DIRECT ACTING ANTIVIRALS IN HEPATITIS C INFECTION: DEVELOPING A PERSONALISED MODEL OF CARE

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I declare that this thesis has not been submitted as an exercise for a degree at this or any other university, and the work described, except where duly acknowledged, is entirely my own work. I acknowledge the work of Dr ZG Jiang for his help with the competing risk analysis and predictive score described in chapter 5.

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Dedicated to my parents, Amal and Ibrahim, my wife Sarah and son Zade.

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Summary

Hepatitis C infection (HCV) is a major global health problem. The HCV treatment landscape has changed beyond recognition in a few short years. The development of HCV direct acting antiviral therapy (DAA) has occurred extremely rapidly from the approval of the first-generation protease inhibitors telaprevir and boceprevir that were combined with peginterferon (IFN) and ribavirin, to all oral single tablet interferon free combination such as ledipasvir-sofosbuvir in less than 4 years. Despite the dramatic success of DAA development, challenges remain on the path to cure.

The aim of this thesis is to generate data related to (i) the clinical pharmacokinetics (PK) of DAA therapy in real-world patients and (ii) DAA treatment outcomes in special populations. PK observational studies were designed to provide quantitative data and develop population PK (PopPK) models for the first-generation protease inhibitor telaprevir, and the second- generation NS5A inhibitor ledipasvir. DAA treatment outcomes and predictors of improvement in liver function in patients with decompensated cirrhosis were investigated through an integrated analysis of clinical trials of sofosbuvir based IFN-free DAA therapy. Treatment outcomes in persons who inject drugs were assessed in a large retrospective study of 1000 patients treated with peginterferon and ribavirin.

The clinical PK studies of telaprevir demonstrated significant inter-individual variability in telaprevir concentrations and exposure, with lower drug exposure in patients with cirrhosis. A one-compartment Pop PK model with first-order absorption and linear elimination best described telaprevir PK with population clearance 32.4L/hr (CV ~ 20%) and volume of distribution 257L. Weight and fibrosis stage were significant covariates for telaprevir exposure.

A highly sensitive, reliable and reproducible bioanalytical method for the simultaneous quantification of sofosbuvir, GS-331007, ledipasvir, daclatasvir and simeprevir was developed and validated as part of this thesis. This method was used to study ledipasvir PK in a cohort of 314 patients with decompensated cirrhosis treated with ledipasvir-sofosbuvir and ribavirin. There was no effect of Child-Pugh-Turcot (CPT) stage on ledipasvir concentrations, and in multivariate analysis, proton pump inhibitor (PPI) use was the most significant predictor of ledipasvir concentrations. Mean ledipasvir trough concentrations were approximately 20% lower in patients on low dose PPI therapy. Relative bioavailability was 34% lower in patients on low dose PPI therapy based on PopPK modelling.

Patients with decompensated cirrhosis can now be considered for DAA therapy. However, patient selection remains a challenge. An integrated analysis of clinical trial data from 620 patients with decompensated cirrhosis was performed. Patients who achieved SVR_{12} had a significantly higher change of achieving compensated liver status (CPT class A) post treatment (HR 2.1, 95% CI 1.0 – 4.3 p = 0.04). A five point BEAAA score (BMI, encephalopathy, ascites, ALT and albumin) was developed to predict patients that would benefit from treatment. A BEAAA score of 4-5 was associated with a HR of 52.28 (95% CI 15.2 – 179.7) of treatment benefit, and may be used to select patients for treatment.

In the study of outcomes in persons who inject drugs (PWID), treatment outcomes and adherence in recent and former PWID were similar to non-drug users, supporting the inclusion of these marginalised patients in national treatment programmes. The WHO target of HCV elimination may be achievable provided treatment moves towards a more personalised model of care to maximise treatment outcomes in all populations.

