Clinical Cancer Research

Cellular but not plasma pharmacokinetics of lometrexol correlate with the occurrence of cumulative hematological toxicity.

T W Synold, E M Newman, M Carroll, et al.

Clin Cancer Res 1998;4:2349-2355. Published online October 1, 1998.

Cellular but not Plasma Pharmacokinetics of Lometrexol Correlate with the Occurrence of Cumulative Hematological Toxicity[1]

Timothy W. Synold,[2] Edward M. Newman, Mary Carroll, Franco M. Muggia, Susan Groshen, Kay Johnson, and James H. Doroshow

Departments of Medical Oncology and Therapeutics Research [T. W. S., M. C., J. H. D.] and Pediatrics [E. M. N.], City of Hope National Medical Center, Duarte, California 91010; and Division of Medical Oncology, USC-Norris Comprehensive Cancer Center, Los Angeles, California 90033 [F. M. M., S. G., K. J.]

ABSTRACT

Lometrexol inhibits the first folate-dependent enzyme in *de novo* purine biosynthesis and is avidly polyglutamated and retained in tissues expressing folylpolyglutamate synthetase. Although clinical studies have been limited by cumulative toxicity, preclinical studies show that pretreatment with folic acid can protect normal tissue while maintaining tumor cytotoxicity. Therefore, a Phase I study of lometrexol every 21 days preceded by i.v. folic acid was initiated. Lometrexol was studied in six patients at 15 mg/m^2, in six patients at 20 mg/m^2, in three patients at 25 mg/m^2, and in nine patients at 30 mg/m^2. Patients received either 5 mg of folic acid 1 h before or 25 mg/m^2 3 h before lometrexol. Blood samples were obtained around the first course and weekly thereafter for determination of plasma and erythrocyte (RBC) lometrexol concentrations. Bioactive folates in plasma and RBCs were determined in a subset of patients. Lometrexol pharmacokinetics were best described by a three-compartment model. Mean clearance and volume of distribution were 1.6 ± 0.6 liters/h/m^2 and 8.9 ± 4.1 liters/m^2. Mean half-lives were 0.23 ± 0.1, 2.9 ± 1.4, and 25.0 ± 48.7 h. Pharmacokinetics were independent of either lometrexol or folic acid dose. In the weekly blood samples, RBC lometrexol levels rose, long after plasma lometrexol was undetectable. RBC lometrexol levels were independent of folic acid or lometrexol dose. Bioactive folates measured in plasma and RBCs during this same time period did not accumulate. Rising RBC levels were correlated with a fall in hematocrit, hemoglobin, and platelet count. This study indicates that the cumulative toxicity of lometrexol is related to tissue concentration and not plasma pharmacokinetics. RBC lometrexol may be an indicator of cumulative drug exposure and effect.

INTRODUCTION

Lometrexol [(6*R*)-5,10-dideaza-5,6,7,8-tetrahydrofolic acid] is an antifolate antimetabolite with a broad spectrum of cytotoxic activity against preclinical murine tumor model systems and human tumor xenografts (1, 2). Unlike classical antifolates, lometrexol acts by inhibiting glycinamide ribonucleotide formyltransferase, the first folate-dependent enzyme in the *de novo* purine biosynthetic pathway (1). In addition to its unique mechanism of action, lometrexol is an excellent substrate for FPGS[3] and is avidly polyglutamated (1, 3). It has been demonstrated previously that the polyglutamates of lometrexol are more potent inhibitors of glycinamide ribonucleotide formyltransferase than lometrexol itself (4, 5). Furthermore, in addition to having a higher affinity for their target enzyme, polyglutamated anabolites are retained intracellularly for prolonged periods of time (3).

