Table 3 Correlations between quantitative indices for liver functional reserve and conventional liver function tests

	ICG R15		LHL 15		HH 15		HC	
	r	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
Platelets (× 10^4/mm^3)	-0.160	0.161	0.235	0.038	-0.185	0.105	0.348	0.002
Prothrombin time (INR)	0.082	0.473	-0.122	0.289	-0.016	0.888	-0.287	0.011
Albumin (g/dL)	-0.044	0.703	0.263	0.020	-0.123	0.285	0.233	0.040
Total bilirubin (mg/dL)	0.204	0.073	-0.217	0.057	0.289	0.010	-0.345	0.002
Cholinesterase (U/L)	-0.113	0.324	0.221	0.052	-0.263	0.020	0.419	0.0001

LHL15 was correlated with platelet count and albumin level. HH15 was correlated with total bilirubin level and cholinesterase level. HC was correlated with all conventional liver function tests. ICG R15: Indocyanine green dye retention at 15 min; HC: Hepatic clearance.

Table 4 Univariate and multivariate analyses of variables predictive of severe fibrosis

Variable	Severe fibrosis		*P* value	
	Yes (*n* = 27)	No (*n* = 51)	Univariate analysis	Multivariate analysis
Gender (male/female)	23/4	Nov-40	0.474	
Age (yr)	66.5 ± 10.0	66.8 ± 10.5	0.950	
HBs-Ag (+/-)	10/17	16/35	0.616	
HCV-Ab (+/-)	10/17	11/40	0.145	
Alcohol abuse (+/-)	2/25	8/43	0.301	
NASH (+/-)	4/23	10/41	0.602	
Platelets (× 10^4/mm^3)	12.7 ± 3.9	18.7 ± 7.4	< 0.001	0.096
Prothrombin time (INR)	1.09 ± 0.12	1.03 ± 0.10	0.032	0.223
Albumin (g/dL)	4.0 ± 0.6	4.1 ± 0.6	0.388	
Total bilirubin (mg/dL)	0.9 ± 0.3	0.7 ± 0.3	0.001	0.354
Cholinesterase (U/L)	234 ± 75	255 ± 68	0.229	
Tumor size (cm)	3.5 ± 1.6	5.7 ± 4.2	0.042	0.137
Tumor number	1.1 ± 0.4	1.2 ± 0.6	0.543	
Tumor vascular invasion (+/-)	5/22	16/35	0.226	
MELD score	5.8 ± 1.1	5.1 ± 1.2	0.009	
CTP score	5.3 ± 0.6	5.2 ± 0.4	0.685	
ICG R15 (%)	14.3 ± 6.1	10.2 ± 5.5	0.019	0.183
LHL 15	0.901 ± 0.044	0.935 ± 0.024	0.042	0.041
HH 15	0.648 ± 0.068	0.556 ± 0.067	0.004	0.053
HC	263.3 ± 90.4	381.1 ± 96.7	< 0.001	0.030

Platelet count, prothrombin time, total bilirubin level, tumor size, MELD score, ICG R15, LHL15, HH15, and HC were significant predictors of severe cirrhosis in the univariate analysis. In the multivariate analysis, HC and LHL15 were the significant independent predictors. HBs-Ag: Hepatitis B surface antigen; HCV-Ab: Hepatitis C virus antibody; NASH: Nonalcoholic steatohepatitis; MELD score: Model for end-stage liver disease score; CTP score: Child-Turcotte-Pugh score; ICG R15: Indocyanine green dye retention at 15 min; HC: Hepatic clearance.

DISCUSSION

In the current study, we demonstrated correlations between the degree of liver fibrosis and ICG R15, HH15, LHL15, and HC. Among these indicators, HC showed the best correlation with conventional liver function tests. HC was the most valuable index for predicting severe cirrhosis. An HC of 298 could be used to predict severe cirrhosis.

The degree of liver fibrosis is a negative predictor of liver regeneration and the restoration of liver function after liver resection[9]. Therefore, estimating the liver functional reserve, which is a reflection of liver fibrosis, is important. Several laboratory variables, such as prothrombin time and cholinesterase, have prognostic value in chronic liver disease[21]. In addition, the Alb level, T-bil level, and prothrombin time are the most useful routine laboratory tests for establishing a prognosis for hepatitis patients[22]. However, none of these laboratory variables reflects liver fibrosis directly. As a result, these variables cannot be used as indices for determining the extent of liver resection for patients with liver tumors. In contrast, several studies have evaluated the liver functional reserve before hepatectomy[23-25]. In particular, the indocyanine green (ICG) clearance test has been widely used to evaluate liver functional reserve[25,26] for liver resection. However, it does not provide quantitative parameters. Moreover, there are occasional discrepancies between the ICG clearance values and histologic findings in the liver because of the imbalance of portal inflow or portasystemic shunts. Such discrepancies make direct assessments of the extent of liver fibrosis difficult. Therefore, a new method to estimate the liver functional reserve that accurately reflects the degree of fibrosis is required.

The asialoglycoprotein receptor (ASGPR) is localized on hepatocytes and is involved in the clearance of glycoproteins containing terminal galactose residues from the circulation[27,28]. The expression of this receptor decreases according to the number of functional hepatocytes. Therefore, liver scintigraphy with ^{99m}Tc-GSA,

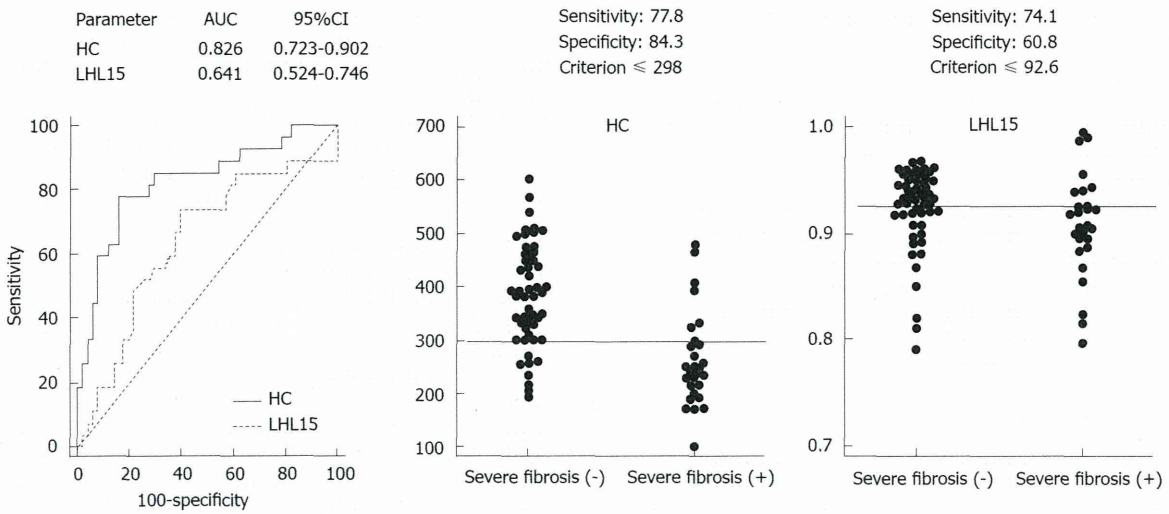

Figure 1 Receiver operating characteristic curve and interactive dot diagrams of hepatic clearance and LHL15 for the diagnosis of severe fibrosis. A: ROC analysis for HC and LHL15. There was a significant difference between the two values (P = 0.0146); B: Interactive dot diagrams showing HC predicts severe cirrhosis. The cutoff value for predicting severe cirrhosis with the highest sensitivity and specificity was 298 (sensitivity, 77.8%; specificity, 84.3%) for HC. The horizontal line indicates the cutoff point with the best separation between the 2 groups (severe fibrosis+, severe fibrosis-); C: Interactive dot diagrams showing LHL15 predicts severe cirrhosis. The cutoff value for predicting severe cirrhosis with the highest sensitivity and specificity was 0.926 (sensitivity, 74.1%; specificity, 60.8%) for LHL15. The horizontal line indicates the cutoff point with the best separation between the 2 groups (severe fibrosis+, severe fibrosis-). AUC: Area under the ROC curve; ROC: Receiver operating characteristic; HC: Hepatic clearance.