CONTENTS

LIST OF FIGURES AND TABLES

Figures

Chapter 6

Tables

LIST OF ABBREVIATIONS

AAG	Alpha-1-acid-glycoprotein
AASLD	American Association for the Study of Liver Diseases
ALT	Alanine Aminotransferase
AUC	Area under the concentration time curve
BCRP	Breast cancer resistance protein
BD	Twice daily
BMI	Body mass index
CL	Clearance
CL_H	Hepatic drug clearance
CL_{int}	Intrinsic Clearance
C_{max}	Maximum Plasma Concentration
C_{min}	Minimum Plasma Concentration
COHP	Countries of High Prevalence
CPT	Child-Pugh-Turcot
C_{trough}	Trough Concentration
CV	Coefficient of variation
CYP	Cytochrome
DAA	Direct Acting Antivirals
DCV	Daclatasvir
EASL	European Association for the Study of the Liver
EC_{50}	Half maximal effective concentration
E_H	Hepatic extraction ratio
EMA	European Medicines Agency

ER	Endoplasmic reticulum
ESLD	End stage liver disease
F	Bioavailability
FDA	Food and Drug Administration
FOCE-I	First order conditional estimation with interaction
fu	Fraction of unbound drug
GFR	Glomerular filtration rate
GLP	Good clinical practice
HBV	Hepatitis B
HCC	Hepatocellular carcinoma
HCV	Hepatitis C
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
IL28B	Interleukin 28B
INF	Interferon
INR	International normalised ratio
IQR	Interquartile range
IS	Internal standard
IU	International unit
IRES	Internal ribosome entry site
kb	Kilo base pairs
kDa	Kilo Dalton
LC/MS-MS	Liquid chromatography/mass spectrometry
LDL	Low density lipoprotein
LDV	Ledipasvir

MELD	Model for end stage liver disease
ml	millilitre
MU	Million Units
NANB	Non-A non-B hepatitis
NCR	Non-coding region
NIH	National Institute of Health
ng/ml	nanogram/millilitre
nm	Nanometer
NONMEM	Non-linear mixed effects modelling
NS	Non-structural
NTP	Nucleoside triphosphate
OFV	Objective function value
Peg	Pegylated
P-gp	P-glycoprotein
PK	Pharmacokinetic
pKa	Partition co-efficient
PopPK	Population Pharmacokinetic Modelling
PPI	Proton pump inhibitor
PWID	Persons who inject drugs
Q_H	Hepatic blood flow
RBV	Ribavirin
RNA	Ribonucleic acid
rpm	Revolutions per minute
RVR	Rapid Virologic Response
SCM	Stepwise covariate modelling

SD	Standard deviation
SIM	Simeprevir
SOF	Sofosbuvir
SVR	Sustained Virologic Response
$t_{1/2}$	Half-life
TBME	Tert-Butyl Methyl Ether
Vd	Apparent volume of distribution
T_{max}	Time to maximum plasma concentration
ULN	Upper limit or normal
VEL	Velpatasvir
VLDL	Very low density lipoproteins
WHO	World Health Organisation
µL	microliter
%RSE	Percentage relative standard error

CHAPTER 1

INTRODUCTION

1.1 Hepatitis C Infection

1.1.1 Global burden of HCV infection

Hepatitis C virus (HCV) infection is a major global health problem. The WHO estimates that in 2015, 71 million persons were living with HCV infection in the world, accounting for 1% of the population [1]. An estimated 2.3 million persons living with HIV also had HCV infection. HCV infection prevalence is unevenly distributed in the world. The European and Eastern Mediterranean regions have higher prevalence rates, but there are also variations in prevalence across and within countries. Deaths due to viral hepatitis (HBV and HCV) are increasing, and will continue to do so unless treatment is scaled up. Mortality from viral hepatitis has increased by 22% since 2000 [1]. It is estimated that viral hepatitis was responsible for 1.34 million deaths worldwide in 2015. This number is comparable with the number of deaths from tuberculosis, and higher than the number of deaths from HIV. Left untreated, HBV and HCV can lead to cirrhosis (720,000 deaths) and hepatocellular carcinoma (470,000 deaths). These long-term complications are life-threatening and account for 96% of the deaths due to viral hepatitis [1].

In Ireland, it is estimated that 20,000-50,000 people have chronic HCV infection, a population prevalence of 0.5-1.2%, with a minority engaged in specialist care [2]. The number of hospital admissions due to end stage liver disease (ESLD) and hepatocellular carcinoma (HCC) in those with HCV infection has been increasing in Ireland [3]. As the infected population ages, the number of patients with HCV-related cirrhosis, hepatic decompensation and HCC is expected to peak by 2025.

