Short-course, direct-acting antivirals and ezetimibe to prevent HCV infection in recipients of organs from HCV-infected donors: a phase 3, single-centre, open-label study

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Summary

Background An increasing percentage of potential organ donors are infected with hepatitis C virus (HCV). After transplantation from an infected donor, establishment of HCV infection in uninfected recipients is near-universal, with the requirement for post-transplant antiviral treatment. The aim of this study was to determine if antiviral drugs combined with an HCV entry blocker given before and for 7 days after transplant would be safe and reduce the likelihood of HCV infection in recipients of organs from HCV-infected donors.

Methods HCV-uninfected organ recipients without pre-existing liver disease were treated with ezetimibe (10 mg; an HCV entry inhibitor) and glecaprevir-pibrentasvir (300 mg/120 mg) before and after transplantation from HCV-infected donors aged younger than 70 years without co-infection with HIV, hepatitis B virus, or human T-cell leukaemia virus 1 or 2. Recipients received a single dose 6–12 h before transplant and once a day for 7 days after surgery (eight doses in total). HCV RNA was assessed once a day for 14 days and then once a week until 12 weeks post-transplant. The primary endpoint was prevention of chronic HCV infection, as evidenced by undetectable serum HCV RNA at 12 weeks after transplant, and assessed in the intention-to-treat population. Safety monitoring was according to routine post-transplant practice. 12-week data are reported for the first 30 patients. The trial is registered on ClinicalTrials.gov, NCT04017338. The trial is closed to recruitment but follow-up is ongoing.

Findings 30 patients (23 men and seven women; median age 61 years (IQR 48–66) received transplants (13 lung, ten kidney, six heart, and one kidney–pancreas) from 18 HCV-infected donors. The median donor viral load was 5·11 $\log_{10}$IU/mL (IQR 4·55–5·63) and at least three HCV genotypes were represented (nine [50%] donors with genotype 1, two [11%] with genotype 2, five [28%] with genotype 3, and two [11%] with unknown genotype). All 30 (100%) transplant recipients met the primary endpoint of undetectable HCV RNA at 12 weeks post-transplant, and were HCV RNA-negative at last follow-up (median 36 weeks post-transplant [IQR 25–47]). Low-level viraemia was transiently detectable in 21 (67%) of 30 recipients in the early post-transplant period but not after day 14. Treatment was well tolerated with no dose reductions or treatment discontinuations; 32 serious adverse events occurred in 20 (67%) recipients, with one grade 3 elevation in alanine aminotransferase (ALT) possibly related to treatment. Non-serious transient elevations in ALT and creatine kinase during the study dosing period resolved with treatment completion. Among the serious adverse events were two recipient deaths due to causes unrelated to study drug treatment (sepsis at 49 days and subarachnoid haemorrhage at 109 days post-transplant), with neither patient ever being viraemic for HCV.

Interpretation Ezetimibe combined with glecaprevir-pibrentasvir given one dose before and for 7 days after transplant prevented the establishment of chronic HCV infection in recipients of different organs from HCV-infected donors. This study shows that an ultra-short course of direct-acting antivirals and ezetimibe can prevent the establishment of chronic HCV infection in the recipient, alleviating many of the concerns with transplanting organs from HCV-infected donors.

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Introduction

Organ transplantation is a life-saving therapy, but access is limited by a shortage of donor organs. In North America, the ongoing opioid epidemic has led to an increase in hepatitis C virus (HCV) transmission, and the overdose crisis has resulted in an increase in the number of organs available from HCV-infected donors.[1] Major advances in HCV therapy have created the possibility of

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Research in context

Evidence before this study

We searched PubMed for studies published from database inception to Jan 1, 2018, with the search terms: "hepatitis C", "entry inhibitor", "entry blocker", and "ezetimibe"; and reviewed all ongoing trials (on "HCV" and "transplant") on ClinicalTrials.gov. All searches were done without language restrictions. No studies with a direct-acting antiviral plus an entry blocker were identified on review of the published literature. Studies have shown that hepatitis C virus (HCV) can be effectively and safely treated after transplantation with DAAs. Initial studies evaluated grazoprevir-elbasvir in HCV-negative patients receiving kidneys from HCV-positive donors. Although effective, the restriction to HCV genotype 1 or 4 limited the application of this approach. Pangenotypic regimens, including sofosbuvir-velpatasvir, have been used successfully, but some difficulties with drug–drug interactions have been noted, and relapses, including with fibrosing cholestatic hepatitis, have been reported. Approaches to prevent HCV transmission would thus be preferable. Consequently, new approaches to preventing HCV transmission are needed. The use of ultraviolet light during ex-vivo organ perfusion has shown only partial success at preventing HCV transmission. Pretransplant treatment with DAAs with the addition of HCV entry blockers has therefore been proposed as an alternative strategy.