an analog of asialoglycoprotein, enables the quantitative evaluation of liver functional reserve. SPECT analysis in ^{99m}Tc-GSA liver scintigraphy, which allows the evaluation of GSA accumulation in the liver, was also developed to investigate liver function[13]. ^{99m}Tc-GSA HC, which is determined based on SPECT data, demonstrates the precise distribution of ASGPR in the liver, thereby providing an accurate calculation of liver functional reserve[29]. In this study, ^{99m}Tc-GSA HC showed a correlation with conventional liver function tests and the extent of liver fibrosis that was better than that of LHL15 or HH15. LHL15 and HH15, which are hepatic uptake and blood clearance ratios in ^{99m}Tc-GSA liver scintigraphy, are the simplest and most commonly used variables. However, they may be insufficient for accurately estimating the degree of liver fibrosis because these indices are calculated from planar scintigraphic images, which do not correctly reflect hepatocyte volume. In contrast, ^{99m}Tc-GSA HC measured by SPECT analysis contains volumetric information and may correctly estimate the hepatocyte volume, thus reflecting the degree of liver fibrosis.

In liver surgery, the risk of perioperative complications is generally believed to increase when the remnant liver volume (RLV) is excessively small[30]. Therefore, reports have advocated preoperatively assessing RLV with CT volumetry[31]. However, CT volumetry can never reflect the function of the remnant liver, especially in patients with parenchymal disease[30,32], such as chronic hepatitis or cirrhosis. Additionally, several reports concerning ^{99m}Tc-GSA SPECT findings have indicated that regional function is not necessarily uniform throughout the liver[33,34], suggesting that an accurate estimation of regional liver function is more important for predicting postoperative liver functional reserve. In this study, ^{99m}Tc-GSA HC strongly reflected the degree of liver fibrosis. Therefore, we believe that using the combined ^{99m}Tc-GSA HC and CT volumetric measurements of the remnant liver can evaluate remnant liver functional reserve after hepatectomy[35]. Further studies are needed to test this hypothesis.

In conclusion, we demonstrated that HC measured with ^{99m}Tc-GSA SPECT showed correlations with the degree of liver fibrosis and conventional liver function tests. ^{99m}Tc-GSA HC was the most valuable index for predicting severe fibrosis. It could yield a more accurate estimation of liver fibrosis compared with currently used measures before hepatectomy for hepatobiliary surgeons.

COMMENTS

Background

Liver fibrosis is a negative predictive factor for postoperative hepatic failure. Therefore, the accurate preoperative estimation of the extent of hepatic fibrosis is essential for successful liver surgery. Although many liver fibrosis indicators have been proposed for preoperative evaluation, the best indicator for evaluating liver fibrosis has not yet been established.

Research frontiers

Technetium-99m-diethylenetriaminepenta-acetic acid-galactosyl human serum albumin (^{99m}Tc-GSA) liver scintigraphy reflects the liver functional reserve and is reported to correlate with several hepatic function tests. In addition, single-photon emission computed tomography analysis in ^{99m}Tc-GSA liver scintigraphy, which can evaluate GSA accumulation in the liver, was also developed to investigate liver function.

Innovations and breakthroughs

Hepatic clearance which was measured with ^{99m}Tc-GSA single-photon emission computed tomography (SPECT) is a reliable index for assessing liver fibrosis.

Applications

Hepatic clearance which was measured with ^{99m}Tc-GSA SPECT could yield a

more accurate estimation of liver fibrosis compared with currently used measures before hepatectomy for hepatobiliary surgeons.

Terminology

^{99m}Tc-GSA liver scintigraphy: Technetium-99m-diethylenetriaminepenta-acetic acid-galactosyl human serum albumin liver scintigraphy. SPECT analysis: Single-photon emission computed tomography analysis.

Peer review

The manuscript evaluates the utility of ^{99m}Tc-GSA SPECT to reliably predict the degree of liver fibrosis in patients for liver resection is planned. Comparisons are made to particularly state that hepatic clearance is superior to other measurements (LHL15 and HH15), other techniques (ICGR15), and clinical parameters of liver function when predicting fibrosis. The study has relevance and is interesting in its concept; however some conclusions are made that need to be justified by more rigorous data analysis.

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ELSEVIER

Impact of Machine Perfusion Preservation of Liver Grafts From Donation After Cardiac Death

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ABSTRACT

Because of the critical shortage of deceased donor grafts, using a donation after cardiac death (DCD) donor is an important resource. However, the ischemic damage of those DCD grafts jeopardizes organ viability during cold storage. Maintaining organ viability after donation until transplantation is important for optimal graft function and survival. This review describes the effective preservation in transplantation for DCD livers. Concepts and development of machine perfusion for DCD liver grafts to reduce ischemia/reperfusion injury are discussed. Despite the fact that hypothermic machine perfusion might be superior to static cold preservation, DCD livers are exposed to hypothermia-induced damage. Recently, some groups introduced the beneficial effects of normothermic or subnormothermic machine perfusion in DCD liver preservation and transplantation.

THE SHORTAGE of donors for transplantation is a universal problem. The wait list for organs has continued to grow. However, the use of marginal donors is a promising way to increase the supply. In particular, use of organs from non–heart-beating donors (NHBD) and donation after cardiac death (DCD) are gaining importance as potential sources of vital organs for clinical transplantation. The two approaches to preservation before transplantation are simple cold storage (SCS) and machine perfusion (MP). The simplicity, lower cost, and need for transport make cold storage the method of choice for the majority of transplantation centers. However, the major principle of simple hypothermic liver preservation is the reduction of metabolic activity. Although MP using hypothermia may have a theoretical advantage in providing metabolic support and oxygenation, its use has not become widespread in clinical practice. Recently, the short- and long-term function of kidneys procured from DCDs by means of normothermic recirculation were reported [1]. The principle of normothermic and subnormothermic perfusion is to recreate the physiological environment by providing the essential substrates for cellular metabolism, oxygenation, and nutrition. In this review, based on the historical background of transplantation from DCD, clinical donor criteria for DCD livers and the progress of MP for DCD livers in cold storage are introduced. Finally, the method of rewarming preservation for DCD liver transplantation is introduced as a challenge using a new MP system.

HISTORICAL BACKGROUND FOR LIVER TRANSPLANTATION FROM DCD DONORS

In March 1995, an international workshop for NHBD was held in Maastricht, Netherlands. DCDs had been classified as the Maastricht classification [2]. Categories 1, 2, and 4 include uncontrolled DCDs, and category 3 includes controlled DCDs. DCDs have come to represent the fastest growing proportion of the donor pool. In some United Network for Organ Sharing (UNOS) regions with limited standard criteria for donors, DCDs comprised up to 16% to 21% of the total donor pool [3]. After successful use of DCD kidney grafts for clinical transplantation, interest has moved toward using extrarenal organs such as the liver, pancreas, and lungs [4]. However, in the early phase, liver transplantations from DCDs did not always show favorable post-transplantation results. The development of ischemic biliary stricture is a major source of morbidity after DCD liver transplantation.

Retransplantation is also associated with a significantly higher mortality risk. The difficulty with using DCD livers has been considered to be that, although the incidence of

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delayed graft function (DGF) in the kidneys is high, it can be treated with hemodialysis until the kidneys recover. In contrast, DGF in the liver often requires retransplantation as rescue therapy. For this reason, there has been great caution in using DCD liver grafts. Recently, the incidences of primary non-function (PNF) and severe DGF have been remarkably reduced due to the use of selected controlled DCD livers, better selection criteria for advanced preservation technologies, and shortened warm and cold ischemic times. However, this strategy does not always lead to a significant increase in donor numbers. Further studies are needed to identify clinical strategies, such as improving organ preservation, and policies to reduce incidences and improve the outcome of PNF and ischemic cholangiopathy in recipients who have DCD liver grafts.