1.1.2 Classification, Structure and HCV replication

Classification

Hepatitis C (HCV), a positive sense single-stranded-RNA virus, is a member of the *Hepacivirus* genus within the *Flaviviridae* family. Related viruses in this family include the classical flaviviruses (e.g. yellow fever, dengue and tick-borne encephalitis viruses) and the animal pestiviruses. *Flaviviridae* are spherical in shape and contain a lipid envelope [4].

Structure

HCV virions are ~ 50nm in diameter, spherical in shape and are inactivated by chloroform. Mature virions consist of a nucleocapsid and an outer envelope composed of a lipid membrane and envelope proteins. The genome is about 9.6kb in size and made up of a 5' non-coding region (NCR) which contains an internal ribosome entry site (~340nt), an open reading frame (~9kb) and a 3'-NCR (~250nt). The virion consists of three core proteins (the nucleocapsid core protein (C), and two envelope glycoproteins, E1 and E2) and seven non-structural proteins. The structural proteins serve essential functions in the viral replication process and are described in Table 1.1 [4].

Hepatitis C can be classified into seven genetic groups, termed genotypes. These genotypes represent the heterogeneity of HCV isolates recovered from around the world, and differ from each other by about 30-35% at the nucleotide level. Each genotype has a number of subtypes (Table 1.2).

Life Cycle

Humans are the natural host and reservoir of the HCV virus. No other natural hosts have been identified, although the virus can be transmitted experimentally to chimpanzees.

Table 1.1 Functions of HCV non-structural proteins

Non-structural protein	Size (kDa)	Function
p7		Ion channel protein important in viral assembly
NS2	21	Zn-dependent cysteine protease mediates autocatalytic cleavage of NS2/NS3 Also involved in viral assembly and release
NS3	70	Serine protease, helicase, and NTPase activities Cleaves the remaining non-structural proteins
NS4A	6	Co-factor for NS3 serine protease activity
NS4B	27	Induces a membranous replication process at the endoplasmic reticulum
NS5A	56-58	Serine phosphoprotein of unknown specific function Critical for viral replication and assembly
NS5B	68	RNA-dependent RNA polymerase

Table 1.2 Hepatitis C genotypes and subtypes

Hepatitis C Virus	**Accession Number**
HCV genotype 1a	M62321
HCV genotype 1b	D90208
HCV genotype 2a	D00944
HCV genotype 2b	D01221
HCV genotype 3a	D17763
HCV genotype 3k	D63821
HCV genotype 4a	Y11604
HCV genotype 4d	DQ516083
HCV genotype 5a	Y13184
HCV genotype 6a	Y12083
HCV genotype 6g	D63822
HCV genotype 7a	EF108306

Although transfusion associated hepatitis was first described in 1975, the hepatitis C virus was only identified in 1989 by immuno-screening an expression library with serum from a patient with post-transfusion non-A, non-B hepatitis [5]. HCV is transmitted by parenteral exposure to blood, blood products or objects contaminated with blood. HCV infection is a highly dynamic process with a viral half-life of only a few hours with the production of an estimated 10^{12} virions per day in an infected individual [6].

After parenteral acquisition, HCV circulates in the infected host and can be associated with low-density lipoproteins (LDL) and very low density lipoproteins (VLDL) [7]. Hepatocytes are the main target cells for infection. The LDL receptor, glycosaminogylcans and other cell surface proteins are involved in HCV binding to cells [8]. HCV enters hepatocytes by clathrin-mediated endocytosis, with transit through an acidified endosome [9]. The 5'-NCR, together with first 20-40 nucleotides of the core-coding region make up the *internal ribosome entry site* (IRES) required for viral translation and polyprotein processing. HCV translation occurs following the formation of a binary complex between the IRES and the 40S ribosomal subunit [10].

IRES-mediated translation initiation of the HCV open reading frame produces a polyprotein precursor that is co- and post-translationally processed by cellular and viral proteases into mature structural and non-structural proteins. The structural proteins and the p7 polypeptide are processed by the endoplasmic reticulum (ER), whereas the non-structural proteins required for viral replication are cleaved by two viral proteases, the NS2-3 protease and the NS3-4A serine protease [8].

