These subtypes have distinctive hematologic and clinical features. Examples include AML-M2 associated with t(8;21), APL with t(15;17), AML with t(9;22) or with monosomy 7, and ANLL with one or several chromosomal abnormalities, often trisomy 8. A partial deletion of 5q has recently been observed in several patients with overt AML.[12] Our cytogenetic findings include examples of most of these subtypes. In addition, three patients, two with AMMoL and one with poorly differentiated AMoL, had either a translocation or a deletion involving chromosome No. 11, with a common breakpoint at band q23, suggesting that a new subtype of ANLL could be identified with high-resolution chromosome techniques. Interestingly, Berger et al. recently reported a chromosomal rearrangement, involving chromosome No. 11 in five of seven patients with poorly differentiated AMoL.[13] With standard techniques, however, the authors found that "the rearrangement of chromosome 11 involved the long arm but was variable from case to case." Four other cases of either AMoL or AMMoL with involvement of 11q in a balanced translocation have been cited in the literature, with no precise definition of breakpoints.[14-17]

Previous work has suggested that patients with ANLL who have a normal karyotype have a better prognosis than those who have an abnormal one, although survival has varied from patient to patient.[1,3] These studies have been based on techniques indicating that 50 per cent of untreated patients have normal chromosomes. Since the high-resolution methotrexate cell-synchronization technique[6] appears to identify abnormalities in all or most patients with ANLL, large-scale studies from various laboratories might reveal the true incidence of ANLL coexistent with a normal karyotype and whether such patients have a distinct clinical course. Our data suggest that the new technology may allow a more precise classification of patients with ANLL into distinct clinical and hematologic subgroups with differing prognoses and responses to treatment.

We are indebted to Dr. Richard Brunning for referring one of the patients to us, and to Dr. Om Prakash for assistance with the illustrations.

References

1. Rowley JD. Chromosome changes in acute leukaemia. Br J Haematol. 1980; 44:339-46.
2. Mitelman F, Levan G. Clustering of aberrations to specific chromosomes in human neoplasms. III. Incidence and geographic distribution of chromosome aberrations in 856 cases. Hereditas. 1978; 89:207-32.
3. Sandberg AA. The chromosomes in human cancer and leukemia. New York: Elsevier/North-Holland, 1980.
4. Yunis JJ. High resolution of human chromosomes. Science. 1976; 191:1268-70.
5. *Idem.* Nomenclature for high resolution human chromosomes. Cancer Genet Cytogenet. 1980; 2:221-9.
6. *Idem.* New chromosome techniques in the study of human neoplasia. J Hum Pathol. 1981; 12:540-9.
7. Bennett JM, Catovsky D, Daniel M-T, et al. Proposals for the classification of acute leukaemias. Br J Haematol. 1976; 33:451-8.
8. Paris Conference, 1971: standardization in human cytogenetics. Cytogenetics. 1972; 11:313-62.
9. Carbonell F, Fliedner TM, Kratt E, Sauerwein K. Crecimiento de las células leucémicas en cultivo: selección de clones citogenéticamente anormales. Sangre (Barc). 1979; 24:1057-60.
10. Knuutila S, Vuopio P, Borgström GH, de la Chapelle A. Higher frequency of 5q-clone in bone marrow mitoses after culture than by a direct method. Scand J Haematol. 1980; 25:358-62.
11. Berger R, Bernheim A, Flandrin G. Absence of chromosome abnormalities and acute leukemia: relationships with normal bone marrow cells. CR Acad Sci [D] (Paris). 1980; 290:1557-9.
12. Van Den Berghe H, David G, Michaux J-L, Sokal G, Verwilghen R. 5q-acute myelogenous leukemia. Blood. 1976; 48:624-6.
13. Berger R, Bernheim A, Weh HJ, Daniel MT, Flandrin G. Cytogenetic studies on acute monocytic leukemia. Leukemia Res. 1980; 4:119-27.
14. Rowley JD, Potter D. Chromosomal banding patterns in acute nonlymphocytic leukemia. Blood. 1976; 47:705-21.
15. Najfeld V, Singer JV, James MC, Fialkow PJ. Trisomy of 1q in preleukaemia with progression to acute leukaemia. Scand J Haematol. 1978; 21:24-8.
16. Van Den Berghe H, Louwagie A, Broeckaert-Van Orshoven A, et al. Chromosome abnormalities in acute promyelocytic leukemia (APL). Cancer. 1979; 43:558-62.
17. Weber TH, Wegelius R, Borgström GH, Gahmberg CG, Anderson LC. A case of pure monocytic leukaemia in a child — characterization of cellular morphology, membrane markers, surface glycoproteins and karyotype. Scand J Haematol. 1979; 22:47-52.

