Reduction of Tissue Iron Stores and Normalization of Serum Ferritin During Treatment With the Oral Iron Chelator L1 in Thalassemia Intermedia

By Nancy F. Olivieri, Gideon Koren, Doreen Matsui, Peter P. Liu, Laurie Blendis, Ross Cameron, Robert A. McClelland, and Douglas M. Templeton

In patients with thalassemia intermedia in whom hyperabsorption of iron may result in serious organ dysfunction, an orally effective iron-chelating drug would have major therapeutic advantages, especially for the many patients with thalassemia intermedia in the Third World. We report reduction in tissue iron stores and normalization of serum ferritin concentration after 9-month therapy with the oral chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L1) in a 29-year-old man with thalassemia intermedia and clinically significant iron overload (SF 2,174 µg/L, transferrin saturation 100%; elevated AST and ALT, abnormal cardiac radionuclide angiogram) who was enrolled in the study with L1 75 mg/kg/day after he refused deferoxamine therapy. L1-induced 24-hour urinary iron excretion during the first 6 months of therapy was (mean ± SD, range) 53 ± 30 (11 to 109) mg (0.77 mg/kg), declining during the last 3 months of L1 to 24 ± 14 (13–40) mg (0.36 mg/kg), as serum ferritin decreased steadily to normal range (present value, 251 µg/L). Dramatic improvement in signal intensity of the liver and mild improvement in that of the heart was shown by comparison of T_1-weighted spin echo magnetic resonance imaging with images obtained immediately before L1 administration was observed after 9 months of L1 therapy. Hepatic iron concentration decreased from 14.6 mg/g dry weight of liver before L1 therapy to 1.9 mg/g liver after 9 months of therapy. This constitutes the first report of normalization of serum ferritin concentration in parallel with demonstrated reduction in tissue iron stores as a result of treatment with L1. Use of L1 as a therapeutic option in patients with thalassemia intermedia and iron overload appears warranted.
© 1992 by The American Society of Hematology.

PROGRESSIVE ORGAN dysfunction, often leading to mortality, is a consequence of transfusional or absorptional tissue iron loading in patients with homozygous β-thalassemia.[1] Regular chelation therapy with deferoxamine reduces hepatic iron and arrests hepatic fibrosis,[2] stabilizes iron-related cardiac disease,[3] and prevents gonadal failure when started before age 10 years.[4] Administration of standard therapy with deferoxamine is problematic, however; the drug requires prolonged nightly subcutaneous (SC) infusion, frequently associated with local irritation. Compliance with this regimen decreases dramatically during adolescence,[5] and many patients with cardiac iron overload still die.[5-8] Moreover, the cost of deferoxamine renders it unavailable to thousands of thalassemia patients in the Third World. It has long been acknowledged[9] that the added convenience of an orally available chelator would simplify treatment of patients with transfusion-dependent thalassemia, and should also simplify management of patients with sickle cell disease who are maintained on a transfusion program in an effort to prevent or arrest specific disease complications.

Although transfusion-dependent anemias have focused attention on the worldwide need for a safe, orally active iron-chelating agent, disorders of increased iron absorption should also benefit from their development. Individuals with nontransfusion-dependent thalassemia (thalassemia "intermedia") exhibit excessive dietary iron absorption that can lead to serious iron loading by the second or third decade of life.[10] The cost to these patients of neglecting iron loading is the development of life-threatening tissue damage, and infrequent infusions of deferoxamine may suffice to prevent iron accumulation and its complications.[11] An orally effective iron-chelating drug would have major therapeutic advantages for these patients, especially for Third World patients with thalassemia intermedia.[12]

Several orally available, iron-chelating compounds have been developed in recent years[9]; few have been evaluated in human trials. Among those that have, 1,2-dimethyl-3-hydroxypyrid-4-one (CP20 or L1) has been demonstrated to promote clinically significant urinary iron excretion in animal studies[13,14] and in human trials in the United Kingdom,[15,16] Europe,[17] India,[18] and Canada.[19] In a short-term randomized, cross-over study, L1's ability to induce urinary iron excretion was demonstrated to be comparable to that of SC deferoxamine and well tolerated[20]; recent studies have demonstrated its continued long-term efficacy.[16,17]

We report reduction in tissue iron in the liver and heart, as demonstrated by magnetic resonance imaging (MRI) of both organs, and percutaneous liver biopsy, after 9 months of therapy with L1 in a patient with thalassemia intermedia at high risk of progressive iron-induced morbidity. This constitutes the first report of normalization of serum ferritin concentration and demonstrated reduction of tissue iron stores as a result of treatment with L1.

