Clinical pharmacokinetics of the antipurine antifolate (6R)-5,10-dideaza-5,6,7,8-tetrahydrofolic acid (Lometrexol) administered with an oral folic acid supplement.

S R Wedge, S Laohavinij, G A Taylor, et al.

Updated Version	Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/1/12/1479

Citing Articles	This article has been cited by 3 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/1/12/1479#related-urls

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, contact the AACR Publications Department at permissions@aacr.org.

Clinical Pharmacokinetics of the Antipurine Antifolate (6R)-5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid (Lometrexol) Administered with an Oral Folic Acid Supplement[1]

**Stephen R. Wedge, Sudsawat Laohavinij,
Gordon A. Taylor, Alan Boddy,
A. Hilary Calvert, and David R. Newell[2]**

Cancer Research Unit, The Medical School, University of Newcastle-upon-Tyne, Framlington Place, Newcastle-upon-Tyne, NE2 4HH, United Kingdom

ABSTRACT

(6R)-5,10-Dideaza-5,6,7,8-tetrahydrofolic acid (lometrexol) is an antipurine antifolate which selectively inhibits glycinamide ribonucleotide formyltransferase. Lometrexol pharmacokinetics were evaluated in 17 patients (32 courses) as part of a Phase I study in which folic acid supplementation was used to improve tolerance to the drug, its clinical utility being previously limited by severe cumulative toxicity. Lometrexol was administered as an i.v. bolus every 4 weeks at a starting dose of 12 mg/m^2, with subsequent interpatient dose escalation to 16, 30, and 45 mg/m^2. p.o. folic acid (5 mg/day) was given for 7 days before and 7 days after lometrexol administration. The disposition of total lometrexol in plasma was best described by a biexponential model for data acquired up to 12 h after drug administration, although triexponential plasma pharmacokinetics were often found to give a more adequate description when data were available at later time intervals (24 h and greater). Mean plasma half-lives (± SD) for model-dependent analysis were $t_{1/2}\alpha$ 19 ± 7 min, $t_{1/2}\beta$ 256 ± 96 min, and $t_{1/2}\gamma$ (where measurable) 1170 ± 435 min. Lometrexol area under plasma concentration *versus* time curve was proportional to the dose administered. Moderate plasma protein binding of lometrexol was evident (78 ± 3%) with an inverse linear relationship between fraction of unbound lometrexol and the concentration of serum albumin. The volume of distribution of lometrexol at steady state was between 4.7 and 15.8 l/m^2. Renal elimination of lometrexol, studied in 19 patients (21 courses), was considerable, accounting for 56 ± 17% of the total dose administered within 6 h of treatment, and 85 ± 16% within 24 h of treatment. These recoveries of unchanged lometrexol indicate that the drug does not appear to undergo appreciable systemic metabolism at the range of concentrations studied.

Lometrexol pharmacokinetics were also examined in seven patients who received 45 or 60 mg/m^2 lometrexol as part of a separate study of the drug given with folinic acid rescue 5–7 days after treatment. No marked differences were evident in lometrexol plasma half-lives, plasma clearance, or the extent of plasma protein binding, indicating that there is not a pronounced pharmacokinetic interaction between lometrexol and folic acid.

INTRODUCTION

Lometrexol is a folate analogue which selectively inhibits GAR[3] formyltransferase, an enzyme essential for *de novo* purine biosynthesis (1, 2). This antipurine antifolate exhibits a broad spectrum of antitumor activity in murine and human xenograft tumor models, in which the established antifolate methotrexate demonstrates little or no effect (3).