Added value of this study

We hypothesised that the use of an entry inhibitor combined with a potent DAA combination would prevent recipients from becoming infected on receipt of an organ from an HCV-infected donor. We treated recipients of different organs with a single dose of ezetimibe, an HCV entry inhibitor, and the potent pangenotypic combination of glecaprevir-pibrentasvir before transplant, followed by 7 days of this combination post-transplant. The protocol was applied in 30 individuals who received organs (13 lung, ten kidney, six heart, and one kidney–pancreas) from 18 HCV-infected donors. All recipients reached at least 14 weeks of follow-up and none developed chronic HCV infection. The treatment was well tolerated with mild reversible alanine aminotransferase and creatine kinase elevations seen during treatment that resolved shortly after treatment completion. This study shows that when given with an entry blocker and before transplantation, DAA regimens of much shorter duration than standard regimens can be used to prevent chronic HCV infection in recipients of organs from HCV-infected donors.

Implications of all the available evidence

Collectively, studies show that organs from otherwise healthy HCV-infected donors can be used safely for transplantation into HCV-uninfected recipients. Although post-transplant treatment of chronic infection is effective, prevention of chronic infection is clearly preferable. This study shows that an ultra-short course of DAAs and ezetimibe can be completed before hospital discharge and prevent establishment of chronic HCV infection in the recipient. This strategy would alleviate most of the concerns with use of HCV-infected organs for transplantation and thus could have a major effect on organ availability across North America and other regions.

transplanting organs from HCV-infected donors to HCV-uninfected recipients.[2,3] Studies from the past few years have shown that recipients of organs from HCV-infected donors can be promptly treated with courses of HCV therapy lasting 4–12 weeks, with a high probability of curing infection.[4–6] Although this approach appears to be safe and effective, challenges have been encountered, including drug–drug interactions and difficulties in accessing medication.[5,7,8] In addition, despite a full course of therapy, cases of relapse with complicated resistance profiles and even fibrosing cholestatic hepatitis have been reported.[7,9,10] To avoid the problems encountered when treating HCV after transplantation, strategies to prevent recipient infection would be preferable.

HCV present in residual blood and fluid in the donor organ at the time of transplantation infects the recipient liver promptly. We have previously shown that light-based therapy during ex-vivo organ perfusion before transplantation can reduce infectivity and lower initial viral load in the recipient, but is inadequate to entirely prevent infection.[7,11] Pre-emptive therapy with direct-acting antivirals (DAAs) that potently inhibit stages in the HCV lifecycle could prevent the replication and spread of HCV after infection; however, the ability to also block or limit HCV entry would be preferable. HCV entry into hepatocytes is a complex process requiring multiple entry factors including CD81,[12] scavenger receptor class B member 1,[13] claudin-1,[14] occludin,[15] and NPC intracellular cholesterol transporter 1 (NPC1).[16] NPC1 silencing or receptor blockade with antibodies potently inhibits HCV entry.[16] Ezetimibe, an approved cholesterol-lowering therapy, is a ligand for the NPC1 receptor, and in cell culture and a humanised mouse model, pre-treatment with ezetimibe has been found to restrict HCV entry.[16]

Preventive therapy with DAAs requires the use of regimens that work against all HCV genotypes and in patients with end-stage organ disease. Glecaprevir-pibrentasvir combines an HCV protease inhibitor with an inhibitor of the non-structural 5A protein and treats all six HCV genotypes, leading to cure rates of 95–99% with 8 weeks of therapy in patients with chronic HCV infection.[3,17] Glecaprevir-pibrentasvir is also safe in patients with chronic kidney disease, including those on dialysis, which is a relevant concern after transplantation.[18]

In this study, we evaluated the use of glecaprevir-pibrentasvir combined with ezetimibe, given as a single

dose before transplant and for 1 week after surgery, as a method to prevent infection after organ transplantation from HCV-infected donors to uninfected recipients.

Methods

Study design and participants

We did a phase 3, single-centre, open-label study based at Toronto General Hospital (Toronto, ON, Canada). The study was approved by the Research Ethics Board of Toronto General Hospital, University Health Network (Toronto) and by Health Canada, and was in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The full study protocol is available in the appendix (pp 9–25).

Eligible donors were aged 70 years or younger (or 45 years or younger for donors of kidney transplants) and showed nucleic acid test (NAT) positivity for HCV. Other donor parameters were evaluated according to local standard metrics for donor selection. Donors positive for hepatitis B virus (NAT or HBsAg positivity), HIV (NAT positivity or positive serology), or human T-cell leukaemia virus 1 or 2 (positive serology) were excluded, but donors positive for HBcAg were eligible. Donors from anywhere in North America were considered for the study. We were notified by organ procurement organisations across North America when they had HCV-positive donors.