MP Preservation of Liver Grafts

The introduction of kidney perfusion preservation in clinical practice started in the late 1960s. Folkert O.Belzer had already been working on the continuous hypothermic isolated perfusion and auto-kidney transplantation with blood [5] and cryoprecipitated plasma [6]. The hypothermic MP (HMP) of the first human kidney became a clinical reality soon thereafter; a patient received a kidney that had been preserved for 17 hours using this preservation circuit, and had acceptable function post-transplantation [6]. In the 1970s, HMP was used by transplantation centers mainly in the United States and Europe to preserve and transport kidneys. Consequently, different perfusion machines were also developed and used clinically for kidney preservation. Currently, there are three commercially available renal perfusion devices: the RM3 from Waters Medical Systems (Rochester, MN, USA) (Fig 1A), the LifePort from Organ Recovery Systems (Fig 1B), and the Kidney Assist by Organ Assist b.v. (Groningen, The Netherlands) (Figs 1C,D). However, in 1980, the development of the University of Wisconsin (UW) solution produced by the same UW group allowed surgeons to preserve kidneys for much longer time, up to 72 hours, by simple cold storage [7]. The development of the UW solution provided an alternative to MP, and most centers abandoned the clinical use of MP. During the last few decades, the success of kidney transplantation as the treatment of choice for end-stage renal failure has led to an increasing shortage of suitable organs. This shortage has forced the transplantation community to (re-) consider the transplantation of organs from marginal donors, such as older donors, hemodynamically unstable donors, and NHBD donors. Thereafter, the MP of kidneys from these marginal donors regained worldwide interest.

The international multicenter trial for HMP during kidney transplantation is a well-designed prospective randomized trial of paired kidneys [8], one preserved with SCS and one with MP. The study examined 672 renal transplantations performed in Europe. MP significantly reduced the risk of DGF, as well as significantly improving the rate of the decrease in the serum creatinine level. The number of use of HMP before kidney transplantation is now increased. Regarding liver preservation, Guarrera et al [9] showed the

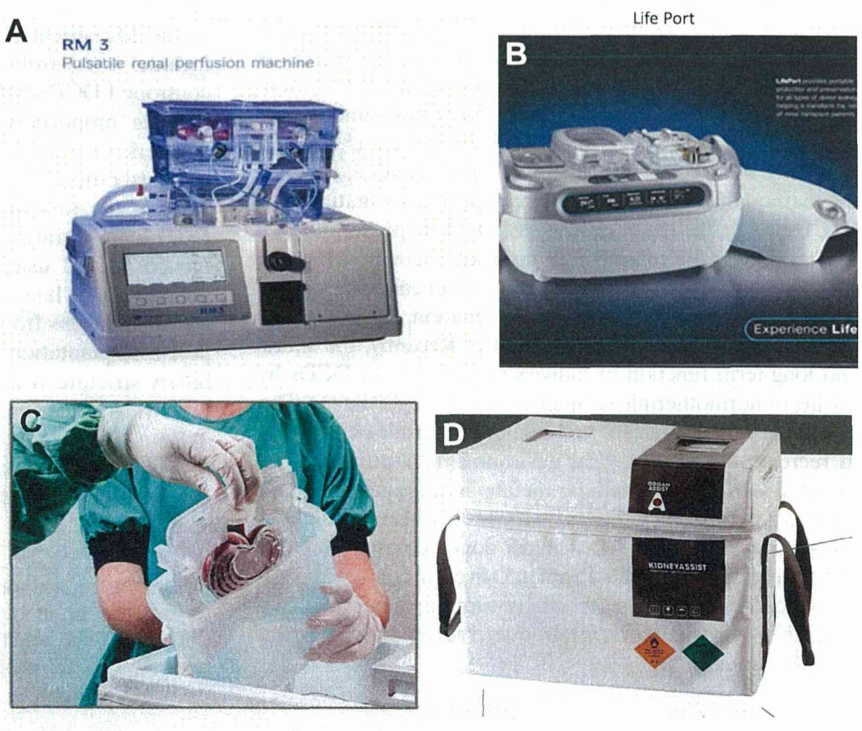

Fig 1. **(A)** RM-3 by Waters Medical System (Rochester, MN, USA). **(B)** LifePort Kidney Transporter by Organ Recovery System Des Plaines. **(C, D)** Kidney Assist by Organ Assist b.v. (Groningen, The Netherlands).

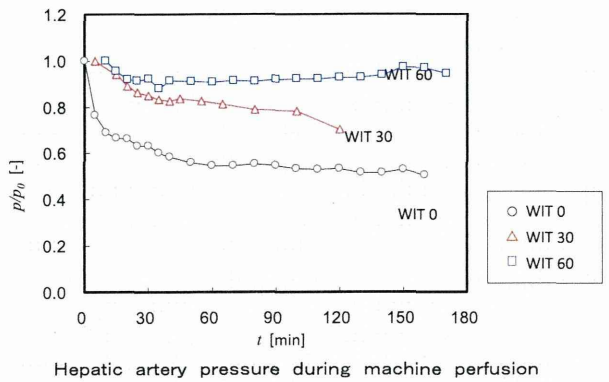

Fig 2. Changes of pressure in hepatic artery during machine perfusion.

outcomes of liver transplantation after 12 hours of HMP or with SCS in a miniature swine model using a new preservation solution, the Vasosolution, which uses a modified Belzer's MP solution. The serum aspartate aminotransferase (AST) and total bilirubin levels were similar in the HMP and SCS groups, indicating that HMP can be used successfully. Later, the Guarrera group reported successful use in human livers. The outcomes of liver transplantation were reported to be satisfactory compared with simple cold storage [10].

Pretransplantation viability testing for a DCD graft is particularly important. An advantage of using MP preservation is that it enables the performance of viability tests on the grafts while they are stored. Preservation by machine enables the physician to judge the acceptability of the graft by registering the flow and pressure characteristics and analyzing the enzymes in the perfusate. Developing a system of MP to establish viability assessments of the liver has not been easy due to the unique blood supply of liver grafts. Predicting viability by evaluating flow in the portal system is not possible because the portal flow is wide ranged and the systems used have found it difficult to generate portal pressure that shows efficient portal flow in the hypothermic stage. Even tissue and vascular resistance, which provide important information in kidney preservation, are particularly low due to easy destruction in the liver. The effluent AST and lactate dehydrogenase (LDH) levels collected in preservation solution have been reported to be useful and predictable biomarkers in previous reports [11–13]. Recently, Obara et al developed a novel liver perfusion system and found that the degree of decreasing hepatic arterial pressure is significantly correlated with the length of warm ischemic time (Fig 2) and the levels of liver enzymes (AST, LDH) in cold perfusate during continuous preservation [14] (Fig 2).

