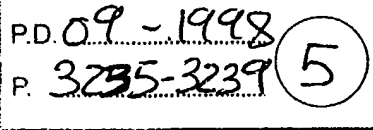


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Role of Folic Acid in Modulating the Toxicity and Efficacy of the Multitargeted Antifolate, LY231514

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Abstract. We studied the effects of folic acid on modulating the toxicity and antitumor efficacy of LY231514. Using several human tumor cell lines adapted to growth in low folate medium, folic acid was shown to be 100- to 1000-fold less active than folinic acid at protecting cells from LY231514-induced cytotoxicity. The lethality of LY231514 was compared in mice maintained on standard diet or low folate diet. The LD50 occurred at 60- and 250-fold lower doses of LY231514 in DBA/2 and CD1 nu/nu mice, respectively, maintained on low folate diet compared to standard diet. The L5178Y/TK-IHX murine lymphoma was much more sensitive to the antitumor action of LY231514 compared to wild type L5178Y-S tumors. For mice on low folate diet, LY231514 at 0.3 and 1 mg/kg (qd x 10, i.p.) produced 100% inhibition of L5178Y/TK-IHX lymphoma growth, and significant lethality occurred at ≥ 3 mg/kg. For mice on standard diet, LY231514 produced >95% inhibition of tumor growth at 30 to 300 mg/kg, but all mice died at 800 mg/kg. Folic acid supplementation was demonstrated to preserve the antitumor activity of LY231514 while reducing toxicity. The combination of folic acid with LY231514 may provide a mechanism for enhanced clinical antitumor selectivity.

LY231514 is a structurally novel antifolate antimetabolite that possesses the unique 6-5-fused pyrrolo[2,3-d]pyrimidine nucleus (1) instead of the more common 6-6-fused pteridine or quinazoline ring structure. The primary mode of antitumor activity for LY231514 has previously been ascribed to inhibition of thymidylate synthase (TS) (1, 2). However, several lines of evidence suggest that multiple enzyme-inhibitory mechanisms are involved in cytotoxicity, hence the acronym MTA (multitargeted antifolate): 1) the reversal pattern for MTA in human leukemia and colon carcinoma cell lines demonstrates that although TS may be a major site

of action for LY231514 at concentrations near the IC50, higher concentrations can lead to inhibition of dihydrofolate reductase (DHFR) and/or other enzymes along the purine de novo pathway (3); 2) MTA is an excellent substrate for polyglutamate synthetase, and the K_i values of the pentaglutamate of LY231514 are 1.3, 7.2, and 65 nM for inhibition against TS, DHFR and glycylamide ribonucleotide formyltransferase (GARFT), respectively (3); 3) intracellular concentrations of LY231514 and its polyglutamates can exceed 40 μ M in CCRF-CEM cells when 3 H-labeled LY231514 was used (R.M. Schultz, unpublished observation); and 4) early clinical studies demonstrated that patients who had previously failed to respond to ZD1694 and 5-fluorouracil/leucovorin treatment responded to LY231514 (4; DA Rinaldi, personal communication).

Several animal studies have indicated that folic acid supplementation in combination with antifolate cancer therapy can prevent delayed toxicity and enhance the therapeutic potential of the GARFT inhibitor lometrexol (5, 6) and the TS inhibitor 1843U89 (7). Unexpected delayed cumulative toxicity was observed in phase I studies with lometrexol, including thrombocytopenia, anemia, and mucositis (8). Additional clinical studies demonstrated the protective effects of folic acid against lometrexol toxicity in humans (9). Morgan and coworkers (10) concluded that a daily supplement of 1 mg of folic acid during low-dose methotrexate therapy in patients with rheumatoid arthritis was useful in lessening toxicity without altering efficacy. In the present communication, we investigated the effects of folic acid on the antitumor activity and lethality of LY231514 in mice.

