New perspectives for preventing hepatitis C virus liver graft infection

Daniel J Felmlee, Audrey Coilly*, Raymond T Chung, Didier Samuel†, Thomas F Baumert†*

Hepatitis C virus (HCV) infection is a leading cause of end-stage liver disease that necessitates liver transplantation. The incidence of virus-induced cirrhosis and hepatocellular carcinoma continues to increase, making liver transplantation increasingly common. Infection of the engrafted liver is universal and accelerates progression to advanced liver disease, with 20–30% of patients having cirrhosis within 5 years of transplantation. Treatments of chronic HCV infection have improved dramatically, albeit with remaining challenges of failure and access, and therapeutic options to prevent graft infection during liver transplantation are emerging. Developments in directed use of new direct-acting antiviral agents (DAAs) to eliminate circulating HCV before or after transplantation in the past 5 years provide renewed hope for prevention and treatment of liver graft infection. Identification of the ideal regimen and use of DAAs reveals new ways to treat this specific population of patients. Complementing DAAs, viral entry inhibitors have been shown to prevent liver graft infection in animal models and delay graft infection in clinical trials, which shows their potential for use concomitant to transplantation. We review the challenges and pathology associated with HCV liver graft infection, highlight current and future strategies of DAA treatment timing, and discuss the potential role of entry inhibitors that might be used synergistically with DAAs to prevent or treat graft infection.

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*Contributed equally as first authors

†Contributed equally as last authors

Inserm, U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, Strasbourg, France (D J Felmlee PhD, Prof T F Baumert MD); **Université de Strasbourg, Strasbourg, France** (D J Felmlee, Prof T F Baumert); **Hepatology Research Group, Peninsula School of Medicine and Dentistry, University of Plymouth, Plymouth, UK** (D J Felmlee); **AP-HP Hôpital Paul Brousse, Centre Hépato-Biliaire, Villejuif, France** (A Coilly MD, Prof D Samuel MD); **University Paris-Sud, UMR-S 1193, Villejuif, France** (A Coilly, Prof D Samuel); **Inserm Unit 1193, Villejuif F-94800, France** (A Coilly, Prof D Samuel); **Gastrointestinal Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA** (Prof R T Chung MD, Prof T F Baumert); **and Institut Hospitalo-Universitaire, Pôle Hépato-digestif, Hôpitaux Universitaires de Strasbourg, Strasbourg, France** (Prof T F Baumert)

Correspondence to:
Prof Thomas F Baumert, Inserm Unit 1110, Hôpitaux Universitaires de Strasbourg, 3 Rue Koeberlé, F-67000 Strasbourg, France
thomas.baumert@unistra.fr

or

Prof Didier Samuel, Inserm Unit 1193, Villejuif F-94800, France
didier.samuel@aphp.fr

Introduction

Hepatitis C virus (HCV) infection causes more than half of all liver transplantations in North America and Europe.[1–5] Furthermore, infection of engrafted livers is universal and accelerates progression to advanced liver disease, with 20–30% of patients having cirrhosis within 5 years of transplantation.[6] The engrafted liver always becomes infected and undergoes rapid progression of fibrosis to serious liver disease. HCV infection is, therefore, associated with the poorest post-transplantation survival rates compared with other causes leading to liver transplantation.[7] The accelerated natural history of allograft HCV infection in patients undergoing re-transplantation makes re-transplantation an ethically challenging proposition because organ shortages lead to selection of the best candidates in terms of survival, and patient and graft survival rates after re-transplantation are inferior to those after primary liver transplantation. Direct-acting antiviral agent (DAA) therapies, developed in the past 5 years, have proved effective in treating chronic HCV infection, and appear more effective in the liver transplant setting than conventional interferon-based treatments in patients with HCV genotype 1. However, treatment options are still limited for patients who need liver transplants as a result of HCV infection, as transplantation requires immunosuppressive drugs to avoid graft rejection with potential drug–drug interactions, the diminished health of this patient population, and the metabolic burden placed on the newly engrafted liver by co-administered pharmaceutical drugs.

The most straightforward means of avoiding the pathogenesis of liver graft infection would be to use preventive measures to avoid graft infection, but the strong efficacy of available DAAs could allow withholding antiviral treatment during the operative stage and addressing HCV infection postoperatively. Here, we review the specific hurdles associated with HCV infection in liver transplantation, evidence supporting treatment strategies of patients needing transplantation, and the outlook for prophylactic measures against liver graft infection.

