bjh review

Advances in understanding the pathogenesis of graft-versus-host disease

John Magenau, Lyndsey Runaas and Pavan Reddy

Blood and Marrow Transplant Program, Division of Hematology/Oncology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

Summary

Allogeneic haematopoietic stem cell transplantation (HCT) is a potent immunotherapy with curative potential for several haematological disorders. Overcoming the immunological barrier of acute graft-versus-host disease (GVHD) remains a fundamental impediment to expanding the efficacy of HCT. GVHD reflects a complex pathological interaction between the innate and adaptive immune systems of the host and donor. Over the past decade there has been a tremendous advancement in our understanding of the cellular and molecular underpinnings of this devastating disease. In this review, we cover several recently appreciated facets of GVHD pathogenesis including novel extracellular mediators of inflammation, immune subsets, intracellular signal transduction, post-translation modifications and epigenetic regulation. We begin to develop general themes regarding the immunological pathways in GVHD pathogenesis, discuss critical outstanding questions, and explore new avenues for GVHD treatment and prevention.

Keywords: GVHD, allogeneic, transplantation.

While increasing numbers of patients receive allogeneic haematopoietic stem cell transplantation (HCT) for aggressive haematological disorders, graft-versus-host disease (GVHD) remains a formidable barrier to maximizing its therapeutic efficacy. Despite the routine administration of immunosuppressive prophylaxis, GVHD is the principle cause of transplant-related mortality (TRM). Clinically significant acute GVHD will occur in approximately 40% of patients undergoing human leucocyte antigen (HLA)-matched related HCT and upwards of 50–70% of patients receiving HLA-matched or -mismatched unrelated donor HCT (Jagasia *et al*, 2012). Unfortunately, less than half of the patients who develop acute GVHD experience a demonstrable response, partly due to the rapid pace of the disease as well as the marginal efficacy of primary treatment with high dose corticosteroids (MacMillan *et al*, 2010; Choi & Reddy, 2014; Magenau & Reddy, 2014). In cases where organ damage is advanced or where high dose corticosteroid therapy is unsuccessful, mortality can exceed 90% (Pasquini, 2008). Many patients who do ultimately respond to intensified immunosuppression must endure significant morbidity from infection, functional impairment and subsequent chronic GVHD. Finally, given that the efficacy of HCT relies heavily upon graft-versus-leukaemia (GVL) responses that are tightly linked to GVHD, intensive immune suppression may increase the risk of relapse and subsequent mortality (Rubio *et al*, 2015).

Correspondence: Pavan Reddy, M.D., Professor of Medicine Blood and Marrow Transplant Program, Division of Hematology/Oncology, Department of Internal Medicine, University of Michigan, 3312 Cancer Center, 1500 E. Medical Center Drive, SPC 5932, Ann Arbor, MI 48109-5932, USA.
E-mail: reddypr@umich.edu

Overview of GVHD biology

The clinical features of acute GVHD manifest as an intense inflammatory injury primarily involving the skin, intestine and liver. Underlying this clinical presentation is an immunologically-mediated process of allogeneic donor cells responding to host tissues expressing polymorphic human leucocyte antigens (Blazar *et al*, 2012). The essential function of allogeneic donor T cells was theorized following an almost complete absence of GVHD in syngeneic and T cell-depleted HCT. However, it is now widely recognized that allogenic GVHD and GVL responses are fundamentally driven by initial interactions between host and donor antigen presenting cells (APCs) that encounter mature T lymphocytes from the donor inoculum (Shlomchik *et al*, 1999; Matte *et al*, 2004; Reddy *et al*, 2005; Koyama *et al*, 2012; Toubai *et al*, 2012). The ensuing inflammatory cascade is self-perpetuating: release of pro-inflammatory cytokines results in expansion of alloreactive T cells with specificity to host tissues that, in turn, secrete additional inflammatory mediators.

Over the past decade our knowledge of the intricacies that govern T cell and APC interactions have grown tremendously. A great number of mechanistic insights are derived from murine models of acute GVHD that sufficiently recapitulate acute GVHD in humans. As a consequence, a bewildering array of cellular immune subsets, extracellular receptors,

First published online 27 March 2016
doi: 10.1111/bjh.13959

mediators of inflammation and molecular signalling pathways are now implicated in GVHD pathogenesis. In this review, we focus on critical advances in the understanding of acute GVHD pathogenesis. Emerging mechanistic themes are highlighted as well as opportunities for clinical translation, i.e. those that shape the allogeneic immune response to suppress GVHD without compromising beneficial anti-tumour and anti-viral immunity (Table I).

