays, freshly isolated mouse bone marrow cells were cultured in MethoCult, containing the cytokines recombinant mouse stem cell factor (rmSCF), recombinant mouse interleukin-3 (rmIL-3), and recombinant human interleukin-6 (rhIL-6; StemCell Technologies). For human CFU-GM assays, the culture medium...
used was MethoCult GF, containing the cytokines human stem cell factor (hSCF), human granulocyte-macrophage colony-stimulating factor (hGM-CSF), and human interleukin-3 (hIL-3; StemCell Technologies).

For experiments, final cell concentrations of $4 \times 10^4$ cells per plate for mouse cells and $8 \times 10^4$ cells per plate for human cells were set up in duplicate 100-cm$^2$ culture dishes for each compound concentration and then placed in a 37°C incubator for 13 days for mouse bone marrow and 15 days for human bone marrow. Human and mouse bone marrow progenitor cells were continuously exposed to a concentration range of Genz-644282, ARC-111, topotecan, or SN-38 in 3 independent CFU-GM colony formation experiments. The compound concentrations were at half-log intervals covering 5 logs (10 concentrations). Colonies were defined as clusters containing 30 or more cells. The IC$_{50}$ and IC$_{90}$ values were expressed as the mean, with 95% CIs, in nanomolar concentrations (18).

**Tumor cell survival CFU assay**

Cell lines MDA-MB-231, HCT-116, HT-29, NCI-H460, and RPMI-8226 were purchased from American Type Culture Collection (ATCC; refs. 28–31). The KB3-1 and KB-V1 cell lines were a gift from Dr. Edmond LaVoie. The MDA-MB-231 is an estrogen receptor, progesterone receptor, and Her2/neu-negative breast cancer cell line. HCT-116 and HT-29 are colorectal carcinoma cell lines. The RPMI-8226 line is a B-lymphocyte plasmacytoma, myeloma cell line, and NCI-H460 is a large-cell lung carcinoma cell line (31). The KB human carcinoma cell line was originally thought to be derived from an epidermal carcinoma of the mouth but was subsequently found to have been established via HeLa cell contamination and is, at present, designated a cervical carcinoma (32). The KB3-1 line was developed by subcloning the KB cell line. The KB-V1 subline was established from the KB3-1 line by continuous exposure to vinblastine (1 μg/mL), which resulted in gene amplification of mdr1 and mdr2 (33, 34). The KB-V1 cells have more...
than 300-fold higher expression of mdr1 mRNA than do KB3-1 cells and are more than 100-fold less sensitive to colchicines, doxorubicin, and vinblastine than are KB3-1 cells.

For human tumor cell colony formation assays, the cell lines which grow as monolayers—MDA-MB-231, HCT-116, HT-29, NCI-H460, KB3-1, and KBV-1—were grown in RPMI medium (Invitrogen/Gibco) supplemented with 5% FBS (Invitrogen/Gibco). RPMI-8226 cells, which are nonadherent, were grown in 0.35% agar in Dulbecco's modified eagle medium (DMEM)-F12 medium supplemented with 10% FBS over a base layer of 0.5% agar. Human tumor cells were plated at a concentration $1 \times 10^5$ per well in 6-well plates in a medium supplemented with 5% or 10% FBS. The compounds were tested over a concentration range from 0.01 to 100 nmol/L in half-log intervals covering 5 logs together with vehicle controls. Each compound concentration was tested in duplicate wells. Cultures were exposed to the compounds continuously for 7 days at 37°C in a humidified atmosphere of 5% CO$_2$. Each experiment was conducted at 3 independent times. Colonies were defined as clusters containing 30 or more cells. For the monolayer cultures, colonies were stained with crystal violet solution containing 0.41% crystal violet, 12% ethanol, and deionized water (Becton Dickinson). The $IC_{50}$ and $IC_{90}$ values were expressed as the mean, with 95% CIs, in nanomoles per liter concentrations.

