Graft-versus-host disease

James L M Ferrara, John E Levine, Pavan Reddy, Ernst Holler

Lancet 2009; 373: 1550–61
Published Online March 12, 2009
DOI:10.1016/S0140-6736(09)60237-3

University of Michigan, Pediatrics and Internal Medicine, Blood and Marrow Transplantation Program, Ann Arbor, MI, USA (Prof J L M Ferrara MD, Prof J E Levine MD, P Reddy MD); and Internal Medicine and Department of Haematology/Oncology, University Hospital, Regensburg, Germany (Prof E Holler MD)

Correspondence to: Prof James L M Ferrara, University of Michigan, 1500 East Medical Center Drive, 6303 CCC, Ann Arbor, MI 48109-5942, USA
ferrara@umich.edu

Haemopoietic-cell transplantation (HCT) is an intensive therapy used to treat high-risk haematological malignant disorders and other life-threatening haematological and genetic diseases. The main complication of HCT is graft-versus-host disease (GVHD), an immunological disorder that affects many organ systems, including the gastrointestinal tract, liver, skin, and lungs. The number of patients with this complication continues to grow, and many return home from transplant centres after HCT requiring continued treatment with immunosuppressive drugs that increases their risks for serious infections and other complications. In this Seminar, we review our understanding of the risk factors and causes of GHVD, the cellular and cytokine networks implicated in its pathophysiology, and current strategies to prevent and treat the disease. We also summarise supportive-care measures that are essential for management of this medically fragile population.

Introduction

The number of allogeneic haemopoietic-cell transplantations (HCTs) continues to rise, with more than 25 000 procedures undertaken annually. The graft-versus-leukaemia or graft-versus-tumour effect during this procedure effectively eradicates many haematological malignant diseases.[1] Development of novel strategies that use donor leucocyte infusions, non-myeloablative conditioning, and umbilical-cord blood transplantation has helped expand the indications for allogeneic HCT over the past few years, especially for older patients.[2] Improvements in infectious prophylaxis, immunosuppressive treatments, supportive care, and DNA-based tissue typing have also contributed to enhanced outcomes after the technique.[1] Yet, the major complication of allogeneic HCT—graft-versus-host disease (GVHD)—remains lethal and limits use of this important procedure.[2] In view of current trends, the number of transplants from unrelated donors is expected to double within the next 5 years, substantially increasing the population of patients with GVHD. In this Seminar, we review advances made in identification of genetic risk factors and pathophysiology of this major HCT complication and its prevention, diagnosis, and treatment.

Cause and clinical features

50 years ago, Billingham formulated three requirements for development of GVHD: (1) the graft must contain immunologically competent cells; (2) the recipient must express tissue antigens that are not present in the transplant donor; and (3) the patient must be incapable of mounting an effective response to eliminate the transplanted cells.[3] We know now that the immunologically competent cells are T cells and that GVHD can develop in various clinical settings when tissues containing T cells (blood products, bone marrow, and solid organs) are transferred from one person to another who is not able to eliminate those cells.[4,5] Patients whose immune systems are suppressed and who receive white blood cells from another individual are at especially high risk for the disease.

GVHD arises when donor T cells respond to genetically defined proteins on host cells. The most important proteins are human leucocyte antigens (HLAs),[2,6,7] which are highly polymorphic and are encoded by the major histocompatibility complex (MHC). Class I HLA (A, B, and C) proteins are expressed on almost all nucleated cells of the body at various densities. Class II proteins (DR, DQ, and DP) are mainly expressed on haemopoietic cells (B cells, dendritic cells, and monocytes), but their expression can be induced on many other cell types after inflammation or injury. High-resolution DNA typing of HLA genes with PCR-based techniques has now largely replaced earlier methods. The frequency of acute GVHD is directly related to the degree of mismatch between HLA proteins,[8,9] and thus ideally, donors and recipients are matched at HLA A, B, C, and DRB1 (referred to as 8/8 matches), but mismatches can be tolerated for umbilical-cord blood grafts (see Clinical features of acute GVHD).[10–12]