Eligible recipients were all patients (of any age) listed on the lung, heart, kidney, kidney–pancreas, or pancreas transplant waitlist at Toronto General Hospital, provided they tested negative for HCV (NAT negativity), did not have pre-existing liver disease (fibrosis >stage 2, METAVIR system;[19] or serum aminotransferase concentration >3 times the upper limit of normal [laboratory reference 40 IU/L]), were not listed for liver transplantation, did not have a known allergy to glecaprevir-pibrentasvir or ezetimibe, were not participating in another interventional clinical trial, and provided written informed consent. Patients were approached during clinic visits or when admitted to hospital. They received educational materials about HCV and were given the opportunity to meet with the hepatology team before transplantation. The study was explained to interested individuals by a study coordinator (IB, NK, or NM) and if they were interested in participating, the patient chart was carefully reviewed and patient eligibility assessed by a study coordinator or a study investigator (MC, JJF, AH, or LGS). For all but kidney transplants, patients provided written informed consent while on the waitlist, with the opportunity to ask questions until the time of transplant, and reaffirmed consent at the time of organ offer. For kidney transplants, patients from the top of the waitlist were offered participation in the trial at the time a kidney from an HCV-infected donor became available to avoid concerns regarding waitlist allocation priority. The kidney recipients spoke to a physician not involved in the transplant process about the study and received written study materials for review. Kidney recipients were given a minimum of 3 h and up to 12 h to review the information and ask questions before signing the informed consent form. For all other transplants, all consenting individuals in the trial remained active on the transplant list and received organs on the basis of their established priority. Consenting to the study did not necessarily mean that participants would receive an HCV-infected organ; if an organ from a HCV-uninfected donor became available first, participants proceeded with a transplant with that organ.

Procedures

Organs from HCV NAT-positive donors were selected on the basis of standard criteria for transplant organ selection. Organs were retrieved and transported in static cold preservation. Lungs were evaluated by the transplant team and if clinically indicated, were treated with 4 h of normothermic ex-vivo lung perfusion (EVLP) with an acellular perfusate solution.[20] On the basis of our previous work showing that ultraviolet-C (UVC) light delivered during EVLP lowers HCV RNA and infectivity, UVC light was delivered to the circulating perfusate for the duration of EVLP.[7,11] Ex-vivo organ perfusion was not done for organs other than lungs.

See Online for appendix

Once the transplant was confirmed, consented recipients received an initial dose of glecaprevir-pibrentasvir (300 mg/120 mg; three fixed-dose combination tablets) and ezetimibe (10 mg), orally at 6–12 h before the anticipated implantation of the donor organ. Recipients continued to receive oral glecaprevir-pibrentasvir and ezetimibe once a day for 7 days after the transplant (eight doses in total). For patients who could not tolerate oral intake, treatment was given by nasogastric tube. The dose of ezetimibe (10 mg once a day) is the approved dose for human use. HCV RNA was measured with the Cobas 4800 HCV RNA assay (Roche Diagnostics, Basel, Switzerland), at the University Health Network Microbiology laboratory, with a lower limit of quantification of 15 IU/mL. Testing was done once a day for the first 14 days, then once a week for 12 weeks, and again 6 months after transplantation. Prevention of established infection was defined as undetectable HCV RNA at 12 weeks after transplantation. Additionally, at 4 weeks and 12 weeks post-transplant, HCV antibodies were measured with a chemiluminescent microparticle immunoassay (Architect anti-HCV; Abbott, Chicago, IL, USA). Tacrolimus was used for all recipients because of a clinically significant interaction with glecaprevir-pibrentasvir and cyclosporin.[21] Other post-operative transplant care and immunosuppression followed standard transplant practice. Patients are currently being followed up with annual HCV RNA testing for 5 years.

Safety monitoring was according to routine post-transplant practice and reported in terms of adverse events regardless of relation to antiviral treatment. Because of the proximity of antiviral treatment to transplantation, documentation of non-serious adverse events focused on laboratory abnormalities, graded according to the Common

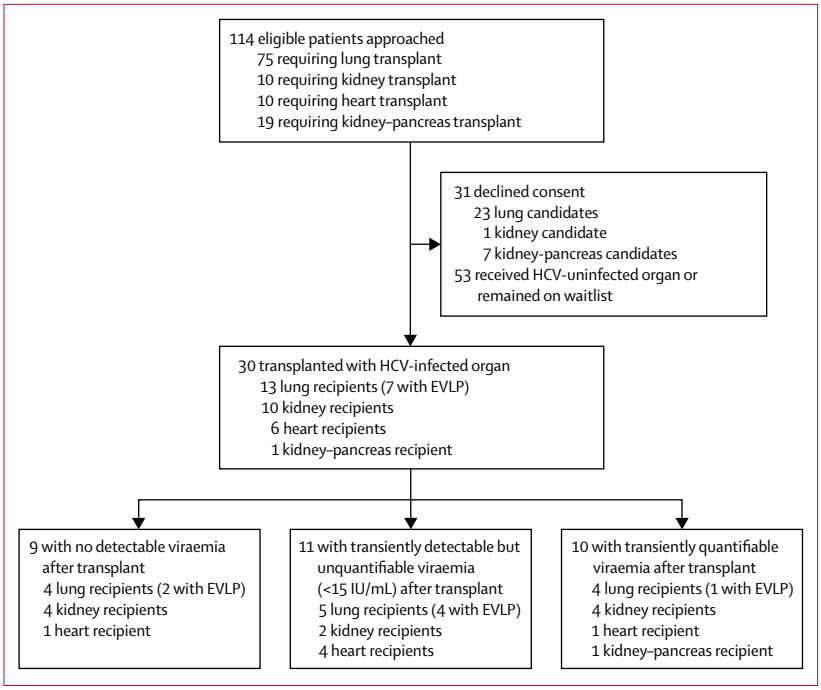

Figure 1: **Study profile**
EVLP=ex-vivo lung perfusion.

