

Disposition and Metabolism of 1-(Tetrahydro-2-furanyl)-5-fluorouracil (Ftorafur) in Humans¹

John A. Benvenuto,² Katherine Lu, Stephen W. Hall, Robert S. Benjamin,³ and Ti Li Loo

Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

ABSTRACT

The pharmacology of high-dose 1-(tetrahydro-2-furanyl)-5-fluorouracil (FT) has been studied by radiochemical and chromatographic techniques in eight patients. Plasma disappearance of FT was exponential, with a half-life of 8.8 hr. Plasma concentrations of 5-fluorouracil (FUra) were sustained at 12.8 nmol/ml (1.7 μ g/ml) for at least 48 hr after FT administration. The concentrations of FUra derived from the administration of FT were considerably greater than were those achieved by constant infusion of FUra at the maximal tolerated dose of 1.1 g/sq m without causing unacceptable mucositis. The cumulative urinary excretion was 20% of the administered dose in 24 hr. FT underwent *in vivo* biotransformation to 2 hydroxytetrahydrofuranyl-5-fluorouracil derivatives in addition to anabolites and catabolites of FUra. High concentrations of FT and FUra were present in the cerebrospinal fluid, which could account for the severe central nervous system toxicity of FT at high doses. We conclude that the antitumor activity of FT is partially attributable to its slow release of FUra.

INTRODUCTION

FT⁴ (NSC 148958) is the 1-(2-tetrahydrofuranyl) derivative of FUra; its antitumor activity is attributed to the slow release of the latter (14). FT is less hematotoxic (8) and less lethal (9) to mice than is FUra. Pharmacology studies in the rat indicate that, after administration of [2-¹⁴C]FT, plasma radioactivity declines with a half-life of 5 hr (5). In another study in the rat, however, plasma radioactivity followed triphasic kinetics, with a terminal half-life of 13.6 hr (10). FT and FUra were identified in the plasma of rats and mice after the administration of FT (15), whereas only FT was detected in the plasma of dogs and monkeys similarly treated (6).

In the rat the majority of given radioactivity was excreted in the expired air as ¹⁴CO₂ (5); the urinary radioactivity consisted of FT, FUra, FdUrd, urea, and unidentified nucleotides. Although unchanged FT predominated in the urine, the proportions of FUra and its metabolites did increase with time. Similarly, FT, FUra, FdUrd, α -fluoro- β -

ureidopropionic acid, and urea were identified in urine of dogs treated with FT; however, FUra and its metabolites were the major urinary excretion products.

In our studies (11, 12) in the dog, the plasma half-life of FT was 6.2 hr and that of FUra derived from FT was 2.6 hr. Further, in dogs with livers damaged by CCl₄, the plasma half-lives of both drugs were increased, indicating the importance of the hepatic metabolism of these agents. These findings are consistent with reports that FT is degraded by rat and mouse microsomal enzyme systems (1, 15).

Clinically, FT has a spectrum of antitumor activity similar to that of FUra, being particularly effective against gastrointestinal and breast cancers (9). At doses of 1 to 2 g/sq m for 5 days, gastrointestinal and central nervous system toxicities of FT were significant (16). The lack of myelosuppression of FT on this dose schedule is reminiscent of that observed with continuous infusion of FUra (13). Clinical pharmacological studies of FT were conducted during Phase 1 trial of this agent at our institution. We now report the results of these studies.

MATERIALS AND METHODS

Patients. Eight patients with metastatic adenocarcinoma (primary tumors of colon, rectum, breast, and lung) were studied after informed consent had been obtained; all had normal liver and renal function as determined by serum chemistries (SMA 12). FT at 5 g/sq m was administered *i.v.* to 7 of these patients over a 2- to 3-hr period; the eighth patient received 4 g/sq m. Four of these patients received 200 μ Ci of [2-¹⁴C]FT. The radioactive FT was sterilized by filtration through a 22- μ m Millipore filter after radiochemical purity (98 to 99%) had been determined by radiochromatography as described below. Blood specimens of 10 ml each, collected at designated intervals in tubes containing heparin, were centrifuged at 2000 \times g in a clinical centrifuge to obtain plasma. Urine was collected as voided for the first day of study and at daily intervals thereafter. Samples of CSF were obtained from one patient by lumbar puncture.