**Growth inhibition assay**

Twenty-nine established human tumor cell lines were exposed to a concentration range of Genz-644282 in 2 to 4 independent experiments. Human tumor cell lines representing a range of histology and potential resistance mechanisms were purchased from ATCC and included MIA PaCa-2, AsPC-1, BxPC-3, CFPAC-1, Hs766T and Capan-1 pancreatic cancers, MEL624, C32, Hs695T and SK-MEL-3 melanomas, NCI-H1299, NCI-H292, NCI-H1915 and SW900 non–small cell lung cancer/carcinoma (NSCLC), HCC1395, HCC1397, HCC202, Hs578T, T-47D and ZR-75-1 breast cancer, ACHN, 769-P, A-498, A-704, SW156, Caki-2 and TK-10 renal cancers, and OVCAR-4 and OVCAR-5 ovarian cancers. Cells were plated at a concentration $4 \times 10^3$ per well in 96-well tissue culture plates in 100 μL RPMI medium supplemented with 5% FBS and 12 concentrations of Genz-644282 ranging from 0.1 nmol/L to 10 μmol/L, with each concentration tested in triplicate. Plates were incubated overnight at 37°C in humidified air with 5% CO$_2$. Plates were incubated with Genz-644282 at 37°C with humidified air/5% CO$_2$ for 72 hours. After the incubation period, the test plates were read utilizing Promega’s Cell Titer-Glo Luminescent Cell Viability Assay according to the manufacturer’s instructions. Luminescence was measured with a Bio-Tek Synergy HT plate reader utilizing the associated Kinetical software (version 3.4). Luminescence data were converted to growth fraction by comparison with the luminescence for the untreated control for each cell line, and $IC_{50}$ and $IC_{90}$ values determined from the graphical data. Each cell line was tested in at least 2 independent experiments.

**Human tumor xenografts**

The efficacy of Genz-644282 was compared with standard anticancer drug therapy in human tumor xenograft studies. All procedures were carried out according to a protocol approved by the Institutional Animal Care and Use Committee in accordance with the Federal Animal Welfare Act (9 CFR, 1992) and were conducted in an AAALAC-accredited facility. Nude/nude (nu/nu) mice from Harlan were implanted subcutaneously with a 4-mm$^3$ tumor fragment, and treatments were initiated when tumors reached 200 mm$^3$. Animals were assigned to treatment ($n = 8–10$) or control groups ($n = 8–10$). Compounds were prepared freshly prior to injection, with Genz-644282 formulated in M/6 lactate, irinotecan in D5W, gemcitabine in saline, and docetaxel in ethanol, Cremophor EL, and saline. The doses, schedules, and routes for each compound were determined in earlier MTD studies. Genz-644282 was tested on 3 schedules, and the most effective, nontoxic regimen was selected for further study.

Genz-644282 was compared with irinotecan in experiments with the human HCT-116, HT-29, HCT-15, and DLD-1 colon carcinoma and 786-O renal cell carcinoma xenografts. Irinotecan was administered at a dose of 60 mg/kg/d by i.v. injection every fourth day for 3 injections. Genz-644282 was compared with docetaxel in the human NCI-H460 NSCLC xenograft. Docetaxel was administered at 12, 16, or 20 mg/kg/d by i.v. injection on alternate days for 3 injections. Genz-644282 was compared with dacarbazine in the human LOX-IMVI melanoma xenograft. Dacarbazine was administered at 90 mg/kg/d by i.p. injection once daily for 5 days. Genz-644282 was administered by i.v. injection at a dose of 1, 1.36, 1.7, 2.7, or 4.1 mg/kg/d by, on alternate days, 3 times per week for 2 weeks in all in vivo experiments.