Despite HLA identity between a patient and donor, about 40% of recipients of HLA-identical grafts develop systemic acute GVHD that needs treatment with high-dose steroids. This disorder is due to genetic differences that lie outside the HLA loci and that encode proteins referred to as minor histocompatibility antigens. Some minor histocompatibility antigens, such as HY and HA-3, are expressed on all tissues and are targets for both GVHD and graft-versus-leukaemia.[13] Others, such as HA-1 and HA-2, are expressed most abundantly on haemopoietic cells (including leukaemic cells) and could, therefore, induce an enhanced graft-versus-leukaemia effect with diminished GVHD.[13,14]

Search strategy and selection criteria

We searched PubMed and Medline with the term "GVHD" and cross-referenced it with the following words: "clinical", "cytokines", "MHC", "HLA antigens", "biology", and "immunology". We included mostly peer-reviewed original and review journal articles published within the past decade, except for seminal articles that initially described the clinical features. All non-peer-reviewed manuscripts, supplements, and textbooks were excluded.

Pharmacyclics Exhibit 2007 - Page 1 of 12
Sandoz v. Pharmacyclics - IPR2019-00865
DOCKET ALARM
Find authenticated court documents without watermarks at docketalarm.com.

Panel 1: **Acute GVHD symptoms**

Skin
- Maculopapular skin rash

Upper gastrointestinal tract
- Nausea, anorexia, or both, and positive histological findings

Lower gastrointestinal tract
- Watery diarrhoea (≥500 mL)
- Severe abdominal pain
- Bloody diarrhoea or ileus (after exclusion of infectious causes)

Liver
- Cholestatic hyperbilirubinaemia

Polymorphisms in both donors and recipients of cytokines that have a role in the classic cytokine storm of GVHD (see Pathophysiology of acute GVHD) have been implicated as risk factors for the disorder.[15] Tumour necrosis factor (TNF) α, interleukin 10, and interferon γ variants have correlated with GVHD in some, but not all, studies.[16–18] Genetic polymorphisms of proteins connected with innate immunity, such as nucleotide oligomerisation domain 2 and keratin 18 receptors, have also been associated with the disorder.[19–22] Future strategies to identify the best possible transplant donor will probably incorporate both HLA and non-HLA genetic factors.

Clinical features of acute GVHD

On the basis of early work, acute GVHD was defined as arising before day 100 post-transplant, whereas chronic disease happened after that time.[23–25] This definition is far from satisfactory, and a National Institutes of Health classification includes late-onset acute GVHD (after day 100) and an overlap syndrome with features of both acute and chronic disorder.[26,27] Late-onset acute GVHD and the overlap syndrome arise with greater frequency after reduced-intensity conditioning, an increasingly widespread technique (see Prevention of GVHD). Panel 1 shows the clinical manifestations of acute GVHD. In a comprehensive review, Martin and colleagues noted that at onset of acute GVHD, affected regions included skin (81% of patients), gastrointestinal tract (54%), and liver (50%).[23]

Skin is most frequently affected and is usually the first organ involved, generally coinciding with engraftment of donor cells. The characteristic maculopapular rash is pruritic and can spread throughout the body, sparing the scalp (figure 1). In severe cases the skin can blister and ulcerate.[28] Apoptosis at the base of epidermal rete pegs is a characteristic pathological finding. Other features include dyskeratosis, exocytosis of lymphocytes, satellite lymphocytes adjacent to dyskeratotic epidermal keratinocytes, and perivascular lymphocytic infiltration in the dermis.[29,30]

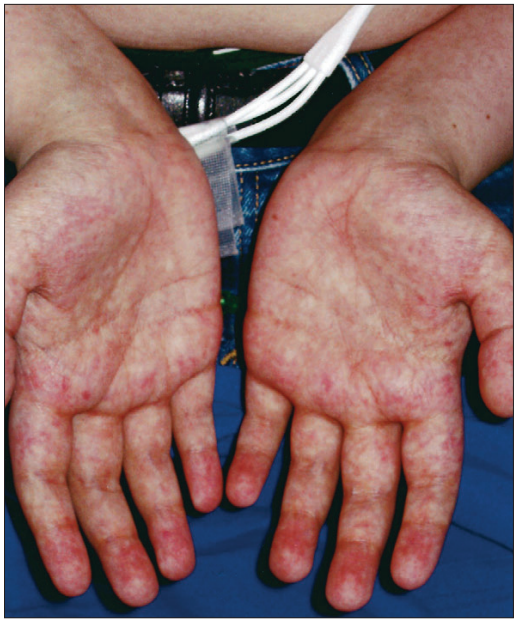

Figure 1: **Acute GVHD of the skin (grade I)**
Photograph courtesy of J Levine.