MEDICAL PROGRESS

THE ANTHRACYCLINE ANTINEOPLASTIC DRUGS

Robert C. Young, M.D., Robert F. Ozols, M.D., Ph.D., and Charles E. Myers, M.D.

PERHAPS no family of chemotherapeutic agents has been so rapidly accepted as a major therapeutic tool in treating patients with cancer as have the anthracyclines. Since the first clinical trials of doxorubicin and daunorubicin little more than a decade ago, these drugs have been extensively studied alone and in combination, and they now have a major role in the effective treatment of acute leukemia, non-Hodgkin lymphomas, breast cancer, Hodgkin's disease, and sarcomas.

The successful use of these drugs has been hampered by conventional toxicities (hematopoietic suppression, nausea and vomiting, and alopecia) as well as unique toxicities (cardiomyopathy); these problems have stimulated an exhaustive analysis of crucial relations of structure and activity in an effort to develop drugs with reduced toxicity. Although hundreds of anthracycline antibiotics have now been isolated, modified, or synthesized, the bulk of the clin-

From the Medicine and Clinical Pharmacology branches, National Cancer Institute. Address reprint requests to Dr. Young at the National Institutes of Health, National Cancer Institute, Bldg. 10, Rm. 12N226, 9000 Rockville Pike, Bethesda, MD 20205.

ical information is confined to doxorubicin and daunorubicin, and to a lesser extent to anthracycline-DNA complexes, zorubicin, carubicin, AD32, and aclacinomycin A.

In this review we concentrate on the recently published information that is most relevant to the clinician interested in the optimal use of this class of chemotherapeutic agents. Therefore, the emphasis is on the clinically related aspects of drug distribution and action, drug toxicity, and clinical trials.

Important Anthracycline Agents

The structural relations of the various anthracyclines are shown in Figure 1.

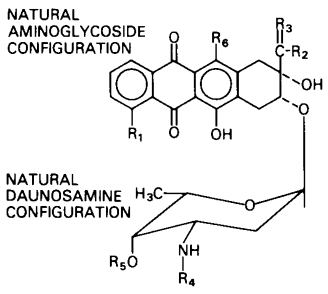
Doxorubicin

Doxorubicin (adriamycin) is a glycoside antibiotic originally isolated from the fungus *Streptomyces peucetius* var. *caesius*.[1] It differs from the other commonly used anthracycline, daunorubicin, by a single hydroxyl group on carbon-14. The doxorubicin molecule contains an aminosugar, daunosamine, linked through a glycosidic bond to adriamycinone, a red-pigmented naphthacenequinone nucleus. Potentially important interactions with commonly employed drugs may occur, and as a result the drug should not be administered directly with hydrocortisone, dexamethasone, fluorouracil, aminophylline, cephalothin, or heparin.[2] The drug can cause serious tissue necrosis if extravasated. Since a major mechanism of drug elimination is through the biliary system, many investigators have advocated a system of progressive reduction of doses based on abnormalities in liver function or on indexes of liver function such as sulfobromophthalein.[3] The drug is cardiotoxic, and total-dose limits of 500 to 550 mg per square meter of body-surface area have been suggested in patients with no underlying cardiac disease. Because of potentially detrimental interactions with thoracic irradiation or cyclophosphamide, total doses of 450 mg per square meter have been recommended in patients receiving combined methods of therapy. The limited information available suggests that the toxicity of all the anthracyclines is additive and that some additional toxicity may also occur from drugs with similar mechanisms of action, such as dactinomycin. All these factors, as well as the extent of any underlying cardiac disease, must be considered in decisions about the relevance of the arbitrary total-dose limitations listed in this section.[4]