**Pharmacokinetic studies**

Liquid chromatography with tandem mass spectrometry was used to quantitate Genz-644282 in blood, plasma, and tumor tissue of nude (nu/nu) mice, both naive and bearing NCI-H460 human tumor xenografts. The pharmacokinetics (PK) of Genz-644282 were evaluated in male nu/nu mice following a single i.v. dose of 2.0 mg/kg administered as the free base prepared in a lactic acid–sodium lactate–water formulation. Noncompartmental PK parameters were determined. The PK/PD (pharmacodynamic) modeling was carried out by the method described by Simeoni and colleagues (35).

**Statistical analysis**

Concentration–response data for bone marrow CFU-GM and human tumor cell line CFU were analyzed using a nonlinear curve fit based on the equation $y = a \times \exp (b \times x)$. The $IC_{50}$ and $IC_{90}$ values and the 95% CI for each compound on human and mouse bone marrow were determined by nonlinear regression analysis using SAS.
(version 8.2). Tumor volumes were calculated using the formula \((\pi \times \frac{d^2}{4}) \times 0.52\). Mouse weight and tumor dimensions were measured twice weekly. The data are presented as mean tumor volume ± SD. Antitumor activity of the compounds was determined by calculating tumor growth delay (TGD, \(T/C\)) in days at a tumor volume of 1,500 mm³. Increase in lifespan was determined as a secondary endpoint, with removal from study due to tumor size. Fold increase in lifespan was calculated from median survival in days for the treated versus control groups. Kaplan–Meier survival curves were prepared using GraphPad Prism software and used to determine the median survival times for each treatment group of mice.

### Results

The chemical structures of the non-camptothecin Top1 inhibitors Genz-644282 and ARC-111 are shown in Figure IA (33–35). The compounds Genz-644282, ARC-111, topotecan, and SN-38 are potent cytotoxic agents with activity toward both mouse and human bone marrow CFU-GM. Exposure to the compounds produced killing of cells in an exponential manner. The concentration–response curves are shown in Figure 1B. Coefficient of determination \((R^2)\) values provide a measure of the goodness of fit of the data to the model ranging between 0.75 and 0.97. For all compounds tested, concentrations killing 50% and 90% of the cells were readily achieved. Human and mouse bone marrow CFU-GM IC\(_{50}\) values were 3- to 4-fold lower than the corresponding IC\(_{90}\) values; thus, the concentration–response curves for these compounds are steep (Fig. 1B). Human bone marrow CFU-GM was more sensitive to the cytotoxicity of the compounds than was mouse bone marrow CFU-GM. Genz-644282 and ARC-111 were more potent cytotoxic agents than the camptothecins, topotecan, and SN-38 (irinotecan). The ratios of the mouse and human bone marrow CFU-GM at the IC\(_{50}\) and IC\(_{90}\) concentrations were calculated. For the camptothecins, the ratios were 10 or more and, for Genz-644282 and ARC-111, the ratios were 4 to 7.

Genz-644282, ARC-111, topotecan, and SN-38 were potent cytotoxic agents, with activity toward the 8 human tumor cell lines included in the colony formation assay. Exposure to the compounds produced exponential killing of human tumor cells as in the bone marrow experiments (Fig. 2). Concentrations killing 50% and 90% of the cells were readily achieved for all compounds. Genz-644282 and ARC-111 were found to be more potent cytotoxic agents than the camptothecins, topotecan, and SN-38 (active metabolite of irinotecan).

KBV-1 cells are engineered to express much higher levels of MDR than parental control KB-3-1 cells, and NCI-H460, HT-29, and RPMI-8226 cells are very high natural expressers of BCRP (19). Cells with high expression of either MDR or BCRP were markedly resistant to killing by topotecan and SN-38 but not resistant to killing by Genz-644282 and ARC-111.