Because of promising preclinical activity, clinical trials of lometrexol began almost 10 years ago; however, the utility of lometrexol has been limited by serious toxicities (thrombocytopenia, leukopenia, and mucositis), which appear to be cumulative and long-lasting (6, 7). It has been speculated that this cumulative toxicity may be related to the formation and retention of polyglutamated anabolites, resulting in longer than expected drug exposures in certain target tissues. In a previous Phase I study of lometrexol (7), unpublished data indicated that RBC folate levels appeared to increase over time and remain elevated for months. Because the RBC folates in that study were measured using a commercially available assay that cross-reacts with lometrexol, it was hypothesized that lometrexol was accumulating in RBCs. Tissue levels of other antifolates, such as methotrexate in erythrocytes, have been used as a measure of long-term drug exposure (8).

Dietary folic acid supplementation has been shown to modulate lometrexol toxicity without diminishing antitumor activity in preclinical models (9). As a result, recent clinical trials of lometrexol have all included the use of either prior or concurrent folic acid treatment. One such trial using folic acid supplementation beginning 1 week prior and continuing for 1 week following lometrexol demonstrated that much higher lometrexol doses could be given without cumulative toxicity (10). However, due to concerns that overly aggressive folic acid supplementation might rescue tumor as well as normal tissues, the current study evaluating the administration of lometrexol every 3 weeks preceded by a single i.v. dose of folic acid was undertaken. Because of its delayed toxicity profile, prolonged

Received 4/14/98; revised 7/9/98; accepted 7/10/98.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by NIH Grants U01 CA 62505 and 5P30 CA 33572.
[2] To whom requests for reprints should be addressed, at City of Hope National Medical Center, 1500 East Duarte Road, Duarte, CA 91010. Phone: (626) 359-8111; Fax: (626) 301-8233.

[3] The abbreviations used are: FPGS, folylpolyglutamate synthetase; AUC, area under the concentration *versus* time curve.

pharmacokinetic and pharmacological monitoring of lometrexol were important correlates of this clinical trial.

MATERIALS AND METHODS

Patients. Patients with a histologically documented diagnosis of a solid tumor were eligible for this study if they met the following inclusion criteria: (*a*) age of ≥21 years, (*b*) life expectancy of at least 4 months and Karnofsky performance status of ≥60%, (*c*) no chemotherapy or radiation therapy for at least 3 weeks prior to study entry, (*d*) evidence of adequate renal and hepatic function, (*e*) evidence of adequate bone marrow function, and (*f*) ability to give voluntary informed consent. Patients were ineligible if they met any of the following exclusion criteria: (*a*) concurrent use of cytotoxic therapy or multivitamins containing folic acid, (*b*) presence of partial bowel obstruction or inflammatory bowel disease, (*c*) concurrent treatment with allopurinol, (*d*) presence of active infection, (*e*) presence of active angina pectoris or other uncontrolled heart disease, or (*f*) presence of other uncontrolled medical or psychiatric condition.

Treatment Protocol. The starting dose level of lometrexol was 30 mg/m^2 by rapid i.v. injection repeated every 3 weeks. Patients originally received 5 mg of folic acid by rapid i.v. injection 1 h before escalating doses of lometrexol. Due to the occurrence of cumulative toxicity at the first dose level, lometrexol dose levels were subsequently lowered to 20 and then 15 mg/m^2. With the continued incidence of cumulative toxicity, the study was amended to increase the folic acid pretreatment dose to 25 mg/m^2 given 3 h before lometrexol. The amended folic acid dose and schedule were chosen based on previously published experience with plasma 5-methyltetrahydrofolate levels (11). With the revised folic acid pretreatment, the starting lometrexol dose was 15 mg/m^2. The dose was subsequently escalated in cohorts of three patients to levels of 20, 25, and 30 mg/m^2.

Pharmacokinetic Sampling. Peripheral blood samples were obtained from all patients around the first course at the following times: immediately prior to treatment and 5 and 30 min and 1, 2, 4, 6, 12, 24, and 48 h after lometrexol. Blood was drawn weekly thereafter for determination of both plasma and RBC lometrexol and bioactive folate concentrations for as long as a patient remained on study. Samples for plasma analysis were centrifuged, and 1-ml aliquots were transferred to tubes containing 1 mg of ascorbic acid. For the weekly blood samples, 0.5 ml of whole blood was removed prior to separation of plasma, diluted 1:10 with 0.1% ascorbic acid, and incubated for 30 min at 37°C to lyse the RBCs and cleave both the lometrexol and endogenous folate polyglutamates to monoglutamates through the action of endogenous conjugase (12). All samples were stored frozen at −70°C or colder until analysis. Ascorbic acid was added to all whole blood and plasma samples to prevent *ex vivo* oxidation of endogenous folates.