Gastrointestinal-tract involvement of acute GVHD usually presents as diarrhoea but can also include vomiting, anorexia, abdominal pain, or a combination when severe.[29] Diarrhoea in GVHD is secretory and usually voluminous (>2 L per day). Bleeding, which has poor prognosis, happens as a result of mucosal ulceration,[31] but patchy involvement of mucosa generally leads to a normal appearance on endoscopy.[32] Radiological findings of the gastrointestinal tract include luminal dilatation with thickening of the wall of the small bowel (ribbon sign on CT) and air or fluid levels suggestive of an ileus.[28] Histological features include patchy ulcerations, apoptotic bodies in the base of crypts, crypt abscesses, and loss and flattening of surface epithelium.[33]

Liver disease caused by GVHD can be difficult to distinguish from other causes of liver dysfunction after bone-marrow transplantation, such as veno-occlusive disease, toxic drug effects, viral infection, sepsis, or iron overload. The histological features of hepatic GVHD are endothelialitis, lymphocytic infiltration of the portal areas, pericholangitis, and bile-duct destruction.[34,35] However, biopsy specimens of liver are taken rarely because thrombocytopenia early after transplantation greatly increases the risks of the biopsy procedure, making the diagnosis of GVHD one of exclusion.

Severity of acute GVHD is ascertained by the extent of involvement of the three main target organs. Overall grades are I (mild), II (moderate), III (severe), and IV (very severe). Severe GVHD has poor prognosis, with 25% long-term survival (5 years) for grade III disease and 5% for grade IV.[36]

Pharmacyclics Exhibit 2007 - Page 2 of 12
Sandoz v. Pharmacyclics - IPR2019-00865
DOCKET ALARM
Find authenticated court documents without watermarks at docketalarm.com.

Panel 2: **Chronic GVHD symptoms**

Skin
Dyspigmentation, new-onset alopecia, poikiloderma, lichen planus-like eruptions, or sclerotic features

Nails
Nail dystrophy or loss

Mouth
Xerostomia, ulcers, lichen-type features, restrictions of mouth opening from sclerosis

Eyes
Dry eyes, sicca syndrome, cicatricial conjunctivitis

Muscles, fascia, joints
Fasciitis, myositis, or joint stiffness from contractures

Female genitalia
Vaginal sclerosis, ulcerations

Gastrointestinal tract
Anorexia, weight loss, oesophageal web or strictures

Liver
Jaundice, transaminitis

Lungs
Restrictive or obstructive defects on pulmonary function tests, bronchiolitis obliterans, pleural effusions

Kidneys
Nephrotic syndrome (rare)

Heart
Pericarditis

Marrow
Thrombocytopenia, anaemia, neutropenia

Prevalence of acute GVHD is directly related to the degree of mismatch between HLA proteins. It ranges from 35–45% in recipients of full-matched sibling donor grafts[8,9] to 60–80% in people receiving one-antigen HLA-mismatched unrelated-donor grafts.[6,37–39] The same amount of mismatch causes diminished GVHD with umbilical-cord blood grafts, and frequency of acute GVHD is low after transplantation of partly matched umbilical-cord blood units (35–65%).[12]

Clinical features of chronic GVHD

Chronic GVHD is the major cause of late non-relapse death after HCT.[40] Its presentation can be progressive (active or acute GVHD merging into chronic), quiescent (acute disease that resolves completely but is followed later by chronic), or de novo. Older recipient age and a history of acute GVHD are the greatest risk factors for chronic disease.[41] Therefore, strategies for acute GVHD prevention could help to prevent chronic disease. Panel 2 shows that manifestations of chronic GVHD are somewhat protean and typically of an autoimmune nature. Clinical signs are generally seen first in the buccal mucosa (figure 2). New consensus criteria for diagnosis and staging of chronic GVHD have been developed.[26]

Pathophysiology of acute GVHD

Two important principles should be considered with respect to the pathophysiology of acute GVHD. First, the disease is indicative of exaggerated but typical inflammatory mechanisms mediated by donor lymphocytes infused into the recipient, in whom they function appropriately in view of the foreign environment they encounter. Second, the recipient's tissues that stimulate donor lymphocytes have usually been damaged by underlying disease, previous infections, and the transplant conditioning regimen.[29] As a result, these tissues produce molecules such as proinflammatory cytokines and chemokines, which increase expression of key receptors on antigen-presenting cells (APCs), thereby enhancing cross-presentation of polypeptide proteins (eg, minor histocompatibility antigens) to the donor immune cells that mediate GVHD.[42–45]