Twenty-nine human tumor cell lines of 6 histologic types were tested for sensitivity to growth inhibition by Genz-644282 in a 72-hour exposure study using an ATP-content readout (Fig. 1C). Exposure to the compound produced exponential killing of cells in a manner consistent with potent inhibition of a critical molecular target. The IC\(_{90}\) concentrations for the 29 human tumor cell lines spread over a 1,000-fold concentration range from 100 nmol/L to more than 10 µmol/L. Four of the 6 pancreatic cancer cell lines tested were among the lines most sensitive to Genz-644282. Three of the 4 melanoma cell lines tested were among the least sensitive to Genz-644282, whereas most of the 6 breast cancer cell lines tended to have moderate response to the compound. The most disparate results were obtained with the 7 renal cell cancer cell lines and ranged from the most sensitive to the least sensitive responders to the compound. Two ovarian cancer lines were tested; the OVCAR-4 cell line, which was from a previously untreated patient, was more sensitive to the compound than was the OVCAR-5 cell line, which was from a patient who had failed multiple chemotherapies. Ten of the 29 tumor cell lines were documented to have originated from metastatic disease. There was no strong trend for these cell lines to be more or less sensitive to the compound; however, half of the metastatic cell lines had IC\(_{50}\) values greater than 50 nmol/L. The median IC\(_{50}\) value for the 29 human tumor cell lines was 33 nmol/L, and the mean IC\(_{50}\) value for the 29 cell lines was 164 nmol/L.

Following i.v. administration of Genz-644282 (2 mg/kg) to nu/nu mice, the mean blood clearance, volume of distribution, and terminal half-life were 21.8 mL/min/kg, 5.44 L/kg, and 5.14 hours, respectively. An i.v. injection of Genz-644282 (2 mg/kg) to nu/nu mice bearing NCI-H460 human tumor xenografts resulted in plasma PK parameters of 104 mL/min/kg mean clearance, a 49.3-L/kg volume of distribution, and terminal half-life of 8.14 hours. The mean area under the curve (AUC) in tumor tissues (9,690 ng·h/mL) was approximately 30-fold higher than that observed in plasma (319 ng·h/mL), showing that Genz-644282 has a high penetration into tumor tissue in the xenograft model. The Genz-644282 IC\(_{50}\) concentration for the human tumor NCI-H460 cell line in the colony formation assay was 5 nmol/L (Fig. 2). For an analysis of the PK/PD relationship of Genz-644282, we compared the exponential growth of tumors in control mice with tumor growth in the Genz-644282–treated mice. The growth decreased by a factor proportional to drug concentration (35). The estimated average tumor concentration of Genz-644282 at steady state ranged from 130 to 519 nmol/L, or 24- to 96-fold higher than the in vitro IC\(_{90}\) (5.4 nmol/L), thus predicting that the tumor would be responsive to treatment.

Many schedules have been described in the literature for the administration of irinotecan in mice (36). Both Genz-644282 and irinotecan were tested on several schedules, and the optimal ones for each compound were selected for doing comparisons in several human tumor xenograft models. In vivo, Genz-644282 was tolerated at doses up...
to 4 mg/kg when administered intravenously on alternate days, and the compound was active at doses from 1 to 4 mg/kg. The efficacy of Genz-644282 was compared with irinotecan in 4 human colon carcinoma xenograft models. In the human HCT-116 colon cancer xenograft, TGD values were 14 days for irinotecan (60 mg/kg) and 34 days for Genz-644282 (2.7 mg/kg), giving maximum test to control ratios (T/Cs) of 23.7% and 16.8%, respectively (Fig. 3A). A similar experiment was conducted with the human HT-29 colon carcinoma xenograft, and the TGD values were 11 days for irinotecan (60 mg/kg) and 27 days for Genz-644282, with maximum T/Cs of 52% and 16.6%, respectively (Fig. 3B). In the human HCT-15 colon carcinoma xenograft the TGD, Genz-644282 was administered at a dose of 2 mg/kg, resulting in a TGD of 33 days as compared with a TGD of 27 days with irinotecan (60 mg/kg), giving maximum T/Cs of 16% and 8.6%, respectively (Fig. 3C). Mice bearing the DLD-1 human colon carcinoma xenograft were treated with Genz-644282 (1 mg/kg), resulting in a TGD of 14 days as compared with a TGD of 11 days with irinotecan (60 mg/kg), with maximum T/Cs of 50.2% and 51.3%, respectively (Fig. 3D).