The NS3-4A complex has been a prime target for drug development. NS3 is a multifunctional protein, with a serine protease located in the N-terminal and an RNA helicase/NTPase in the C-terminal of the protein. The NS4A polypeptide serves as a co-

factor for the NS3 serine protease [11]. NS5A is a phosphoprotein involved in specific protein-protein interactions that are essential for the formation of a functional HCV replication complex [12]. The NS5B RNA dependent RNA polymerase is also essential for replication [8]. The NS3-4A serine protease, the NS5A, and NS5B polymerase are critical in the formation of a membrane associated replication complex, composed of HCV viral proteins, replicating RNA and altered cellular membranes. Virions form by budding into the ER or an ER-derived compartment and exit the cell through the secretory pathway to begin the cycle of infection again [8].

Pathogenicity and clinical course of infection

The clinical course of HCV infection ranges from subclinical to acute and chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Persistence of the virus after acute infection occurs in 60-80% of cases, depending on the population studies [4]. Long term follow-up studies demonstrate the progression to cirrhosis in 20-30% of patients after 20-30 years of infection [13]. Risk factors for rapid progression include obesity and the metabolic syndrome, HIV co-infection and significant alcohol intake.

1.1.3 Evolution of HCV therapy

The first pilot study for the treatment of non-A non-B (NANB) hepatitis was undertaken at the NIH in 1984 [14]. Interferon-α, which had shown to have some efficacy in studies with hepatitis B, was subcutaneously administered to 10 patients with risk factors for NANB hepatitis at a dose of 5 million units daily for 16 weeks. Within a month of starting therapy, serum ALT levels were normal and values remained normal throughout the 16 weeks of therapy. ALT levels returned to baseline pre-treatment values within a month of stopping treatment. However, this pilot study led to the initiation of two randomised controlled trials

of IFN-α therapy. Both trials extended the treatment duration from 12 to 24 weeks of subcutaneous INF-α at a dose of either 1, 2 or 3 MU three times weekly with a placebo control arm [15-16]. A dose dependent improvement in ALT values and liver histology was observed, although the efficacy endpoint was measured at the end of treatment, rather than determining a sustained virologic response (SVR). Sustained virologic response was later defined as the absence of HCV RNA in serum 24 weeks after the completion of therapy, and this was shown to be associated with improvements in liver enzymes and histology in follow-up. The SVR rate with IFN-α monotherapy was only 6%. With subsequent trials extending treatment duration to 48 weeks, SVR rates improved to 13-19% [17-18]. The addition of ribavirin to IFN resulted in progressive improvements in SVR rates of 43% [18].

The development of pegylated-interferon represented the next major advance in treatment allowing for once weekly administration of interferon. Prior to the development of direct-acting antivirals (DAA) for HCV, dual therapy with peginterferon and ribavirin had been the standard of care [19]. Peginterferon-α was administered at a dose of 180mcg as a weekly subcutaneous injection. Weight-based dosing of ribavirin was used – with a starting dose of 1000mg/day for patients $\leq$ 75kg and 1200mg/day for patients > 75kg split over two oral doses. SVR rates for dual therapy ranged from 40-50% for genotype 1 and more than 75% in patients with genotype 2 and 3 infection [20-21]. However, patients with "difficult to treat" infection (advanced fibrosis or cirrhosis, patients of African-American ethnicity, individuals co-infected with HIV, and patients with co-morbidities such as fatty liver and diabetes) continued to demonstrate low SVR rates in response to peginterferon and ribavirin combination therapy [22-23].

Personalisation of therapy during the peginterferon era evolved with the use of "on treatment" viral kinetics allowed for the modulation of treatment duration, so-called "response guided therapy". HCV RNA level measurement at baseline, weeks 4, 12 and 24, at the end of treatment, and 24 weeks after treatment withdrawal were used to characterise the virologic response. The rapid virologic response (RVR) was defined as undetectable HCV RNA at weeks 4 of therapy; the early virologic response (EVR) was defined as detectable HCV RNA at week 4 but undetectable at week 12. Patients with a low baseline HCV RNA and an RVR needed 24 weeks of therapy, while patients with a high baseline viral load required extension to 48 weeks [19].