	Summary measures
Recipient factors (n=30)	
Age, years	61 (48–66)
Sex	
Male	23 (77%)
Female	7 (23%)
Ethnicity	
White	22 (73%)
Asian	4 (13%)
Black	3 (10%)
Hispanic	1 (3%)
Organ received	
Lung	13 (43%)
Kidney	10 (33%)
Heart	6 (20%)
Kidney–pancreas	1 (3%)
Time from consent to transplant, days	8 (1–34)
Lung	31 (10–49)
Kidney	NA*
Heart	1 (1–18)
Kidney–pancreas	NA*
Follow-up post-transplant, weeks	36 (14–54; 25–47)
Donor factors (n=18)	
Age, years	36 (31–39)
Sex	
Male	14 (78%)
Female	4 (22%)
HCV RNA level, log_{10}IU/mL	5·11 (1·18–7·13; 4·55–5·63)
HCV genotype	
1a	7 (39%)
1b	1 (6%)
1 unspecified	1 (6%)
2	2 (11%)
3	5 (28%)
Unknown†	2 (11%)

Data are n (%), median (IQR), or median (range; IQR). NA=not applicable. HCV=hepatitis C virus. *Kidney recipients were consented at the time an organ became available. †Genotyping was unsuccessful in two donor samples.

Table 1: **Baseline characteristics of organ donors and recipients**

Terminology Criteria for Adverse Events version 5.0. In particular, creatine kinase concentrations were measured on the same day of transplant (pretransplant) and on day 3, day 7, and day 14 post-transplant because of the association of rhabdomyolysis with ezetimibe use.[22]

Outcomes

The primary endpoint was prevention of HCV infection as evidenced by undetectable serum HCV RNA at 12 weeks after transplantation. Secondary endpoints were: graft and patient survival; in-hospital mortality; development of anti-HCV antibodies; development of HCV-specific immune responses (to be reported elsewhere); HCV viraemia with and without EVLP; incidence of HCV donor to recipient transmission; correlation between donor viraemia and recipient infection; interval of time from transplantation to viraemia development; HCV cure rates after treatment for infected patients; incidence of acute liver dysfunction (including fibrosing cholestatic hepatitis); incidence of biopsy-proven acute rejection requiring treatment (an addition to the original protocol but planned a priori to study initiation); and organ function at 3 months, 6 months, and 12 months post-transplant as measured by 6-min walk and forced expiratory volume in 1 s (FEV_1; lung), estimated glomerular filtration rate (eGFR; kidney), ejection fraction (heart), and freedom from exogenous insulin (pancreas).

Statistical analysis

All HCV RNA values were log-transformed and values that were detectable but below the limit of quantification were arbitrarily assigned a value of 7·5 IU/mL. The primary and secondary endpoints were analysed as the proportions of the population who met each endpoint and are expressed in terms of descriptive statistics. We compared median donor HCV RNA between recipients with no viraemia, those with detectable but unquantifiable HCV RNA, and those with quantifiable viraemia using the Kruskal-Wallis test. As post-hoc secondary analyses, we evaluated baseline factors associated with transient recipient viraemia and associations between recipient infection and donor viraemia, HCV antibody development, and alanine aminotransferase (ALT) elevation using exact logistic regression with both dichotomous (viraemic *vs* non-viraemic) and ordinal (undetectable,

detectable <15 IU/mL, and quantifiable viraemia) models. Because some recipients received organs from the same donors, the regression analysis was also fit to a generalised estimating equation (GEE) model, to account for the fact that the samples were not independent. In testing for an association of transient viraemia with donor viral load and HCV genotype, the bivariate analysis was done with both exact logistic regression and the GEE model to ensure that results were concordant. The study was designed with a target sample size of 40 patients to assess safety and efficacy. Efficacy and safety were evaluated by intention-to-treat analysis. The study was designed with futility rules to extend therapy with glecaprevir-pibrentasvir and ezetimibe to 14 days if more than one patient in the first five to receive a transplant became infected, and to extend to 4 weeks if more than one in the first five to receive a 14-day treatment regimen became infected. An independent data safety and monitoring board from the University Health Network, composed of five individuals with expertise in transplant care or HCV management, reviewed the results of the study after the first five patients completed their treatment course, and then every 3 months or on reporting of an unexpected adverse event as decided by any members of the study team. 12-week data are reported for the first 30 recipients. Analyses were done with SAS (version 9.4), with two-sided p values of less than 0·05 considered to indicate statistical significance. The study was registered on ClinicalTrials.gov, NCT04017338; however, there was a delay between initial submission and final posting of the trial due to an administrative error.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The lead authors (JJF, MC, and AH) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 114 eligible transplant recipient candidates approached for the study, 83 (73%) consented to participate, of whom 30 (27%) ultimately received an organ from an HCV NAT-positive donor (figure 1). 18 HCV NAT-positive donors provided the 30 transplant organs to HCV-uninfected recipients between Feb 4 and Nov 11, 2019. Total transplanted organs were 13 lungs (six single, seven double), ten kidneys, six hearts, and one kidney–pancreas. EVLP with UVC was used in seven of the 13 lung cases. A maximum of four organs (heart, kidney, and kidney–pancreas) were used from any single donor. Characteristics of donors and recipients are shown in table 1. Median time from informed consent to transplantation was 31 days (IQR 10–49) for lung recipients and 1 day (1–18) for heart recipients, compared with 50 days (17–103) and 66 days (21–123) from listing to lung and heart transplants from HCV-uninfected donors, during the study period.