Challenge in DCD Liver Grafts Using MP

Despite successful MP for DCD kidney grafts, DCD liver transplantation has been challenging. There are important limitations of basic research using small animals because of the difficulties associated with assessment of the hepatic artery flow. In large-animal and clinical studies, successful transplantation was achieved by Brettschneider et al after 24 hours of MP in a canine model [15]. Starzl et al preserved the first 11 human livers up to 7.5 hours by the same method [16]. However, the use of fresh diluted blood is inconvenient in the clinical setting. Low-pressure HMP was applied via the hepatic artery in porcine livers for 2 hours before transplantation and compared to similar grafts stored in cold Euro-Collins solution for the same period. Both the LDH and AST levels were consistently lower in the HMP group compared with the SCS group [17]. A new preservation solution, Polysol, was developed for MP by the Amsterdam group in 2005. Polysol solution contains many vitamins and a protein-like, enriched tissue culture medium for functional recovery during preservation, which is expensive [18,19]. As for DCD liver grafts in large animals, most groups agree that 30 minutes of warm ischemic time (WIT) plus 4 to 5 hours of cold preservation results in primary loss of function in the pig liver [20,21]. Dutkowski used a large animal model to test whether short-term hypothermic oxygenated perfusion (HOPE) – treated DCD livers could experience the same benefits as those noted in the previous report using a rat model. The porcine DCD liver with 60 minutes of WIT preserved with SCS for 6 hours could be rescued by a 1 hour short-term HOPE treatment [22]. Lower values of AST and LDH after reperfusion, and a higher survival rate up to 30 hours in the HOPE group were shown. We developed a new preservation machine with a temperature-controlled system (NES) (Fig 3A). We reported beneficial functional recovery in the HMP group after 30 minutes of WIT plus 4

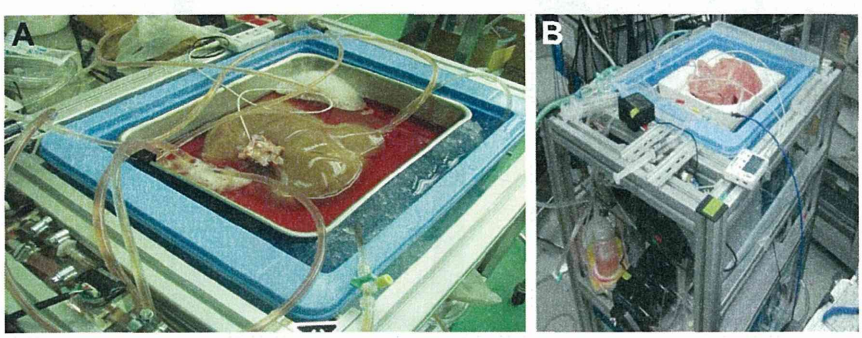

Fig 3. (A, B) Liver Perfusion System (NES).

to 5 hours of total ischemic time compared to the SCS-only group in a porcine liver transplantation model [23]. On the other hand, the concept for DCD graft has been changed and reported in recent years. Experimental studies have shown that even brief periods of cold preservation will cause injury to hepatocytes, Kupffer cells, and endothelial cells in DCD livers, even those later recirculated under normothermia. The use of normothermic extracorporeal membrane oxygenated (NECMO) perfusion is based on experimental studies which have shown that the recirculation of oxygenated blood at 37°C improves the cellular energy load, reduces tissue injury, and improves the post-transplantation graft function in livers damaged by the period of warm ischemia caused by cardiac arrest [24,25]. In 2002, the Hospital Clinic in Barcelona developed a clinical protocol to resuscitate organs from donors and to maintain viability for transplantation [26]. The protocol includes cannulation of the femoral vessels to establish an NECMO circuit. NECMO is used to reperfuse and oxygenate abdominal organs after cardiac arrest while the potential DCD is evaluated and consent for organ donation is obtained. In 2007, the first 10 human liver transplantations were performed with uncontrolled DCDs in which the donor was maintained with NECMO before organ retrieval. Ten DCD livers were transplanted with only 1 graft lost to PNF and 1 to hepatic artery thrombosis. In March 2013, two cases of human warm liver perfusion were successfully transplanted in Kings College Hospital group. The great advantage of normothermic preservation, including the use of NECMO, is the ability to overcome the disadvantaged aspects of hypothermic cellular physiology [27]. However, the use of blood-based perfusates may increase the risk of microvascular failure and sinusoidal plugging and bacterial growth. Normothermic preservation requires full metabolic support with a large machine. Additionally, any equipment failures result in unexpected warm ischemic injury. Therefore, achieving normothermic liver preservation remains troublesome and expensive. The reality of clinical organ retrieval might require a period of cold preservation due to transport between institutions. Some studies have investigated the perfusion temperature. For example, subnormothermic MP performed at 20°C resulted in reduced vasoconstriction, as well as lower metabolic requirements in DCD [28] and steatotic [29] rat models. Shigeta et al successfully transplanted porcine livers with 60 minutes of WIT plus 4 hours of total ischemic time by rewarming preservation from 4°C to 22°C using MP [30]. Development of liver perfusion system in the world is shown in Figure 4.

CONCLUSION

Traditional methods of hypothermic preservation based on both static and machine storage may not be best for DCD liver grafts because liver organs from DCDs have already suffered severe tissue damage secondary to hypoxia and hypoperfusion before the initial period of warm ischemia. Additional cold storage damage to the organ caused by hypothermic conditions may limit the ability to improve cellular function because metabolic activity is decreased in the cold storage. Ideally, these livers will be continuously perfused ex vivo with warm or subnormothermic oxygenated preservation solution. Rewarming preservation during perfusion may become practically available and useful.

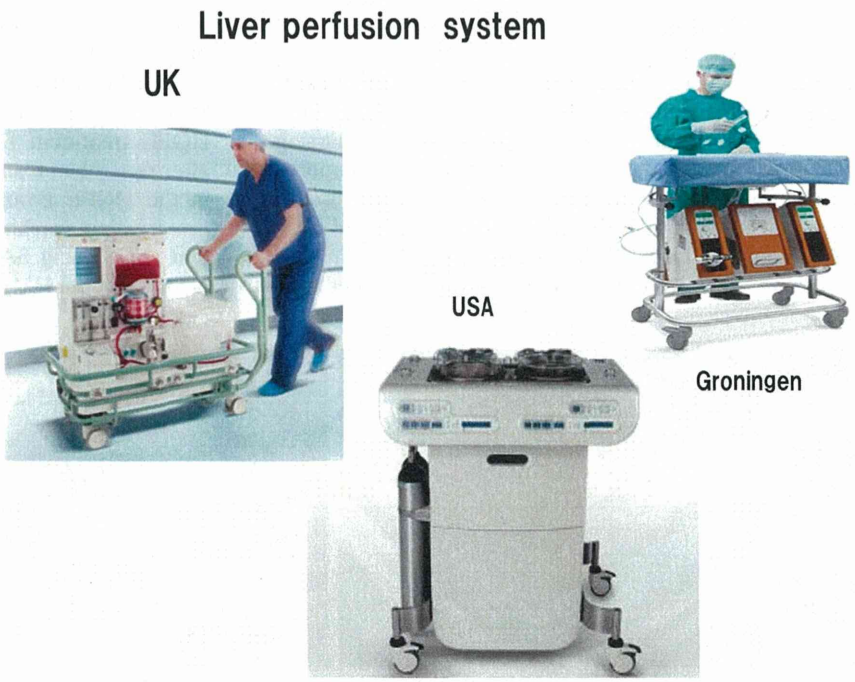

Fig 4. Development of liver perfusion system.

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エクソーム解析に着手し，解析を進めている．しかし，トリオ解析と比較して，大量のシークエンスデータや遺伝子変異情報を適切かつ効率的に処理する必要があり，あらたな計算手法・解析手法の開発が期待される．

一方で，エクソーム解析を利用して，対象とする疾患の原因遺伝子変異が特定されれば，そこからあらたな薬剤や治療法が開発される可能性がある．そればかりか，遺伝的背景に応じて最適な医療を選択する“オーダーメイド医療”の実現へ向けて大きく前進するであろう．

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免疫学

免疫抑制療法の進歩と展開

Current progress in immunosuppression for transplantation

移植医療の歴史は拒絶反応の機序解明と免疫抑制剤による拒絶抑制の歴史ともいえる．免疫抑制剤の進歩は臓器移植の成績に大きく貢献しており，とくに1980年代のカルシニューリン阻害剤(CNIs)の登場は移植成績を大きく向上させ，現在では移植後1年以内の臓器生着率は90%以上である．