In early Phase I clinical studies with lometrexol, significant clinical toxicity was evident, characterized by severe mucositis and myelotoxicity (thrombocytopenia and leucopenia), which limited drug administration to only one or two courses (4–7). These toxicities were unexpected, occurring at drug concentrations which were approximately one hundredth of the 10% lethal dose in mice (8). A number of clinical responses were documented, including activity against malignant fibrous histiocytoma (5), non-small cell lung cancer, breast cancer, and colonic adenocarcinoma (7), which stimulated studies aimed at the pharmacological amelioration of lometrexol toxicity. Experimentation in mice revealed that the therapeutic index of lometrexol was highly dependent on dietary folic acid intake (9, 10), and suggested that folic acid administration could reduce toxicity, without ablating antitumor activity. To enable the development of a tolerable and effective schedule for the routine clinical use of lometrexol, a Phase I study was initiated in which 5 mg folic acid/day were given for 7 days before and after lometrexol administration. After 7 days, this dose of folic acid resulted in an increase in plasma folate levels from 3 to 64 ng/ml to 6 to 180 ng/ml in the patients studied.

The Phase I study of lometrexol with folate supplementation provided an opportunity, for the first time, to conduct detailed clinical pharmacokinetic studies with lometrexol: a comprehensive pharmacological examination in humans being previously prohibited by the lack of clinical utility and availability of a suitable assay. The principal objectives of this

[3] The abbreviations used are: GAR, glycinamide ribonucleotide; CV, coefficient of variation; AUC, area under the lometrexol plasma concentration *versus* time curve; Cl$_{TOT}$, total plasma clearance; GFR, glomerular filtration rate; Vd$_{ss}$, volume of distribution at steady state; FBP, folate-binding protein.

clinical pharmacokinetic study were: (*a*) to determine the plasma pharmacokinetics in patients receiving multiple courses of lometrexol, and thereby define the relationship between lometrexol AUC and dose, and intra- and interpatient variability in AUC; (*b*) to investigate the extent of lometrexol plasma protein binding; (*c*) to measure urinary excretion of lometrexol; and (*d*) to evaluate the effect of folic acid supplementation on lometrexol pharmacokinetics to determine whether the improved tolerance of lometrexol produced by folic acid administration is a consequence of a pharmacokinetic interaction.

MATERIALS AND METHODS

Patient Eligibility. Patients eligible for this study had a histologically documented malignant solid tumor, which was either refractory to established therapies, or for which no standard therapy existed. All patients had a predicted life expectancy of at least 12 weeks, and had recovered from the toxic effects of previous treatment before entering into the study, *i.e.*, they had not received any major therapy or investigational drug for at least 4 weeks (6 weeks if prior therapy included chemotherapy with a compound known to have delayed toxicity, *e.g.*, a nitrosourea). Exclusion criteria included factors which could have interfered with lometrexol disposition/toxicity or folic acid absorption and comprised: (*a*) concomitant medication with probenecid, trimethoprim, co-trimoxazole, pyrimethamine, prednisolone, antiepileptics, or allopurinol; (*b*) extensive radiotherapy; and (*c*) inflammatory, ulcerative bowel disease or malabsorption syndrome.

All patients were required to have adequate organ function prior to treatment, with hepatic function characterized by bilirubin levels of <25 μmol/liter, and renal function by a creatinine measurement of <120 μmol/liter and a ^{51}Cr-EDTA clearance of >50 ml/min. Informed written consent was given according to local regulatory requirements.

Study Design. Folic acid (Approved Prescription Services Ltd., Leeds, United Kingdom) was given daily as a single 5-mg tablet for 7 days before and 7 days after lometrexol administration at 4-week intervals. Lometrexol (Lilly Research Centre, Surrey, United Kingdom) was reconstituted in 0.9% (v/v) saline and administered as a rapid i.v. bolus over 0.5–1.0 min at a concentration of 1–10 mg/ml. Patients were admitted to the Department of Medical Oncology, Newcastle General Hospital, to receive lometrexol and were observed for an additional 24 h after drug administration to ensure that acute toxicity was not apparent. The performance status of patients was assessed at least once a week, for a period of 4 weeks, following lometrexol therapy.

The trial design required three patients, previously untreated with lometrexol, to be treated at each dose level. The first patient entered at each dose level was followed up for 3 weeks before the next patient was entered. At least two patients per dose level received two courses before dose escalation. Toxicities were evaluated according to the WHO criteria. If repeated courses at a given dose level were tolerated without toxicity greater than WHO grade II, doses were escalated according to the clinical judgment of the investigator with approval of the Medicines Control Agency (London, United Kingdom) and the Local Ethics Committee. The starting dose of lometrexol was 12 mg/m², with subsequent escalation to 16, 30,

and 45 mg/m². Dose escalation increments were determined by clinical experience at the previous dose level and by data from a parallel study of lometrexol given with folinic acid (11). No intrapatient dose escalation occurred.