Chemicals. FT, FUra, FdUrd, FUrd, and [2-¹⁴C]FT (46.5 μ Ci/mg) were supplied by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Md; 5-fluoro-2'-deoxyuridine 5'-monophosphate was obtained from Terra-Marine Bioresearch, La Jolla, Calif. Other reagents and chemicals were purchased from regular commercial suppliers.

Sample Preparation. Urine samples (5 ml) were lyophilized, reconstituted with water (1 ml), and chromatographed on Dowex 1 \times 8 formate resin (200 to 400 mesh, 1- \times 20-cm

¹ Supported by Contracts N01-CM-53773 and N01-CM-43801 and Grant CA-14528 from the Division of Cancer Treatment, National Cancer Institute, NIH, USPHS.

² To whom requests for reprints should be addressed.

³ Junior Faculty Fellow, American Cancer Society.

⁴ The abbreviations used are: FT, 1-(tetrahydro-2-furanyl)-5-fluorouracil; FUra, 5-fluorouracil; FdUrd, 5-fluoro-2'-deoxyuridine; CSF, cerebrospinal fluid; FUrd, 5-fluorouridine; HPLC, high-pressure liquid chromatography; HFT, hydroxyftorafur.

Received April 14, 1978; accepted August 10, 1978.

column) with formic acid buffer as the eluent, as described by Chadwick and Rodgers (3). Fractions of 5 ml each were collected, and the radioactivity of 0.2-ml portions of each fraction was determined by liquid scintillation counting (see below). Those fractions that corresponded to the radioactive peaks were lyophilized and dissolved in 0.7 ml of water. Plasma samples were deproteinized by ultrafiltration (Model 12 stirred ultrafiltration cell, Diaflow type PM30 ultrafiltration membrane; Amicon Corporation, Lexington, Mass.).

Analytical Techniques. FT and its metabolites in biological fluids were identified and quantitated by HPLC and radiochromatography as previously described (2). A Waters Associates ALC Model 202/401 liquid chromatograph equipped with a variable wavelength UV detector (Schoeffel Instrument Corp., Westwood, N. J.) operating at 270 nm was used. The separations were achieved with a Waters μ -Bondapak C₁₈ column (30 cm x 4.0 mm inside diameter) with filtered, deionized water as eluent at a flow rate of 2.0 ml/min. Peak areas were determined by a Columbia Scientific Model CSI 38 integrator. Peak areas and drug concentrations were linearly correlated for both plasma and urine. Urine samples of 10 μ l each and plasma ultrafiltrates of 50 μ l each were analyzed by HPLC. For radiochemical analysis 200- μ l samples of the fractions from column chromatography were applied to Whatman No. 1 paper, and the chromatogram was developed with ethylacetate/formic acid/water (65/5/5, v/v/v). The paper was cut into 2-cm strips and placed in vials containing 11 ml PCS (Amersham Corp., Arlington Heights, Ill.), and the radioactivity was determined by liquid scintillation counting (Packard Tri-Carb 3855). Quenching was determined by the automatic external standard ratio method; the counting efficiency for ¹⁴C was 83%.

Protein Binding. The binding of FT to human plasma was determined by a rapid ultrafiltration method with an Amicon Diaflow Model 52 ultrafiltration cell equipped with a UM-2 membrane. A fresh 10-ml plasma solution of [2-¹⁴C]FT was filtered under 50 psi of nitrogen. The ultrafiltrate collected after the first 10 min was discarded. The samples were collected, and radioactivity was determined by liquid scintillation counting. The membrane showed no binding to FT.

Isolation and Degradation of HFT's. The HPLC effluent corresponding to the urinary HFT peak was collected and divided into 5 aliquots, and each aliquot was evaporated to dryness. The residues were redissolved in 2 ml of one of the following: 0.1 M acetate buffer, pH 5; ultrafiltered plasma; 0.05 M carbonate buffer, pH 9; or deionized water. Each solution was incubated for 4 hr, and 50 μ l were analyzed by HPLC.