Materials and Methods

Reagents. Folic acid, folinic acid (leucovorin), and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTI) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The disodium salt of LY231514 was synthesized at Eli Lilly and Co. (1).

Cell lines. Human CCRF-CEM leukemia cells were obtained from St. Jude Children's Research Hospital (Memphis, TN, USA). Human IGROV1 ovarian carcinoma cells were generously supplied by Dr.

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Barton Kamen (Univ. of Texas Southwestern Medical Center, Dallas, TX, USA). GC3 human colon carcinoma cells were obtained from Dr. Janet Houghton, St. Jude Children's Research Hospital. Human KB epidermoid carcinoma cells were purchased from the American Type Culture collection (ATCC, Rockville, MD, USA). The human LX-1 lung carcinoma cell line was established at Lilly from xenograft tissue. These cell lines were adapted to folic acid-free RPMI-1640 medium containing L-glutamine and 25 mM HEPES buffer (Whittaker Bioproducts, Walkersville, MD, USA) and supplemented with 10% dialyzed fetal calf serum (Hyclone Laboratories, Inc. (Logan, UT, USA) and 2 nM folic acid. The L5178Y/TK-/HX- murine lymphoma cell line was obtained from Eli Lilly Department of Genetic Toxicology (Greenfield, IN, USA). The tumor is a double mutant, deficient in thymidine kinase and hypoxanthine phosphoribosyl transferase. It was cultured in RPMI-1640 medium supplemented with 10% horse serum. The L5178Y-S wild type lymphoma cell line was obtained from ATCC and routinely cultured in Fischer's medium (Whittaker Bioproducts) supplemented with 10% horse serum and 1 mM sodium pyruvate. All cell lines were tested and found free of mycoplasma contamination by the ATCC.

In vitro cytotoxicity testing. We used a modification of the original MTT colorimetric assay described by Mosmann (11) to measure cell cytotoxicity. The human tumor cells (previously adapted to growth in low folate (2 nM folic acid) medium) were seeded at 1×10^4 cells in 80 μ l of assay medium/well in 96-well flat-bottom tissue culture plates (Costar, Cambridge, MA, USA). Assay medium consisted of folic acid-free RPMI-1640 medium supplemented with 10% dialyzed fetal calf serum and 2 nM folic acid. Well 1A was left blank (100 μ l of growth medium without cells). Various levels of folic or folinic acid (0.1 to 100 μ M) were added to the wells and incubated for 2 hours prior to addition of LY231514. LY231514 was prepared in Dulbecco's phosphate-buffered saline (PBS) at 1 mg/ml, and a series of two-fold dilutions were subsequently made in PBS. Aliquots (10 μ l) of each concentration were added to triplicate wells. Plates were incubated for 72 hours at 37°C in a humidified atmosphere of 5% CO₂-in-air. MTT was dissolved in PBS at 5 mg/ml. Following incubation of plates, 10 μ l of stock MTT solution was added to all wells of an assay, and the plates were incubated at 37°C for two additional hours. Following incubation, 100- μ l dimethyl sulfoxide was added to each well. Following thorough formazan solubilization, the plates were read on a Dynatech MR600 reader, using a test wavelength of 570 nm and a reference wavelength of 630 nm.

Mice. Female CD 1 nu/nu mice were purchased from Charles River Laboratories (Wilmington, MA, USA). Female DBA/2 mice were purchased from Taconic (Germantown, NY, USA). Mice weighed 20 to 25 grams at the beginning of the studies. Mice were housed in temperature and humidity controlled rooms. Mice were fed either standard laboratory rodent chow (Purina Chow #5001) or folic acid-deficient diet containing 1% succinylsulfathiazole (Purina Chow #5831C-2); both diets were purchased from Ralston Purina Co. (St. Louis, MO, USA). The average content of folates from natural sources in both diets was found to be 0.03 ppm, whereas the standard diet was analyzed to contain 7.3 ppm of added folic acid. It was estimated that mice on a standard diet ingested 1 to 2 mg/kg/day of folates, while mice on a low folate diet ingested 0.001 to 0.008 mg/kg/day. In some studies, mice received solubilized folic acid once a day by oral gavage. Food and water were provided ad libitum.