Determination of Plasma and RBC Lometrexol. Lometrexol in plasma and whole blood was determined using a previously published high-performance liquid chromatography assay (13). Briefly, following a solid-phase extraction step using cation-exchange cartridges, chromatographic separation was achieved isocratically across a phenyl column. Using UV and electrochemical detectors in series for low and high sensitivity, respectively, lometrexol was determined over the entire range of clinically achievable concentrations with a single injection. This method has a percentage recovery of >85% and a limit of quantitation of 20 ng/ml and is accurate and precise to within 5.5%.

Determination of Plasma and RBC Bioactive Folates. Bioactive folates in plasma and whole blood were determined using a previously published microbiological assay (14). The assay is based on the growth of *Lactobacillus rhamnosus* (ATCC 7469, NCIB 10463), formerly *Lactobacillus casei*. Previous investigators have shown that longer chain folate polyglutamates are inactive in the *L. casei* microbiological assay (15). Therefore, whole blood samples were first incubated in the presence of endogenous conjugase to convert folate polyglutamates to monoglutamates (12). Moreover, because lometrexol appeared to inhibit the growth of *L. rhamnosus*, even when hypoxanthine or inosine was added to the assay medium, an extraction method was devised to separate bioactive folates from lometrexol. Blood was processed for both plasma and whole blood analysis, as described previously for the determination of lometrexol. For plasma, 0.3 ml of sample was diluted with 0.6 ml of 0.1% ascorbic acid and 0.3 ml of 0.5 M acetic acid was added. For whole blood lysate, 0.1 ml of 0.5 M acetic acid was added to 1.0 ml of the lysate. The resulting pH was in the range of 4–5 for both plasma and whole blood lysate. Bakerbond C_{18} columns (VWR Scientific, Cerritos, CA) were preconditioned with 3 ml of methanol, followed by 3 ml of distilled water and 3 ml of 0.1% ascorbic acid, without allowing the columns to dry. The acidified whole blood lysate and plasma samples were loaded onto preconditioned columns, and folates were eluted with 1.0 ml of 0.1% ascorbic acid and 10 ml of 7.5% acetonitrile in 0.1% ascorbic acid. The sample flow-through, ascorbate wash, and 10 ml of 7.5% acetonitrile in 0.1% ascorbic acid were pooled together. The pooled eluate for each sample was analyzed for total bioactive folates. The solid-phase extraction method developed for use in this study resulted in recovery of 81 ± 11% of 5-methyltetrahydrofolate from spiked samples.

Pharmacokinetic Modeling. Plasma lometrexol data were fit with either a two- or three-compartmental pharmacokinetic model using ADAPT II software (16). The compartmental model that best described lometrexol disposition was identified and used to determine the primary (*e.g.*, V_c, K_{10}, K_{12}, K_{21}, K_{13}, and K_{31}) pharmacokinetic parameters. Secondary pharmacokinetic parameters (Cl_{plasma}, V_d, half-lives, and AUC) were derived from the model-generated primary parameters.

Statistical Methods. The Akaike Information Criterion (17) was used to identify the compartmental pharmacokinetic model that best described the disposition of lometrexol. Distribution of the pharmacokinetic parameters were expressed as means ± SD. For analysis of the plasma pharmacokinetic data, the natural logarithm transformation was used to correct for heteroscedasticity. After transformation, a general linear regression model was used to evaluate the association between each pharmacokinetic parameter and either the lometrexol or folic acid dose. Significance testing was done using an F test based on the regression model, with $P < 0.05$ being considered indicative of a significant difference.

