Antiviral Chemistry & Chemotherapy 16:69–90

Review

Host cell targets in HCV therapy: novel strategy or proven practice?

Bert M Klebl[1]*, Alexander Kurtenbach[1], Kostas Salassidis[1], Henrik Daub[1] and Thomas Herget[2]

[1]Axxima Pharmaceuticals AG, Munich, Germany
[2]New Business Chemicals Bio, Merck KGaA Germany, Darmstadt, Germany

*Corresponding author: Tel: +49 89 55065 460; Fax: +49 89 55065 461; E-mail: bert.klebl@axxima.com

The development of novel antiviral drugs against hepatitis C is a challenging and competitive area of research. Progress of this research has been hampered due to the quasispecies nature of the hepatitis C virus, the absence of cellular infection models and the lack of easily accessible and highly representative animal models. The current combination therapy consisting of interferon-α and ribavirin mainly acts by supporting host cell defence. These therapeutics are the prototypic representatives of indirect antiviral agents as they act on cellular targets. However, the therapy is not a cure, when considered from the long-term perspective, for almost half of the chronically infected patients. This draws attention to the urgent need for more efficient treatments. Novel anti-hepatitis C treatments under study are directed against a number of so-called direct antiviral targets such as polymerases and proteases, which are encoded by the virus. Although such direct antiviral approaches have proven to be successful in several viral indications, there is a risk of resistant viruses developing. In order to avoid resistance, the development of indirect antiviral compounds has to be intensified. These act on host cell targets either by boosting the immune response or by blocking the virus host cell interaction. A particularly interesting approach is the development of inhibitors that interfere with signal transduction, such as protein kinase inhibitors. The purpose of this review is to stress the importance of developing indirect antiviral agents that act on host cell targets. In doing so, a large source of potential targets and mechanisms can be exploited, thus increasing the likelihood of success. Ultimately, combination therapies consisting of drugs against direct and indirect viral targets will most probably provide the solution to fighting and eradicating hepatitis C virus in patients.

Keywords: HCV, interferon, ribavirin, indirect (cellular) and direct (viral) antiviral targets, (in)direct antiviral agent/compound, protein kinase, signal transduction, glutathione peroxidase

Introduction

Hepatitis C is a serious threat to a significant percentage (1–2%) of the global population. This review summarizes the course of the disease and existing treatments, describes products under investigation and explains their mode of action on direct and indirect antiviral targets. Direct viral targets are those that are encoded by the viral genome. Indirect viral targets are those encoded by the host cell genome and are functions that are either usurped by the virus to allow its propagation or play important roles in the immune response and immunomodulation. We discuss here some exciting approaches to discovering and exploiting novel, cellular or indirect antiviral targets as potential therapies. These targets might be exploited to serve as a basis for the generation of new and powerful medications against hepatitis C.