In vivo antitumor drug testing. L5178Y-S and L5178Y/TK-/HX were established and characterized *in vivo* for tumor growth in syngeneic DBA/2 mice. Cells derived from *in vitro* culture were washed twice by centrifugation (300 g for 10 minutes) in serum-free medium. Recipient DBA/2 mice were shaved and inoculated subcutaneously in the axillary region with 2×10^6 cells in 0.5 ml serum-free RPMI-1640 medium. LY231514 treatment was administered *ip.* on a daily schedule for ten days and initiated on the day after tumor implant. LY231514 was dissolved in 0.9% sodium chloride solution. All animals were weighed at

the beginning and end of drug treatment. Two-dimensional measurements (width and length) of all tumors were taken using digital electronic calipers interfaced to a microcomputer (12). Tumor weights were calculated from these measurements using the following formula:

$$\text{Tumor weight (mg)} = \text{tumor length (mm)} \times \text{tumor width (mm)}^2 / 2$$

Percent inhibition of tumor growth was determined by comparing the tumor weight in treated groups to that of controls. No group was included in the analysis for therapeutic activity in which deaths attributable to drug toxicity exceeded 20% of the treated group.

Results

In vitro protective effect of folic or folinic acid for the cytotoxic activity of LY231514. We tested the ability of folic and folinic acid to protect human carcinoma and leukemia cells from LY231514-induced cytotoxicity. Previous studies demonstrated that the antiproliferative activity of LY231514 for CCRF-CEM leukaemia cells was completely reversed by the addition of leucovorin (0.05 to 16 μ M) in a competitive manner (1). This suggested that LY231514 competed with natural reduced folate cofactors both at transport and intracellular folate levels and acted as a pure folate antagonist. In addition, we have reported that LY231514 is primarily transported via the reduced folate carrier (RFC) in human cell lines (3). For the current studies, we utilized tumor cell lines that had been adapted over >4 weekly passages to growth in low folate (2 nM folic acid) media. Varying concentrations of folic and folinic acid were added to these adapted cells 2 hours prior to LY231514 exposure. As shown in Table I, the sensitivity to LY231514 cytotoxicity (IC₅₀) of low folate medium-adapted cells ranged from 3.6 nM (CCRF-CEM leukemia) to 44 nM (IGROV1 ovarian carcinoma). In addition, Table I shows the ability of folic acid and folinic acid to modulate the cytotoxic activity of LY231514 in five different human tumor cell lines. Folic acid was approximately 100- to 1000-fold less active than folinic acid at protecting cells from LY231514-induced cytotoxicity. Folic acid required concentrations of 10 μ M or greater to exert significant protection.

Enhanced lethality of LY231514 to mice with dietary restriction of folic acid. Dietary folate deprivation has previously been shown to markedly enhance the toxicity of lometrexol (5). To assess the importance of dietary folate in modulating the toxicity of LY231514, LD₅₀ values were determined in mice maintained on standard diet (normal rodent laboratory chow) or on a special low folate diet (LFD). LFD mice have been shown to be significantly folate deficient in plasma and several tissues including liver and implanted tumors (13). Mice maintained on LFD for two weeks before intraperitoneal administration of LY231514 daily for 10 days were extremely sensitive to the toxic effects of LY231514 with LD₅₀ values of 1.6 and 10 mg/kg for CD1 nu/nu and DBA/2 mice, respectively (Figure 1). In contrast, the LD₅₀ values for CD1 nu/nu and DBA/2 mice maintained on standard diet

Table I. *In vitro* protective effects of folic or folinic acid on LY231514-induced cytotoxicity.