In addition to the more conventional schedules of administration as a single bolus or three times daily, several unique clinical approaches have been tested. Continuous-infusion regimens may reduce the gastrointestinal and myelopoietic toxicities while retaining the antitumor effect.[5] However, their postulated reduction of cardiotoxicity is more difficult to document. Intra-arterial doxorubicin has been used in instances in which regional perfusion of the tumor could be well defined. Whether this approach is substantially better than the simpler intravenous approach has not been established. One comparative trial[6] in hepatocellular carcinoma demonstrated little therapeutic difference between the two approaches. Topical bladder instillation of the drug in patients with superficial bladder cancer has produced clinical regression of tumors, with limited or no systemic toxicity reported.[7] Finally, intraperitoneal doxorubicin in a large-volume dialysate is being studied as an approach to the treatment of minimal residual ovarian cancer.[8,9]

Daunorubicin

Daunorubicin (daunomycin or rubidomycin) was originally isolated from *Str. peucetius* in 1963.[10] Its solubility characteristics are similar to those of doxorubicin; like doxorubicin, it has some important incompatibilities with commonly used agents, including heparin and dexamethasone. Daunorubicin also causes serious local tissue necrosis if it is extravasated. Total-dose limitations of 500 to 600 mg per square meter have been recommended to reduce the risk of



	DERIVATIVES	R_1	R_2	R_3	R_4	R_5	R_6
1	ADRIAMYCIN	OCH_3	CH_2OH	O	H	H	OH
2	DAUNOMYCIN	OCH_3	CH_3	O	H	H	OH
3	CARMINOMYCIN	OH	CH_3	O	H	H	OH
4	RUBIDAZONE	OCH_3	CH_3	$NNHCOC_6H_5$	H	H	OH
5	AD32 (N-TRIFLUOROACETYL-ADRIAMYCIN-14-VALERATE)	OCH_3	$CH_2O\overset{O}{\overset{\|}{C}}(CH_2)_3CH_3$	O	$\overset{O}{\overset{\|}{C}}-CF_3$	H	OH
6	ACLACINOMYCIN A	OH	OCH_3	O	$(CH_3)_2$	*	H

*2-DEOXYFUCOSE-CINERULOSE

Figure 1. Structural Characteristics of Some of the Important Anthracycline Compounds in Clinical Use.
Adriamycin denotes doxorubicin, daunomycin daunorubicin, carminomycin carubicin, and Rubidazone zorubicin.

cumulative cardiotoxicity, and the toxicity appears to be additive with other anthracyclines, other related compounds, irradiation of the thorax, and perhaps cyclophosphamide. Like that of doxorubicin, the primary route of elimination of this drug is biliary, and dose modifications are suggested in patients with hepatic compromise.

Zorubicin

Zorubicin (Rubidazone or daunorubicin benzoylhydrazone hydrochloride), a semisynthetic anthracycline, is produced by the reaction of benzoyl hydrazine with the ketone group of carbon-13 of daunorubicin. The drug is available for investigational use, primarily in acute leukemia. This anthracycline is less stable at room temperature than the other anthracyclines, and it should be used immediately after preparation. Extravasation is associated with the same local tissue reactions seen with the parent compounds. Chills, fever, and urticarial reactions have been noted with the drug.[11] Cardiotoxicity can occur with this agent, and although the data on this reaction are limited, cumulative doses should not exceed 3500 mg per square meter. Prior therapy with other anthracyclines or related compounds, as well as thoracic irradiation, requires some dose reduction. In spite of the higher total doses of zorubicin that can be given without cardiac toxicity (in comparison with daunorubicin), the clinically active single dose is also higher (approximately 200 to 450 mg per square meter), and it is unclear whether the therapeutic ratio is improved.

Carubicin

This anthracycline antibiotic (also known as carminomycin) was isolated from *Actinomadura carminata* in the Soviet Union and is biochemically closely related to doxorubicin.[12] Carubicin I has been purified from a complex containing seven colored compounds, which differ according to the number of attached sugars. Carubicin I has a single sugar, daunosamine, but contains a different aglycone, a desmethyldaunomycin known as carminomycinone. This anthracycline, soluble in water and saline, is well absorbed after oral or subcutaneous administration. No tissue necrosis has been reported from subcutaneous administration or from extravasation. Alopecia is also rare with this drug, and cardiomyopathy from the drug has not been reported, although electrocardiographic alterations and unexplained arrhythmias have been described in 5 per cent of patients. Extensive Phase I and II trials have been performed in the Soviet Union, and considerable activity in soft-tissue sarcomas has been noted.[13]