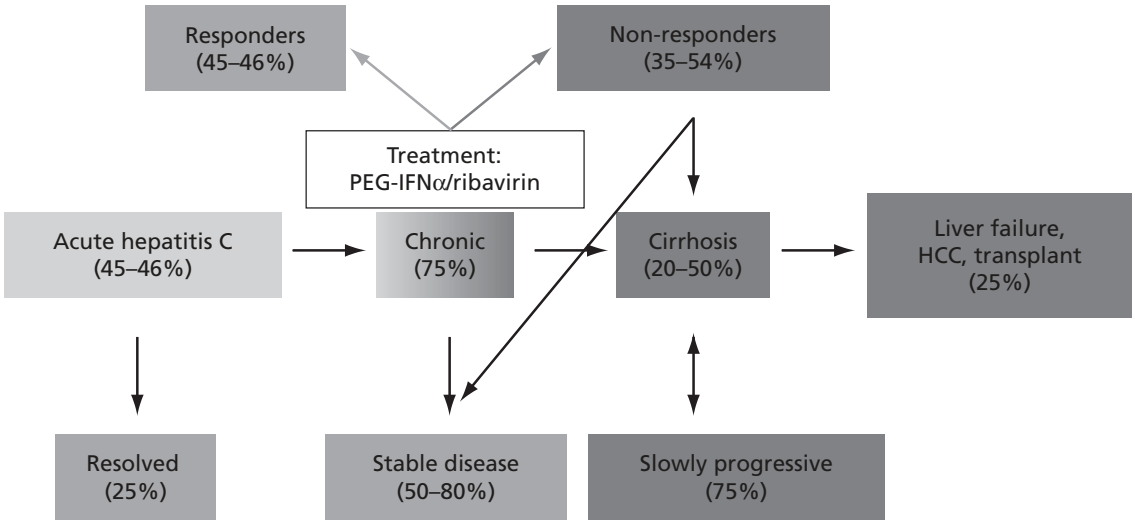
HCV: the disease and its consequences

Chronic viral hepatitis is a common disease. More than 500 million people suffer from chronic viral hepatitis worldwide, due to chronic infection with hepatitis B virus, hepatitis D virus or hepatitis C virus (HCV). Chronic viral hepatitis is the main cause of cirrhosis and hepatocellular carcinoma (HCC), which are responsible for major morbidity and mortality worldwide. There are 350 million cases of chronic hepatitis B infection and 170 million cases of chronic hepatitis C infection (Marcellin & Boyer, 2003). With no prophylactic or therapeutic vaccines available to date, this will create a major health crisis during the next decade. HCV escapes the immune system and establishes a persistent infection in approximately 75% of cases. These chronic carriers are at risk of developing life-threatening liver disease, such as cirrhosis and HCC. Five million

people in Europe and 4 million people in the USA are chronically infected, with an estimated 20–50% of these patients likely to develop cirrhosis within the next 20–30 years (Vogel, 2003). The entire spectrum of outcome ranges from a very slow progression over 50 years to an accelerated progression to cirrhosis within 5 years (Zoulim *et al.*, 2003). In retrospective studies of post-transfusion hepatitis C, some 20–50% developed cirrhosis and 5–25% developed HCC after 10–30 years (Durantel *et al.*, 2003) (Figure 1). Cases of decompensated liver cirrhosis ultimately undergo liver transplantation. Even after liver transplantation, the rate of relapses is high, which is most probably due to extrahepatic infection, subsequently resulting in re-infection of the transplanted liver. Several reports indicate that HCV can also infect organs and cell types other than the liver, particularly lymphoid cells (Dammacco *et al.*, 2000). Furthermore, HCV RNA can persist at very low levels in the serum and peripheral lymphoid cells and can persist in peripheral blood monocytes for many years after spontaneous or antiviral therapy-induced resolution of chronic hepatitis C (Pham *et al.*, 2004). Negative-strand HCV RNA has been reported in the brain, providing evidence that the central nervous system is an additional site of HCV replication (Forton *et al.*, 2004). This may explain the cerebral dysfunction found in chronically HCV-infected patients. These extrahepatic infections might contribute to the immune-mediated pathogenesis of chronic liver disease and/or the development of auto-immune diseases, including mixed cryoglobulinaemia. The presence of abnormal serum levels of cryoglobulins can damage the kidneys and cause glomerulonephritis, a kidney disease affecting the capillaries of the glomeruli (the compact cluster of capillaries in the kidney that filter blood). The protein is characterized by oedema, raised blood pressure and excess protein in the urine.

A prognosis for chronically infected HCV patients can be made, relying on biochemical and histological parameters (Marcellin & Boyer, 2003). Important parameters for the long-term prognosis of chronically infected patients are the initial level of viral load in the serum and the early virological response towards interferon (IFN) treatment (Durantel *et al.*, 2003). In general, ~25% of patients have normal serum alanine aminotransferase (ALT) levels despite detectable HCV RNA in serum; liver histological lesions are generally mild, cirrhosis is rare and their prognosis is good. The majority, ~50%, of chronically infected patients progress very slowly and the long-term risk of developing cirrhosis is low. Approximately 25% of the chronically infected patients have moderate to severe chronic hepatitis. Liver biopsies are used to diagnose