Challenges of HCV liver graft infection

Universal graft infection in HCV RNA positive patients

Because of the burden of HCV on transplants, new, potent DAAs are anticipated to decrease transplantation activity, thereby reducing the number of patients presenting with hepatocellular carcinoma and decompensated cirrhosis.[8] To achieve this goal, however, comprehensive screening is necessary, because most patients with chronic HCV infection only seek medical care after liver-related complications.[9] A positive outlook is warranted given that findings from a 2013 analysis showed that a decline of more than 90% in total infections could be achievable by 2030, though this result will require a 3–5-times increase in diagnosis and treatment.[10] However, the public health strategy to address this widespread problem must hope for the best while planning for the worst.

HCV recurrence after liver transplantation remains universal in all patients with detectable serum HCV RNA before transplantation. 30% of patients who have undetectable levels of serum HCV RNA on therapy before transplantation relapse, excluding patients who have sustained virological response (SVR) to therapy for an extended period.[11] HCV recurrence is an important medical problem and is responsible for an increased risk of death and graft failure. Detection of HCV RNA in transplant recipients before transplantation is associated with diminished 5-year patient survival (69·9% *vs* 76·6%, p<0·0001) and allograft survival (56·8% *vs* 67·7%, p<0·0001) compared with patients who had a transplant for an indication other than HCV.[12]

Reinfection is not only a serious problem for the recipient, but also impacts negatively on the precious resource of suitable donated organs.

Rapid fibrosis progression after liver transplantation

The diminished 5-year survival rate in patients who had a transplant because of HCV infection is attributed to accelerated development of pathology due to the immune-suppressive drugs given to prevent graft rejection. Whereas the average time of progression from initial HCV infection to cirrhosis is about 30 years, 20–30% of transplant recipients develop cirrhosis within 5 years.[6] Whereas only 30% of non-transplant patients with cirrhosis have liver decompensation after 10 years of cirrhosis, more than 40% of graft recipients decompensate within 12 months of liver transplantation, of whom less than 50% survive the following year. Progression to fibrosis in the context of HCV recurrence varies widely depending on individual patient characteristics, whereas the average time of progression to cirrhosis after liver transplantation is 10–12 years.[13] Re-transplantation is the only therapeutic option to achieve long-term survival of patients with decompensated cirrhosis after transplantation. Because of poor patient and graft post-transplant survival rates and the paucity of suitable organ donations, re-transplantation is not a sustainable option in most countries.[14]

An important clinical challenge is to identify scenarios of early and rapid fibrosis development in which to use early intervention while minimising liver damage, which highlights the importance of the development of diagnostics. The previous consensus was that interferon-based antiviral therapy should be initiated after detection of chronic hepatitis of the liver graft, usually greater than F1 on the METAVIR fibrosis scoring system. The diagnosis of HCV recurrence is typically based on liver biopsy detection, because biopsies can reveal severity of disease progression and exclude other possible diagnoses. Substantial periportal sinusoidal fibrosis in early biopsies (<6 months) has been shown to be a good predictor of severe HCV recurrence.[15] Use of serum markers that decisively indicate fibrosis progression and other non-invasive techniques that measure liver stiffness will contribute to future decision making in post-transplantation HCV treatment. Liver stiffness values of less than 8·7 kPa have a 90% negative predictive value and can be used as a threshold to define substantial fibrosis.[16] Pressure levels exceeding 6 mm Hg of hepatic venous pressure likewise indicate fibrosis.[17]

Robust recurrence of HCV RNA levels soon after transplantation is associated with poor prognosis, so early monitoring of HCV levels is crucial. Robust recurrence occurs in 2–8% of patients and often results in fibrosing cholestatic hepatitis. Fibrosing cholestatic hepatitis is characterised by high levels of cholestatic enzymes and the presence of extensive dense portal fibrosis with immature fibrous bands extending into the sinusoidal spaces, ductular proliferation, cholestasis, and moderate mononuclear inflammation detected in liver graft biopsies.[18] If symptoms do not improve with antiviral drug treatment, fibrosing cholestatic hepatitis typically proceeds to complete liver failure.

Several risk factors that contribute to rapid and severe fibrosis progression have been identified. High HCV RNA levels in either serum or liver tissue are associated with increased progression to cirrhosis, graft loss, and death.[19] Recipient and donor characteristics associated with poor outcome include female sex, donor age, and graft steatosis, while HLA matching and *IL28B* (also known as *IFNL3*) genotype negatively associate with poor outcome.[4,20,21] Although some of these factors can be selected for before transplantation, others are unpredictable and only antiviral treatment can improve the prognosis of transplant recipients.

The strategic options of HCV treatment with liver transplantation can be divided based on timing of treatment, HCV clearance before transplantation, inhibition of graft infection concomitant with transplantation, or antiviral treatment after graft infection (figure 1).