Initiating events

According to Billingham's postulates made almost 50 years ago, immunocompetent donor cells must experience histocompatibility differences in the host for the development of GVHD (Billingham, 1966). It is now well established that disparities in major histocompatibility complex (MHC) loci on chromosome 6, as occurs in HLA-mismatched HCT, are directly proportional to the severity of acute GVHD (Flomenberg *et al*, 2004; Petersdorf *et al*, 2015). Today, the majority of HCT occurs with HLA-matched unrelated donors, thus differences in other polymorphic genes, so called minor histocompatibility antigens (mHA), provide the necessary tissue disparity to produce GVHD. Furthermore, given that adaptive immune responses require additional signalling events to initiate GVHD, the importance of MHC independent events is becoming increasingly appreciated.

Table I. Select pathways of GVHD pathogenesis with potential therapeutic approaches

Aberrant pathway	Approach	Proposed mechanism(s)	Clinical trial (phase I-II)	Reference(s)
Inhibiting costimulation				
CTLA4-Ig (Abatacept)	CTLA4-Ig (Abatacept)	Decreased activation of T cells	+	Miller *et al* (2010)
Modulate cytokine(s)/chemokine(s)				
IL6	anti-IL6 receptor mAb (Tocilizumab)	↓Th1, Th17, Tissue Damage; ↑Treg;	+	Drobyski *et al* (2011);Kennedy *et al* (2014)
IL21	anti-IL21 mAb	↓ Th1, Th17; ↑ iTreg	–	Hippen *et al* (2012)
CCR5	Inhibit CCR5 coreceptor (Maraviroc)	↓ T cell homing to Gut	+	Reshef *et al* (2012)
Decrease DAMPs/PAMPs				
↑ protease inhibition	Exogenous Alpha-1-antitrypsin (AAT/SERPINA1)	Suppress release of DAMPs; Limit cytokine production	+	Marcondes *et al* (2011); Tawara *et al* (2012); Brennan *et al* (2012)
Activate Siglecs	CD24-Fc	Sequester DAMPs, ↓DAMP-mediated DC activation	–	Chen *et al* (2009a); Toubai *et al* (2014)
Alter microbiome	Antibioltics, probiotics, other	Gut protection through modifying microbiome and byproducts	–	Atarashi *et al* (2013); Jenq *et al* (2015)
Improve peripheral tolerance				
Expand/induce Tregs	Treg graft engineering, Graft Selection, Pharmacological (several)	Inactivate/delete alloreactive T cells	+	Di Ianni *et al* (2011); Brunstein *et al* (2011)
iNKT	*ex vivo* iNKT expansion, adoptive transfer	Promotes Treg expansion	–	Schneidawind *et al* (2015)
Naive T cell depletion	Graft Engineering (select memory T cells)	Reduce alloreactive T cells	+	Bleakley *et al* (2015)
Intracellular signalling				
Tyrosine kinases	JAK-1/2 inhibition	Increase Treg:Teff ratios, ↓STAT	+	Spoerl *et al* (2014)
NF-κB	c-Rel inhibition, proteasome inhibition (Velcade), neddylation inhibition (MLN4924)	↓transcription of multiple inflammatory cytokines	+	Shono *et al* (2014); Mathewson *et al* (2013)
STATs	STAT3 inhibition	↑ FOX P3 Expression (iTREG)	–	Laurence *et al* (2012)
Epigenetic				
DNA methylation	Azacitidine	Promotes iTreg expansion	+	Choi *et al* (2010); Goodyear *et al* (2012)
Histone acetylation	Vorinostat	Increase IDO, decrease inflammatory cytokines	+	Reddy *et al* (2008); Choi *et al* (2014)
Bromodomain (BET) proteins	I-BET-151, JQ1	↓DC activation; ↓T-cell proliferation; NF-kB signalling	–	Sun *et al* (2015a)

DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns.