Figure 1. Progression of HCV infection



Flow chart for the progression and prognosis of acute hepatitis C infection. Patients suffering from acute hepatitis C have a 25% chance of resolving the disease and a 75% chance of developing chronic hepatitis C. Without treatment, chronic hepatitis C patients develop either stable disease or cirrhosis. Of cirrhotic patients, 75% are slowly progressive and 25% develop hepatocellular carcinoma (HCC), ultimately undergoing liver failure requiring transplantation. Of chronic patients, 46–65% who are treated with PEG-IFN-α/ribavirin combination are long-term responders. The remaining 35–54% are non-responders and subject to the same conditions as non-treated chronic patients.

moderate or severe chronic hepatitis. These patients are at elevated risk of developing HCV-related cirrhosis, which can lead to mortality due to portal hypertension, hepatic failure or HCC. These numbers are representative of non-treated HCV patients. Approximately 250000 chronically infected HCV patients receive therapy per year. Only 46–65% of these patients respond to the current drug regimen. The remaining 35–54%, the so-called non-responders, face disease comparable with non-treated chronically infected patients, leading to a significant number of cirrhotic patients with an elevated risk of developing HCC and liver failure (Figure 1).

Incidences and transmission

De novo infections are still considered to occur at the rate of 20–25 cases per 100000 persons per year (Vogel, 2003). HCV is mainly transmitted through contact with blood and blood products, with blood transfusions and sharing of non-sterilized needles and syringes being the main routes (Poynard *et al.*, 2003). With the advent of routine blood screening for HCV antibodies in 1991, transfusion-related hepatitis C has almost disappeared and the incidence has been in decline (Marcellin & Boyer, 2003).

Treatments and perspectives

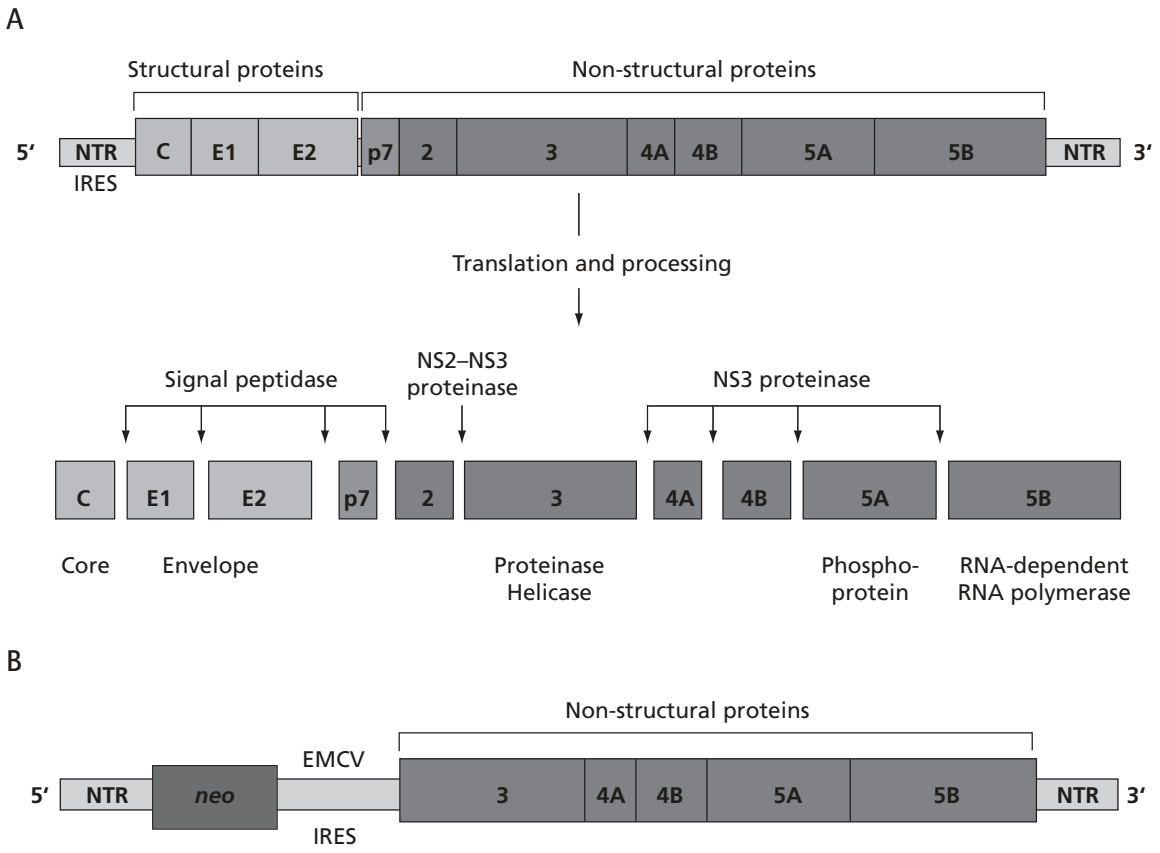
Because of the small number of symptomatic patients, randomized trials to identify new regimens in acute hepatitis C have been rare. So far, the standard treatment for chronic HCV infection, IFN-α, has been found to be effective for acute hepatitis C (Poynard *et al.*, 2003). The aim of the antiviral therapy is to cure viral infection and thereby prevent the progression of liver disease towards cirrhosis and HCC. Ten years ago, IFN-α monotherapy was approved by the Food and Drug Administration (FDA) as the basis of therapies for chronic viral hepatitis (Tan *et al.*, 2002). Subsequently, a combination treatment (Davis *et al.*, 1998; McHutchinson *et al.*, 1998), consisting of IFN-α and ribavirin (Sidwell *et al.*, 1972) was introduced 6 years ago. The IFN-α/ribavirin combination has been considerably improved by the introduction of pegylated interferons (PEG-IFNs) (Vogel, 2003). Using either PEG-IFN-α2b (PEG-Intron®; Schering-Plough, Kenilworth, NJ, USA) or PEG-IFN-α2a (Pegasys®; Hoffmann-LaRoche, Basel, Switzerland) in combination with ribavirin (Rebetol®; Schering-Plough or Copesus®; Hoffmann-LaRoche), sustained viral responses (SVRs) as high as 46–65% have been achieved for chronically infected patients (Figure 1). These clinically manifested results have turned the combination treatment into the most efficacious therapy for HCV currently available (Vogel, 2003).