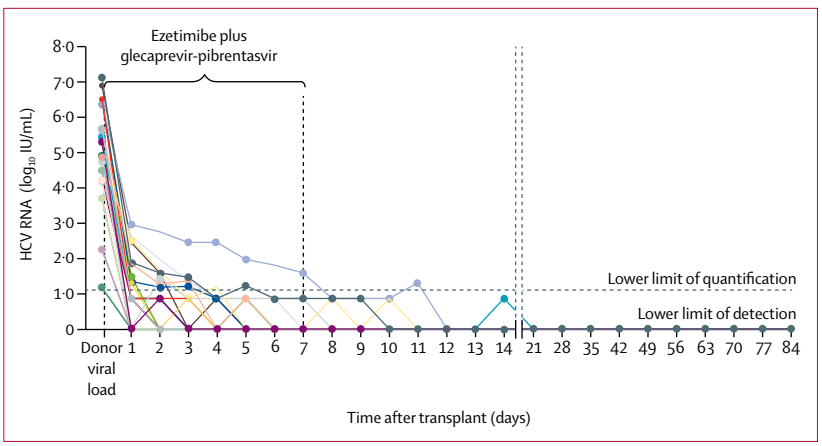

Figure 2: **HCV RNA concentrations in donors and recipients post-transplant**
HCV RNA concentrations are shown for the 18 organ donors and for each of the 30 organ recipients over time (each coloured line represents an individual recipient). HCV RNA concentrations that were detectable but lower than the limit of quantification (15·0 IU/mL) were arbitrarily set to 7·5 IU/mL. A dose of ezetimibe and glecaprevir-pibrentasvir was given before and for 7 days after transplantation. HCV=hepatitis C virus.

All 30 (100%) transplant recipients met the primary endpoint of undetectable HCV RNA at 12 weeks post-transplant, and all were HCV RNA-negative at last follow-up (median 36 weeks post-transplant [IQR 25–47]; minimum follow-up 14 weeks). All heart, lung, and kidney–pancreas recipients and five kidney recipients completed HCV treatment before discharge from hospital.

Among the 30 recipients, nine (30%) never had detectable viraemia, 11 (37%) had transiently detectable but unquantifiable HCV RNA, and ten (33%) had transiently quantifiable viraemia (figure 1) with peak HCV RNA on day 1 after transplantation (median 1·87 $\log_{10}$IU/mL [range 1·30–2·96; IQR 1·49–2·55]; mean 0·88 $\log_{10}$IU/mL [SD 0·89]). Individual HCV RNA concentrations are shown in figure 2. Four (13%) patients had detectable HCV RNA after the eight doses of antiviral therapy. Of these, three patients had a single detectable HCV RNA result that was lower than the limit of quantification, on day 9, day 10, and day 14 post-transplant, respectively, and HCV RNA subsequently remained undetectable beyond 12 weeks of follow-up. The other individual had an HCV RNA concentration of 2·96 $\log_{10}$IU/mL on postoperative day 1 that decreased daily, reaching unquantifiable but detectable concentrations on postoperative day 8. On postoperative day 11, 4 days after completing therapy, HCV RNA was 1·3 $\log_{10}$IU/mL, but has been undetectable since (last follow-up at 39 weeks post-transplant). All other patients had undetectable HCV RNA by the end of treatment and have remained negative for HCV RNA during follow-up.