DAA-based strategies for prevention and treatment of liver graft infection

HCV cure before transplantation

The optimum strategy is, clearly, to prevent reinfection early by eliminating HCV infection prior to transplantation. This strategy had been difficult to apply until approval of DAAs, because interferon-based therapies have limited effectiveness for those with advanced disease while on a transplantation waiting list with SVR being achieved in only 8–39% of cases. Tolerability of interferon treatment is generally poor in these patients and contraindicated in patients with decompensated cirrhosis who require either dose reduction (70%) or early discontinuation (30%) of treatment. The results of a phase 2 clinical study[28] of sofosbuvir with ribavirin in 61 patients on waiting lists for liver transplants show that this approach with DAAs is efficacious. Of 43 patients who had a viral response prior to transplantation, 70% had sustained viral clearance 12 weeks after transplantation. However, the efficacy of this strategy is genotype-dependent and managing DAA combinations in the pre-transplant period is challenging. The use of sofosbuvir and ribavirin in advanced cirrhosis could contribute to lactic acidosis in approximately 14% of patients.[29] In the SOLAR-1 trial,[30] Charlton and colleagues investigated the NS5A inhibitor ledipasvir in combination with sofosbuvir and ribavirin for patients with cirrhosis and moderate or severe hepatic impairment due to genotype 1 and 4 infections. SVR12 was achieved in 86–89% in this difficult-to-treat cohort. The inclusion of ledipasvir with sofosbuvir and ribavirin has been investigated in the SOLAR-2 study[31,32] and inclusion of daclatasvir with sofosbuvir and ribavirin was studied in

the ALLY-1[33] trial, focusing on patients with advanced liver disease pre-transplantation or with recurrent HCV post-transplantation. In the SOLAR-2 study, patients with the sofosbuvir-sensitive HCV genotypes 1 or 4 were treated for 12 or 24 weeks with sofosbuvir, ledipasvir, and ribavirin. Preliminary results revealed high SVR rates of 85–88%, irrespective of treatment duration in genotype 1. Longer treatment duration was superior (SVR rate of 86% *vs* 57%) for patients with genotype 4 infection, and effective antiviral therapy was associated with improvement in liver histology, Model for End Stage Liver Disease (MELD), and Child-Pugh (CP) scores.[32] For some patients with decompensated cirrhosis, however, MELD or CP scores increased. Searching for prognostic factors of clinical or biological response instead of only viral response is an ongoing and needed area of investigation.

The ALLY-1 phase 3 study[33] included 60 patients with advanced cirrhosis who were given daclatasvir, sofosbuvir, and ribavirin. Although overall the SVR12 was 83%, response depended on severity of liver disease (92% for CP A [lowest severity], 94% for CP B, and 56% for CP C [highest severity]). These findings suggest that further studies are needed to define the best therapy management for CP C patients.

Although the inclusion of sofosbuvir has had an effect on the management of genotype 1 infection, use of this drug did not substantially improve treatment for genotype 3 infection by comparison with genotype 1. Foster and colleagues[34] analysed the addition of NS5A inhibitors to sofosbuvir and ribavirin treatment in patients with decompensated cirrhosis caused by genotypes 1 or 3.[32] The response rates varied between 44% and 88%, depending on genotype, NS5A inhibitor, and the use of ribavirin. Addition of ledipasvir to sofosbuvir and ribavirin was inferior to daclatasvir plus sofosbuvir in patients with genotype 3 infection. Over 40% of patients had improved liver function with a mean improvement of more than 2 points on the MELD score. Overall, these combinations showed good efficacy results and safety profiles, although some patients had worsening of their MELD scores. However, severity of cirrhosis remains an impediment to response, even with the new combinations of DAAs. Although the combination of sofosbuvir and NS5A inhibitor velpatasvir for 12 weeks provides an SVR in over 95% of patients without cirrhosis,[35,36] SVR rate was 83% (n=90) in patients with decompensated cirrhosis.[37] However, the addition of ribavirin to this combination improves the SVR rate to 94%, even in patients with cirrhosis.[37]

The crucial argument for treatment before transplantation is the prospect of avoiding liver transplantation altogether for individuals with liver disease that has not progressed to hepatocellular carcinoma. About two-thirds of patients achieve clinical and biological improvement during treatment in studies that enrolled patients with decompensated cirrhosis.[30,33,37,38] However, our own analysis of the data revealed that a third of patients do not improve or worsen during treatment, regardless of antiviral response. For patients who do improve, the difference is often modest with variations of only 1 or 2 points in MELD score. In a meta-analysis[39] involving five studies and including 533 patients, 28% had an improvement of MELD score over 3. Some patients do improve to the point where liver transplantation is avoidable. A French cohort study[38] of 183 patients awaiting transplantation showed that of 53 patients with decompensated cirrhosis, 36% had a complete clinical and biological response, meaning a CP A at the end of treatment. The best improvement was in patients with the least disease progression—those with baseline CP with an area under the curve of 0·81; CP threshold of improvement 7·5. This raises further questions, because some patients keep improving over longer periods of follow-up. When considering longer follow-up times for patients while on waiting lists for liver transplantation, comorbidities need to be considered. Optimum conditions and thresholds have not been defined for removing patients from waiting lists. Although we could expect significant improvement for a third of patients, this improvement is more likely to be seen in patients with less severe disease. For individuals with more severe disease, caution should be taken