Only a minor fraction of chronically infected patients receive medication, indeed, less than 1%. The costs of complications, including decompensated cirrhosis and liver transplantation, may far exceed the medication costs for PEG-IFN-α/ribavirin treatment. In the absence of a more affordable combination treatment, we expect a pharmacoeconomic health crisis for the industrialized world, let alone in the major areas of virus dissemination in Asia, with a total of 9 million people infected with HCV in the USA and Europe (Vogel, 2003). Since 35–54% of patients are still non-responders to the currently existing and expensive therapies, it has become obvious that there is a huge need for novel treatment alternatives and medications. Without effective treatment strategies, HCV-related morbidity and mortality are expected to increase nearly threefold by the year 2015. The age-adjusted death rate in 1999 was 1.8 per 100000 persons in the USA (Kim, 2002). The development of small molecule drugs will become particularly important as they are typically associated with lower production and development costs. Novel treatment options should not only address the patient population of IFN non-responders, but also try to shorten the overall treatment period, which currently takes up to 24 months. Equally important, although difficult to achieve due to the quasispecies nature of HCV (a quasispecies is a family of closely related, but slightly different, viral genomes; viral genetic variants, derived from the original infecting virus, which are present during an infection) and the large number of HCV genotypes, is the development of preventive and therapeutic vaccines, which are currently being tested in Phase II clinical trials (Pawlotsky & McHutchison, 2004).