HCV antibody became detectable in 14 (47%) of 30 patients. In ten patients, HCV antibody was first detected at week 4. In two of these patients, HCV was no longer detectable at week 12. In the other four patients, antibodies were first detected at week 12. We found no association between transient viraemia and development

	Recipients (n=30)
Serious adverse events*	
Total events	32
Number of patients	20 (67%)
Treatment-related serious adverse events	1/32 (3%)
Adverse events requiring treatment discontinuation	0
Transplants with at least one episode of acute rejection requiring treatment*	
Lung	3/13 (23%)
Kidney	0
Heart	4/6 (67%)
Kidney–pancreas	0
Episodes of acute rejection requiring treatment	
Lung	3
Kidney	0
Heart	7
Kidney–pancreas	0
Laboratory adverse events out of total recipients†	
ALT elevation*	
Grade 1	9 (30%)
Grade 2	2 (7%)
Grade 3	4 (13%)‡
Grade 4	1 (3%)
Bilirubin elevation*	
Grade 1	1 (3%)
Grade 2	6 (20%)
Grade 3	1 (3%)
Grade 4	2 (7%)
Creatine kinase elevation*	
Grade 1	2 (7%)
Grade 2	14 (47%)
Grade 3	1 (3%)
Grade 4	0

ALT=alanine aminotransferase. *Appendix (pp 6–8) provides further details on serious adverse events, rejection episodes, and ALT, creatine kinase, and bilirubin elevations. †Laboratory abnormalities were graded with the Common Terminology Criteria for Adverse Events version 5.0. ‡Including one patient with serious elevation in ALT (appendix pp 6, 8).

Table 2: **Safety data**

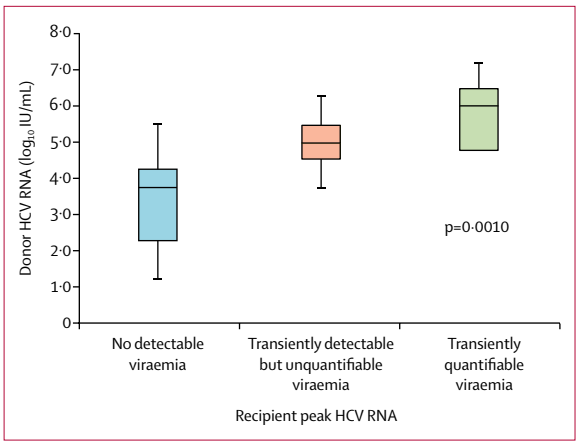

Figure 3: **Recipient HCV RNA concentration on day 1 post-transplant compared with donor viral load**
Median, IQR, and range of HCV RNA concentrations in the organ donors are shown (for transiently quantifiable viraemia, the lower range value was within the IQR). HCV RNA concentrations were compared between groups with the Kruskal-Wallis test. HCV=hepatitis C virus.

of HCV antibodies (data not shown).

Regarding in-hospital mortality, two lung transplant recipients died due to causes unrelated to study drug treatment (sepsis at 49 days and subarachnoid haemorrhage at 109 days post-transplant; appendix p 8). Neither patient was ever viraemic for HCV. The other 28 recipients were alive at last follow-up with functioning grafts. Including the deaths, 32 serious adverse events occurred in 20 patients (table 2; appendix pp 6–7). One serious adverse event that was deemed possibly related to the therapy was prolongation of hospital admission in a kidney recipient, for transiently elevated liver enzymes (peak ALT 650 U/L on postoperative day 11; international normalised ratio and bilirubin normal). Laboratory investigations to identify causes of acute hepatitis were unrevealing, including persistently undetectable HCV RNA. Liver biopsy showed non-specific acute hepatitis with moderate steatosis but no steatohepatitis and no fibrosis. ALT returned to normal on day 27 after transplantation.

In terms of non-serious adverse events, no laboratory abnormalities (table 2; appendix p 8) led to dose reductions or study discontinuation. Transient ALT elevations occurred in 16 (53%) recipients during dosing of study medications, but resolved with treatment completion. Post-hoc analysis showed no association between ALT elevation and transient HCV viraemia by logistic regression (data not shown). No cases of acute liver failure or fibrosing cholestatic hepatitis were observed. Bilirubin elevations occurred in ten (33%) patients, with grade 2 or higher elevations seen only in lung or heart recipients, which were associated with hepatic congestion on clinical evaluation, but resolved in all patients by day 30 after transplantation. Creative kinase was elevated during follow-up in 17 (57%) patients on therapy, but none of these patients presented with symptomatic myopathy or myositis or any renal consequences attributed to rhabdomyolysis (appendix p 8). The transient elevations in creatine kinase resolved on treatment completion.

Organ function was evaluated at the 12-week follow-up, with a median left ventricular ejection fraction of 58% (IQR 56–60) in heart recipients, a median 6-min walk of 480 m (IQR 403–538) and median FEV_1 of 76% of predicted (IQR 57–78) for lung recipients, and a median eGFR of 74 mL/min per 1·73 m² (IQR 65–92) for kidney recipients. The pancreas recipient did not require insulin injections. Graft function was similar to that in patients who received organs from HCV-uninfected donors during the study period (data not shown). At least one and up to four episodes of biopsy-proven acute

rejection requiring treatment were documented in four heart recipients and three lung recipients (table 2), all of whom responded to pulse steroids and increased immunosuppression. Rejection was not observed in kidney or kidney–pancreas recipients.