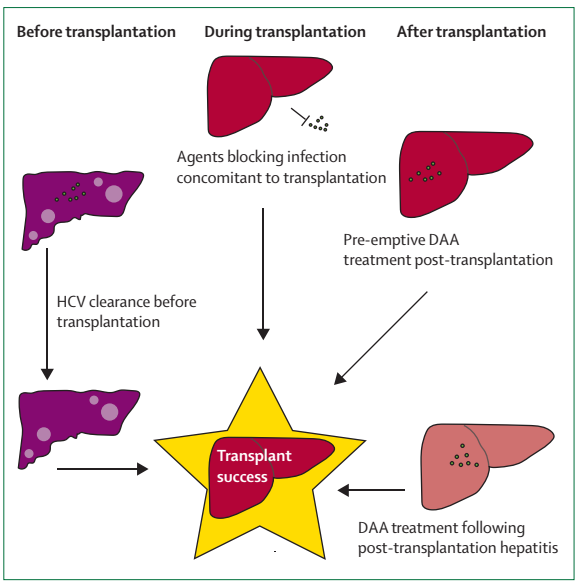

Figure 1: **Timing of antiviral strategies for successful liver transplantation in HCV-infected patients**
After a patient presents with a cirrhotic liver (upper left) with HCV infection (green dots), multiple strategies have been proposed for a successful, non-infected transplantation. Successful treatment that eliminates the virus before transplantation (left) has been successful in approximately 70% of patients given DAAs.[21] Evidence has shown that immunoprevention and HCV entry antagonists can impair infection concomitant to transplantation (centre, top).[22–25] Allowing infection and treating later with DAAs (right, top and bottom) has proven successful in 70–97% of cases depending on the study and drug used.[26,27] DAA=direct-acting antiviral. HCV=hepatitis C virus.

	Type of study	n	Genotype	Cirrhosis (percentage of patients with cirrhosis, %)	SVR12	Reference, trial name
Sofosbuvir+ribavirin 24 weeks	Prospective, multicentre, open-label	40	All (83% G1)	Yes (40%)	70%	Charlton[56]
Sofosbuvir+daclatasvir+ribavirin 12 weeks	Prospective, multicentre, open-label	53	All (77% G1)	Yes	94%	Poordad,[33] ALLY-1
Sofosbuvir+daclatasvir±ribavirin 12 or 24 weeks	Prospective, multicentre, real-life cohort	130	All (82% G1)	Yes (31%)	96%	Coilly,[58] ANRS CO23 CUPILT
Paritaprevir+r-ombitasvir +dasabuvir+ribavirin 24 weeks	Prospective, multicentre, open-label	34	Only G1	No	97%	Kwo,[59] CORAL-I
Sofosbuvir+ledipasvir+ribavirin 12 or 24 weeks	Prospective randomised phase 2 study	444	G1 (>95%) and G4	Yes (about 50%)	92%	Charlton,[30] SOLAR I; Manns,[31] SOLAR II
Sofosbuvir+simeprevir±ribavirin 12 weeks	Prospective, multicentre, open-label	109	Only G1	F3–F4 (29%)	90%	Pungpapong[60]
Sofosbuvir+simeprevir±ribavirin 12 weeks	Prospective, multicentre, real-life cohort	143	All (80% G1)	Yes (56%)	90% (SVR4)	Brown,[61] HCV-TARGET

r=boosted with ritonavir. SVR12=sustained virological response at 12 weeks. G1=genotype 1. F3–F4=METAVIR fibrosis score 3–4. SVR4=sustained virological response at 4 weeks.

Table: **Available results of DAA-based regimens to treat HCV recurrence after liver transplantation**

because treating HCV in patients only to achieve partial biological improvement might be deleterious.

Patients on transplantation waiting lists with hepatocellular carcinoma, who normally present with compensated cirrhosis, have several approved regimens available, albeit regimens with limited efficacy. In fact, treatment can be unsuccessful in about 30% of patients considering the rate of dropout before transplantation and early hepatocellular carcinoma recurrence after liver transplantation.[40] The severe disease state of patients on the transplantation waiting list can limit treatment options and the 12 weeks needed to confirm SVR status is not always afforded before transplantation. Patients with severe end-stage liver disease prior to liver transplantation or who require complicated post-operative treatment are frequently ineligible for pre-emptive interferon therapy.