In Vitro Metabolism of FT. Mouse (BALB/c x DBA/2 F₁) and rat (Sprague-Dawley) livers were homogenized in 2 volumes of 0.15 M KCl/0.05 M Tris/0.01 M MgCl₂ buffer, pH 7.4, and the homogenate was centrifuged at 9750 x g for 20 min. The pellet was discarded, and the supernatant was ultracentrifuged at 105,000 x g for 60 min. The supernatant was removed, and the pellet was resuspended in half the volume of the above buffer containing 0.01 M EDTA and recentrifuged at 105,000 x g for 30 min. The pellet that resulted was resuspended in the KCl/Tris/MgCl₂ buffer so that each g wet weight of liver gave 1 ml of microsomal suspension.

[2-¹⁴C]FT (0.2 mM) was incubated for 1 hr at 37° in a

mixture containing NADP⁺ (0.4 mM), glucose 6-phosphate (5.0 mM), glucose 6-phosphate dehydrogenase (0.6 units/ml), and 1 ml of microsomal suspension in a total volume of 5 ml. Protein was precipitated by the addition of 2 ml of 20% trichloroacetic acid. After centrifugation to remove protein, the supernatant was analyzed by HPLC and radiochromatography.

RESULTS

Plasma FT concentrations decreased slowly, as shown in Chart 1. The plasma disappearance of FT was exponential for the initial 48 hr after drug administration, with a *t*_{1/2} of 8.8 hr. In 4 patients who received FT (5 g/sq m), drug concentrations reached a plateau at approximately 48 hr and then slowly decreased but remained detectable at 94 hr. The apparent volume of distribution of FT, estimated from the exponential phase, is 0.66 liter/kg, about the D₂O space in humans. In 5 patients the average plasma concentrations of FUra derived from the FT remained nearly constant (Chart 1) despite large individual variations. No anabolites or catabolites of FUra were detected in the plasma.

The average cumulative urinary excretion of radioactivity in 4 patients receiving [2-¹⁴C]FT amounted to 19.5% of the administered dose in 24 hr, as shown in Chart 2. In 2 of these patients, 20.8% of the administered radioactivity was excreted in 48 hr. In the first 2 hr, the urinary radioactivity was mostly in FT (*R_f* 0.81); however, FUra (*R_f* 0.50), FUr

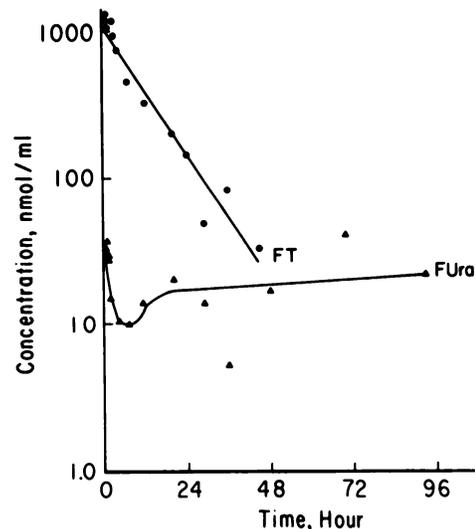


Chart 1. Plasma concentrations of FT and FUra after administration of FT, 4 to 5 g/sq m. FT (●), average of 8 patients; FUra (▲), average of 5 patients.

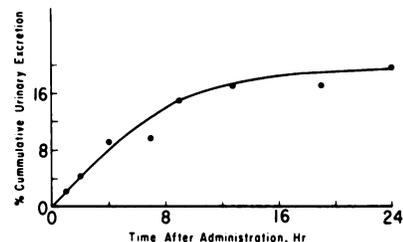


Chart 2. Cumulative 24-hr urinary excretion of radioactivity after administration of [2-¹⁴C]FT, 5 g/sq m. Average of 4 patients.

(R_f 0.21), FdUrd (R_f 0.38), 5-fluoro-2'-deoxyuridine 5'-monophosphate (R_f 0.04), urea (R_f 0.30), α -fluoro- β -ureidopropionic acid (R_f 0.50), and an unknown metabolite (R_f 0.63) were also observed (Chart 3). Identification was made by comparison of chromatographic R_f values and, when applicable, retention times with those of authentic samples. By 24 hr only 25% of the radioactivity resided in FT, with significant increases in the percentage of excretion of all the metabolites except FdUrd.