MATERIALS AND METHODS

Case Report

In 1989, a 29-year-old Italian male was evaluated for liver disease, presumed to be secondary to iron overload. The son of

nonconsanguinous parents from Calabria, he had been diagnosed with homozygous β thalassemia and had undergone a splenectomy in 1964 at age 3 years. In 1980, he underwent cholecystectomy after repeated episodes of abdominal pain associated with radiologic demonstration of gallstones; RBC transfusions were administered before both operations. He had never received transfusions since then. In 1980, observation of abnormal levels of AST and ALT and an increased serum ferritin concentration prompted initiation of a program of nightly SC deferoxamine therapy; the patient complied with this erratically and discontinued it after 6 months, although the risk of potential iron-related morbidity had been explained to him. He administered no iron-chelating therapy in the next 10 years.

In 1990, he sought medical attention because of worsening fatigue. Physical examination showed him to be deeply pigmented and icteric, with mild muscle wasting. The liver was enlarged and firm. Height was at the fiftieth percentile for an adult male[21]; external genitalia were fully developed. Laboratory hematologic studies were consistent with previous assessments of steady-state hemoglobin concentrations of 75 to 85 g/L, increased total WBC and platelet counts, and a hemoglobin electrophoresis demonstrating 98% hemoglobin F and 2% hemoglobin A_2, consistent with β^0 thalassemia. Fasting glucose, testosterone, TSH, thyroxine, calcium, phosphate and PTH measurements were within normal limits. Serum AST and ALT levels were increased at 139 and 98 U/L, respectively (normal for both <40 U/L); prothrombin time, PTT, total protein, and albumin were within normal limits. Transferrin saturation was 95% to 100%; serum ferritin concentration was 2,000 µg/L (normal <300 µg/L). Analysis of DNA from peripheral blood lymphocytes (PBL) demonstrated homozygosity for the codon 39 C-T mutation responsible for β^0 thalassemia, a normal α-globin gene cluster, and homozygosity for the presence of the C to T substitution at position 158 5' to the Gγ gene.[22] Resting electrocardiogram demonstrated atrial bigeminy; radionuclide angiogram of the heart showed a normal resting ejection fraction (EF), but no increase in EF with exercise, and mild abnormalities of diastolic function.

The evidence for and risk of progression of iron-related organ damage was explained in full to the patient, who nevertheless declined to resume SC deferoxamine or to begin a program of continuous intravenous (IV) deferoxamine infusions; 9 months later, he was offered enrollment in the Canadian trial of the oral iron chelator L1. At this time, serum ferritin concentration was increased at 2,174 µg/L; transferrin saturation was increased at 100% (normal <30%); serum AST and ALT were increased at 126 and 94 U/L, respectively; and serum triglyceride level was increased at 3.74 mmol/L (normal 0.34 to 1.58 mmol/L). Antinuclear antibody and rheumatoid factor were negative. ACTH stimulation testing demonstrated normal baseline ACTH and cortisol, normal cortisol levels 30 and 60 minutes after ACTH stimulation, and two normal urinary free cortisol quantitations. Baseline 24-hour urinary iron excretion was 2.9 mg. After undergoing baseline MRI of the liver and heart and percutaneous liver biopsy, the patient began therapy with L1 at 75 mg/kg body weight per day, administered in three divided doses at 8-hour intervals in the fasting state.