Cell line ^a	IC50 (nM) ^b	Relative (-fold) Change in IC50						
		Folic acid conc. in media ^c			Folinic acid conc. in media			
		1 μ M	10 μ M	100 μ M	0.1 μ M	1 μ M	10 μ M	100 μ M
IGROV1	44	1	14	25	28	370	>970	>970
KB	34	2	3	17		6	78	>1270
GC3	12	1	3	9		105	47	640
LX-1	4	1	3	6		6	82	1460
CCRF-CEM	4	1	4	22	2	22	130	4600

^aCells were adapted to >4 weekly passages in low folate (2 nM folinic acid) medium.

^bCytotoxicity was determined by MTT assay with 72 h exposure to LY231514. Data represent mean of triplicate determinations.

^cFolic or folinic acid was added two hours prior to LY231514 addition.

were approximately 250- and 60-fold greater, respectively than mice on LFD.

Role of folic acid in the antitumor activity of LY231514 against the L5178Y murine lymphoma. High circulating thymidine levels in mice decrease the efficacy and toxicity of TS inhibitors in mice (14, 15). Unless a tumor model which cannot salvage thymidine is utilized in mice, only limited antitumor effects for specific TS inhibitors have been observed. LY231514 treatment (i.p., qd x10) produced modest activity against the wild type L5178Y-S murine lymphoma (Table II). In contrast, similar treatment of a variant of this line, L5178Y/TK-/HX-, produced potent tumor suppression (100% tumor inhibition on the day following the last drug treatment at 30 and 100 mg/kg per day) with 11 of 14 mice tumor-free on day 100 after tumor implantation. This tumor is deficient in both thymidine kinase as well as hypoxanthine-guanine phosphoribosyl transferase and consequently, cannot salvage either thymidine or the purines hypoxanthine and guanine. The exquisite sensitivity of the L5178Y/TK-/HX- tumor model to LY231514 treatment allowed us to evaluate the effect of low folate diet on the therapeutic activity of this compound. For mice on LFD, LY231514 at 0.3 and 1.0 mg/kg/day (i.p. qd x10) produced 100% inhibition of tumor growth for tumors measured one day after the completion of a single course of drug treatment (Figure 2). As noted in Figure 1, higher drug levels yielded unacceptable toxicity. For mice on LFD that received a folate supplement of 15 mg/kg/day via oral gavage, significant inhibition of tumor growth was noted over a broad dose range (10 - 1000 mg/kg/dose). Moreover, 100% inhibition of tumor growth was observed at 30 to 1000 mg/kg/dose without any lethality. This antitumor dose response (with folate supplementation) was virtually identical to that observed for mice receiving standard diet. However, the lethality was significantly greater for the mice on standard diet (lethality at

Table II. LY231514 antitumor activity against L5178Y/S wild type and L5178Y/TK-/HX-lymphoma.

	Tumor Dose ^a (mg/kg)	% Tumor Inh. ^b	# Tumor-free/total	
			day 10 ^c	day 100
L5178Y/S	10	0	0/10	-
	30	8	0/10	-
	100	68	0/10	-
L5178Y/TK-/HX-	10	90	0/7	0/7
	30	100	5/7	6/7
	100	100	7/7	5/7

^aLY231514 was administered i.p. on a qd x 10 schedule.

^bTumors were measured on the day following the last drug treatment.

^cDays represent the number of days since therapy was initiated.

400 and 800 mg/kg/day of 10% and 100%, respectively). Mice on standard diet received approximately one-tenth of the amount of daily folic acid as the mice on LFD with 15 mg/kg/day supplemental folic acid.