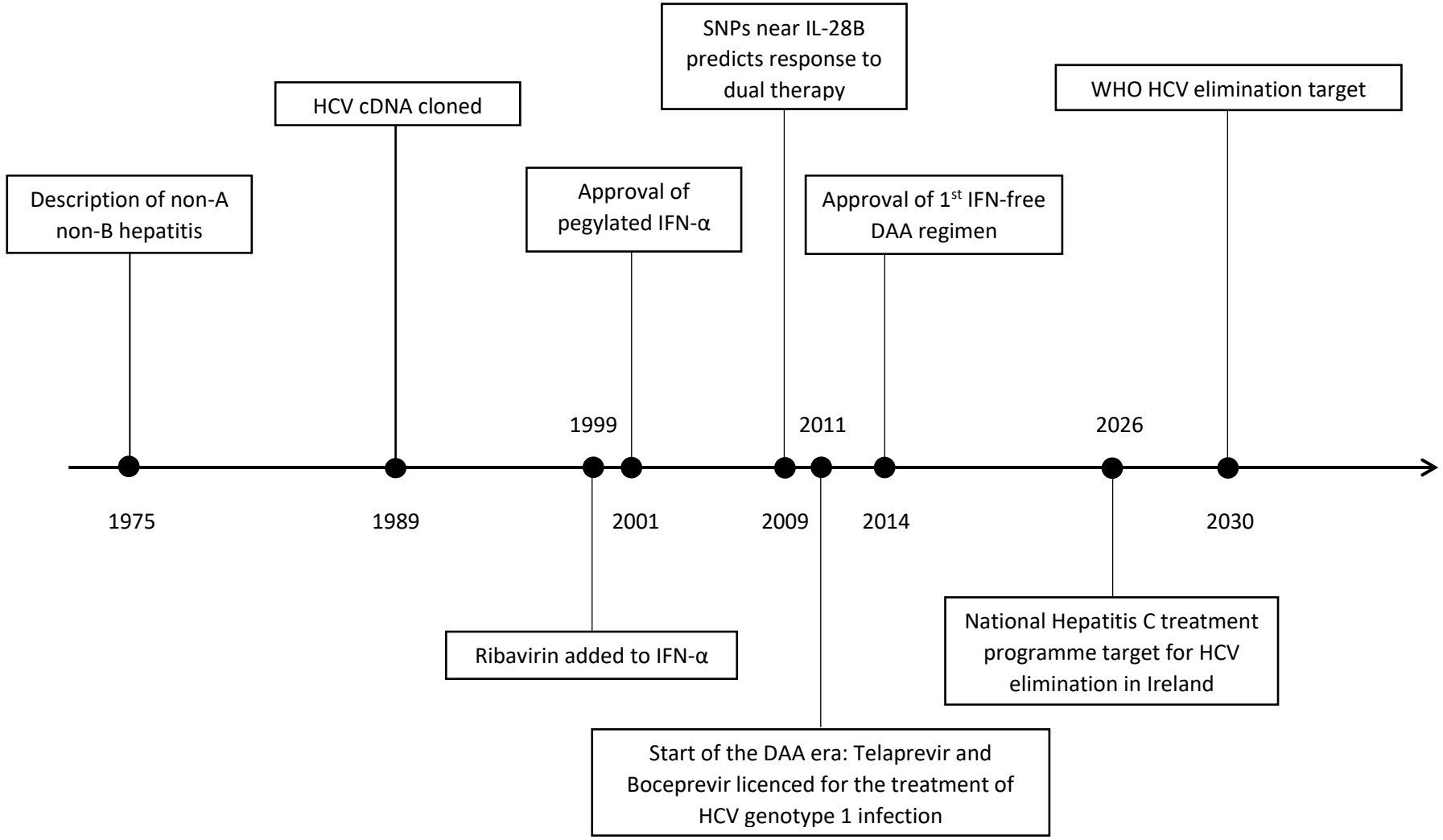
Another important advance towards the end of the interferon era was the discovery by means of genome-wide association studies, of a single nucleotide polymorphism located upstream of the IL28B gene (interferon-λ4) which was strongly associated with the likelihood of SVR with dual therapy. Single nucleotide polymorphism rs12979860 was the single best predictor of response to interferon, with the cc genotype associated with SVR rates of up to 75% in genotype 1 patients [24]. IL-28B genotyping became part of the pre-assessment standard of care for interferon therapy. IL-28B genotyping advanced the personalisation of HCV therapy in combination with response guided therapy – patients with unfavourable genotypes were shown to benefit from extended therapy with pegylated interferon and ribavirin compared to those with favourable genotypes in whom treatment duration could be reduced [25].