The unknown metabolite, which predominated at 24 hr, was isolated by HPLC. It liberated FUra when incubated in plasma or buffer, pH 9; however, there was no conversion when the metabolite was incubated in buffer, pH 5, or deionized water (Chart 4).

Although the metabolite appeared as a single peak on HPLC, thin-layer chromatography on silica gel ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$, 3/1/0.04) showed 2 spots (R_f 0.37 and 0.43). The 2 components were isolated by preparative thin-layer chromatography and were identified by mass spectrometry and nuclear magnetic resonance spectroscopy as 3'- and 4'-hydroxyftorafur. The details of detection, isolation, and identification by spectral interpretation are to be published.

For determination of the mode of formation of the HFT metabolites, $[2-^{14}\text{C}]\text{FT}$ was incubated with rat and mouse hepatic microsomal enzymes. Rat microsomes caused a 2% decrease in FT, whereas those from mice degraded FT by about 10%. The products were FUra and a component with the same paper chromatographic R_f (0.64) and HPLC reten-

tion time (850 sec) as the HFT urinary metabolite.

In one patient high concentrations of FT and FUra were observed in the CSF (Chart 5). By 8 hr the FT content of the CSF had surpassed that of the plasma. The FUra concentrations in the CSF were also high but never exceeded those of the plasma.

In the range of 6.25 to 100 $\mu\text{g}/\text{ml}$, FT was 30 to 50% bound to human plasma protein.

DISCUSSION

FT is thought to owe its antitumor activity to the slow release of FUra, and therefore it should mimic the constant infusion of FUra. Current Phase 1 clinical trials of FT consist of the daily infusion of 1.0 to 2.5 g/sq m for 5 days. Our pharmacology studies were undertaken to determine whether intermittent high doses (4 to 5 g/sq m) of FT would give sustained, therapeutic plasma concentrations of FUra.

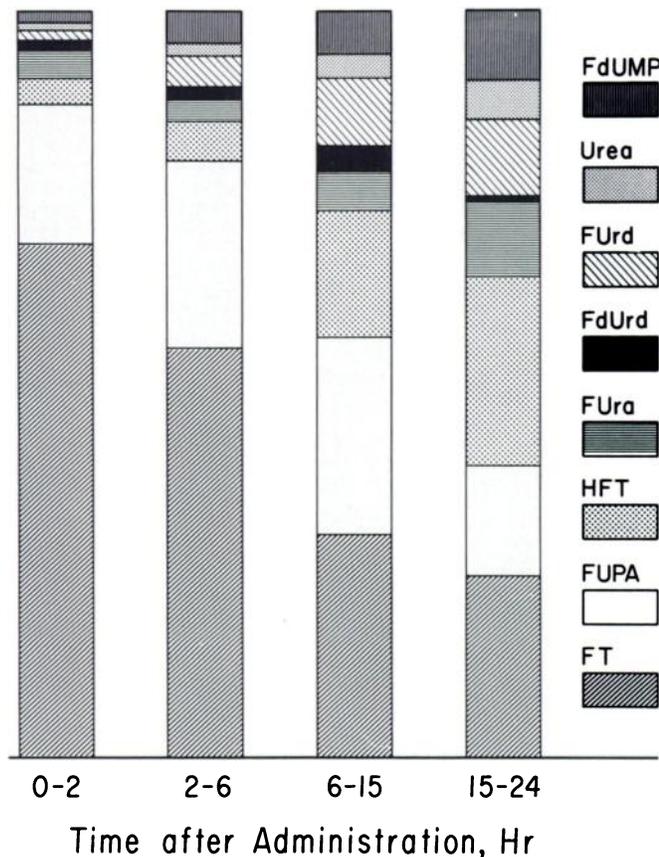


Chart 3. Normalized percentage of distribution of urinary FT and metabolites in 4 patients after FT, 5 g/sq m. FdUMP, 5-fluoro-2'-deoxyuridine 5'-monophosphate; FUPA, α -fluoro- β -ureidopropionic acid.