Discussion

The poor predictive value of mouse models for antifolate toxicity may be partially due to the fact that standard laboratory mouse diets contain high levels of folic acid. Previous data demonstrated that serum and RBC folate levels of mice maintained on a diet formulated without added folic acid fall to levels considered normal in humans (5, 13). In this paper, we demonstrate that mice fed a low folate diet for a short period (2 weeks) became 60- to 250-fold more sensitive

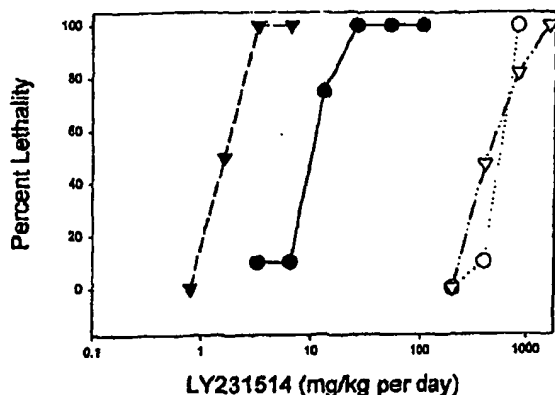


Figure 1. The toxicity of LY231514 in mice is increased by a folate-deficient diet. DBA/2 and CD1 *nu/nu* mice were fed either a standard laboratory diet (○ and ▽, respectively) or a folate-deficient diet for 2 weeks prior to the first dose of LY231514 (● and ▼, respectively) and for the duration of the study. Groups of mice (> 10 animals/group) on each diet were given 10 daily doses of LY231514 *i.p.* at the indicated doses. The data present the percent lethality within 3 weeks after the last dose of LY231514.

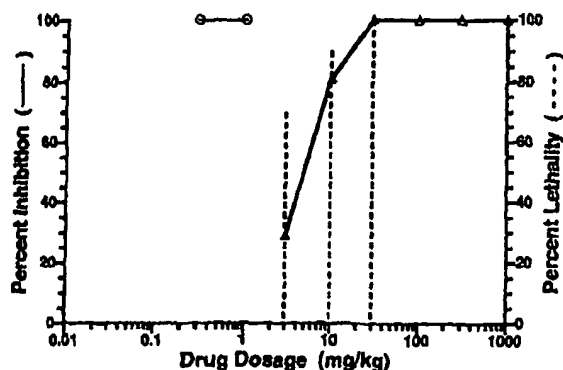


Figure 2. Antitumor activity of LY231514 therapy (*i.p.*, *qd* × 10) against LS178Y/TK-*/HX*- lymphoma for mice on low folate diet with no folate supplementation (○) and for mice on low folate diet that received 15 mg/kg/day daily folate supplementation (△). Vertical dashed lines represent percent lethality in mice on low folate diet with no folate supplementation. No lethality was observed in mice that received folate supplementation.

to the lethality of LY231514 than observed in mice fed standard laboratory diet (Figure 1). The antifolate GARFT inhibitor, lometrexol has previously been shown to accumulate in the livers of folate-deficient mice, and this accumulation was diminished by the administration of folic acid to these animals (16). These investigators hypothesized that the substantial and unexpected toxicity of lometrexol in humans not given concurrent folic acid and in folate-deficient mice is due to the sequestration of drug in hepatic tissue, with the subsequent slow release of drug to the circulation at toxicologically relevant concentrations. The mechanism for this accumulation of lometrexol in liver probably involves metabolism to polyglutamate forms by the enzyme folylpoly- γ -glutamate synthetase (FPGS). In this regard, Mendelsohn and coworkers (6) demonstrated that liver produced the greatest response in elevated FPGS to low dietary folate of all tissues tested. A similar mechanism probably exists for the potentiation of LY231514 toxicity by folate-deficient diet, since this compound is an extremely efficient substrate for mouse liver FPGS (1). In addition, LY231514 requires polyglutamation for cytotoxic potency (3).