Other Anthracyclines of Clinical Interest

AD32

AD32 is an *N*-trifluoroacetyl-adriamycin-14 valerate derivative of doxorubicin[14] with pharmacologic properties different from those of the other anthracyclines. It is water insoluble, and it does not appear to intercalate with DNA. The ester linkage between doxorubicin and valeric acid is cleaved by in vivo esterases, and the metabolite formed, AD41, shows antitumor activity in vivo.[15]

Aclacinomycin A

This anthracycline antibiotic has been isolated from *Str. galilaeus* by Japanese investigators.[16] It consists of an aglycone, aklavinone, and a trisaccharide moiety, consisting of L-cinerulose, 2-deoxy-L-fucose, and L-rodosamine. This agent has a broad spectrum of activity against tumors in animals and has been examined in a clinical trial.[17] Detailed studies in animals indicate substantially less cardiac toxicity than doxorubicin has at equivalent therapeutic doses.[18] In addition, it is much less mutagenic than doxorubicin. These findings make it a drug of considerable clinical interest.

Anthracycline-DNA Complexes

Anthracyclines complexed with DNA have been developed as an innovative approach to selective drug delivery. These complexes are based on the hypothesis that tumor cells have a higher rate of endocytosis than does normal tissue, leading to greater uptake of the complex and subsequent release of the free drug within the tumor cell.[19] Unfortunately, the conditions necessary for such an effect are better established for daunorubicin-DNA than for doxorubicin-DNA, in which the complex is unstable and acts primarily as a slow-release form of doxorubicin. Although several clinical trials have shown activity of these complexes in acute leukemia, alopecia, stomatitis, and bone-marrow suppression appear with a frequency similar to that occurring with the free drug. Although there is some evidence that cardiotoxicity may be lessened, this side effect has certainly not been eliminated.[20]

Pharmacokinetics and Disposition of the Anthracyclines

Doxorubicin and Daunorubicin

The study of the pharmacokinetics of doxorubicin and daunorubicin has been rendered difficult by their complex metabolism and by the lack of satisfactory assays for these drugs in biologic fluids. Most assays depend on the intense fluorescence of the compounds, and initial pharmacokinetic studies used such measurements in extracts of plasma or tissue. These assays are inadequate because they give no information about the pattern of metabolites found. Assays using high-pressure liquid chromatography can separate doxorubicin from its metabolites with a sensitivity (1 to 100 μg per milliliter) sufficient for clinical application.[21-26]

The pharmacokinetics of doxorubicin and daunorubicin are characterized by substantial plasma protein binding (50 to 90 per cent) and tissue binding.[26] Tissue:plasma ratios of 20:1 to 550:1 indicate that tissue concentrations are much higher than simultane-

ously measured plasma levels.[27] The majority of the tissue-bound drug appears to be localized to the nucleus, with smaller amounts in mitochondria, and to be bound to tissue proteins.[28] From this observation it is apparent that plasma concentrations have a complex and not necessarily direct relation to the actual concentration at sites of specific drug action, such as DNA.

Renal clearance appears to be quantitatively unimportant in human beings, and dose modification is not required in renal failure. The role of liver clearance is less obvious. In one study that measured hepatic clearance, 45 to 50 per cent of the drug was cleared by this mechanism.[29] Dose modification has been recommended in persons with hepatic dysfunction; however, no reliable quantitative relation has been found among any given test of liver function, doxorubicin clearance, and toxicity.

After intravenous administration, doxorubicin has a triphasic plasma-disappearance curve, with approximate half-lives of 10 to 30 minutes, 10 hours, and 24 to 48 hours.[27] The initial phase is considered to represent tissue uptake, the second phase metabolism, and the slow final phase the gradual release of the drug from multiple sites of binding, the most prominent of which is probably DNA. The pharmacology of continuous infusion of doxorubicin has been studied; although marrow and gastrointestinal toxicity were prominent, cardiac toxicity was not noted, even though the cumulative dosages reached in several instances were in excess of 1 g per square meter.[6,29]