本稿では，近年注目されている免疫抑制剤の使用と新しい免疫抑制法について解説する．

免疫抑制法の進歩

臓器移植後の免疫抑制法は作用機序の異なる免疫抑制剤を併用し，それぞれの副作用を軽減する多剤併用療法が一般的になりつつある．細胞の核酸合成を阻害するmycophenolate mofetil(MMF)とCNIsの併用，あるいは抗IL-2レセプター抗体とCNIsの併用，導入療法にalemtuzumab(抗CD52抗体)を用いるなどの工夫により，肝移植においてCNIsが引き起こす腎機能障害を軽減するとされている[1]．近年，mTOR阻害剤が免疫抑制剤として注目されており，腎機能障害を引き起こさないこと，免疫抑制効果に加えて抗腫瘍効果をもち合わせることが特徴である．肝細胞癌に対する脳死肝移植において，mTOR阻害剤は移植後の肝細胞癌再発を抑制することが報告されており，現在国外で臨床試験が行われている[2]．さらに，

表1　免疫抑制剤の種類とその作用機序

	免疫抑制剤	作用
カルシニューリン阻害剤	Cyclosporine A Tacrolimus	IL-2産生抑制/T細胞活性化抑制
M-TOR阻害剤	Sirolimus(Rapamycin)	M-TORを阻害して細胞周期G1期からS期への誘導抑制
	Everolimus	Sirolimusの誘導体
モノクローナル抗体	Alemtuzumab	Anti-CD52/T，B，NK細胞除去
	Basiliximab	Anti-CD25/T細胞活性化抑制
	Belatacept Abatacept	Anti-CD80/86/T細胞のco-stimulation阻害 (CD80.86-CD28 interaction)
	Alefacept	Anti-CD2/T細胞のco-stimulation阻害 (LFA3-CD2 interaction)
	Rituximab	Anti-CD20/B細胞除去
ポリクローナル抗体	Thymoglobulin	種々の細胞表面マーカーに反応/T，B，NK細胞除去
アポトーシス抑制蛋白阻害剤	ABT-737	Bcl-2阻害剤/骨髄移植でドナー反応性リンパ球除去
プロテアソーム阻害薬	Bortezomib	プロテアソーム阻害/形質細胞除去
プリン体合成阻害剤	Mycophenolate mofetil	ミコフェノール酸に加水分解されプリン合成系を選択的に阻害/リンパ球分化抑制
	Azathioprine	チオイノシン酸に変換され，イノシン酸と拮抗して核酸の生合成を阻害/リンパ球分化抑制
ステロイド剤	グルココルチコイド	さまざまな機序による免疫抑制

mTOR阻害剤は移植心冠動脈病変の進行を軽減することから心臓移植の分野で，また腎毒性をもたないことから腎移植においてラ島に対して毒性をもたないことからラ島移植にも有効とされている．1990年代からT細胞活性化における副シグナルの役割の研究が盛んとなった．とくに抗原提示細胞に発現するCD80/86からTリンパ球上のCD28へのシグナル伝達を抑制することにより，抗原特異的に免疫寛容を誘導できることが動物実験で明らかになった．CD28のシグナル伝達を抑制するbelataceptはヒトTリンパ球の活性化を抑制し，腎障害を引き起こすことなく免疫抑制効果を発揮する新しい免疫抑制剤である．海外で2011年6月に腎移植で使用が認可され，アメリカ第Ⅲ相臨床試験では腎移植後1年生存率，臓器生着率はcyclosporine Aと差がなかったものの，移植後3年では腎機能の改善と心血管系のリスク軽減をbelatacept投与群に認めたとしている[3]．副作用としては移植後リンパ増殖症(PTLD)の頻度が有意に高く，とくにEBウイルス未感染の腎移植患者に多くみられたため，現在EBウイルス既感染患者のみへの投与とされている．また，肝移植においてのアメリカ第Ⅱ相臨床試験で術後合併症に伴う死亡例がbelatacept投与群に比較的多く認めたために肝移植まで適応が広げられなかった．Belataceptに関してはさらなるデータの蓄積が必要である[4]．

免疫寛容の誘導

混合キメラの誘導は移植臓器特異的な免疫寛容誘導に重要である．ABT-737はアポトーシス抑制蛋白であるBcl-2の活性阻害剤であり，動物実験でABT-737を骨髄移植後に短期間投与したところ，ABT-737がドナー反応性のリンパ球を除去し，安定した混合キメラが誘導されて，免疫寛容を誘導することができたと報告された[5]．免疫寛容における臨床試験においては国際的な組織であるImmune Tolerance Network(ITN)主導で行われた臨床試験の結果が大きなインパクトを与えた．ハーバード大学ではレシピエントの胸腺への放射線照射，T細胞除去(抗CD2抗体)，rituximab(抗CD20抗体)，cyclophosphamideに加えてドナーの骨髄移植をすることにより10人の腎移植患者のうち7人に免疫寛容を誘導することに成功し，そのうち4人は免疫抑制剤なしに最長11年半の生着を認めている[6]．また，スタンフォード大学では16人の腎不全患者に対して，10回のtotal lymphoid irradiation，腎移植後に5回のthymoglobulin投与，そしてドナーの$CD34^+$細胞と1×10^6個のドナーT細胞を術後11日目に投与したところ，15人の患者でGVHDを起こさずにキメラが確認でき，11例で免疫抑制剤からの離脱に成功している[7]．興味深いことに，レシピエントの血液中においてnatural killer T細胞と$CD4^+CD25^+$制御性T(Treg)細胞の割合が増加しており，それらの細胞がドナー細胞の活性を抑制するとともに，移植された臓器の拒絶を抑制していると考えられている．$CD4^+CD25^+$Treg細胞の発見により自己免疫疾患のメカニズム解明，癌免疫，免疫寛容誘導などのさまざまな分野に新しい道が開かれた[8]．King's College(London, UK)においては肝移植における自己Treg細胞を用いた細胞療法の単独施設臨床研究“ThRIL”が予定されており，その新しい免疫抑制法が注目されている．2005年にはレシピエントの脾細胞をドナー抗原と抗CD80/86抗体とともに培養し，ドナー抗原特異的な免疫不能状態へ誘導した後，その細胞を腎移植後のレシピエントに移入し免疫寛容を誘導するというinfectious toleranceを応用した画期的な方法が考案され，サルを用いた腎移植で成功している[9]．この方法は現在，腎移植，肝移植での臨床試験がはじまっており，その結果が期待されている．

おわりに

iPS細胞やES細胞の発見により近い将来拒絶を起こすことのない細胞や臓器をつくることが現実になりつつある．Treg細胞による細胞療法や免疫寛容の誘導も免疫抑制剤を減量して免疫抑制剤の副作用を軽減しようとする点では同じである．今後，それぞれの患者に合った免疫抑制剤を組み合わせるテーラーメイド免疫抑制が可能となり，さらには免疫寛容へ誘導できるように，この分野におけるさらなる研究が期待されている．

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Sofosbuvir plus ribavirin in Japanese patients with chronic genotype 2 HCV infection: an open-label, phase 3 trial

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SUMMARY. Genotype 2 hepatitis C virus (HCV) accounts for up to 30% of chronic HCV infections in Japan. The standard of care for patients with genotype 2 HCV – peginterferon and ribavirin for 24 weeks – is poorly tolerated, especially among older patients and those with advanced liver disease. We conducted a phase 3, open-label study to assess the efficacy and safety of an all-oral combination of the NS5B polymerase inhibitor sofosbuvir and ribavirin in patients with chronic genotype 2 HCV infection in Japan. We enrolled 90 treatment-naïve and 63 previously treated patients at 20 sites in Japan. All patients received sofosbuvir 400 mg plus ribavirin (weight-based dosing) for 12 weeks. The primary endpoint was sustained virologic response at 12 weeks after therapy (SVR12). Of the 153 patients enrolled and treated, 60% had HCV genotype 2a, 11% had cirrhosis, and 22% were over the aged 65 or older. Overall, 148 patients (97%) achieved SVR12. Of the 90 treatment-naïve patients, 88 (98%) achieved SVR12, and of the 63 previously treated patients, 60 (95%) achieved SVR12. The rate of SVR12 was 94% in patients with cirrhosis and in those aged 65 and older. No patients discontinued study treatment due to adverse events. The most common adverse events were nasopharyngitis, anaemia and headache. Twelve weeks of sofosbuvir and ribavirin resulted in high rates of SVR12 in treatment-naïve and previously treated patients with chronic genotype 2 HCV infection. The treatment was safe and well tolerated by patients, including the elderly and those with cirrhosis.