Pharmacokinetic Studies. Lometrexol pharmacokinetics was determined in 17 patients (32 courses) receiving folic acid supplementation and in an additional 7 patients (7 courses) who did not receive folic acid. Plasma samples from patients receiving lometrexol without folate supplementation were kindly provided by Drs. C. Sessa and F. Cavalli (Ospedale San Giovanni, Bellinzona, Switzerland), who were responsible for an alternative Phase I study that involved folinic acid administration (15 mg every 6 h for 12 doses), starting 5–7 days after treatment with lometrexol (11).

Blood samples were collected by venipuncture into vacutainer tubes placed on ice and containing the sodium salt of EDTA as an anticoagulant, and were taken before treatment and at 5, 15, 30, and 45 min and at 1, 1.5, 2, 4, 6, 8, 12, and 24 h, and in some patients at 48, 72, and 96 h, after lometrexol administration. Samples were immediately centrifuged (1000 × *g*, 8 min, 4°C), and plasma was removed by aspiration with a Pasteur pipette. Plasma was stored at -20°C prior to analysis.

The plasma lometrexol concentration was measured by the HPLC method of Wedge *et al.* (12), which uses derivitization and fluorescence detection. Briefly, patient samples were thawed at room temperature and diluted to 1 ml with control human plasma (Red Cross Transfusion Service, Newcastle-upon-Tyne, United Kingdom) to contain 10–250 ng/ml lometrexol. Samples were further diluted (1:1) with aqueous formic acid [1% (v/v); pH 3.7] containing 100 ng C^{10}-desmethylene lometrexol (Lilly Research Centre) as an internal standard. Following rotary mixing and centrifugation, each sample was subjected to solid-phase extraction using a C8 (1 cm) Bondelut cartridge (Analytichem International, Harbour City, CA). Eluted samples were evaporated to dryness using a Speedvac concentrator (Savant Ltd., Farmingdale, New York) and reconstituted in 13% (v/v) aqueous formic acid. Oxidation of lometrexol and the internal standard was achieved by incubation (37°C, 90 min) with a suspension of manganese dioxide (0.2 mg/ml) in water and terminated by the addition of a 1:1 mixture of 5 M NaOH and 1% (w/v) ammonium carbonate (pH 5) to samples on ice. Samples were centrifuged (13,000 × *g*, 10 min), and 100 μl of the supernatant were analyzed chromatographically. Chromatographic analysis was achieved using an Apex II (C18, 3 μm; 150 × 4.6 mm) analytical column (Jones Chromatography, Hengoed, Glamorgan, South Wales, United Kingdom) and a mobile phase of 12% (w/v) acetonitrile in 1% (v/v) aqueous acetic acid (pH 5) containing tetramethylammonium hydrogen sulfate (0.171 g/liter) as an ion pair reagent. Elution was isocratic, at a flow rate of 1 ml/min, and analyte measurement was by fluorescence detection (E_x, 325 nm; E_M, 450 nm).

To assess intraassay variation, each assay was calibrated using a five-point standard curve of duplicate lometrexol standards in the range 10–250 ng/ml, prepared in control human plasma, and extracted/analyzed at the same time as patient samples. Quantitation was achieved using internal standardization by a comparison of peak height ratios, with peak heights being quantified using Minichrom Software (VG Data Systems Ltd., Altrincham, Cheshire, United Kingdom). All calibration

curves were linear ($r^2 > 0.997$), the lower limit of determination of the assay was 10 ng/ml, and the intraassay CV at nominal concentrations of 10, 50, and 250 ng/ml was always <8.5%. In addition, three lometrexol quality assurance samples (10, 50, and 250 ng/ml), prepared every 2–3 months in bulk, were assayed in triplicate to assess the interassay CV, which was always <6%.