The uptake of natural reduced folate compounds and folate analogues into cells appears to involve membrane protein receptors of two different classes: a reduced folate/methotrexate carrier (RFC), which binds reduced folate in the micromolar range, and a high-affinity folate binding protein (mFBP), which preferentially binds to oxidized folate and other analogs with an affinity <1 nM (17). Studies using a panel of ZR-75-1 human breast sublines with differing transport properties have demonstrated a predominant role for the RFC in intracellular transport of

LY231514 (3). Similarly, we now report that folic acid only weakly modulates the cytotoxic activity of LY231514 for various human leukemia and carcinoma cells adapted to low folate conditions (Table I). Some of these cells (KB and IGROV1) have previously been demonstrated to possess elevated levels of mFBP (18), further suggesting a minor role for mFBP in LY231514 transport.

LY231514 produced potent antitumor activity against the LS178Y/TK-*/HX*- lymphoma at 100-fold lower dose levels (0.3 and 1 mg/kg/day, Figure 2) in LFD mice relative to 30 and 100 mg/kg (Table II) in mice on standard diet. It is interesting to note that the LD₅₀ was reduced 3000-fold for lometrexol in LFD animals, and antitumor activity could not be demonstrated even at low dose levels (5). In contrast, the shift in both LD₅₀ and antitumor activity for mice on LFD compared to standard diet were of a similar magnitude (approximately 100-fold) for LY231514. However, LFD animals with high levels of folate supplementation demonstrated decreased lethality to LY231514 compared to conventional diet animals, suggesting that folate intake can be manipulated to achieve greater therapeutic effects. Oral folic acid dramatically decreased the toxicity of LY231514 and preserved antitumor activity (albeit at higher dose levels) in these mice (Figure 2).

Previous studies have demonstrated that the multitargeted antifolate, LY231514 has a unique biochemical and pharmacological profile. Exciting antitumor activity has been observed in phase I and II clinical trials, including responses in colon, breast, non-small cell lung and pancreatic cancers. More advanced and extensive clinical trials of LY231514 are currently in progress. The combination of folic acid with

LY231514 may provide a mechanism for enhanced clinical antitumor selectivity.