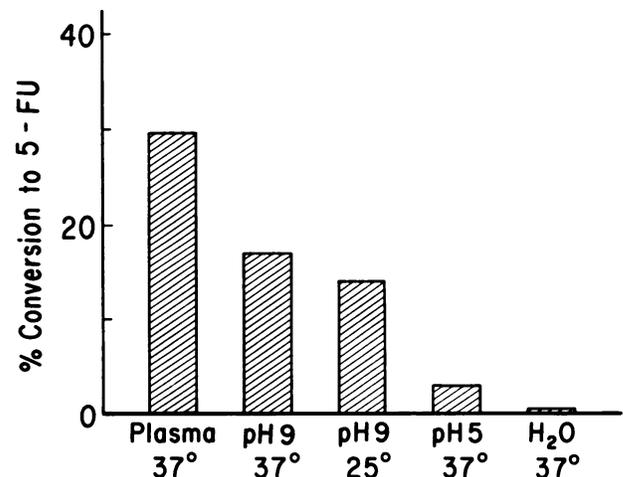


Chart 4. Percentage of conversion of a mixture of HFT metabolites to FUra after incubation for 4 hr in plasma, buffer (pH 9), buffer (pH 5), and H₂O.

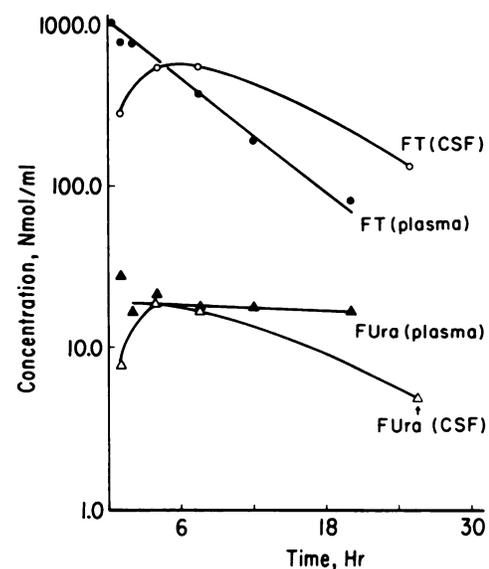


Chart 5. Plasma and CSF concentrations of FT (●, plasma; ○, CSF) and FUra (▲, plasma; △, CSF) in one patient after FT, 5 g/sq m.

The apparent plateau in FT plasma concentrations after the initial rapid plasma clearance suggests the slow exit of FT from body compartments. This is consistent with its distribution in total-body water, including poorly permeated tissue from which prolonged release is expected.

Although variable the high, sustained plasma levels of FUra produced after FT administration suggest that the mechanism for FUra generation from FT is saturated at this dose of FT. The average plasma FUra concentration 2 to 48 hr after a single i.v. FT injection is 12.8 nmol/ml (range, 5.1 to 19.2), considerably higher than the average plasma FUra concentration of 2.1 nmol/ml (range, 1.3 to 3.0) obtained from daily continuous infusion of 1.1 g of FUra per sq m (4).

Because of severe gastrointestinal mucosal toxicity, this is taken as the maximal tolerated dose of FUra by continuous infusion for 4 to 5 days (13). Although at 5 g/sq m acute gastrointestinal toxicity was dose limiting for FT, mucositis was infrequent, suggesting that despite high plasma concentrations of FUra derived from FT, FT or its metabolites somehow lessen FUra toxicity. The mechanism of this protective effect is currently under investigation.

In addition to gastrointestinal toxicity, severe central nervous system toxicity was observed after FT administration (7). This is attributable to the high CSF concentrations of FT and FUra but not necessarily to active metabolites of FUra.

The presence of HFT metabolites in urine of patients treated with FT suggests that FUra may not be formed directly from FT. The facile conversion of these urinary metabolites to FUra implicates them as intermediates in this conversion. Furthermore, the low but significant hydroxylation of FT by mouse hepatic microsomal enzymes suggests that the liver is the site of initial biotransformation.

Thus, administration of high doses of FT produces high plasma concentrations of FUra. The conversion of FT to FUra is mediated by the liver, with hydroxylated FT derivatives as intermediates. The subsequent formation of cytotoxic FUra metabolites suggests that FT owes its antitumor activity principally to its conversion of FUra. Additionally, the

large apparent FT distribution and slow conversion to FUra allow for prolonged release of the parent drug.

ACKNOWLEDGMENTS

We thank Mary Myers for her capable technical assistance.