Pharmacokinetic Parameters. Plasma lometrexol data were analyzed using both model-independent and model-dependent analyses. In model-independent analyses, the AUC was calculated using the log trapezoidal rule (13) with extrapolation to infinity, using the terminal phase rate constant calculated by the compartmental analysis. For model-dependent analyses, a biexponential or triexponential equation was fitted to the concentration *versus* time data using a nonlinear-weighted least-squares parameter estimation program (ADAPT II, kindly provided by Dr. S. D. Z. D'Argenio and A. Schumitzky, Biomedical Simulations Resource, Los Angeles, CA). Data were weighted as the reciprocal of the estimated variance, where the SD of the output was assumed to be proportional to the estimated concentration (constant CV). The model providing the best fit to each data set was determined using the precision of the parameter estimates and consideration of the Akaike information criterion (14). The parameters derived were used to calculate model-independent (AUC, Cl_{TOT}, and Vd_{ss}) or model-dependent (AUC, Cl_{TOT}, $t_{1/2}\alpha$, $t_{1/2}\beta$, and $t_{1/2}\gamma$) pharmacokinetic parameters (13, 15).

Plasma Protein Binding. Lometrexol plasma protein binding was determined using [^{14}C]lometrexol (specific activity, 13 μCi/mg), which was kindly provided by Dr. M. D'Incalci (Istituto Mario Negri, Milano, Italy). This compound was radiolabeled at the carbonyl group of the benzyl moiety, and had a radiochemical purity of >88%, as determined by HPLC. Each patient plasma sample (1 ml) was spiked with 3.5 μg [^{14}C]lometrexol, rotary mixed, and an aliquot (100 μl) was removed. The remainder was then subjected to ultrafiltration using an Amicon Centrifree Micropartition Unit (Amicon Corp., Upper Mill, Stonehouse, Gloucestershire, United Kingdom) and centrifugation (1000 × g, 10 min, 4°C), after which an aliquot (100 μl) of ultrafiltrate was removed. [^{14}C]Lometrexol in the ultrafiltrate and prefiltered plasma was determined by liquid scintillation counting, and the ratio was used to calculate the percentage of unbound lometrexol. Nonspecific binding of [^{14}C]lometrexol to the filter was determined to be <4% by ultrafiltering solutions of [^{14}C]lometrexol in PBS (0.1 M, pH 7.4; 1 ml) and was therefore ignored. Samples of plasma from each patient, taken at <1 h and at 12–120 h after lometrexol administration were examined to assess any potential concentration dependency of lometrexol plasma protein binding, but no significant differences in binding were found ($P = 0.11$, paired *t*-test). The mean of each pair of analyses was therefore used to: (*a*) relate the unbound fraction of lometrexol with serum albumin and plasma protein concentrations and (*b*) calculate the unbound Vd_{ss} (15).

Urinary Excretion. Urinary excretion was studied in 13 patients (14 courses) for which there was evaluable plasma pharmacokinetics available, and in an additional 6 patients (7 courses) for which plasma pharmacokinetics was not measured. Urine samples for lometrexol analysis were collected for 24 h after drug administration at 6-hour intervals, and 20 ml aliquots

Table 1 Patient characteristics

Evaluable patients (courses)	17 (32)
Sex	
Male	6
Female	11
Median age, yr (range)	49 (30–67)
WHO performance status	
0	4
1	5
2	8
Primary tumor	
Breast	4
Ovarian	3
Malignant melanoma	3
Others	7
Prior treatment	
Chemotherapy	14
Radiotherapy	9