Mouse models have been central to identification and understanding of pathophysiological mechanisms of GVHD, and work undertaken in dogs has been vital for development of clinically useful strategies for GVHD prophylaxis and treatment advances in donor leucocyte infusions.[36,46,47] Largely on the basis of these experimental data, progression of acute GVHD can be summarised in three sequential steps or phases: (1) activation of APCs; (2) donor T-cell activation, proliferation, differentiation, and migration; and (3) target tissue destruction (figure 3).

The first step entails activation of APCs by the underlying disease and the HCT conditioning regimen. Damaged host tissues respond by producing so-called danger signals, including proinflammatory cytokines (eg, TNFα and interleukins 1 and 6), chemokines, and amplified expression of adhesion molecules, MHC antigens, and costimulatory molecules on host APCs.[42,48–50] Findings of a report showed that 1 week after HCT, increased amounts of TNFα receptor 1—a surrogate marker for TNFα—correlated strongly with later development of GVHD.[51] Injury to the gastrointestinal tract from conditioning is especially important because it allows for systemic translocation of additional inflammatory stimuli, such as microbial products including lipopolysaccharide or other pathogen-associated molecular patterns, that further enhance activation of host APCs.[49]

The secondary lymphoid tissue in the gastrointestinal tract is probably the initial site of interaction between activated APCs and donor T cells.[52] These observations have led to an important clinical strategy to diminish acute GVHD by reducing the intensity of the conditioning regimen.[53,54] Experimental GVHD can also be decreased

Pharmacyclics Exhibit 2007 - Page 3 of 12
Sandoz v. Pharmacyclics - IPR2019-00865
DOCKET ALARM
Find authenticated court documents without watermarks at docketalarm.com.

by manipulation of distinct subsets of APCs.[55,56] Furthermore, non-haemopoietic stem cells, such as mesenchymal stromal cells, can reduce allogeneic T-cell responses and ameliorate GVHD, although the mechanism for such inhibition remains unclear.[57]

The idea that amplified activation of host APCs increases the risk for acute GVHD unifies several seemingly disparate clinical associations with that risk, such as advanced stages of malignant disease, more intense transplant conditioning regimens, and history of viral infection. APCs detect infections with receptors on their cell surfaces, such as Toll-like receptors, which recognise conserved molecular patterns of microbes.[27,58] Toll-like receptors specific for viral DNA or RNA activate APCs and could boost GVHD, providing a potential mechanistic basis for enhanced disease associated with viral infections such as cytomegalovirus.[59]

The core of the graft-versus-host reaction is the second step, in which donor T cells proliferate and differentiate in response to host APCs (figure 3). The danger signals generated in the first phase augment this activation, at least in part, by increasing expression of costimulatory molecules.[60] Blockade of costimulatory pathways to prevent GVHD is successful in animal models, but this approach has not yet been tested in large clinical trials.[2]

In mouse models, in which genetic differences between donor and recipient strains can be tightly controlled, CD4+ cells induce acute GVHD to MHC class II differences and CD8+ cells induce acute disease to class I differences.[61,62] In most HLA-identical HCTs, both CD4+ and CD8+ subsets respond to minor histocompatibility antigens and can cause GVHD in HLA-identical procedures.

Regulatory T cells can suppress proliferation of conventional T cells and prevent GVHD in animal models when added to donor grafts containing conventional T cells,[63] but use of regulatory T cells in clinical acute GVHD will need enhanced techniques to identify and expand them. Natural killer T-cell 1.1+ subsets from the host and donors have also been shown to modulate acute GVHD.[64] In a clinical trial of total lymphoid irradiation (as conditioning), GVHD was reduced significantly and host natural killer T-cell function was amplified.[65]