Methods

This study was approved by The Hospital for Sick Children's Human Subject Review Committee and the Health Protection Branch, Health and Welfare Canada (File No. 9427-H1117-41C, HPB, Ottawa, Canada). Written informed consent was obtained from the patient. L1 was synthesized according to previously published methods[23] by the direct reaction in aqueous solution of methylamine and maltol, as described previously.[20,24]

Hepatic iron quantitation. Percutaneous liver biopsy specim were divided with a wooden splint and weighed immediatel determine fresh weight. One portion was then overdried (105° hours), reweighed and wet-ashed with hot concentrated HI (Suprapur; Merck, Darmstadt) in a screw-cap Teflon vessel, analysis of total iron by Zeeman-corrected electrothermal ato absorption (Varian SpectrAA-300; Techtron Ply, Ltd, Malgr Australia). The other portion was homogenized with a pla pestle in a microcentrifuge tube in 0.50 mL cold 0.25 m(sucrose/3 mmol/L imidazole HCl, pH 7.2. Total iron recovere the homogenate, as compared with that determined in the d sample, was 89% to 90%. After sonication (Branson Sor W-350; power setting 3, 15 pulses at 20% duty cycle), a 100 aliquot was applied to a carboxymethylcellulose column and el in stepwise fashion according to the method of Selden and Pete Fractions eluting in the order of transferrin, ferritin, hemoprote and hemosiderin were analyzed for iron by atomic absorption.

Histologic examination of the liver. Cores of liver tissue v fixed in 10% buffered formalin, embedded in paraffin, and stai with Prussian blue for iron. Tissue was fixed in universal fixa (2% glutaraldehyde plus 4% formaldehyde) and processed u standard methods for transmission electron microscopy usii Phillips 400 electron microscope.

Magnetic resonance imaging. The patient underwent magr resonance imaging (MRI) in a 1.5T superconducting magnet u a spin-echo technique.[26] The patient was positioned supine simultaneous cardiac and respiratory gating. Initial coronal transverse images were taken to determine the Euler angle image the heart, liver, and peripheral muscle simultaneously a the cardiac short axis. T_1-weighted spin echo was performed u cardiac gated sequence consisting of R-R interval/20/2 [TR (of pulse repetition)/TE (time to echo)/number of acquisiti spanning from the apex to the base of the heart at 1-cm center center separations and 7-mm slice thickness.

A midheart slice position was then selected as the referenc the T_2-weighted spin echo acquisition. This was a gated multi(sequence consisting of [x R-R/20-40-60-80/2 (TR/TE)/numb(acquisitions]. Finally, the slice was angled so that it also trave both left and right lobes of the liver and the shoulder muscles.

Image analysis. The signal intensities from the cardiac sep posterior wall, liver, and peripheral muscle were calculated f identically positioned slices of the T_1-weighted spin echo gradient echo images. A 0.25-cm^2 of the region of interest applied over these organs, and an average signal intensity obtained. The ratio of signal intensities between the heart peripheral muscle and the liver and peripheral muscle were subsequently for comparison. Image-derived T_2 values were tained by fitting the signal intensities from each echo of multiecho sequence to an exponential function: $SI = S0 \cdot e^{-T}$ where SI is signal intensity, S0 is nondecayed signal intensity, TE is time to echo. The signal intensities were first corrected variation in image field size and receiver attenuation when app ble.[27]

RESULTS

Efficacy of L1

Urinary iron excretion. Urinary iron excretion during first 6 months of L1 therapy varied between 11.3 and 10ʂ iron excreted in 24 hours, with a mean excretion of 53.3 equal to a mean urinary iron of 0.77 mg/kg body weight day. During the next 6 months of L1 administration, patient's urinary iron excretion varied between 4.6 and mg in a 24-hour period, with a mean excretion of

mg/day, or 0.27 mg/kg/day. The decrease in urinary iron excretion is shown in Fig 1A.

Serum ferritin concentration. Serum ferritin concentration decreased steadily during 6 months of L1 therapy, from a prechelation level of 2,174 to 251 µg/L after 6 months of L1 therapy. The decreases in urinary iron excretion and serum ferritin concentration are shown in Fig 1B.

MRI of Cardiac and Hepatic Iron

Image interpretation. On the MRI before the start of L1 therapy (Fig 2A), the initial T_1-weighted spin echo image in the coronal view identified an enlarged liver, with a vertical span of 18 cm in the right midclavicular line. The most notable feature was complete absence of signal from the liver, compatible with significant field inhomogeneity created by iron deposition. This corresponds to heavy iron deposition in the liver. Similarly, on the cardiac short axis view (Fig 3A) before initiation of chelation therapy, the liver signal was severely depressed. The myocardial signal also was depressed in both views, corresponding to iron deposition in the cardiac septum and posterior wall of the heart.