DAA treatment after HCV graft infection

At present, two therapeutic approaches can be considered after transplantation: the pre-emptive strategy involving treatment in the first month following transplantation, or not to start treatment until chronic hepatitis is observed. Despite the clear benefits of early treatment, the pre-emptive strategy is historically not used because of safety and efficacy limitations of initiating interferon-based antiviral therapy during the postoperative period.[41–45] New DAA, interferon-free combination therapy could revive this strategy, although the evidence regarding efficacy is scarce because of these therapies are new. Factors affecting the future use of the pre-emptive strategy will depend on safety, cost, and tolerability of next-generation DAAs in relation to typical liver graft damage incurred before assessment of HCV recurrence.

Conversely, treating HCV recurrence has been the standard therapy and, until 2011, involved 48 weeks of PEG-interferon and ribavirin treatment. Three systematic reviews[26,27,46] have reported an SVR rate in these conditions of only approximately 30% with limitations of tolerability including bacterial infections, haematological toxicity, and graft rejection. Early virological response (EVR) is a major predictive factor associated with SVR.[47,48] However, effective antiviral treatment after transplantation has clear benefits in preventing disease progression.[49–54] First-generation protease inhibitors, telaprevir or boceprevir, were the first drugs to be tested in the treatment of recurrent HCV after transplantation. Their inclusion with PEG-interferon and ribavirin improved SVR rates by 50–65% in recipients infected with genotype 1 HCV, but with a worse safety profile and potent drug–drug interactions.[55,56] Although feasible, these regimens required close monitoring and expertise of caregivers leading to their withdrawal. The development of sofosbuvir added to ribavirin further changed the treatment landscape in post-transplantation antiviral administration, which is shown in two studies[28,56] where initiation of treatment took place a year after liver engraftment. Sofosbuvir plus ribavirin treatment has a 70% efficacy rate in yielding SVR that is roughly equal to the virological response seen when clearing the virus before transplantation.[28] Although this response efficacy is not optimum, it shows efficacy and tolerability even in the most severe patients.[57] However, a more complex combination of DAAs can be more efficacious, and several studies describing this finding have already been published (table). Data come from both open-label studies and real-life cohorts (HCV-TARGET[61] and CUPILT[58,62] studies). The SVR12 rates are usually greater than 90%,

better than SVR12 rate treating patients with decompensated cirrhosis prior to liver transplantation, and well tolerated. In the SOLAR-1 study[30] assessing post-transplantation treatment, progressive liver disease was associated with lowered response; however, all six individuals who had fibrosing cholestatic hepatitis achieved SVR 12 weeks after the end of treatment. Although the treatment of HCV in transplant patients has been substantially improved and simplified, several issues remain to be clarified. The efficacy of these studies shows the promise of DAAs and combinatorial therapy; multiple targets and mechanisms of action synergise to eliminate the virus.

The optimum duration of therapy remains to be defined. Although some risk factors of treatment failure were identified for interferon-based regimens, no risk factors have been identified for new DAAs, except in HCV genotype 3 infection. In the non-transplant setting, most studies comparing different treatment durations did not show any benefit of longer treatment, and better adherence, fewer side-effects, and lower cost were associated with a shorter duration. In the transplant setting, robust data are scarce and many studies conservatively use 24 weeks of treatment in this special population until more evidence is collected.

Benefits of the use of ribavirin in future regimens are not yet established and use of ribavirin could be abandoned once next-generation DAAs with higher efficacy are added. There remains a great benefit of ribavirin in patients with severe liver disease and recurrent HCV post-transplantation.[34,63,64]

In liver transplant patients, renal impairment is common and should be properly assessed before initiating antiviral therapy, especially sofosbuvir-based regimens.[65] The metabolism of sofosbuvir takes place in the kidney and its use is not recommended in patients with creatinine clearance below 30 mL/min until an appropriate dose is determined. A phase 2b, open-label study of 200 mg or 400 mg sofosbuvir and ribavirin for 24 weeks in patients infected with HCV genotype 1 or 3, and ledipasvir or sofosbuvir in individuals with genotype 1 and 4 infection with renal insufficiency is ongoing (ClinicalTrials.gov, number NCT01958281). For other available DAAs, the metabolism takes place in the liver. Although no detrimental effect is expected in ribavirin-free combination, the ANRS C023 CUPILT group reported a slight, but significant, reduction in creatinine clearance during treatment (from 72·7 [SD 29·0] to 66·3 [25·7] mL/min between baseline and end of treatment, p<0·0001) using the combination of sofosbuvir and daclatasvir.[58] Further studies are needed to clarify if this decrease in clearance is seen in all patients or one subgroup that should be identified and monitored closely. In a multicentre trial[66] of liver transplant recipients with recurrent HCV infection treated with sofosbuvir based regimens, renal improvement was observed in 58% of patients. Patients with SVR at 12 weeks post-treatment were more likely to have renal improvement, indicating that HCV affects renal health.[66]