The antitumor efficacy of Genz-644282 was compared with the antitumor activity of dacarbazine in the human LOX-IMVI melanoma xenograft (Fig. 4A). Administration of Genz-644282 (2 mg/kg) produced a TGD of 28 days in mice bearing LOX-IMVI melanoma compared with a TGD of 14 days after administration of dacarbazine (90 mg/kg), giving maximum T/Cs of 66.2% and 80.6%, respectively. The antitumor efficacy of Genz-644282 was compared with the antitumor activity of irinotecan in the human 786-O renal cell carcinoma xenograft (Fig. 4B). Administration of

Figure 2. Concentration–response curves in a colony formation assay for each of the 4 Top1 inhibitors, Genz-644282 (●), ARC-111 (○), SN-38 (■), and topotecan (□), in human HCT-116 and HT-29 colon carcinoma cells, NCI-H460 NSCLC cells, MDA-MB-231 breast carcinoma cells, RPMI-8226 multiple myeloma cells, and KB3-1, KBV-1, and KBH5.0 cervical carcinoma cells as representative human tumor cell lines. The red dotted curves fit the nonlinear exponential model for the Genz-644282 data.
Genz-644282 (1.7 mg/kg) produced a TGD of 23 days in mice bearing 786-O tumors compared with a TGD of 16 days after administration of irinotecan (60 mg/kg), with maximum T/Cs of 8.7% and 20.7%, respectively.

The antitumor activity of Genz-644282 was compared with the antitumor activity of docetaxel in NCI-H460 and NCI-H1299 human NSCLC xenografts. Administration of Genz-644282 (2.7 mg/kg) to mice bearing the NCI-H460 human NSCLC produced a TGD of 27 days compared with a TGD of 21 days by the administration of docetaxel (20 mg/kg), giving maximum T/Cs of 4.6% and 19.5%, respectively (Fig. 4C). In the NCI-H1299 human NSCLC xenograft, treatment with Genz-644282 (1.7 mg/kg) resulted in a TGD of 33 days whereas treatment with docetaxel (20 mg/kg) produced a TGD of 15 days, with maximum T/Cs of 8.7% and 20.7%, respectively (Fig. 4D).

As an initial combination chemotherapy regimen, administration of Genz-644282 (1.36 mg/kg) together with docetaxel (12 mg/kg) was tested in mice bearing NCI-H460 human NSCLC xenografts (Fig. 5). The TGD with docetaxel (12 mg/kg) alone was 12 days, the TGD obtained following administration of Genz-644282 (1.36 mg/kg) was 24 days, and the TGD achieved with the combination regimen was 27 days.

Discussion

Top1 is a fully validated target in cancer, with 2 drugs specifically targeted to the enzyme in clinical use. Top1 inhibition became an important target in cancer with the discovery that camptothecin, a very potent anticancer plant alkaloid, specifically targets Top1 (37). Top1 remains a target of active interest in the development of new anticancer agents because Top1 inhibitors are
clearly active and effective anticancer drugs and because the current Top1 inhibitors are molecules that can be improved upon. There are camptothecin derivatives and non-camptothecin Top1 inhibitors in preclinical and clinical development. Each of these investigational molecules may have properties that lead to improved therapeutic benefit to patients. Furthermore, there are very active preclinical efforts based on protein phosphorylation levels and mRNA levels in tumor and blood samples to define biomarkers that can select patients who are most likely to benefit from treatment with specific Top1 inhibitors and to guide clinical investigators toward definition of the lowest effective dose and optimal schedule for administration of these agents (38).