HCV biology

HCV has been classified as the sole member of a distinct genus, Hepacivirus, in the family Flaviviridae, which also includes flaviviruses and pestiviruses. Originally cloned in 1989 (Choo *et al.*, 1989), the viral genome is now well characterized. HCV is an enveloped particle harbouring a plus-strand RNA molecule that is ~9600 nucleotides in length (Bartenschlager & Lohmann, 2000; Bartenschlager, 2002). The initiation of translation is mediated by the interplay of host and viral factors. An internal ribosome entry side (IRES), a complex RNA structure located at the 5′ non-coding region, serves to bind directly to ribosomes to initiate protein synthesis (Durantel *et al.*, 2003; Bartenschlager, 2002). The open reading frame (ORF) encodes a polyprotein of ~3000 amino acids in length, which is processed by both cellular and viral proteases into at least 10 discrete polypeptides (Figure 2A). The structural proteins (core or capsid, gpE1 and gpE2) are used for the assembly of new-progeny virus particles, whereas most of the non-structural (NS) proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) participate in the replication of

Figure 2. Schematic representation of **(A)** the HCV genome and **(B)** the sub-genomic replicon



(A) The major structural proteins include the core (C) and the envelope proteins (E1 and E2). The non-structural proteins p7, NS2, NS3, NS4A/NS4B and NS5A/NS5B are indicated. At the 5′ non-translated region (NTR) resides the internal ribosomal entry site (IRES), which is highly conserved and represents a site for development of translation inhibitors such as antisense oligonucleotides, ribozymes and small interfering RNAs (siRNAs). NS3 encodes a protein with a specific protease and helicase activity. The NS5A region encodes for a phosphoprotein and the NS5B for an RNA-dependent RNA polymerase enzyme, both important for viral replication. They also represent sites for the development of specific viral enzyme inhibitors. Other potential enzyme targets include the HCV-specific proteases (NS2/3 and NS3). These enzymes are involved in processing the viral polyprotein at specific sites as indicated. **(B)** The subgenomic replicon has been generated by replacing the region that encodes the core protein up to the NS2-encoding region by the neomycin phosphotransferase gene (*neo*) and the IRES of the encephalomyocarditis virus (EMCV). This IRES drives the translation of the HCV polypeptide from NS3 to NS5B, whereas the selectable marker *neo* is expressed under the control of the original HCV IRES of the 5′ non-translated region.

the viral genome. During viral replication, the viral genome acts as a template for the synthesis of negative-strand RNA, which, in turn, is a template for the production of excess amounts of positive-strand RNA progeny. Details concerning the initiation of the synthesis of the positive-strand RNA are not clearly known and only sparse information is available in terms of initial and late phases, for example, entry and morphogenesis of viral particles, since the field lacks a reproducible and efficient cell culture system for viral replication (Bartenschlager & Lohmann, 2000; Bartenschlager, 2002; Dymock *et al.*, 2000).

HCV consists of six different genotypes (genotypes 1–6). Knowledge of the genotype or serotype is helpful for prediction of SVR and the choice of treatment duration. Genotypes do not change during the course of an infection. Response rates to treatment with the combination of PEG-IFN and ribavirin are 88% for genotypes 2 and 3, and 48% for genotypes 1, 4, 5 and 6 (Poynard *et al.*, 2003). Unfortunately, genotype 1 is the most frequent genotype in Europe and the USA and is present in 60–80% of cases (Marcellin & Boyer, 2003). These insufficient response rates underscore the need for the development of novel and efficient genotype-

independent treatments and also argue for the further improvement of existing cellular and animal models.

Tissue culture model: the replicon system

Many aspects of HCV disease can only be addressed *in vivo*, for example, the influence of the immune system. For many investigatiors, however, it is sufficient and desirable to work with a less complex, well-controlled system *in vitro*. The recently generated HCV-replicon system, in which expression of the HCV non-structural proteins drives the replication of a subgenomic HCV RNA, fulfills these criteria (for reviews, see Bartenschlager & Lohmann, 2001; Bartenschlager, 2002).