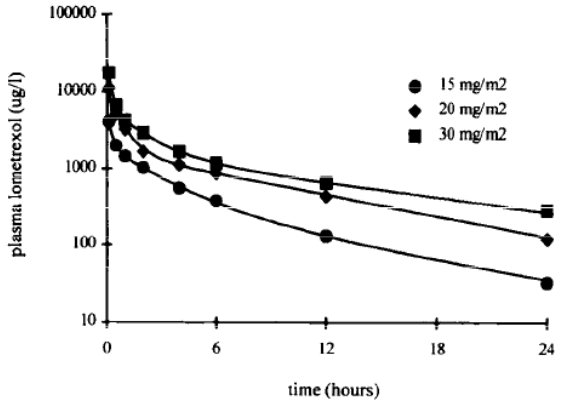

Fig. 1 Representative plasma *versus* time curves for lometrexol in patients receiving 15, 20, or 30 mg/m^2 lometrexol with i.v. folic acid supplementation. Curves were generated in ADAPT II using a three-compartment pharmacokinetic model.

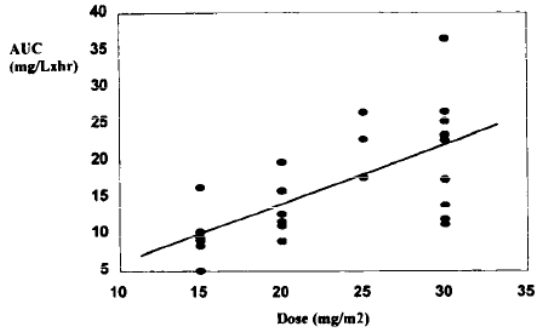

Fig. 2 Relationship between lometrexol dose and AUC calculated by dividing the dose by the model-derived systemic clearance. Line was generated by linear regression analysis.

Table 1 Plasma pharmacokinetics by lometrexol dose

Lometrexol dose (mg/m^2)	n^a	$Cl_{systemic}$ (liters/h/m^2)	V_d (liters/m^2)	$t_{1/2_\alpha}$ (h^{-1})	$t_{1/2_\beta}$ (h^{-1})	$t_{1/2_\gamma}$ (h^{-1})
15	6	1.7	11.0	0.19	2.9	30.7
20	6	1.4	6.8	0.25	2.9	7.9
25	3	1.4	10.2	0.22	2.9	6.5
30	9	1.7	8.5	0.24	2.8	38.7
	24^b	1.6±0.6^c	8.9±4.1^c	0.23±0.1^c	2.9±1.4^c	25.0±48.7^c

[a] Number of patients per dose level.
[b] Total number of patients.
[c] Mean ± SD.

Table 2 Effect of folic acid pretreatment on lometrexol plasma pharmacokinetics

Pharmacokinetic parameter	5 mg i.v. folic acid 1 h before lometrexol^a (n = 9)	25 mg/m^2 i.v. folic acid 3 h before lometrexol^a (n = 15)	P^b
$Cl_{systemic}$ (liters/h/m^2)	1.7 ± 0.6	1.5 ± 0.6	0.30
V_d (liters/m^2)	8.8 ± 1.6	9.0 ± 5.1	0.54
$t_{1/2_\alpha}$ (h^{-1})	0.2 ± 0.2	0.2 ± 0.4	0.86
$t_{1/2_\beta}$ (h^{-1})	2.1 ± 1.1	3.3 ± 1.4	0.05
$t_{1/2_\gamma}$ (h^{-1})	32.1 ± 73.7	20.7 ± 27.2	0.43

[a] Mean ± SD.
[b] F test based on regression model.