Drug–drug interactions between DAAs and immunosuppressive drugs, mainly calcineurin inhibitors, remain a concern with these regimens. Simeprevir, a second-generation protease inhibitor, is a partial cytochrome P450 (CYP) 3A inhibitor. Because the immunosuppressant drug ciclosporin is likewise a partial CYP3A inhibitor, combination results in accumulation of both drugs in the blood, and coadministration is discouraged.[67] Ombitasvir, paritaprevir, ritonavir, and dasabuvir require dosing modifications for calcineurin inhibitors tacrolimus and ciclosporin.[59,68] Conversely, sofosbuvir, ledipasvir, and daclatasvir do not seem to interact with calcineurin inhibitors.[68] However, close monitoring before, during, and after DAA therapy is essential. In the ANRS C023 CUPILT study,[58] 59% of 130 patients treated with sofosbuvir and daclatasvir after liver transplantation had to change dose of one immunosuppressive drug during therapy.

Finally, the optimum timing for initiation of therapy post-transplantation remains to be determined. Antiviral therapy is usually initiated only when histologically proven recurrent HCV occurs (fibrosis stage 2 or higher on the METAVIR score or severe and rapid progression of fibrosis as observed in cholestatic hepatitis). This decision was based on the tolerability of the classic interferon-based regimen, which required post-transplantation recovery time to restore health. Development of interferon-free therapy allows treatment of patients earlier after liver transplantation without waiting for disease markers indicating HCV recurrence. This strategy is reasonable, but scientific evidence of its efficacy is not available. Nonetheless, earlier treatment seems safe and effective and the potential risk of allowing fibrosis progression on liver graft could raise ethical issues. Treatment of patients early after liver transplantation could help to overcome the issue of differentiating HCV recurrence and rejection and it could also prevent graft rejection induced by viral clearance while immunosuppressive drug concentrations are still high at early stages after liver transplantation. Immunosuppressive drug concentrations have been reported to decrease substantially in patients responding to antiviral therapy as viral clearance improves hepatic microsomal function and elevated regulatory T-cell levels could decline.[69]

Prevention of graft infection concomitant with transplantation: HCV entry inhibitors

Viral entry has been shown to play an essential role during reinfection of the graft after liver transplantation.[70,71] Thus, concomitant treatment of safe and effective entry inhibitors, including virus-targeting neutralising antibodies (nAbs), during and immediately after transplantation could prove an effective means of preventing graft infection without allowing allograft

damage.[72] This strategy is supported by the experience in prevention of hepatitis B virus (HBV) graft infection where hepatitis B immune globulin in combination with nucleoside or nucleotide analogues can reduce HBV recurrence in liver transplant patients to 4%.[71,73–75] Entry inhibitors have been shown to effectively inhibit HCV infection, work synergistically with DAAs, and have proved to be safe and effective in humanised mice.[76] Although most of these drugs are in preclinical development, the results of the first clinical trials with anti-envelope antibodies[22,23,77] and a small molecule host-targeting inhibitor[24] suggest that they could be future instruments in the antiviral arsenal during transplantation. Strategies for blocking viral entry during liver graft infection can target either the virus or host entry factors.

Anti-envelope antibodies

Viral diversity, high rate of glycosylation of HCV glycoproteins, and association with apolipoproteins helps the escape of HCV from nAbs.[78–84] During HCV infection, nAbs develop that mostly target regions of E2 that interact with the host receptor CD81.[79,85–87] The crystal structure of the core of glycoprotein E2[88,89] defined the area of the protein to which most nAbs bind. Polyclonal and monoclonal nAbs isolated from patients with chronic HCV infection or given by gene therapeutic approaches are capable of inhibiting infection of human liver chimeric mice.[90–95] In patients, nAbs targeting the HCV envelope glycoprotein (MBL-HCV1) effectively delayed viral rebound, proving the principle that immunotherapy will be an effective addition to the synergistic antiviral arsenal.[22] Studies are underway that combine MBL-HCV1 with DAAs to optimise therapeutic efficacy of this approach with the latest tools. Clinical trials of human HCV immune globulin in combination with DAAs show that administration of the immune globulin is safe and more efficacious than DAAs alone.[23] However, a potential challenge in the use of nAbs for prevention of infection is identical to the problem during chronic infection—ie, genetic adaptation enabling viral escape.[22] Complementing anti-envelope antibodies, small molecules have been identified to interfere with viral entry.[96–98]