Keywords: Hepatitis C virus, HCV genotype 2, direct-acting antiviral agents, nucleotide polymerase inhibitor.

INTRODUCTION

Approximately two million people in Japan – nearly 2% of the population – are chronically infected with the hepatitis C virus (HCV) [1]. The population of patients with chronic HCV infection in Japan differs from that of other countries; patients are generally older, have more advanced liver disease and are more likely to have received previous treatment for HCV infection [2,3]. It is estimated that 15–30% of Japanese patients with HCV will develop serious complications, including liver cirrhosis, end-stage liver disease and hepatocellular carcinoma [4]. Although genotype 1 HCV is currently the most prevalent strain of the virus in Japan, genotype 2 HCV, which now accounts for up to 30% of infections, is rising in prevalence [5]. The current standard of care regimen for the treatment of chronic genotype 2 HCV infection in Japan is 24 weeks of pegylated interferon alpha (Peg-IFNα) and ribavirin (RBV) [6]. Although relatively high rates of SVR

Abbreviations: CI, confidence interval; GCP, Good Clinical Practice; HCV, hepatitis C virus; ICH, International Conference on Harmonization; Peg-IFNα, pegylated interferon alpha; PK, pharmacokinetics; RBV, ribavirin; SVR12, 12 weeks after therapy.

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have been reported in clinical trials with this regimen (71–86%), the use of Peg-IFNα+RBV in an ageing population with progressive liver disease is limited by safety and tolerability issues. Moreover, a substantial number of patients have absolute or relative contraindications to interferon. As a result, many Japanese patients with chronic genotype 2 HCV infection have no available treatment options and are thus at risk for worsening of liver disease and complications of cirrhosis, including hepatocellular carcinoma.

Sofosbuvir (Gilead Sciences) is an oral nucleotide analogue inhibitor of the HCV-specific NS5B polymerase that has recently been approved in the United States and Europe for the treatment of chronic HCV infection [7]. The labelled use for patients with chronic genotype 2 HCV infection is sofosbuvir and RBV for 12 weeks. In phase 3 studies, 12 weeks of treatment with sofosbuvir plus RBV in patients infected with genotype 2 HCV resulted in rates of SVR12 of 97% in treatment-naïve patients, 93% in patients ineligible to receive interferon and 86–90% in previously treated patients [8–10].

We conducted a phase 3 trial to determine the efficacy and safety of 12 weeks of sofosbuvir and RBV in treatment-naïve and previously treated Japanese patients with chronic genotype 2 HCV infection with and without compensated cirrhosis.

METHODS

Patients

Patients were enrolled between 16 July 2013 and 30 September 2013 at 20 sites in Japan. Eligible patients were aged 20 years or older with a body weight of at least 40 kg. Patients were required to be chronically infected with genotype 2 HCV and with HCV RNA levels $\geq 10^4$ IU/mL at screening. Planned enrolment was for approximately 84 treatment-naïve and 50 previously treated patients. See Supplement for definitions of types of response to prior treatment.

Up to 40% of enrolled subjects in each group (i.e. treatment naive or treatment experienced) could have evidence of compensated cirrhosis at screening (Child-Pugh A). Cirrhosis was defined as liver biopsy showing a Metavir score of 4 or Ishak score ≥5 or a FibroScan score of >12.5 kPa. Patients were required to have ALT and AST ≤10 × upper limit of the normal range, platelet count ≥50 000 per μL, haemoglobin ≥11 g/dL for women and ≥12 g/dL for men and albumin ≥3 g/dL. There were no upper limits on age or body mass index. Similarly, no restriction was applied to white blood cell or absolute neutrophil count at screening.

Study design

In this multicenter, open-label trial, all patients received 12 weeks of treatment with 400 mg of sofosbuvir, administered orally once daily, and ribavirin (Copegus®, Chugai Pharmaceutical Co., Ltd, Tokyo, Japan), administered orally twice daily, with doses determined according to body weight (600 mg daily in patients with a body weight of ≤60 kg, 800 mg daily in patients weighing >60 and ≤80 kg, and 1000 mg daily in patients with a body weight of >80 kg).

In addition to the main study of efficacy and safety, sparse PK samples were collected from all patients over the course of the study for population PK analyses and all patients were eligible to participate in an optional substudy to determine the steady-state pharmacokinetics (PK) of sofosbuvir (and its predominant circulating metabolite GS-331007). The target enrolment per treatment group was approximately 15 patients. For the PK substudy, intensive serial pharmacokinetic samples were collected (samples obtained over 24 h postdose) at either the week 2 or week 4 treatment visits.

Study assessments

Screening assessments included serum HCV RNA levels and IL28B (rs12979860) genotyping, as well as standard laboratory and clinical tests. Serum HCV RNA was measured with the COBAS® TaqMan® HCV Test, version 2.0 for Use with the High Pure System (Roche Molecular Systems, West Sussex, UK), which has a lower limit of quantification (LLOQ) of 25 IU/mL. HCV genotype and subtype were determined at screening using the Siemens VERSANT HCV Genotype INNO-LiPA 2.0 assay.

On-treatment assessments included standard laboratory testing, serum HCV RNA, vital signs, electrocardiography and symptom-directed physical examinations. All adverse events were recorded and graded according to a standardized scale (see Supplementary Table S7).

NS5B amplification and deep sequencing was performed at DDL Diagnostics Laboratory (Rijswijk, The Netherlands) for all subjects who did not achieve SVR12. Deep sequencing of HCV NS5B was performed at the first virologic failure time point if a plasma/serum sample was available and HCV RNA was >1000 IU/mL, along with the respective baseline samples. Amino acid substitutions in NS5B in the samples collected at virologic failure were compared with the genotype 2 reference and the respective baseline sequence for each patient.

The population pharmacokinetic parameters for sofosbuvir and GS-331007 were computed for all subjects from concentration data from intensive and/or sparse samples using the previously established sofosbuvir and GS-331007 population PK models [11].

Statistical analysis

For treatment-naïve patients without cirrhosis, the SVR12 rate was compared to an adjusted historical SVR rate of 69%, using a two-sided exact one-sample binomial test. The historical control rate was calculated from the weighted average of historical SVR rates for noncirrhotic,

treatment-naïve Japanese patients with genotype 2 HCV infection receiving 24 weeks of Peg-IFNα+RBV (79% with a 10% discount applied due to the expected improvement in safety profile and shorter treatment duration – see Supplementary Table S2 for further details). We calculated that a sample size of 50 patients would provide 80% power to detect an 18% improvement in the SVR12 rate over the adjusted historical rate at a significance level of 0.05. For SVR12 rates for the overall population, for treatment-naïve patients with cirrhosis, and for previously treated patients, statistical hypothesis testing was not performed. For these outcomes, we calculated point estimates of SVR12 rates with two-sided 95% exact confidence interval using the binomial distribution (Clopper–Pearson method).

Study oversight

This trial was approved by the institutional review board or independent ethics committees at all participating sites and was conducted in accordance with local regulations and with recognized international scientific and ethical standards, including the International Conference on Harmonization (ICH) guideline for Good Clinical Practice (GCP) and the original principles embodied in the Declaration of Helsinki. The study was designed and conducted according to protocol by the sponsor (Gilead Sciences) in collaboration with the principal investigators. The sponsor collected the data, monitored study conduct and performed the statistical analyses. The manuscript was prepared by Gilead Sciences with input from all authors.

RESULTS

Baseline characteristics

Of the 188 patients who were initially screened, 153 (90 treatment-naïve and 63 previously treated patients) were enrolled and began treatment (Table S1 and Figure S1). The demographic and baseline clinical characteristics of the patients are provided in Table 1. Overall, the majority of patients were female (54%), and all were Japanese. The mean age was 57 years (ranging from 25 to 74 years) and 22% were aged 65 or older.