Activation of immune cells results in rapid intracellular biochemical cascades that induce transcription of genes for many proteins, including cytokines and their receptors. T-helper 1 cytokines (interferon γ, interleukin 2, and TNFα) are released in large amounts during acute GVHD. Production of interleukin 2 by donor T cells remains the main target of many current clinical therapeutic and prophylactic approaches to GVHD, such as cyclosporine, tacrolimus, and monoclonal antibodies directed against this cytokine and its receptor.[9] However, emerging data indicate an important role for interleukin 2 in the generation and maintenance of CD4+CD25+ regulatory T cells, suggesting that prolonged interference with this cytokine could unintentionally stop development of long-term tolerance after allogeneic HCT.[66]

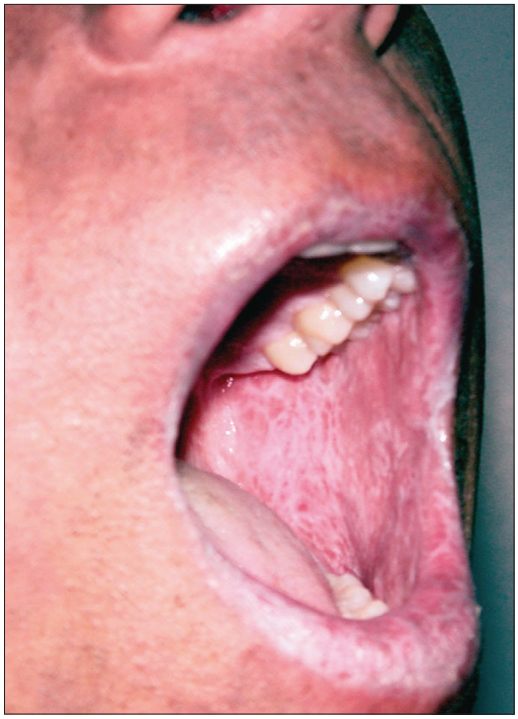

Figure 2: **Lichenoid changes of buccal mucosa in chronic GVHD**
Photograph courtesy of J Ferrara and J Levine.

Interferon γ has many functions and can either amplify or reduce GVHD.[67,68] It could boost disease by increasing expression of molecules such as chemokine receptors, MHC proteins, and adhesion molecules; it also raises the sensitivity of monocytes and macrophages to stimuli such as lipopolysaccharide and accelerates intracellular cascades in response to these stimuli.[69] Early polarisation of donor T cells so that they secrete less interferon γ and more interleukin 4 can also attenuate experimental acute GVHD.[70] Interferon γ might amplify GVHD by direct damage to epithelium in the gastrointestinal tract and skin and by induction of immunosuppression by generation of nitric oxide.[71] By contrast, this cytokine could suppress GVHD by hastening apoptosis of activated donor T cells.[68,72] This complexity means manipulation of interferon γ could have diverse effects in vivo, making the cytokine a challenging target with respect to therapeutic intervention.

Interleukin 10 has a key role in suppression of immune responses, and clinical data suggest it might regulate acute GVHD.[17] Transforming growth factor β, another suppressive cytokine, can subdue acute GVHD but exacerbate chronic disease.[73] Thus, timing and duration of secretion of any given cytokine could establish the specific effects of that molecule on GVHD severity.

DOCKET ALARM
Pharmacyclics Exhibit 2007 - Page 4 of 12
Sandoz v. Pharmacyclics - IPR2019-00865
Find authenticated court documents without watermarks at docketalarm.com.