Our post-hoc analyses showed that detection of viraemia in the recipient was associated with HCV RNA concentration in the donor (figure 3). Recipients who never had detectable viraemia had a median donor viral load of 3·71 $\log_{10}$IU/mL (range 1·18–5·46; IQR 2·25–4·21), compared with 4·93 $\log_{10}$IU/mL (3·71–6·52; 4·49–5·42) in recipients with transiently detectable but unquantifiable HCV RNA, and 6·04 $\log_{10}$IU/mL (4·73–7·13; 4·92–6·52) in recipients with transiently quantifiable HCV RNA (Kruskal-Wallis p=0·0010).

Ten patients received organs from donors infected with genotype 3 HCV, and all ten recipients had detectable viraemia post-transplant (p=0·049), which remained significant after controlling for donor viral load by exact or GEE regression (exact regression model odds ratio 7·09 [95% CI 1·05–48·0], p=0·045). Organ type, recipient and donor age and sex, and nasogastric drug delivery were not associated with recipient viraemia (appendix pp 3–5). Recipients of organs from the same donor had similar outcomes, with all or none developing viraemia. EVLP with UVC during perfusion was done in seven of 13 lung transplants on the basis of clinical indications. Of these, one had quantifiable viraemia, whereas three of six with no EVLP had quantifiable viraemia, although these transmission rates with and without EVLP did not differ significantly. The donor virus was not sequenced, and thus we did not establish if any transmitted virus had pre-existing substitutions associated with resistance to antiviral treatment.

Discussion

The use of ezetimibe as an HCV entry blocker combined with the potent DAA therapy glecaprevir-pibrentasvir was able to prevent the establishment of chronic HCV infection when given immediately before and for 7 days after organ transplantation from HCV-infected donors to HCV-uninfected recipients. With this short course of therapy, most patients completed antiviral therapy before hospital discharge, and remained free of HCV infection at last follow-up.

The ability to use HCV-infected organs for transplantation has become increasingly important, particularly in North America given the negative consequences of the ongoing overdose crisis.[1] The prevalence of HCV has been steadily increasing among potential organ donors who die of overdose, many of whom are young without medical comorbidities.[4] Studies have documented the safety of transplanting organs from HCV-infected donors when antiviral therapy is initiated shortly after transplant. However, post-transplant treatment can be challenging because of drug interactions, postoperative complications, and logistical challenges such as securing coverage for a full course of DAA therapy.[8,10,23] Although most studies have reported high cure rates, relapses after therapy have been documented with complex resistance profiles and even fibrosing cholestatic hepatitis, the most severe form of HCV infection.[7,9,10] In addition, other complications such as HCV-related glomerulonephritis and increased acute cellular rejection have been reported in studies with long delays before starting antiviral treatment.[10,24] As such, prevention of transmission would be preferable.

From our study, clarifying whether transmission was truly prevented or if patients were infected but rapidly cured is difficult. Although 21 patients had transiently detectable viraemia, viral load was lower than the 15 IU/mL limit of quantification in 11 (52%) patients, and viral load steadily decreased in all patients with initial viraemia. These results might reflect the presence of residual viral RNA from donor plasma in the allograft at the time of implantation, rather than active infection and replication. Consistent with this observation, donor viral load was significantly associated with viraemia in the organ recipient, as previously observed.[5] Detection of negative-strand HCV RNA would confirm active viral replication; however, viral loads were too low for negative-strand virus detection.[25] Genotype 3 HCV was also associated with recipient viraemia. Although infection with genotype 3 HCV has generally proven harder to cure with DAAs than infection with the other genotypes, glecaprevir-pibrentasvir is active against this genotype;[3,26] however, the small population limits strong conclusions as evidenced by the wide confidence intervals in our regression analysis. Clearance of low residual viraemia after treatment completion might reflect immune control of infection or measurement of non-infectious virions, as has been observed with short-course DAA therapy for chronic HCV infection.[27] The appearance of HCV antibodies in nearly half of the recipients did not appear to correlate with viraemia. In at least some patients, the antibodies might reflect adoptive transfer from the donor, which has been observed in recipients of HCV antibody-positive, NAT-negative organs in the absence of transmission.[28]

The strategy we used has some notable advantages over other approaches. The ultra-short course of antivirals allowed completion of therapy during the initial inpatient stay for heart and lung recipients, and at a substantially reduced cost compared with a standard course of treatment. Thus, with such a short-term approach, incorporation of HCV treatment cost into the overall cost of transplantation could be feasible, similar to the scenario of prophylactic therapy for other infections (eg, cytomegalovirus and hepatitis B virus). Early studies used genotype-specific regimens, which can be problematic because the donor HCV genotype is rarely known.[4,29] The pangenotypic combination of sofosbuvir-velpatasvir has been used successfully, but sofosbuvir is contraindicated in patients who receive amiodarone, a relevant consideration for management of post-operative arryth-

mias, especially after heart or lung transplantation.[30] Furthermore, although the label for sofosbuvir-velpatasvir was updated by the US Food and Drug Administration for use in renal impairment in November, 2019, sofosbuvir metabolites accumulate in patients with severe kidney impairment with unknown clinical significance.[31] Glecaprevir-pibrentasvir is pangenotypic and can be safely used in people on dialysis.[18] In this study, although treatment was well tolerated, 16 (53%) patients had ALT elevations during treatment. Notably, these elevations did not appear to correlate with HCV RNA and resolved after therapy was completed, raising the possibility that some ALT elevations were due to mild hepatotoxicity. Bilirubin elevations, although associated with hepatic congestion in heart and lung transplant recipients, resolved in all patients within 30 days of transplantation.