The MR image after 9 months of L1 therapy demonstrated a distinct improvement in signal intensity from the liver region by T_1-weighted spin echo imaging, observed in both the coronal (Fig 2B) and cardiac short axis (Fig 3B) views. Cardiac muscle signal intensity also improved. All these are indications that the iron content in the organs imaged was reduced as compared with that of the previous study.

Quantitative MR image-derived parameters. The signal intensity ratio between the liver and the peripheral muscle was less than 0.01 (normal > 1.0) before therapy with L1, owing to the very low-to-absent signal intensity in the liver. After L1 therapy, the same signal ratio improved to 0.72. Before therapy, image-derived T_2 relaxation time for the liver was also very short at 9.2 ms (normal 32 to 36 ms). After completion of 9 months of L1 therapy, the T_2 relaxation values increased to 17.4 ms. This near doubling of relaxation time can be attributed to a reduction of liver iron content, owing to a fixed relationship between iron concentration and 1/T2.

The myocardial/peripheral muscle ratio was 0.90 before L1 therapy (normal > 1.3). This improved to nearly normal values of 0.96 after 9 months of L1 therapy. Myocardial T_2 values before therapy was 33 ms, improving to 35 ms after 9 months of therapy. The less severe iron deposition in the myocardium before chelation therapy rendered the improvement in the signal from this organ less dramatic than that observed in the liver.

Hepatic Iron Stain and Histology

Histologic examination of the liver biopsy specimen taken before the start of treatment with L1 (Figs 4 and 5) showed more than 90% of hepatocytes strongly positive for cytoplasmic iron by Prussian blue staining, with iron also abundant in portal macrophages. The liver architecture was altered with moderate portal fibrosis. Light microscopic examination of the liver biopsy specimen taken after 9 months of L1 therapy showed approximately 30% of hepatocytes staining positively for iron, with a marked reduction in the amount of iron within each hepatocyte (Figs 4 and 5B).

Hepatic Iron Concentration of Liver Biopsy Specimens

Hepatic iron concentration after 9 months of L1 therapy was 1.9 mg/g dry weight of liver tissue, substantially reduced from the value of 14.6 mg/g dry weight of liver immediately before initiation of chelation therapy. The pattern of iron loading was unchanged, however. Before therapy, 83% of hepatic iron was associated with hemosiderin, 16% was ferritin, and a very small portion was observed in other fractions. After 9 months of chelation, 85% of hepatic iron was still associated with hemosiderin and most of the remainder was associated with ferritin.

Cardiac Function

Radionuclide angiography before L1 therapy showed an abnormal resting electrocardiogram with atrial bigeminy,
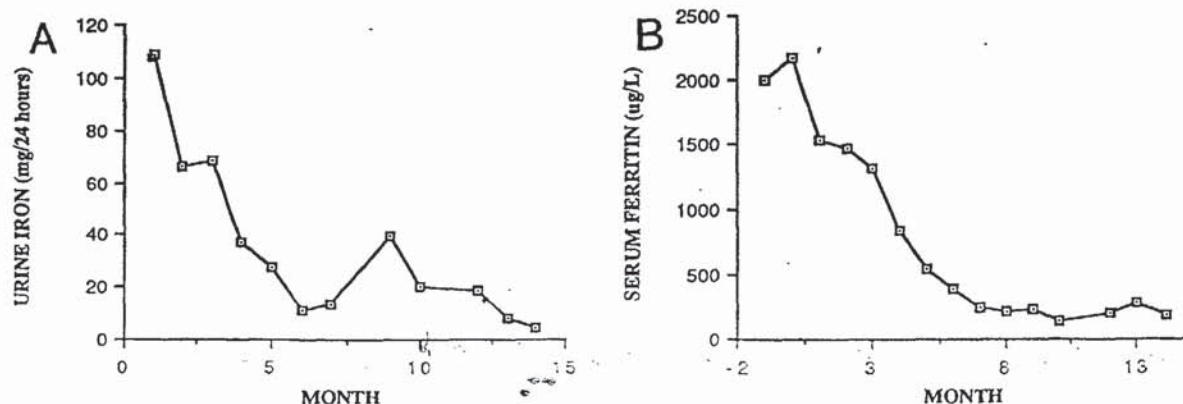