Previously treated patients were slightly older than the treatment-naïve patients, with a higher percentage of males, higher baseline viral load, with a higher prevalence of cirrho-

Table 1 Baseline Demographic Characteristics

Characteristic	Overall (N = 153)	Treatment naïve (n = 90)	Previously treated (n = 63)
Mean age, years (range)	57 (25, 74)	55 (25, 73)	60 (34, 74)
Mean BMI, kg/m^2 (range)	24 (16.5, 34)	24 (17, 34)	24 (16.5, 34)
Male, n (%)	70 (46)	33 (37)	37 (59)
Mean HCV RNA, $\log_{10}$ IU/mL ± SD	6.3 (0.84)	6.2 (0.92)	6.5 (0.66)
HCV RNA ≥5 $\log_{10}$ IU/mL, n (%)	140 (92)	78 (87)	62 (98)
HCV genotype, n (%)			
2a	92 (60)	52 (58%)	40 (63%)
2b	61 (40)	38 (42%)	23 (37%)
Cirrhosis, n (%)			
No	136 (89)	82 (91)	54 (86)
Yes	17 (11)	8 (9)	9 (14)
IL28B genotype, n (%)			
CC	121 (79)	73 (81)	48 (76)
CT	28 (18)	17 (19)	11 (17)
TT	4 (3)	0	4 (6)
Median baseline ALT, U/L (range)	34 (12, 412)	32 (12, 412)	36 (12, 232)
Baseline ALT >1.5 × ULN, n (%)	43 (28)	28 (31)	15 (24)
Interferon eligibility, n (%)*			
Interferon eligible	72 (80)	72 (80)	Not applicable
Interferon ineligible	5 (6)	5 (6)	Not applicable
Interferon unwilling	13 (14)	13 (14)	Not applicable
Response to prior HCV treatment, n (%)			
Nonresponse	15 (24)	Not applicable	15 (24)
Relapse/breakthrough	45 (71)	Not applicable	45 (71)
Interferon intolerant	3 (5)	Not applicable	3 (5)
Median eGFR, mL/min (range)	85 (51, 209)	86 (52, 175)	84 (51, 209)

*Interferon eligibility was determined by the site investigator based on whether or not, in their judgment, the patient had contraindications to interferon therapy.

sis and non-CC IL28B genotype. Overall, 11% of participating subjects had cirrhosis. The proportions of patients infected with genotype 2a and 2b HCV were 60% and 40%, respectively, which is similar to previous reports of HCV subtype distribution in the Japanese population [4]. Most (80%) of the treatment-naïve patients were considered eligible for interferon therapy, with 6% having contraindications to interferon therapy and 14% unwilling to receive this treatment. Most (71%) of the previously treated patients had experienced virologic breakthrough or relapse after previous treatment, with 24% reporting nonresponse to prior therapy.

Efficacy

Overall, 148 of the 153 patients (97%, 95% confidence interval [CI] 93–99%) achieved SVR12 (Table 2). By prior treatment history, 88 of the 90 treatment-naïve patients (98%, 95% CI, 92–100%) and 60 of the 63 previously treated patients (95%, 95% CI, 87–99%) achieved SVR12. Of the 82 treatment-naïve patients without cirrhosis, 80 (97%, 95% CI 91–100%) achieved SVR12, thus meeting the primary efficacy endpoint for this group of superiority to the adjusted historical control rate of 69% ($P < 0.001$). Of note, all eight treatment-naïve patients (100%) with cirrhosis and eight of the nine previously treated patients with cirrhosis (89%) achieved SVR12. Overall, 16 of the 17 patients with cirrhosis (94%, 95% CI 71–100%) achieved SVR12.

Patient responses according to baseline characteristics are shown in Supplementary Table S3. Rates of SVR12 were high in all subgroups of patients. Patients with characteristics historically associated with poor response to interferon-based treatment – non-CC IL28B genotype, high baseline viral load, elderly patients, cirrhosis – had rates of SVR12 similar to those in patients without these characteristics.

Relapse accounted for all cases of virologic failure; there were no patients with virologic breakthrough or nonresponse during treatment. Among all patients treated, 97% had HCV RNA <LLOQ by treatment week 2, and 100% achieved HCV RNA <LLOQ by treatment week 4. Overall, five patients experienced virologic relapse after the end of therapy: two (2%) treatment-naïve patients and three (5%) treatment-experienced patients. Four patients relapsed by post-treatment week 4, and one patient relapsed between post-treatment weeks 4 and 12. Characteristics of patients who relapsed are provided in Table S4. There were no consistent host or viral characteristics in the five subjects who relapsed; however, the number of virologic failures is too small for any conclusions to be drawn concerning predictors of virologic failure. No patient relapsed after post-treatment week 12. All 148 SVR12 patients (100%) also achieved SVR24.

Viral resistance testing

The NS5B region was deep sequenced in samples collected from the five relapsers at baseline and at the time of relapse. No S282T variant – known to be associated with reduced susceptibility to sofosbuvir – or any other nucleotide inhibitor resistance-associated variants were detected in any patient at relapse. Phenotypic analysis of the NS5B gene showed no change in susceptibility to either sofosbuvir or ribavirin.

Pharmacokinetics

Population pharmacokinetic analysis was performed to estimate the pharmacokinetics of sofosbuvir and its major circulating nucleoside metabolite, GS-331007. The mean (CV%) of steady-state AUC_{0-24} and C_{max} were 973 (31.2) ng*h/mL and 544 (33.6) ng/mL for sofosbuvir ($N = 45$), respectively, and 10 400 (27.2) ng h/mL and 818 (27.9) ng/mL for GS-331007 ($N = 153$), respectively. Within the Japanese study population, there were no clinically relevant differences in the pharmacokinetics of GS-331007 and sofosbuvir, based on age, sex, BMI, cirrhosis status, prior treatment experience or SVR12 outcome.

Safety

Overall, 73% of patients experienced at least one adverse event; however, the majority of patients experiencing

Table 2 Response during and after Treatment

Response	Overall ($N = 153$)	Treatment naïve ($n = 90$)	Previously treated ($n = 63$)
HCV RNA <LLOQ during treatment, *n* (%)*			
At week 2	148 (97%)	88 (98%)	60 (95%)
At week 4	153 (100%)	90 (100%)	63 (100%)
HCV RNA <LLOQ after end of treatment, *n* (%)			
SVR4	149 (97%)	89 (99%)	60 (95%)
SVR12	148 (97%)	88 (98%)	60 (95%)
95% confidence interval	92.5–99%	92–>99%	87–99%
On-treatment failure	0	0	0
Relapse, *n*/*n* (%)	5 (3%)	2 (2%)	3 (5%)

*LLOQ denotes lower limit of quantification, which is 25 IU/mL. SVR denotes sustained virologic response.

adverse events (84%) had only mild (grade 1) events. The most common treatment-emergent adverse events were nasopharyngitis (upper respiratory viral illness), anaemia, headache, malaise and pruritus (Table 3). No patient in the study discontinued treatment prematurely due to adverse events (or for any other reason). Twenty-two patients (14%) had adverse events that led to modification or interruption of a study drug; 20 patients had ribavirin dose reductions to manage anaemia, and one patient interrupted sofosbuvir and RBV for 1 day because of an event of nasopharyngitis. All but one of the 22 patients with modification or interruption of study drugs achieved SVR12. Two patients experienced treatment-emergent serious adverse events: one treatment-experienced 63-year-old woman had a worsening of anaemia for which she was hospitalized, and one treatment-naïve 36-year-old woman had a severe anaphylactic reaction to a bee sting. No patient experienced a life-threatening (grade 4) adverse event, and only three patients experienced severe (grade 3) events, two of which were deemed to be related to study treatment, the above-mentioned case of anaemia and one case of transient, ribavirin-associated hyperbilirubinaemia in a treatment-experienced 65-year-old man, which resolved during follow-up.