Costimulation

While the primary interaction occurs between the MHC/allopeptide on APCs and the T cell receptor (TCR) of donor T cells, this signal alone is insufficient to induce T cell activation. Several studies in HCT and related haematological fields have focused on the effect of the required second or co-stimulatory signal on T cell activation and GVHD (McDonald-Hyman *et al*, 2015). Second signals between APCs and T cells may positively or negatively influence the immune response (Fig 1A). CTLA4 is one of several co-stimulatory molecules expressed on differentiated T cells which functions as a key negative regulator of T cell activation. CD28, a structural homolog of CTLA4, provides a positive co-stimulatory signal to T cells. By competing with greater affinity than CD28, CTLA4 preferentially binds ligands on APCs (B7-1/2 or CD80/86) thereby constraining T cell proliferation (Mueller, 2010; Fig 1B). CTLA4-immunoglobulin or Abatacept is a US Food and Drug Administration (FDA) approved therapy for rheumatoid arthritis that fuses a modified Fc portion of human immunoglobulin-G1with CTLA4. Abatabcept increases the availability of CTLA4 which probably minimizes activating interactions with CD28. Based on encouraging pre-clinical evidence of GVHD suppression and pilot studies in humans (Miller *et al*, 2010), abatacept is currently being tested in a phase II multi-centre randomized study. This mechanism is notably distinct from the alternate strategy of directly inhibiting CTLA4, which activates T cells (e.g. ipilimumab; Fig 1C). Interestingly, toxicity related to ipilimumab (without allogeneic HCT) may resemble GVHD.

Programmed cell death-1 (PD-1, also termed PCDC1) is another distinct inhibitory signal that has been implicated in maintaining peripheral tolerance (Mueller, 2010). Because PD-1 and PD-1 ligand (also termed CD274) interactions promote T cell exhaustion, this pathway may be physiologically important for eliminating chronically self-reactive T cells. While pre-clinical studies after HCT suggest interrupting these interactions may aggravate acute GVHD, delaying PD-1 blockade or selective interruption of PD ligands with differing tissue distribution may promote graft-versus-tumour (GVT) responses without aggravating GVHD (Koestner *et al*, 2011; Saha *et al*, 2013).

Therapeutic interventions surrounding the TCR:MHC immune synapse must account for orchestrating co-stimulatory and co-inhibitory signals, but also time-dependent effects specific to immune cells. As an example, the B7-H3 (also termed CD276) membrane protein, widely expressed on T cells, natural killer cells, dendritic cells (DCs) and macrophages, is an activating co-stimulatory signal that mediates transplant rejection (Wang *et al*, 2005). However, B7-H3 can also effectively curtail alloreactive T cells and GVHD in the early HCT period (Veenstra *et al*, 2015) and donor lymphocyte infusion with T cells lacking B7-H3 promotes GVL responses without GVHD. These studies illustrate the time dependence of co-stimulatory pathways, thus agonism or antagonism both may present viable therapeutic strategies after HCT.