Of the known metabolites of doxorubicin, doxorubicinol (adriamycinol) is the most prominent in plasma. Deoxyadriamycin aglycone is also found to a variable degree.[30] The toxicity and antitumor activity of these metabolites have only recently been investigated.[31-35] Both adriamycinol and daunorubicinol have some antitumor activity. Although various other metabolites are detected in urine and in bile, their cytotoxicity and concentration in relevant tissue have not been reported.[33]

Carubicin and Zorubicin

Carubicin closely resembles doxorubicin in structure. The molecular pharmacology, antitumor activity, and toxicity of carubicin have recently been reviewed.[36] Its major metabolite in plasma is carminomycinol, which appears rapidly in the plasma after intravenous administration of the parent compound. In contrast to doxorubicin, in which adriamycinol is present at less than 1/10 the concentration of doxorubicin, plasma carminomycinol levels are higher than those of the parent compound.

Zorubicin is a hydrazone analogue of doxorubicin, and it hydrolyzes in vivo to doxorubicin. The pharmacokinetics of zorubicin are consequently similar to those of doxorubicin, with the exception that there is quantitatively less adriamycinol detected after zorubicin administration than after doxorubicin.[37] This finding suggests that zorubicin inhibits doxorubicin reductase. The major route of excretion of zorubicin is the biliary system.

Dose-Response Effects

Dose-response relations in cancer chemotherapy are of theoretical and practical concern, but they have been difficult to define clinically in tumors in human beings.[38-40] The clonogenic human-tumor assay is an interesting experimental system with which to study anthracycline dose-response relations in multiple myeloma, acute leukemia, and ovarian cancer.[41] In ovarian carcinoma, three different patterns of doxorubicin sensitivity have been observed,[31] and they are summarized in Figure 2. Effective suppression of clonogenicity with low doses of doxorubicin was observed in cells from most of the untreated patients. In cells obtained from patients who had a relapse after treatment with non-doxorubicin-containing combinations, suppression was observed only at doxorubicin concentrations 10 times greater than those achievable with intravenous administration. In contrast, cells obtained from most of the patients who had a relapse after therapy with doxorubicin did not have a dose-dependent decrease in colony formation at any dose. These results demonstrate that the slope of the dose-response curve for doxorubicin can vary from patient to patient and that it is dependent in part on the nature of the patient's previous treatment.

Mechanisms of Action

The anthracyclines produce a wide range of biochemical effects that have potentially toxic consequences for mammalian cells. This complexity has made it difficult to assign a given biochemical action to specific host-tissue toxicity or to tumor-cell killing. However, there is growing evidence that the mechanisms of tumor-cell killing and of certain host-tissue toxicities may be sufficiently different to be dissociated. It is hoped that we will soon be better able to relate the biochemical actions described below to specific organ toxicity or to tumor-cell killing. At present, there are three mechanisms of action ascribed to anthracyclines: DNA intercalation, membrane binding, and lipid peroxidation.

DNA Intercalation

The first described action of these agents, and certainly the best documented, is their interaction with DNA. Daunorubicin inserts itself into the DNA double helix in such a fashion that the aglycone moiety is between the adjacent base pairs and parallel to them.[42] The aminosugar portion of the anthracycline is then available to bind strongly through the ionic process with the sugar-phosphate backbone of DNA. This combination of binding forces is thought to explain the high affinity of daunorubicin for DNA (association constant, 6×10^5 m^{-1}).

Once binding to DNA occurs, several consequences may ensue. Blockage of synthesis of DNA,