The overall rates of adverse events in younger (<65 years) and older (≥65 years) patients did not differ substantially (72% *vs* 76%, respectively), although there was a higher incidence of anaemia and pruritus in older patients (Table S5). The incidence and severity of adverse events in patients with and without cirrhosis at baseline were similar (Table S6).

Table 3 Discontinuations, Adverse Events and Laboratory Abnormalities by Age

Parameter	Overall (N = 153)
Discontinuation of any study drug due to adverse event	0
Serious adverse events	2 (1%)
Anaemia	1 (1%)
Anaphylactic reaction	1 (1%)
Any adverse event	112 (73%)
Common adverse events*	
Nasopharyngitis	45 (29%)
Anaemia	18 (12%)
Headache	15 (10%)
Malaise	11 (7%)
Pruritus	9 (6%)
Laboratory abnormalities, *n* (%)	
Decreased haemoglobin concentration	
<10 g/dL	19 (12%)
<8 g/dL	1 (1%)
Neutropenia (500–<750 per mm^3)	1 (1%)
Hyperglycaemia (>250–500 mg/dL)	3 (2%)
Hyperbilirubinaemia (>2.5–5.0 × ULN)	2 (1%)

ULN, upper limit of normal.
*Adverse events occurring in at least 5% of patients.

Overall, the mean change in haemoglobin from baseline to week 12 of treatment was −1.2 g/dL. For patients aged 65 and older, the mean change in haemoglobin was −1.7 g/dL, as compared with 1.0 g/dL in patients under the age of 65. Of all 153 patients enrolled and treated, 19 (12%) had at least one postbaseline haemoglobin value of <10.0 g/dL, and one (1%) had a postbaseline haemoglobin value of <8.5 g/dL. Two patients (1%) had grade 3 hyperbilirubinaemia; no grade 4 hyperbilirubinaemia occurred. One patient, who had grade 2 neutropenia at baseline, had transitory grade 3 neutropenia.

DISCUSSION

In this phase 3 trial, twelve weeks of treatment with sofosbuvir and RBV resulted in high rates of sustained virologic response (>95%) in treatment-naïve and previously treated Japanese patients with chronic genotype 2 HCV infection. Patients with host and viral characteristics that have historically been predictive of lower rates of SVR – older age, presence of cirrhosis, high viral load, non-CC IL28B alleles – had rates of SVR12 similar to patients without these characteristics. In patients who had been previously treated for HCV infection, the nature of the prior response was not associated with significant differences in rates of SVR following treatment with sofosbuvir and ribavirin; patients who had nonresponse to prior treatment had similar response rates as patients who had previously experienced relapse or viral breakthrough. No clear or consistent baseline predictors of treatment failure were evident among the five patients who relapsed after treatment.

The current standard-of-care treatment for Japanese patients with chronic genotype 2 HCV infection is 24 weeks of Peg-IFNα+RBV. Although patients who received this regimen in clinical trials achieved SVR12 rates ranging from 72% to 86%, these studies were restricted to patients <65 years of age [12,13]. However, the Japanese population chronically infected with genotype 2 HCV includes many patients with characteristics that make the use of interferon-based therapy problematic – older age, progressive liver disease, prior treatment experience and comorbid conditions such as diabetes and cardiovascular disease [14]. Moreover, many patients cannot receive interferon therapy due to relative or absolute contraindications. The interferon-free combination of sofosbuvir and ribavirin may represent a promising treatment option for these patients.

Given the characteristics of the patient population in Japan with HCV infection – generally older, and more likely to have advanced liver disease – safety and tolerability of therapeutic regimens is an important issue. In the present study, 22% of patients were aged 65 or older and 11% had cirrhosis. Analyses of safety data by age (<65 *vs*

≥65 years) showed increases in reported adverse events and laboratory abnormalities in older patients, but these differences did not present a barrier to treatment as no premature discontinuation of study treatment occurred in any patient. Analysis of safety data according to the presence or absence of cirrhosis did not indicate clinically important differences in safety or tolerability of the 12-week sofosbuvir plus ribavirin regimen.

Consistent with previous reports, the results of this study confirm the high barrier to resistance afforded by the sofosbuvir plus RBV treatment regimen. Rapid viral suppression was observed with all patients achieving HCV RNA undetectable status by week 4, with no virologic breakthrough observed during treatment in any of the 153 patients. The percentage of patients who relapsed after treatment was low (3%), and none of the subjects who relapsed had S282T or other nucleoside inhibitor resistance-associated variants. No change in susceptibility to sofosbuvir or ribavirin compared with the corresponding baseline or wild-type reference was observed at the relapse time point.

The main limitation of this study was the lack of a control arm to allow direct comparison with interferon-based regimens. Several considerations guided our choice of an uncontrolled study design. Adding an interferon-based control arm would have required exclusion of patients who were ineligible to receive or intolerant of interferon – an important and substantial proportion of patients – as well as previously treated patients, for whom further interferon treatment is not an option. Moreover, given that Peg-IFNα is administered by subcutaneous injection, blinding of treatment arms would not have been possible.

In conclusion, treatment with the all-oral, interferon-free combination of sofosbuvir and RBV resulted in high rates of sustained virologic response in both treatment-naïve and previously treated Japanese patients with chronic genotype 2 HCV infection. The degree of antiviral efficacy coupled with a favourable safety and tolerability profile, including patients with cirrhosis and those aged 65 and older, suggest that this combination may fill an important unmet medical need in Japan.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Patient disposition.

Table S1. Reasons for screen failure.

Table S2. Calculation of the adjusted historical control rate.

Table S3. SVR12 by subgroup.

Table S4. Characteristics of patients who relapsed.

Table S5. Common adverse events by age group.

Table S6. Common adverse events by cirrhosis status.

Table S7. Gilead sciences grading scale for severity of adverse events and laboratory abnormalities.

Hepatology Research

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Original Article

Simeprevir (TMC435) once daily with peginterferon-α-2b and ribavirin in patients with genotype 1 hepatitis C virus infection: The CONCERTO-4 study

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Aim: The efficacy and safety of simeprevir in combination with peginterferon-α-2b and ribavirin (PEG IFN-α-2b/RBV) were investigated in patients infected with hepatitis C virus (HCV) genotype 1 who were treatment-naïve or had previously received interferon (IFN)-based therapy.

Methods: CONCERTO-4 (NCT01366638) was an open-label, non-comparative, multicenter study of once-daily simeprevir (TMC435) 100 mg in combination with PEG IFN-α-2b/RBV in treatment-naïve and -experienced patients (prior relapsers or non-responders to IFN-based therapy) with chronic HCV genotype 1 infection. Twelve-week combination treatment was followed by 24/48-week response-guided PEG IFN-α-2b/RBV therapy for treatment-naïve patients and prior relapsers, and 48-week PEG IFN-α-2b/RBV therapy for prior non-responders. Patients were followed for 72 weeks after treatment initiation. The proportions of patients with sustained viral response (SVR; undetectable HCV RNA) at treatment end and 12 weeks after the last treatment (SVR12) were among the major efficacy end-points. Safety, including adverse events (AE), was monitored.

Results: Of the 79 patients treated, the proportion achieving SVR12 was highest among treatment-naïve patients (91.7%) and prior relapsers (100%) versus 38.5% of prior non-responders. All treatment-naïve patients and prior non-responders who achieved SVR12 also achieved SVR at treatment end and 24 weeks after last dose; 96.6% of prior relapsers achieved both end-points. Most AE were of grade 1 or 2 severity. Grade 3 AE occurred in 17 patients, most frequently neutropenia (6.3%).

Conclusion: Simeprevir combined with PEG IFN-α-2b/RBV was effective in patients infected with HCV genotype 1, both for initial treatment of naïve patients and for retreatment of patients in whom previous IFN-based therapy had failed.

Key words: chronic hepatitis C, direct-acting antiviral, protease inhibitor, simeprevir (TMC435), sustained virologic response

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