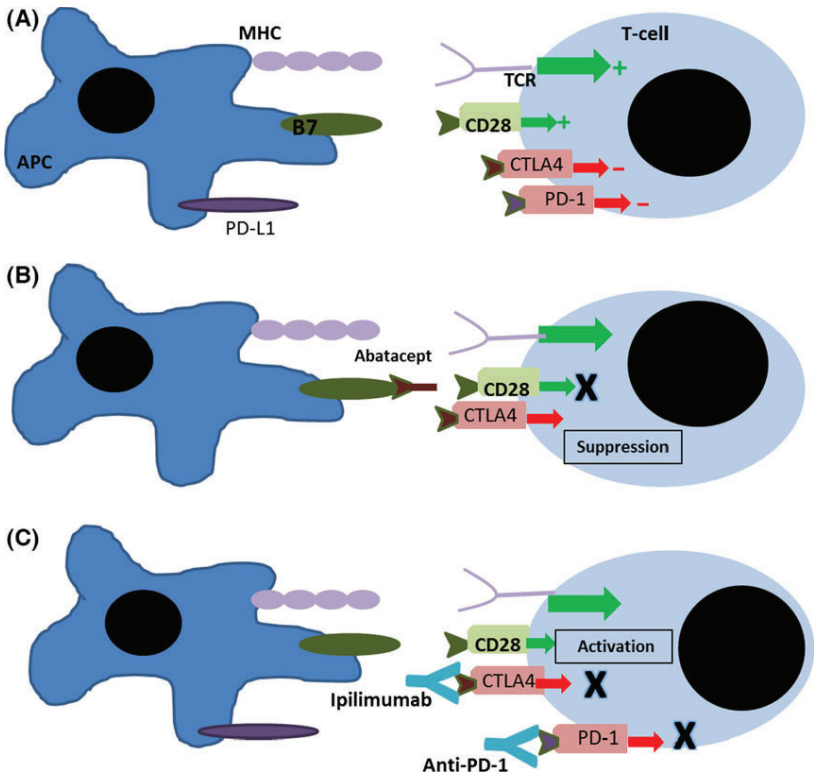

Fig 1. Co-stimulatory interactions between APCs & T cells. Co-stimulatory ligands and receptors (**A**) provide positive or negative second signals together with the TCR that determine the activation status of T cells. Clinically available pharmacological agents modulate co-stimulatory interactions on APCs and T cells. Interrupting B7:CD28 interactions (**B**) can suppress T cells through loss of activating second signals, potentially impeding graft-versus-host disease. Conversely, targeting CTLA4 or PDCD1 (**C**) can activate T cells through blunting inhibitory second signals. MHC, major histocompatibility complex; TCR, T-cell receptor; APC, antigen presenting cell.

Mediators of inflammation: cytokines and chemokines. Once activated, T cells initiate transcriptional programmes that result in the massive release of pro-inflammatory mediators [tumour necrosis factor α (TNF-α, TNF), interleukin 1 (IL1), γ interferon (IFN-γ, IFNG)] that amplify the immune response and result in tissue damage (Paczesny *et al*, 2010). In addition, cytokines and chemokines influence proliferation, differentiation and homing of effector cells to GVHD target tissues. This knowledge laid the foundation for attempts at blocking single cyto/chemokines to ameliorate GVHD. This approach has been met with modest clinical success, but is gaining renewed interest from pre-clinical studies suggesting that early interruption of novel cytokines can impede multiple cellular pathways in GVHD.

Interleukin 6 (IL6): There is compelling evidence that IL6 promotes GVHD by divergent mechanisms including increasing inflammatory T-helper cells types 1 and 17 (Th1 and Th17, respectively) subsets, decreasing regulatory T cells (Tregs) and by direct cytotoxicity (Chen *et al*, 2009b; Tawara *et al*, 2011). In models of HCT, levels of IL6 are elevated, following cytotoxicity from conditioning or GVHD. IL6 blockade suppresses experimental GVHD without impairing the GVL response. Given the clinical availability of the humanized anti-IL6 receptor antibody, tocilizumab, for rheumatoid arthritis, IL6 blockade was piloted in steroid-refractory acute GVHD without untoward toxicity (Drobyski *et al*, 2011). Because IL6 is elevated early after HCT, a single centre phase I/II trial evaluated whether early intervention with tocilizumab might reduce GVHD in HLA-matched related and unrelated donors (Kennedy *et al*, 2014). Correlative studies revealed that downstream STAT3 was reduced in monocytes and T cells from patients receiving tocilizumab, implying successful IL6 blockade. The rate of grade II-IV GVHD was 12% and severe grade III-IV GVHD was 4%, supporting additional studies in GVHD prevention.

Interleukin-21 (IL21): is capable of promoting Th1 and Th17 differentiation, NK cell expansion and formation of inducible Tregs (iTregs) in addition to impacting a wide range of lymphoid and myeloid cells including APCs. Administration of IL21 inhibitors or IL21-deficient T cells mitigates gastrointestinal (GI) GVHD without impairing GVL responses, perhaps due to its tissue-specific effects (Bucher *et al*, 2009). In humans, IL21 expression was increased in the GI tracts of patients with GVHD. In a xenograft model, prophylactic administration of anti-human IL21 reduced GVHD lethality (Hippen *et al*, 2012). Exogenous IL21 is currently being evaluated in immunotherapy trials for cancer, while IL21 blocking strategies are being developed for autoimmune conditions.