Bone marrow CFU-GM assays comparing the toxic effects on bone marrow by investigational agents across species have been useful in predicting the blood levels that might be reached in patients in comparison with blood levels in preclinical efficacy and safety species (27). Frequently, mouse bone marrow is less sensitive than human bone marrow to investigational agents and allows blood levels to be achieved in mouse preclinical efficacy testing that cannot be reached in patients. The bone marrow toxicity of 4 Top1 inhibitors was examined (Fig. 1B; ref. 18–21). Murine bone marrow is 4- to 28-fold less sensitive to the Top1 inhibitors than human bone marrow. The differential sensitivity between mouse and human bone marrow to investigational agents and blood levels in preclinical efficacy and safety species have been useful in predicting the blood levels that might be reached in patients in comparison with blood levels in preclinical efficacy and safety species (27).
sensitivity across species may have greater potential for reaching similar blood levels in patients as in mice. From these data, we infer that Genz-644282 and ARC-111 may have promise as development candidates for antitumor agents.

A hallmark of malignant cells is the capacity for unlimited renewal or proliferation. The cancer stem cell hypothesis postulates that malignant disease is maintained by a rare subset of cells with stem cell properties (39, 40). The colony formation assay is the gold standard for determination of the cytotoxicity of compounds toward malignant cells because it measures the survival/killing of cancer cells capable of proliferating to form colonies or clones. Each colony originates from a single cell capable of self-renewal with the ability to generate malignant disease. Genz-644282 cytotoxicity was assessed in 8 human tumor cell lines of varied histology and resistance mechanisms by colony formation. Compared with topotecan and SN-38, Genz-644282 and ARC-111 were more potent cytotoxic agents. Genz-644282 and ARC-111 remained highly effective in cells expressing efflux pumps. Meng and colleagues (41) reported that, similar to other Top1 inhibitors, the antiproliferative effects of ARC-111 were oxygen independent, which is distinguishable from inhibition of hypoxia-inducible factor-1α accumulation by ARC-111—observed only under hypoxia and supporting the potential anticancer efficacy of ARC-111 and similar compounds toward hypoxic tumors.

In earlier reports, ARC-111 was shown to be an effective antitumor agent against several standard human tumor xenografts. ARC-111 had activity equal to irinotecan in the HCT-8 colon carcinoma and topotecan or irinotecan in the SKNEP anaplastic Wilm’s xenograft (24). Furthermore, ARC-111 was a very effective antitumor agent in animals bearing the SJ-BT45 medulloblastoma cell line (26). In subsequent studies, ARC-111 was superior or equally effective as an anticancer agent compared with irinotecan in the HCT-116 and HT-29 human colon carcinoma xenografts, docetaxel in the NCI-H460 human NSCLC xenograft, and gemcitabine in the MiaPaCa2 human pancreatic carcinoma xenograft (19). In the present study, Genz-644282 showed greater or equal antitumor efficacy as irinotecan in 4 human colon carcinoma xenografts, greater antitumor efficacy than dacarbazine in the human LOX-IMVI melanoma xenograft, greater antitumor efficacy than irinotecan in the human 786-O renal cell carcinoma xenograft, and greater than or equal antitumor efficacy than docetaxel in 2 human NSCLC xenografts. In an initial combination chemotherapy regimen, Genz-644282 and docetaxel together showed a modest increase in tumor response in the NCI-H460 NSCLC xenograft compared with Genz-644282 as a single agent.

Top1 inhibitors are active and effective anticancer drugs. The FDA-approved Top1 inhibitors are molecules that have flaws that newer compounds can improve upon. For this reason, Top1 remains a target of active interest in the development of new anticancer agents. Investigational molecules, both camptothecin derivatives and non-camptothecin Top1 inhibitors in preclinical and clinical development, have properties that lead to improved therapeutic benefit to patients. There is an active search in the Top1 inhibitor field.
for biomarkers such as gene signatures or markers based on protein phosphorylation and mRNA levels in tumor or blood samples that can identify patients who are most likely to benefit from treatment with a specific Top1 inhibitor.

Genz-644282 is a promising non-camptothecin Top1 inhibitor that, based on preclinical activity and safety, was selected for development and is currently undergoing a phase 1 clinical trial.

References

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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