RESULTS

Patient Characteristics. Between March and December of 1995, 24 patients were entered into this study. There were 12 men and 12 women, whose tumor types included colorectal (n = 11), lung (n = 6), breast (n = 4), and miscellaneous (n = 3) cancers. Six patients each were treated with 15 and 20 mg/m^2 lometrexol, whereas three patients received 25 mg/m^2, and nine patients were treated with 30 mg/m^2. Of the patients treated with 15 and 20 mg/m^2 lometrexol, three received 5 mg of i.v. folic acid 1 h before whereas the other three received 25 mg/m^2 3 h before the lometrexol. All of the patients treated at the 25 mg/m^2 dose level received 25 mg/m^2 of folic acid 3 h before the lometrexol. At the 30 mg/m^2 lometrexol dose level, three patients received 5 mg of i.v. folic acid 1 h before whereas the other six received 25 mg/m^2 3 h before the lometrexol. As reported previously (18), dose-limiting toxicities were encountered on cycles 2 and 3 in most patients, regardless of the lometrexol dose level. Grade 3 and 4 toxicities included neutropenia, thrombocytopenia, anemia, mucositis, and fatigue. Anemia was the most common toxicity encountered, with 5 of 24 patients requiring transfusions.

Plasma Pharmacokinetics. First-dose lometrexol plasma pharmacokinetics were determined in all 24 patients entered on study. Fig. 1 shows representative plasma concentration *versus* time curves for each of three lometrexol doses. Lometrexol elimination showed a triexponential pattern of decay, and the pharmacokinetics were best described by a three-compartment model in 16 of 24 patients, according to the Akaike Information Criterion. As shown in Table 1, the mean lometrexol Cl_{plasma} and V_d were 1.6 ± 0.6 liters/h/m^2 and 8.9 ± 4.1 liters/m^2, respectively. Mean α, β, and γ half-lives were 0.23 ± 0.1, 2.9 ± 1.4, and 25.0 ± 48.7 h, respectively. Cl_{plasma} was independent of dose, as evidenced by a linear increase in AUC with increasing dose (Fig. 2).

The effect of folic acid pretreatment on lometrexol plasma pharmacokinetics was explored, and the results are shown in Table 2. A total of 9 patients received 5 mg of i.v. folic acid 1 h before lometrexol, and 15 patients received 25 mg/m^2 3 h before lometrexol. As shown in Table 2, with the exception of $t_{1/2\beta}$, there were no significant differences in any of the lometrexol pharmacokinetic parameters in patients receiving either of the pretreatment regimens. Fig. 2 illustrates the significant interpatient variability observed in plasma AUC, with a 4-fold range in patients treated at the 30 mg/m^2 dose level. Despite this wide variation in plasma lometrexol exposure, pharmacodynamic analyses were unable to identify any relationships between plasma pharmacokinetics on the first course and the occurrence of dose-limiting toxicity.

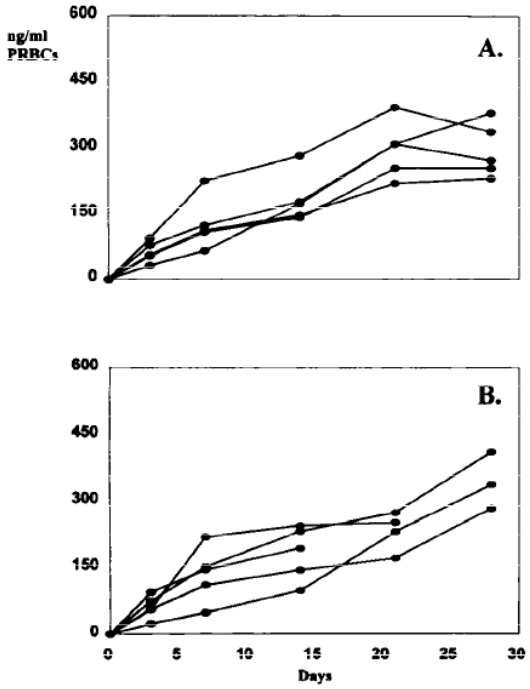

Fig. 3 Representative RBC lometrexol *versus* time plots for patients receiving either 5 mg i.v. folic acid 1 h before (*A*) or 25 mg/m^2 3 h before (*B*) lometrexol. RBC lometrexol was expressed as ng/ml of packed RBCs.