Fig 1. (A) Urinary iron excretion during 9 months of L1 therapy in a patient with thalassemia intermedia, approximately 0.77 mg/kg/day at the start of L1 therapy, decreased during the last months as serum ferritin normalized. (B) Decrease in serum ferritin concentration during 9 months of L1 therapy, from a prechelation level of 2,174 to 251 µg/L, is shown.
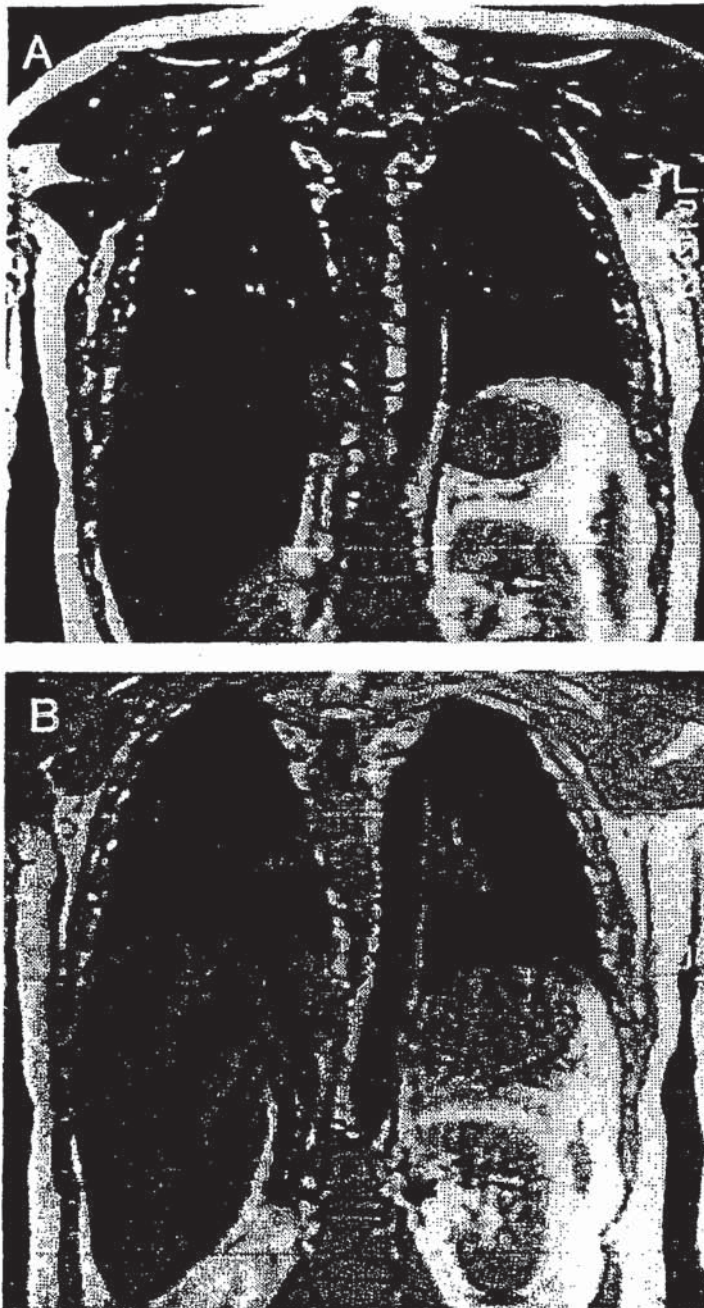
Fig 2. MRI of hepatic iron (A) before L1 therapy. Initial T_1-weighted spin echo MRI in the coronal view identified an enlarged liver with a vertical span of 18 cm in the right midclavicular line. Complete absence of signal from the liver is compatible with significant field inhomogeneity created by iron deposition. (B) After 9-month L1 therapy, T_1-weighted spin echo MRI in the coronal view demonstrated improvement in signal intensity from the liver, indicating that the iron content was reduced as compared with that in the previous study.

reverting to normal sinus rhythm during exercise. The left ventricular EF (LVEF) was 61% at rest, with no increase at peak exercise. Mild right ventricular dilatation, and mild abnormalities in diastolic function (peak filling rate, time to peak filling, and atrial contribution) were observed.

After 9 months of L1 therapy, the resting LVEF remained normal (57%), with a normal increase (to 63%)

now observed at peak exercise. The abnormalities of right ventricular dilatation and abnormalities in diastolic function did not change during L1 therapy, except for a slight improvement in atrial contribution (14% after L1 therapy, as compared with 17% before therapy).