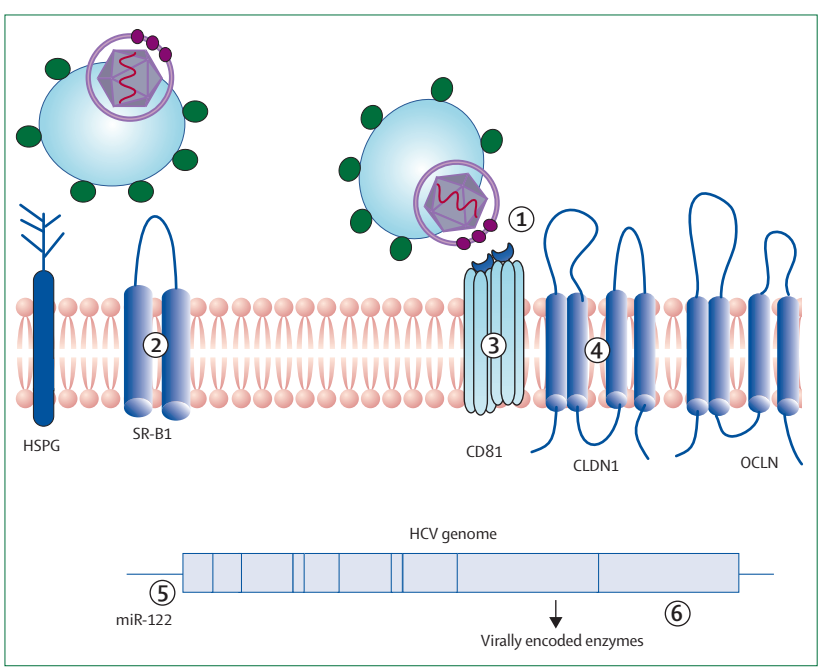

***Figure 2*: HCV entry factors as targets to prevent graft infection**

Several points of HCV entry can be effective targets to prevent initial or ongoing liver graft infection. (1) The HCV glycoprotein E1/E2 is crucial for HCV entry, and nAbs binding to E1/E2 have proved effective in animal models and clinically.[22,23,90,91,94] (2) Early steps of HCV entry probably involve initial attachment of apolipoprotein E to HSPG and utilisation of SR-B1. SR-B1 inhibitors have been effective in animal models and in the clinic in the context of liver transplantation.[24,25,99,100] (3) HCV E2 directly binds to host entry factor CD81, and antibodies binding CD81 prevent HCV infection in animal models.[101,102] (4) Antibodies recognising CLDN1 have proved effective in preventing and curing HCV infection in animal models.[103–105] Furthermore, small molecules such as erlotinib targeting epidermal growth factor receptor,[106] a kinase promoting CD81-claudin-1 co-receptor formation, and ezetimibe targeting cholesterol transporter NP1CL1 (not shown) have been shown to inhibit HCV infection in humanised mouse models.[107] (5) Downstream of cell entry, miR-122 antagonists (antagomirs) have been shown to be effective and safe for use in animals and patients.[108,109] (6) DAAs targeting virally encoded enzymes have revolutionised HCV treatment. nAbs=neutralising antibodies. HSPG=heparan sulfate proteoglycans. SR-B1=scavenger receptor B1. CLDN1=claudin 1. OCLN=occludin. miR=microRNA. HCV=hepatitis C virus.

Host-targeting entry inhibitors

One solution that could feasibly prevent viral escape from antiviral antibodies would be to target host entry factors (figure 2).[72,110] Infection with HCV variants that escape host anti-envelope antibodies or are resistant to DAAs are effectively blocked by host-targeting entry inhibitors,[70,76,111–113] Host-targeting agents have been investigated for several steps of viral entry. HCV virions circulate in dynamic complex with lipoproteins and apolipoproteins.[114,115] The first step of HCV attachment is mediated by apolipoprotein E binding to heparan sulfates on the basolateral surface of the hepatocyte.[116] Inhibitors of heparan sulfate attachment, such as the green tea polyphenol epigallocatechin-3-gallate, are generally safe and can impair infection in cell culture systems,[117,118] although in HCV mouse models the addition of epigallocatechin-3-gallate adds no observable advantage compared with anti-envelope antibodies alone.[119] The next step of the HCV entry process is interaction of the virion with scavenger receptor B1 (SR-B1). Antibodies against SR-B1 substantially inhibit HCV infection in small animal models[99,100] and prevent antiviral resistance to DAAs.[120] Inhibition of the lipid transfer activity of SR-B1 is sufficient to inhibit infection.[121] A small chemical inhibitor of SR-B1, ITX5061, has been tested in patients undergoing transplantation with HCV infection.[24] Patients with HCV genotype 1 infection on treatment had sustained reduction of viral load, and the genetic variation of the quasispecies was limited.[24] After this initial attachment, a sequence of events takes place including the triggering of signalling pathways involving host kinases such as epidermal growth factor receptor to cluster essential entry factor claudin 1 (CLDN1) and CD81.[106,122] Erlotinib, a small molecule inhibitor of epidermal growth factor