REFERENCES

1. Benvenuto, J. A., Farquhar, D., Freireich, E. J., and Loo, Ti Li. Biotransformation of Ftorafur (FT, NSC-148958) in Man. *Proc. Am. Assoc. Cancer Res.*, **17**: 145, 1977.
2. Benvenuto, J. A., Lu, K., and Loo, Ti Li. High-Pressure Liquid Chromatographic Analysis of Ftorafur and Its Metabolites in Biological Fluids. *J. Chromatography*, **134**: 219-222, 1977.
3. Chadwick, M., and Rodgers, W. I. The Physiological Disposition of 5-Fluorouracil in Mice Bearing Solid L1210 Lymphocytic Leukemia. *Cancer Res.*, **32**: 1045-1056, 1972.
4. Clarkson, R., O'Connor, A., Winston, L., and Hutchinson, D. The Physiologic Disposition of 5-Fluorouracil and 5-Fluoro-2'-Deoxyuridine in Man. *Clin. Pharmacol. Therap.*, **5**: 581-610, 1964.
5. Cohen, A. M. The Disposition of Ftorafur in Rats After Intravenous Administration. *Drug Metab. Disposition*, **3**: 303-308, 1974.
6. Freudenthal, R. I., and Emmerling, D. C. The Metabolism of Ftorafur in the Beagle Dog and Rhesus Monkey. *Xenobiotica*, **7**: 757-764, 1977.
7. Hall, S. W., Valdivieso, M., and Benjamin, R. S. Intermittent High Single Dose Ftorafur: A Phase I Clinical Trial with a Pharmacologic-Toxicity Correlation. *Cancer Treat. Rept.*, **61**: 1495-1498, 1977.
8. Hrsak, I., and Pavicic, S. Comparison of the Effects of 5-Fluorouracil and Ftorafur on the Hematopoiesis in Mice. *Biomedicine*, **21**: 164-167, 1974.
9. Kavev, N. I., Blokhina, N. G., Vozny, E. K., and Pershin, M. P. Experience with Ftorafur in Breast Cancer. *Neoplasma*, **19**: 347-350, 1972.
10. Kozhukhov, A. N., Meiren, D. V., and Gilev, A. P. Pharmacokinetics of Ftorafur-2-¹⁴C in Rats with Walker's Carcinosarcoma. *Bull. Exptl. Biol. Med. - Engl. Tr.*, **83**: 872-975, 1977.
11. Lu, K., Benvenuto, J. A., Benjamin, R. S., and Loo, Ti Li. Pharmacokinetics of Ftorafur and 5-Fluorouracil in Dogs. *In: Proceedings of the 12th International Cancer Congress*, in press, 1978.
12. Lu, K., Loo, Ti Li, Benvenuto, J. A., Benjamin, R. S., Valdivieso, M., and Freireich, E. J. Pharmacologic Disposition and Metabolism of Ftorafur. *Pharmacologist*, **17**: 202, 1975.
13. Seifert, P., Baker, L. H., Reed, M. L., and Vaitkevicius, V. K. Comparison of Continuously Infused 5-Fluorouracil with Bolus Injection in Treatment of Patients with Colorectal Adenocarcinoma. *Cancer*, **36**: 123-128, 1975.
14. Smolyanskaya, A. Z., and Tugarinov, O. A. The Biological Activity of Antitumor Antimetabolites "Ftorafur". *Neoplasma*, **19**: 341-345, 1972.
15. Toide, H., Akiyoshi, H., Minato, Y., Okuda, H., and Fujii, S. Comparative Studies on the Metabolism of 2-(Tetrahydrofuryl)-5-Fluorouracil and 5-Fluorouracil. *Gann*, **68**: 553-560, 1977.
16. Valdivieso, M., Bodey, G. P., Gottlieb, J. A., and Freireich, E. J. Clinical Evaluation of Ftorafur (Pyrimidine-deoxyribose N₂-2'-Furanidyl-5-fluorouracil). *Cancer Res.*, **36**: 1821-1824, 1976.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Disposition and Metabolism of 1-(Tetrahydro-2-furanyl)-5-fluorouracil (Ftorafur) in Humans

John A. Benvenuto, Katherine Lu, Stephen W. Hall, et al.

Cancer Res 1978;38:3867-3870.

Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/38/11_Part_1/3867

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/38/11_Part_1/3867.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.