were stored at −20°C prior to analysis. Samples were thawed at room temperature and diluted in control urine to ensure that the lometrexol concentration would be between 2 and 25 μg/ml (*i.e.*, the dilution factor used was estimated according to the dose of lometrexol administered and the volume of urine produced in a 6-h period). The method for sample preparation was as described for plasma samples, except C^{10}-desmethylene lometrexol was added at a concentration of 15 μg/ml, and evaporated samples were not oxidized, but resuspended in 150 μl of the mobile phase and 50 μl were analyzed chromatographically. Chromatographic analysis (12) involved a Spherisorb C6 (5 μm; 150 × 4.6 mm) analytical column (Jones Chromatography) and isocratic elution with a mobile phase of 13% (w/v) acetonitrile in aqueous phosphoric acid [1% (v/v), pH 3.2] at a flow rate of 1.5 ml/min. Each assay included a five-point standard curve (duplicate samples within the range 0.2–10 μg/ml), and quality assurance standards at 0.2, 2, and 10 μg/ml were assayed in triplicate. The intraassay and interassay CVs for these assays were found to be <2% and <3%, respectively. Lometrexol excretion in urine was expressed as a percentage of the administered dose, and renal clearance was calculated as the ratio of the total amount excreted and the AUC.

Statistical Methods. All values expressed with a margin of error represent the mean ± SD. Levels of significance were calculated using Student's *t* test, where $P < 0.05$ was considered indicative of a significant difference between groups. The relationship between lometrexol dose and pharmacokinetic parameters was assessed by linear regression analysis and/or a Spearman rank correlation.

RESULTS

Pharmacokinetics of Lometrexol Administered with a Folic Acid Supplement. The characteristics of the patients studied are shown in Table 1, and plasma lometrexol concentration-time profiles from representative patients receiving 12, 16, 30, or 45 mg/m^2 lometrexol are shown in Fig. 1. Data collected within the first 12 h of lometrexol administration were found to be best described by a biexponential equation when evaluated by compartmental analysis. However, at doses of 30 and 45 mg/m^2 lometrexol and where data were available at later
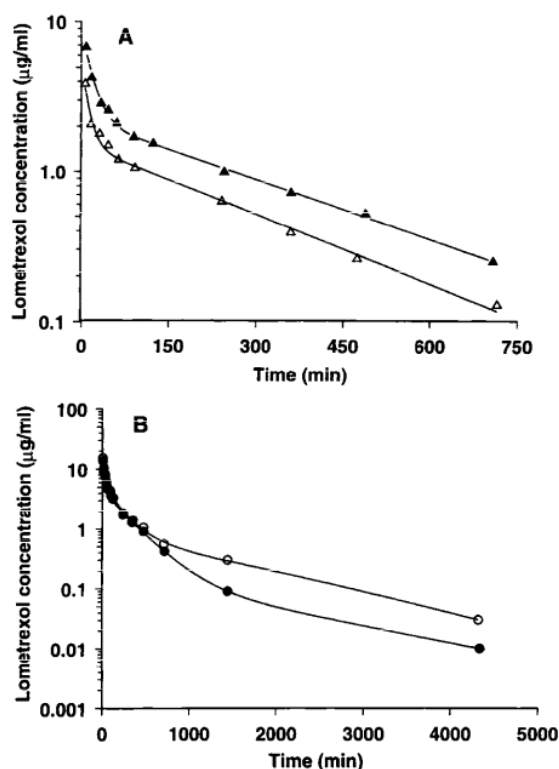
Fig. 1 Representative plasma disposition curves for total lometrexol in patients receiving folic acid supplementation: A, 12 mg/m² (△), 16 mg/m² (▲); B, 30 mg/m² (○), 45 mg/m² (●) lometrexol. The *lines* are those generated by compartmental analysis.

time points (≥24 h), plasma elimination could be described more accurately by a triexponential equation. The most appropriate equations (biexponential or triexponential) fitted to each data set all had coefficients of determination (r^2) of >0.92, *i.e.*, 0.99 median (range, 0.92–0.99).

Pharmacokinetic parameters are shown in Table 2 with parameters for patients receiving more than two courses of lometrexol being represented as the mean and SD of all courses studied in that patient. Model-dependent analysis of lometrexol pharmacokinetics resulted in a $t_{1/2}\alpha$ and $t_{1/2}\beta$ of 19 ± 7 min and 256 ± 94 min, respectively, and a $t_{1/2}\gamma$ (where measurable) of 1170 ± 435 min.