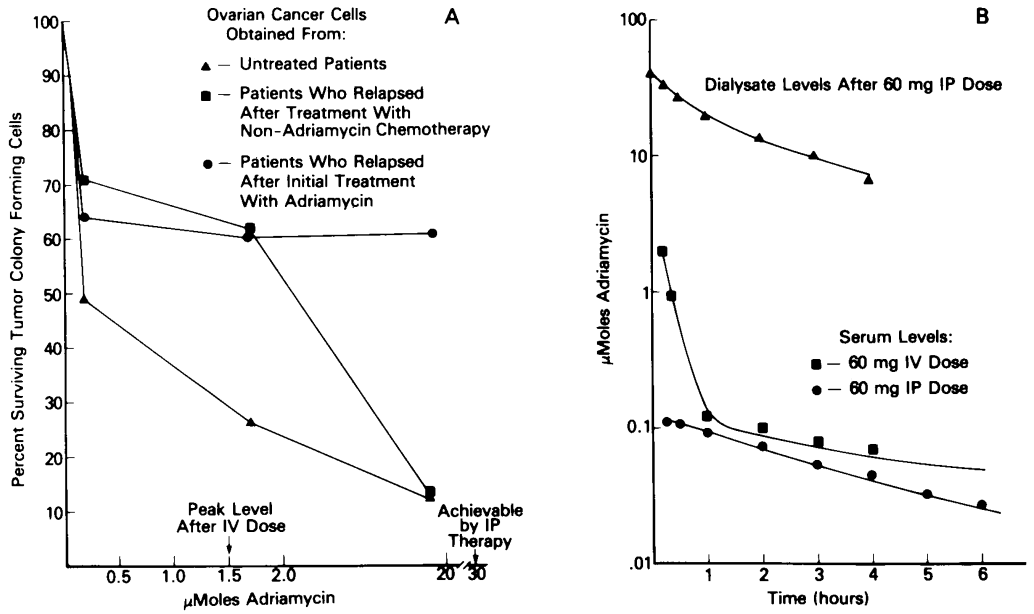


Figure 2. Comparison of Doxorubicin (Adriamycin) in Vitro Dose-Response Relations with Doxorubicin Pharmacokinetics in Intravenous (IV) and Intraperitoneal (IP) Administration.

Panel A shows the in vitro dose-response relations in human ovarian carcinoma. Human ovarian cancer cells were exposed for one hour to various concentrations of doxorubicin, and the effect on clonogenicity was compared with untreated controls. Each curve represents the mean percentage of surviving colonies obtained from patients in the different groups. Adapted from Ozols et al.[31]

Panel B shows the pharmacokinetics of IP and IV doxorubicin. The top curve represents the mean drug concentrations in the dialysate after IP administration of 60 mg in 2 liters. The lower curves represent the mean serum levels in patients receiving 60 mg, either IV or IP, as adapted from Ozols et al.[8]

Note the lack of suppression of colony formation at clinically achievable serum levels after IV administration in cases refractory to either doxorubicin or a combination without doxorubicin. At concentrations achievable through IP administration, suppression of colony formation was observed in cases refractory to a combination without doxorubicin, whereas patients in whom IV doxorubicin failed were more resistant and were not likely to benefit even from IP administration.

RNA, and protein, fragmentation of the DNA, and inhibition of DNA repair have all been reported.[43]

Among the various species of RNA, synthesis of ribosomal RNA appears to be most sensitive to these agents.[44] Analogues have been developed that preserve this blockade of ribosomal-RNA synthesis, but they are generally ineffective as inhibitors of DNA synthesis. These analogues remain effective antineoplastic agents, strongly suggesting that this inhibition of ribosomal-RNA synthesis may be more important than direct inhibition of DNA synthesis.

Doxorubicin and daunorubicin are mutagenic and have long been known to cause fragmentation of DNA.[45] However, two observations now suggest that DNA fragmentation may not be necessary for an antitumor response. In the first place, analogues such as aclacinomycin A have reduced mutagenicity but still have antitumor activity.[46] Secondly, there appears to be no relation between the frequency of single strand breaks caused by doxorubicin and cell killing.[47] Thus, there seems little indication that fragmentation of DNA is required for tumor-cell killing, and this observation suggests that clinically effective anthracyclines that lack the mutagenic properties of daunorubicin and doxorubicin may be developed.

Membrane Binding

It has been shown that daunorubicin and doxorubicin bind to cell membranes and alter membrane function at or below the concentrations that affect DNA function. Although all membrane-binding sites have not been identified, binding to spectrin and cardiolipin has been demonstrated.[48] The latter substance is of interest because an increased cardiolipin content in membranes appears to be a shared characteristic of malignant cells and cardiac mitochondria, leading Tritton et al.[49] to speculate that this property explains why anthracyclines kill tumor cells and damage cardiac tissue. Other studies have shown that doxorubicin alters concanavalin A receptors,[50] increases sodium permeability,[51] and alters calcium handling by guinea-pig atriums.[52] These observations provide an explanation for the altered sodium and calcium concentrations observed in cardiac tissue that are associated with the development of chronic cardiomyopathy.[53]

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.