This study has some limitations. The small sample size might limit generalisability; however, no virological failures occurred despite high donor viral loads and different HCV genotypes across several organ types, increasing confidence that our approach was safe and effective. Although our follow-up was generally short-term, 25 patients have been followed up for more than 6 months and eight for more than 1 year. Furthermore, no late relapses in this study or other studies on organ transplant from HCV-positive donors to HCV-negative recipients have been reported, giving us confidence that establishment of chronic infection can be prevented. Our short-course strategy to prevent infection requires that the recipient liver is not already infected with HCV, and thus this approach should not be used in HCV-discordant liver transplantation. Meanwhile, the specific contribution of ezetimibe is unclear. However, in studies of short-course sofosbuvir-velpatasvir for 1 day or 4 days, transmission was seen in three (30%) of 10 patients after 1 day and two (13%) of 15 patients after 4 days, with the need for retreatment in these patients with one and sometimes a second full course of salvage DAA therapy.[32] Bethea and colleagues[33] used a similar protocol for heart transplants, starting with a pretransplant dose of glecaprevir-pibrentasvir (300 mg/120 mg) but without ezetimibe. They treated all patients for a standard 8-week course and all achieved a sustained virological response. Notably, the mean peak HCV RNA concentration in recipients was 1·76 $\log_{10}$ IU/mL (SD 1·46) after a dose of glecaprevir-pibrentasvir,[33] compared with 0·88 $\log_{10}$ IU/mL (0·89) after a dose of glecaprevir-pibrentasvir with ezetimibe in the present study (Wilcoxon rank sum p=0·029). With the short treatment course used in the present study, we felt we should maximise the chance of preventing transmission, and thus ezetimibe was given to all recipients. Fortunately, ezetimibe is inexpensive, has few drug interactions, and is well tolerated, but it would be reasonable to consider a randomised trial to clarify its utility.

In conclusion, in this proof-of-concept study, ezetimibe combined with glecaprevir-pibrentasvir, given one dose before and for 7 days after transplantation, prevented the establishment of chronic HCV infection in recipients of different organs from HCV-infected donors. Our findings suggest that short-course therapy might be adequate to prevent HCV transmission from infected donors to uninfected recipients in organ transplantation.

Contributors

JJF, MC, DK, HD, KT, SJK, MM, SK, LGS, and AH contributed to the study design and concept. JJF, MC, DK, RVPR, NM, NK, IB, FQO, OC, TWR, JS, CA, TKW, GS, MS, LGS, and AH acquired study data. JJF, MC, DK, NM, NK, IB, MAZ, OC, BEH, LGS, and AH analysed and interpreted data. JJF, MC, and AH drafted the manuscript. JJF, MC, HD, NK, IB, MAZ, OC, CA, HLAJ, BEH, and AH contributed to statistical analyses. JJF, MC, DK, LGS, and AH supervised the study. All authors contributed to critical review and revision of the manuscript. All authors reviewed the final manuscript and approved the decision to submit for publication.

Declaration of interests

JJF has received personal fees for consulting from AbbVie, Enanta, Gilead, Janssen, and Roche, and research funding from AbbVie, Abbott, Gilead, and FUJIFILM Wako Chemicals. HD has received personal fees for consulting from Cocrystal Pharma and for speaking from Replicor, and has received research support from Eiger Biorpharmaceuticals. SJK has received personal fees for consulting from Astellas and for speaking from Alexion. MM has received personal fees for consulting from Novartis and Servier. TKW has received personal fees for consulting from Lung Bioengineering. HLAJ has received personal fees for consulting from Arbutus, Gilead, Janssen, MedImmune, Merck, Roche, Vir Biotechnology, Viral Clinics, Enyo, Arena, and GlaxoSmithKline, and research support from AbbVie, Arbutus, Bristol Myers Squibb, Gilead, Janssen, MedImmune, Merck, and Roche. BEH has received personal fees for consulting from Intercept, Cymabay, Mirum, Albireo, Chemomab, and Caliditas, and research support from Intercept, Cymabay, Mirum, and Albireo. LGS has received research support from Gilead. AH has received personal fees for consulting from Astellas, Merck, and Sanofi, and research support from Astellas, Roche, and Qiagen. All other authors declare no competing interests.

Data sharing

Deidentified participant-level data will be available on publication of the study. Requests for data should be sent to Jordan.feld@uhn.ca and, on review of the proposed protocol and signing of a data sharing agreement, the data will be made available. The protocol and consent form will also be available on email request.

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