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References

- 1 Taylor EC, Kuhnt D, Shih C, Rinzel SM, Grindey GB, Barredo J, Jannatipour M and Moran RG: A dideazatetrahydrofolate analogue lacking a chiral center at C-6, N-(4-[2-(2-amino-1,7-dihydro-4-oxopyrrolo [2,3-d]pyrimidine-6-yl)ethyl]benzoyl) glutamic acid, a new and potent inhibitor of thymidylate synthase. *J Med Chem* 35: 4450-4454, 1992.
- 2 Grindey GB, Shih C, Barnett CJ, Pearce HL, Engelhardt JA, Todd, GC, Rinzel SM, Worzalla JF, Gossett LS and Everson TP: LY231514, a novel pyrrolopyrimidine antifolate that inhibits thymidylate synthase (TS). *Proc Am Assoc Cancer Res* 33: 411, 1992.
- 3 Shih C, Chen VJ, Gossett LS, Gates SB, MacKellar WC, Habeck LL, Shackelford KA, Mendelsohn LG, Soose DJ, Patel VF, Andis SL, Bewley JR, Rayl EA, Moroson BA, Beardsley GP, Kohler W, Ratnam M and Schultz RM: LY231514, a pyrrolo[2,3-d]pyrimidine-based antifolate that inhibits multiple folate-requiring enzymes. *Cancer Res* 57: 1116-1123, 1997.
- 4 Rinaldi DA, Burris HA, Dorr FA, Woodworth JR, Kuhn JG, Eckardt JR, Rodriguez G, Corso SW, Fields SM, Langley C, Clark G, Faries D, Lu P and Van Hoff DD: Initial phase I evaluation of the novel thymidylate synthase inhibitor, LY231514, using the modified continual reassessment method for dose escalation. *J Clin Oncol* 13: 2842-2850, 1995.
- 5 Alati T, Worzalla JF, Shih C, Bewley JR, Lewis S, Moran RG and Grindey GB: Augmentation of the therapeutic activity of lometrexol [(6-R)-5,10-dideazatetrahydrofolate] by oral folic acid. *Cancer Res* 56: 2331-2335, 1996.
- 6 Mendelsohn LG, Gates SB, Habeck LL, Shackelford KA, Worzalla J, Shih C and Grindey GB: The role of dietary folate in modulation of folate receptor expression, folypolyglutamate synthetase activity and the efficacy and toxicity of lometrexol. *Advan Enzyme Regul* 36: 365-381, 1996.
- 7 Smith GK, Amyx H, Boytos CM, Duch DS, Ferone R and Wilson HR: Enhanced antitumor activity for the thymidylate synthase inhibitor 1843U89 through decreased host toxicity with oral folic acid. *Cancer Res* 55: 6117-6125, 1995.
- 8 Ray MS, Muggia FM, Leichman CG, Grunberg SM, Nelson RL, Dyke RW and Moran RG: Phase I study of (6R)-5,10-dideazatetrahydrofolate: a folate antimetabolite inhibitory to de novo purine synthesis. *J Natl Cancer Inst* 85: 1154-1159, 1993.
- 9 Luohavinij S, Wedge SR, Lind MJ, Bailey N, Humphreys A, Proctor M, Chapman F, Simmons D, Oakley A, Robson L, Gumbrell L, Taylor GA, Thomas HD, Boddy AV, Newell DR and Calvert AH: A phase I clinical study of the antipurine antifolate lometrexol (DDATHF) given with oral folic acid. *Invest New Drugs* 14: 325-335, 1996.
- 10 Morgan SL, Baggott JE, Vaughn WH, Young PK, Austin JV, Krumdieck CL and Alarcon GS: The effect of folic acid supplementation on the toxicity of low-dose methotrexate in patients with rheumatoid arthritis. *Arthritis Rheum* 33: 9-18, 1990.
- 11 Mosmann T: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 65: 55-63, 1983.
- 12 Worzalla JF, Bewley JR and Grindey GB: Automated measurement of transplantable solid tumors using digital electronic calipers interfaced to a microcomputer. *Invest New Drugs* 8: 241-251, 1990.
- 13 Schmitz JC, Grindey GB, Schultz RM and Priest DG: Impact of dietary folic acid on reduced folates in mouse plasma and tissues: relationship to dideazatetrahydrofolate sensitivity. *Biochem Pharmacol* 48: 319-325, 1992.
- 14 Duch DS, Banks SD, Dev IK, Dickerson SJ, Ferone R, Heath LS, Humphreys J, Knick V, Pendergast W, Singer S, Smith GK, Waters K and Wilson HR: Biochemical and cellular pharmacology of 1843U89, a novel benzoquinazoline inhibitor of thymidylate synthase. *Cancer Res* 53: 810-818, 1993.
- 15 Jackman AL, Taylor GA, Gibson W, Kimbell R, Brown M, Calvert AH, Judson IR and Hughes LR: ICI D1694, a quinazoline antifolate thymidylate synthase inhibitor that is a potent inhibitor of L1210 cell growth *in vitro* and *in vivo*; a new agent for clinical study. *Cancer Res* 51: 5579-5586, 1991.
- 16 Pohland RC, Alati T, Lantz RJ and Grindey GB: Whole-body autoradiographic disposition and plasma pharmacokinetics of 5,10-dideazatetrahydrofolic acid in mice fed folic acid-deficient or regular diets. *J Pharm Sci* 83: 1396-1399, 1994.
- 17 Antony AC: The biological chemistry of folate receptors. *Blood* 79: 2807-2820, 1992.
- 18 Schultz RM, Andis SL, Shackelford KA, Gates SB, Ratnam M, Mendelsohn LG, Shih C and Grindey GB: Role of membrane-associated folate binding protein in the cytotoxicity of antifolates in KB, IGROV1, and L1210A cells. *Oncology Res* 7: 97-102, 1995.

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