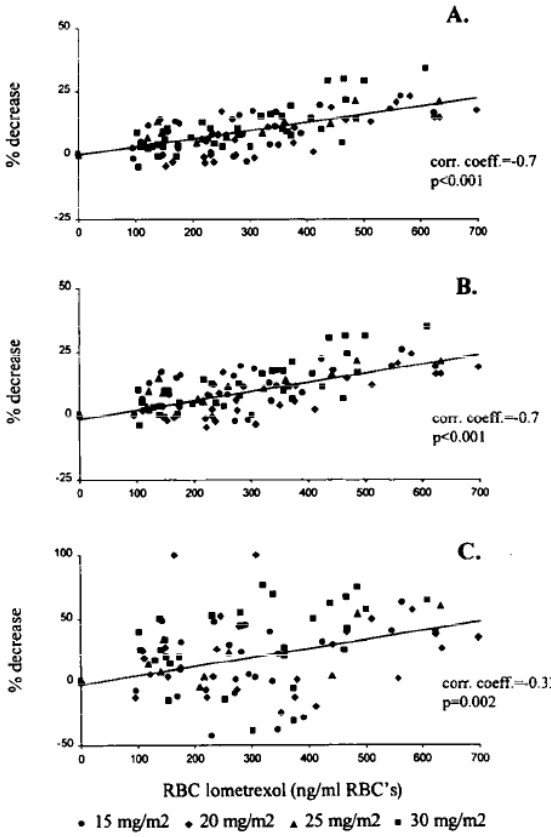

Fig. 4 Relationship between RBC lometrexol and percentage decrease in hemoglobin (*A*), hematocrit (*B*), and platelets (*C*). Different symbols represent different lometrexol dosages. Percentage decrease was calculated by subtracting the measured value on the given day from the pretreatment baseline measurement, then dividing by the baseline value and multiplying by 100.

RBC Pharmacokinetics. Weekly RBC lometrexol data were available in all 24 patients enrolled on study, with a median follow-up of 39 days (range, 14–111). Fig. 3 shows the RBC lometrexol levels *versus* time for each patient receiving either 5 mg of i.v. folic acid 1 h before (Fig. 3*A*) or 25 mg/m^2 3 h before (Fig. 3*B*) lometrexol. Although plasma lometrexol was undetectable in all patients within 72 h after a dose and remained undetectable throughout the follow-up period, RBC lometrexol levels measured in weekly blood samples rose between courses. RBC lometrexol levels were detectable starting at around 4 days following the first dose and continued to rise weekly in every patient. Furthermore, the rate and extent of apparent lometrexol accumulation in RBCs are independent of plasma pharmacokinetics and either the lometrexol or folic acid dose.

Although no relationship between plasma pharmacokinetics and toxicity could be identified, a significant linear relationship existed between the accumulation of lometrexol in RBCs and the percentage fall in hemoglobin, hematocrit, and platelet count. Fig. 4 shows the relationship between the measured RBC lometrexol level and the percentage fall in hemoglobin (Fig. 4*A*), hematocrit (Fig. 4*B*), and platelet count (Fig. 4*C*). The correlation coefficients between the change in RBC lometrexol and the fall in these hematological parameters were -0.68 ($P < 0.001$), -0.66 ($P < 0.001$), and -0.33 ($P = 0.002$), respectively. No clear trends or differences in these relationships could be identified when the different lometrexol dosage levels were compared.

Plasma and RBC Bioactive Folates. Bioactive folates in plasma and RBCs were determined in a subset of six patients over the same time period as that for lometrexol. Although the data confirm that significantly higher plasma folate levels were achieved with the higher folic acid pretreatment dose (data not shown), analysis of weekly blood samples failed to show accumulation of folates in the RBCs. Fig. 5 demonstrates the failure of RBCs to accumulate bioactive folates in patients receiving either 5 mg or 25 mg/m^2 i.v. folic acid prior to lometrexol.

DISCUSSION

Initial Phase I studies of lometrexol demonstrated that drug-induced toxicity was related to the cumulative dose rather than the result of a single dose. These toxicities were severe and long-lasting, usually occurring following the second or third course of therapy (6, 7). Laboratory investigations performed after the initial Phase I studies found that lometrexol-induced

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.