The human hepatoma cell line (Huh-7) was transfected with subgenomic HCV RNAs called replicons (Lohmann *et al.*, 1999). These were derived from a cloned full-length HCV genome of genotype 1b (Figure 2A) by replacing the reading frame for the N-terminal proteins (including p7 or NS2) with the neomycin phosphotransferase gene (*neo*) downstream of the HCV IRES. Translation of the HCV NS2-5B or NS3-5B region was directed by the IRES of the encephalomyocarditis virus (EMCV) inserted downstream of the *neo* gene (Figure 2B). Upon transfection of Huh-7 cells, only those in which HCV RNA replication occurs develop continuous resistance against the drug geniticin due to *neo* expression. Cell lines obtained from such resistant colonies contain high levels of replicon RNAs and viral proteins (Lohmann *et al.*, 1999; Pietschmann *et al.*, 2001). This allows the study of HCV RNA replication as well as of translation and processing of those HCV proteins present in the system (Figure 2).

The development of subgenomic replicons containing HCV non-structural proteins that replicate in human hepatoma cells, now provides a system to test or screen candidate drugs that target the non-structural gene products. Thus, the antiviral efficacy of BILN 2061, a NS3 protease inhibitor, the development of which was put on hold after entering Phase II clinical trials (see Table 1), was determined in the HCV replicon cell culture system. BILN 2061 has an IC_{50} of 4 nM (genotype 1a) and 3 nM (genotype 1b), while cytotoxicity analysis in parental Huh-7 cells produced a cytotoxic concentration (CC_{50}) of 16–35 μM (Pause *et al.*, 2003; Lamarre *et al.*, 2003). As these subgenomic replicons do not produce infectious virions, the option of studying encapsidation or cell-to-cell viral transmission is not provided. Furthermore, the replicon cell lines are of clonal origin and have, apart from adaptive mutations, identical HCV sequences (Krieger *et al.*, 2001). However, the genetic heterogeneity of HCV suggests that future drugs should display activity against a broad range of HCV genotypes, subtypes and quasispecies.

In summary, the HCV-replicon RNA replicates to fairly high levels in Huh-7 cells and provides, for the first time, a genetic system to study HCV RNA replication and a cell-based assay screen for HCV inhibitors. Such a replicon-based screen for anti-HCV substances has been described in a recent study on the nucleoside antimetabolite-mediated reduction of HCV RNA (Stuyver *et al.*, 2003). In this study, a specific anti-HCV replicon effect was defined as minimal interference with the exponential cell growth, minimal reduction in cellular host RNA levels and reduction of the HCV RNA copy number per cell compared with that of the untreated control (Stuyver *et al.*, 2003).

Animal models

Developing robust animal model systems for HCV is highly desirable and some progress has recently been achieved (reviewed by Pietschmann & Bartenschlager, 2003). Besides the fact that ethical considerations do not permit experiments with humans, an animal system may allow for monitoring of the entire cycle of viral replication, from infection of naive tissues to full-blown viraemia. Since acute HCV infection often happens without any obvious symptoms, these patients do not consult a physician. Thus, valuable information about early events of HCV infection and remission is lacking. A model system will allow the study of each phase of the disease under controlled conditions.

Establishing a model system is hampered by the fact that HCV infects only humans and chimpanzees, primarily targeting hepatocytes. The determinants of the restricted host and tissue specificity are not understood. Despite the extremely robust replication rate of HCV in humans, efforts to propagate this virus in cell culture have been frustratingly unsuccessful. Therefore, the use of surrogate virus models closely related to HCV, such as the bovine viral diarrhoea virus (BVDV) and the tamarin GB virus-B (GBV-B), both which belong to Flaviviridae, provides alternative approaches. GBV-B is most closely related to HCV and is, therefore, a good surrogate model. For instance, one potential mechanism of action of the pleiotropic antiviral agent, ribavirin, was verified using the GBV-B model. The antiviral effect of ribavirin does not seem to be solely based on the inhibition of inosine 5′-monophosphate dehydrogenase (IMPDH) and reduction of intracellular pools of GTP and dGTP, but also by the incorporation of ribavirin triphosphate into viral RNA and induction of error-prone replication (Lanford *et al.*, 2001). However, the biological activity of a tested compound in these systems does not necessarily translate into efficacy for human HCV infection.

Transplant mouse models

Mice are the preferred models for scientific studies for a number of reasons (for example, short breeding cycles,

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.