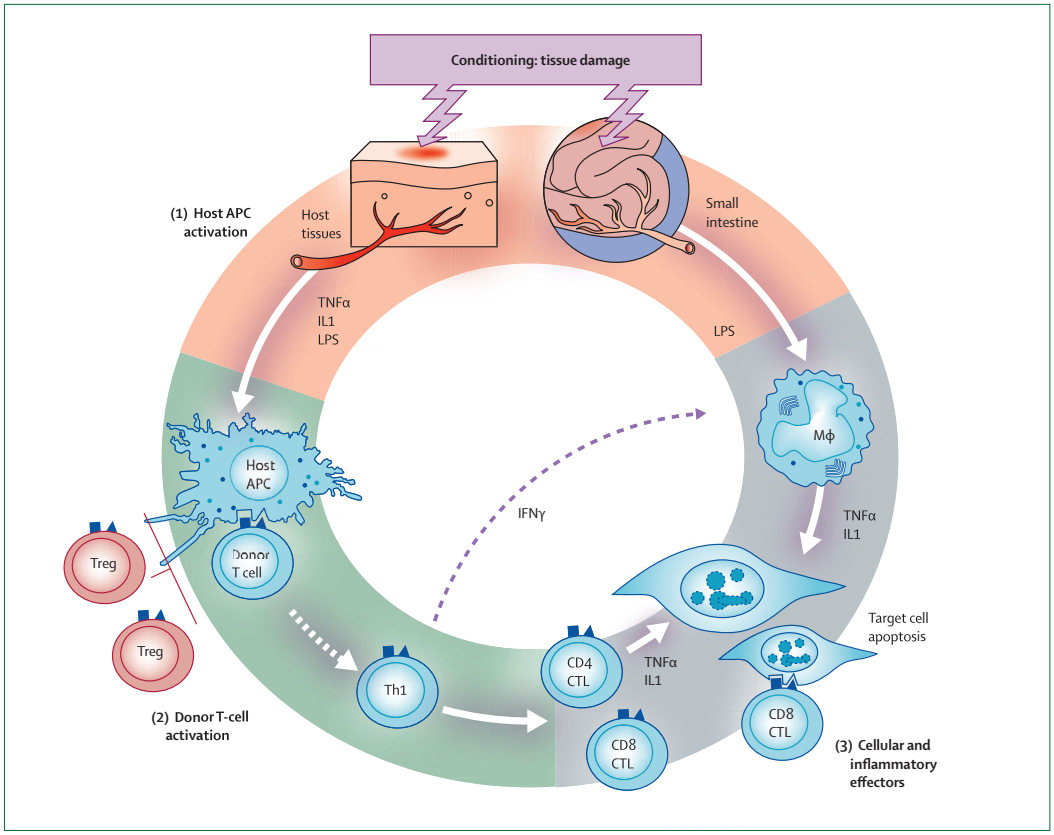

Figure 3: **Pathophysiology of acute GVHD**
IL 1=interleukin 1. IFN γ=interferon γ. LPS=lipopolysaccharide. Treg=regulatory T cell. Th1=T-helper 1 cell. CTL=cytotoxic T lymphocyte.

The third effector phase of the graft-versus-host process (figure 3) is a complex cascade of cellular mediators (such as cytotoxic T lymphocytes and natural killer cells) and soluble inflammatory agents (eg, TNFα, interferon γ, interleukin 1, and nitric oxide).[2,29] These molecules work synergetically to amplify local tissue injury and further promote inflammation and target tissue destruction.

The cellular effectors of acute GVHD are mainly cytotoxic T lymphocytes and natural killer cells.[49] Cytotoxic T lymphocytes that prefer to use the Fas and FasL pathway of target lysis seem to predominate in GVHD liver damage (hepatocytes express large amounts of Fas) whereas cells that use the perforin and granzyme pathways are more important in the gastrointestinal tract and skin.[2,74] Chemokines direct migration of donor T cells from lymphoid tissues to the target organs in which they cause damage. Macrophage inflammatory protein 1α and other chemokines (such as CCL2–CCL5, CXCL2, CXCL9, CXCL10, CXCL11, CCL17, and CCL27) are overexpressed and enhance homing of cellular effectors to target organs during experimental GVHD.[75] Expression of integrins, such as α4β7 and its ligand MADCAM1, is also important for homing of donor T cells to Peyer's patches during intestinal GVHD.[52,76,77]

Microbial products such as lipopolysaccharide, which leak through damaged intestinal mucosa or skin, can stimulate secretion of inflammatory cytokines through Toll-like receptors.[49,78] The gastrointestinal tract is especially susceptible to damage from TNFα, and the gastrointestinal tract has a major role in amplification and propagation of the cytokine storm characteristic of acute GVHD.[49] TNFα can be produced by both donor and host cells and it acts in three different ways: (1) it activates APCs and enhances alloantigen presentation; (2) it recruits effector cells to target organs via induction of inflammatory chemokines; and (3) it directly causes tissue necrosis (as its name suggests).[79–81]

Prevention of GVHD

On the basis of evidence from animal models for the central role of T cells in initiation of GVHD, many clinical studies of T-cell depletion as prophylaxis for the disease were undertaken in the 1980s and 1990s. Three main depletion strategies were studied: (1) negative selection

Pharmacyclics Exhibit 2007 - Page 5 of 12
Sandoz v. Pharmacyclics - IPR2019-00865
DOCKET ALARM
Find authenticated court documents without watermarks at docketalarm.com.

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.