Safety of L1. The patient was reviewed in the clinic weekly. He tolerated L1 well, and reported no joint pain or
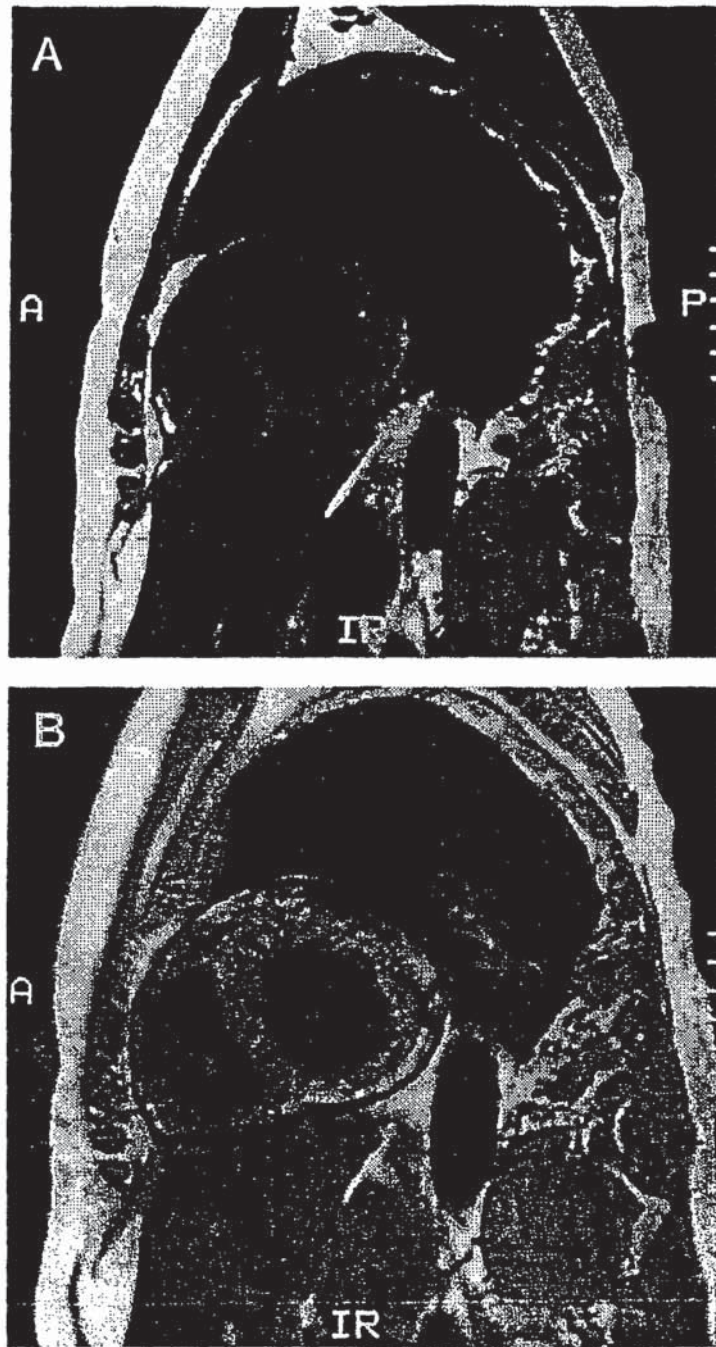
Fig 3. MRI of cardiac iron before L1 therapy (A). Initial T_1-weighted spin echo MRI in the cardiac short axis view demonstrated a depressed myocardial signal. (B) After 9-month L1 therapy, T_1-weighted spin echo MRI in the cardiac short axis view demonstrated improvement, indicating that the iron content was reduced as compared with that in the previous study.

stiffness.[28,30] No change in hematologic or biochemical parameters was observed during 9 months of therapy. ACTH testing repeated 4 months after baseline showed no change in the values observed before L1 administration. Antinuclear antibody and rheumatoid factor have remained negative.

DISCUSSION

Thousands of Third World patients with major thalassemia syndromes, including those who develop iron-related toxicity in association with gastrointestinal hyperabsorption of iron,[11] exhibit serious iron-related morbidity and mortality. For these patients, an orally active iron-

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.