Interleukin-2 (IL2): Interrupting IL2 secretion by donor T cells, via the calcineurin inhibitors (CNIs), ciclosporin and tacrolimus, is an established standard of care in GVHD prevention. However, subsequent randomized trials directly targeting the IL2 receptor (i.e. daclizumab) have been unsuccessful in acute GVHD treatment, partly due to high rates of relapse (Lee *et al*, 2004). While CNIs reduce effector T cell function, an unintended consequence of prolonged IL2 inhibition may be that Treg generation is constrained. In support of this hypothesis, murine data and a recent prospective trial demonstrated that administration of low dose IL2 in chronic GVHD results in regression of disease (Koreth *et al*, 2011). The mechanism of IL2 may relate to differential effects on STAT5 proteins in dividing lymphocytes that restore a more favourable balance between Tregs and conventional T cells (Matsuoka *et al*, 2013).

Chemokine (C-C motif) receptor-5 (CCR5): CCR5 is upregulated in T lymphocytes upon allogeneic stimulation and directs homing to target tissues such as the GI tract. HCT recipients with a missense mutation of *CCR5* (Delta32) appear less susceptible to developing clinical GVHD (Bogunia-Kubik *et al*, 2006). A phase I/II clinical trial using a small molecule antagonist of CCR5 (maraviroc) demonstrated promising results for GVHD prevention (Reshef *et al*, 2012) and is now being prospectively evaluated in a larger clinical trials network (CTN) study (NCT02208037).

Interleukin-22 (IL22): Our understanding of cytokines has expanded to include identification of molecules capable of tissue repair. IL22 acts upon intestinal stem cells (ISCs) that are critically involved in epithelial repair. Because the GI tract is exquisitely sensitive to the cytotoxic effects of conditioning, its damage responses are instrumental for propagating GVHD. Deficiency of IL22 promotes loss of ISCs, greater intestinal damage and more severe experimental GVHD (Hanash *et al*, 2012).

Suppression of Tumourigenicity 2 (ST2): *ST2* functions as both a soluble (sST2) and membrane bound receptor (ST2L) for IL33, a cytokine in the IL1 receptor family with diverse immunological roles depending on disease, cell type or model system. Using proteomic methods to compare plasma levels prior to and during GVHD, elevations in sST2 have been identified as an independent biomarker of treatment resistance and mortality (Vander Lugt *et al*, 2013). While high ST2 levels predict GVHD mortality, non-specific tissue damage or the genetic background of the host could also influence plasma concentrations (Ito & Barrett, 2015). High levels of ST2 and IL33 are produced by non-haematopoietic cells in the GI tracts of animals with GVHD (Reichenbach *et al*, 2015; Zhang *et al*, 2015). IL33 binding to ST2 aggravates GVHD, which can be reversed in IL33(−/−) deficient hosts or in T cells lacking *ST2* expression. In pre-clinical models, minimizing IL33/ST2 interactions by administering an ST2-Fc fusion protein reduced GVHD.

FTY720 (FTY): FTY is an immunomodulator derived from a metabolite of the fungus *Isaria sinclairii*. It binds with high affinity to sphingosine 1-phosphate receptors found on all cell types and is thought to exert its immunomodulatory effects through sequestering of lymphocytes within secondary lymphoid organs. Given this, there was interest in evaluating FTY to prevent or treat GVHD as an oral FTY (fingolimod) was approved by the FDA in 2010 to treat relapsing multiple

sclerosis. Taylor *et al* (2007) were able to demonstrate that FTY significantly inhibited but did not completely prevent GVHD in a model of GVHD. Similarly, administration of FTY slightly mitigated but did not eliminate GVL effect (Taylor *et al*, 2007).

Retinoic acids: Retinoic acid (RA) is produced by intestinal cells and is known to have a role in intestinal immune homeostasis; however, its exact role in the pathophysiology of GVHD remains uncertain. Nishimori *et al* (2012) demonstrated that administration of a RA analogue ameliorated cGVHD. The effects on acute GVHD appear more nuanced with administration of a vitamin-A deficient diet reducing gut acute GVHD but worsening liver GVHD (Koenecke *et al*, 2012). Similarly, Aoyama *et al* (2013) found that inhibiting donor T-cell RA receptor signalling reduced GVHD while preserving graft-versus-lymphoma effects.

Modulating innate immune responses

As DCs are primary sensors of initial inflammatory signals, their regulation can significantly shape GVHD. Currently, several efforts are underway to understand if modulating DC function can be utilized for treating GVHD.