receptor, has been shown to inhibit HCV infection in both cell culture and animal models.[106] Antibodies that recognise CD81 have also been shown in small animal models to inhibit HCV infection.[101,102] Anti-CLDN1-specific antibodies are not only effective in preventing HCV infection, but can also prevent and cure HCV infection in humanised mice in monotherapy without resistance and observable side-effects.[103–105] The anti-CLDN1 antibody has been shown to be highly synergistic with DAAs,[76] and to prevent antiviral resistance by impairing viral spread.[112] There are several other host entry factors such as occludin,[123,124] Niemann-Pick C1-like 1,[107] transferrin receptor 1,[125] and serum response factor binding protein 1.[126] A clinically approved small chemical inhibitor of Niemann-Pick C1-like 1 has likewise proved effective in small animal models and to synergise with DAA.[107,127] Future research will enable the discovery and development of host-targeting entry inhibitors of optimum safety in administration and efficiency in synergising with DAAs to play a key role in increasing the cure rates in liver transplantation.

Advantages and disadvantages

The success of next-generation DAAs in treatment raises the debate of whether HCV entry inhibitors have a place in future clinical practice.[127,128] Advantages and disadvantages of using entry inhibitors exist.[72,129] Strengths include their capacity to be used in targeting intervention around transplantation with a short duration of treatment. The barrier for resistance, inhibiting the potential adaptation of viral mutant strains resistant to treatment, appears to be higher for host-targeting entry inhibitors than for DAA when used in monotherapy.[103,112] Given their complementary mechanism of action to DAAs[110] and efficacy against DAA-resistant viruses,[112,130] entry inhibitors could be used in patients who do not respond to pre-emptive therapy, while preventing costly post-transplant therapy. The high level of synergy of entry inhibitors with DAAs seen in cell culture and animal models suggests that these agents could shorten treatment time and circumvent the development of antiviral-resistant variants.[76,103,120] One drawback is that a number of entry inhibitors are only now reaching clinical development stages. There are more DAAs in the drug development pipeline that might not be limited by the current safety issues of resistance and complications of renal failure. Targeting host factors will require careful surveillance of side-effects[110] and viral escape has been described.[22,130–133] Furthermore, the numbers of individuals with serious HCV-related liver disease will decline, as will prices for the DAA regimens. Other approaches targeting host factors downstream of HCV cell entry[110,134] include microRNA (miRNA) antagonists (antagomirs) or cyclophilin inhibitors.[135,136] A key miRNA that boosts HCV replication, miR-122, acts by shifting HCV genome activity away from translation and toward replication.[137] Antisense agents targeting miR-122 have been shown to be safe and efficient in primate models and patients.[108,109,138] Cyclophilin, a host factor required for viral replication, is efficiently inhibited by alisporivir, a host-targeting agent in clinical development.[139] Whether these strategies are feasible in liver transplantation remains to be shown.

Search strategy and selection criteria

We searched PubMed for articles published from January, 1995, to January, 2016, with the terms "hepatitis C", "transplantation", "HCV", "liver graft", and "cirrhosis". Relevant presentations of upcoming publications were identified at the European Association for the Study of the Liver International Liver Congresses (2012–15) and the American Association for the Study of Liver Diseases Liver Meetings (2012–16). Articles resulting from these searches and relevant references cited in those articles were reviewed. Articles published in English, French, and German were included.

Summary and conclusion

Treatment of patients in need of liver transplant as a result of HCV-associated advanced disease is a sensitive and complicated tree of decision making. The successful use of DAAs as prophylactic and therapeutic agents against HCV infection, both before and after transplantation, promises to assist increasing numbers of individuals who find themselves in this historically difficult-to-treat population. Randomised clinical trials are ongoing to define the role of entry inhibitors in the prevention of graft infection.

Contributors

All authors contributed to the writing and editing of the manuscript. DJF and AC contributed equally. DS and TFB contributed equally.

Declaration of interests

AC has served as a consultant and has received speaker fees from Astellas, Novartis, AbbVie, Bristol-Myers Squibb, Gilead, and Janssen-Cilag. RTC reports grants from from Gilead, Merck, Bristol-Myers Squibb, Mass Biologics, and Janssen; grants and personal fees from AbbVie; and personal fees from Idenix. DS has served as a consultant for Astellas, Bristol-Myers Squibb, Gilead, LFB, MSD, Novartis, Roche, Biotest, and AbbVie. TFB reports personal fees from Gilead and Vironexx; grants and personal fees from Biotest; and has a patent WO2010034812, US 8,518,408 issued for anti-host cell factor antibodies and small molecules for prevention and treatment of HCV infection. DJF declares no competing interests.

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