Calculation of both lometrexol AUC and Cl_{TOT} by either a model-independent or model-dependent analysis was not found to result in any significant difference ($P = 0.14$ and $P = 0.33$, respectively, paired t test), and there were strong linear relationships for both parameters for both analyses ($r > 0.98$).

Model-independent analysis indicated that lometrexol AUC was linearly related to dose ($r = 0.88$, $P < 0.001$; Fig. 2), *i.e.*, plasma clearance of lometrexol was not dose dependent. However, two patients had a lometrexol AUC and Cl_{TOT} which differed markedly from that observed in other patients treated at the same dose level. One patient (patient 12) receiving 30

mg/m² lometrexol had a consistently greater AUC than for others receiving the same dose, *i.e.*, 2.08–2.28 mg/ml · min compared with 1.38 and 1.50 mg/ml · min (Table 2 and Fig. 2). Reduced lometrexol clearance in this patient may have been due to an underlying early left ventricular failure, which could have decreased cardiac output and thereby reduced tissue perfusion, combined with a relatively low pretreatment GFR of 75 ml/min, which may have influenced the renal excretion of lometrexol. In contrast, at a dose of 45 mg/m² lometrexol, one patient (patient 19) had a much lower AUC than was measured in others (1.27 mg/ml · min compared with values of 1.60–2.20 mg/ml · min), with a correspondingly higher Cl_{TOT} (35.8 ml/min/m² compared to values of 23.1–27.7 ml/min/m²). This may be attributable to an unusually large lometrexol Vd_{ss} in this patient of 15.8 liters/m², which in turn could have been caused by the presence of a bilateral pleural effusion, combined with a high pretreatment GFR of 165 ml/min which would have promoted extensive renal excretion of lometrexol.

No consistent change in the model-independent AUC was evident following more than one course of lometrexol, with the possible exception of three patients receiving 45 mg/m² lometrexol every 4 weeks (patients 13, 16, and 17), in whom small increases in AUC after the second and third courses of lometrexol were observed. In patients receiving more than three courses of lometrexol, no consistent change in clearance could be found, *i.e.*, the intrapatient CV of plasma clearance was 14% (median; range, 3–21%; $n = 5$).

The lometrexol Vd_{ss} varied between 4.7 and 15.8 l/m², and the unbound lometrexol Vd_{ss}, calculated from measurements of lometrexol plasma protein binding (see below), varied between 18.1 and 67.1 l/m². The larger Vd_{ss} of two patients receiving 45 mg/m² lometrexol may be attributed to the fact that both patients had pleural effusions. Even if data from these two patients are excluded, rank correlations are observed between dose level (mg/m²) and both Vd_{ss} ($r_s = 0.84$, $P < 0.001$) and unbound Vd_{ss} ($r_s = 0.66$, $P < 0.01$).

Plasma Protein Binding of Lometrexol. The percentage of unbound lometrexol in plasma was 22 ± 3%, indicating plasma protein binding of 78 ± 3%. Protein binding determined at two concentrations of lometrexol (3.5–4.7 and 7.7–21.1 µg/ml) revealed that binding was not concentration dependent ($P = 0.11$, paired t test). An inverse linear relationship was apparent between the lometrexol binding and serum albumin concentration ($r = 0.88$; $P < 0.001$; Fig. 3). The relationship between unbound lometrexol and total serum protein was not found to be significant ($r = 0.38$; $P > 0.05$), when one patient with particularly low total serum protein (55 g/liter) was removed from the analysis.

Urinary Excretion of Lometrexol. The 0–24-h cumulative urinary excretion data (Fig. 4) for 19 patients (21 courses) indicated that the major elimination route for lometrexol was renal excretion, with 85 ± 16% of the administered dose being excreted within 24 h of drug administration and 56 ± 17% within the first 6 h. Urinary excretion studied in two patients who received two consecutive courses of lometrexol did not reveal any consistent change in excretion following the second course of treatment. Two patients had 0–24-h lometrexol urinary recoveries of >100% of the administered dose, which was likely to be due to inaccuracies in the measurement of urine

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.