Damage-associated molecular patterns (DAMPs)

Immune and myeloablative conditioning therapy preceding HCT provides important anti-tumour effects, but also causes direct immune activation by tissue injury and release of pro-inflammatory mediators. Large scale observational studies have confirmed a modest yet significant increase in acute GVHD incidence with higher conditioning intensity, especially in regimens containing total body irradiation (TBI) (Nakasone *et al*, 2015). A growing body of evidence suggests that highly conserved Toll-like receptors (TLRs) and other pattern recognition receptors on innate immune cells acutely sense endogenous 'danger' signals from DAMPs, such as ATP, HMGB1, uric acid (UA) and heat-shock proteins, that provide a crucial initiating step in alloreactive T cell responses (Wilhelm *et al*, 2010; Jankovic *et al*, 2013; Brennan *et al*, 2015; Fig 2).

The purine nucleoside ATP, when present in the extracellular space following tissue injury, is one of several early danger signals or DAMPs. ATP released into peritoneal fluids of humans and mice following radiation interacts with P2X7 causing expression of co-stimulatory molecules, phosphorylation of signal transducer and activator of transcription (STAT) proteins and production of inflammatory cytokines. Antagonizing ATP:P2X7 interactions limits mortality in models of GVHD (Wilhelm *et al*, 2010). Other DAMPs are increasingly being recognized as initiators of the alloimmune response. For example, tissue injury releases the extracellular matrix component UA, which, in turn, activates the NLRP3 inflammasome (Jankovic *et al*, 2013). Mice under conditions of less UA or deficient in components of the NLRP3 complex demonstrate less severe GVHD. Heparin sulfate (HS), also a DAMP, is similarly capable of activating alloreactive T cell responses through binding TLR4 receptors on DCs and elevated levels correlate with GVHD (Brennan *et al*, 2012). While inhibiting single DAMPs is effective in suppressing

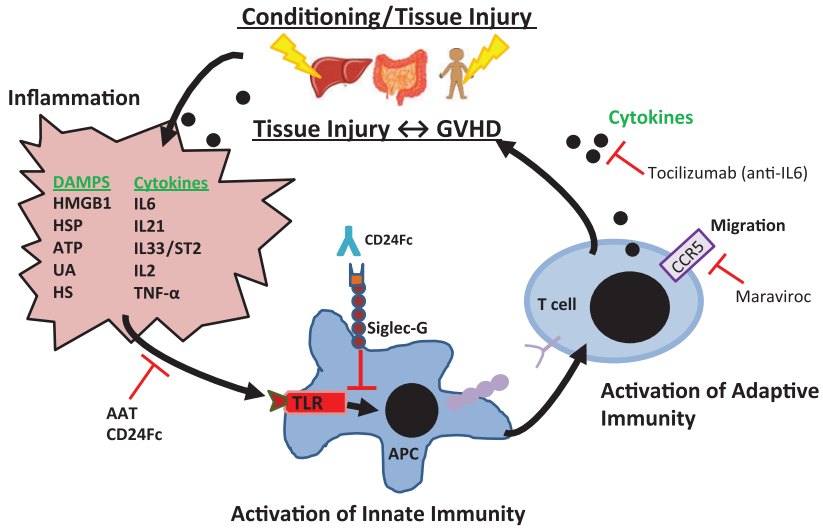

Fig 2. Role of DAMPs & Novel Cytokines in GVHD Pathogenesis. Tissue damage following conditioning (chemotherapy/radiation) results in the release of numerous sterile inflammatory mediators, termed damage-associated molecular patterns (DAMPs), that together with cytokines contribute to the initiation of acute GVHD. Inflammatory mediators activate innate immunity through interactions with toll-like receptors (TLR) and cytokine receptors (not depicted) on APCs. These interactions promote activation of the adaptive immune response characterized by T cell differentiation, proliferation, and migration that perpetuates GVHD and worsens tissue damage. Certain investigational agents (e.g. AAT, CD24Fc) may mitigate GVHD by either sequestering DAMPs or regulating APC-mediated responses to DAMPs. GVHD, graft-versus-host disease; APC, antigen presenting cell; AAT, alpha-1-antitrypsin; CD24Fc, CD24 fusion protein; HS, heparin sulfate; UA, uric acid; HS, heat shock protein.

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.