Significant advances in the understanding of the molecular virology and replication of HCV came with the development of genotype 1 subgenomic and genomic replicon systems and the identification of the genotype 2a JFH1 clone, which leads to productive infection in cell culture after infection. Using a combination of compound screening and drug

design, small molecule agents with potent activity against various HCV enzymes were discovered. Two first-wave first generation HCV NS3-4A protease inhibitors were approved in combination with peg-interferon and ribavirin. Telaprevir based triple therapy (combined with peginterferon alfa and ribavirin) was associated with an SVR rate of 75% in treatment naïve patients and 83% in previous relapsers with dual therapy [26-27]. Response rates were lower in prior partial and null responders [27]. Anaemia, gastrointestinal sides effects, and skin rashes occurred at a higher incidence with telaprevir compared to patients receiving dual therapy alone. [26]. The pharmacokinetics and clinical efficacy of telaprevir are described in section 1.3.

The HCV drug pipeline continued with the approval of the first NS5B inhibitor, sofosbuvir in combination with peginterferon and ribavirin in 2013. Ledipasvir, the first NS5A inhibitor to receive regulatory approval in late 2014 heralded the dawn of the interferon-free DAA era. There was extensive consolidation of drugs in the pipeline over the following 24 months with the approval of the triple therapy combination of paritaprevir/ritonavir, ombitasvir and dasabuvir for genotypes 1 and 4 infection, the approval of daclatasvir in combination with sofosbuvir for genotypes 1-6 and the approval of the fixed dose combination of grazoprevir-elbasvir for genotypes 1 and 4. A schematic of the HCV journey is outlined in Figure 1.1.

Figure 1.1 – Schematic of the hepatitis C treatment journey



1.1.4 Lessons learned from HIV

Although there are some important differences, many aspects of the early experience with HCV DAA draws strong parallels to HIV therapy. A number of lessons have been learnt from the experience of HIV physicians with early antiretroviral therapy that directly apply to treatment of HCV including antiviral resistance, drug-drug interactions, and the need for new models of care with new treatments.

HIV physicians knew that antiviral monotherapy would result in the selection of resistant viruses. The risk of resistance was mitigated by using multiple antiviral agents with differing mechanisms of action. This knowledge has informed HCV DAA drug development. Although first generation protease inhibitors were used as single agents in combination with pegylated interferon and ribavirin, interferon-free DAA regimens used combination therapy.

Patients that developed resistance to HIV antiretrovirals were treated with complex salvage regimens to achieve viral suppression. Additionally, resistant HIV viruses could be transmitted, necessitating the need for pre-treatment resistance testing. These lessons apply to the HCV DAA era where resistance to all DAA classes and the transmission of resistant viruses have both been described.

New treatments bring new challenges, including new side-effects and the potential for drug-drug interactions (DDI). HIV physicians are experienced in assessing and managing drug-drug interactions to prevent the development of drug resistance or side effects as a result of DDI. The first generation HCV protease inhibitors added to the side effect profile of HCV interferon therapy and necessitated regular patient review during treatment.

Complex algorithms for side effect management were developed (e.g. the management of rash with telaprevir). Interferon-free DAA has improved the tolerability of treatment but drug-drug interactions remain an important consideration.

New treatments require new models of care

Many lessons can still be learned from HIV care especially in the area of access to care for DAA therapy and the need for new treatment models to access difficult to reach patients. HCV therapy has historically been provided in specialist hospital based centres. The HV mantra of "seek, test, and treat" needs to be applied to HCV to reduce the disease burden of advanced HCV liver disease in the community and achieve the WHO eradication targets.

Simply translating the current interferon treatment paradigm to the IFN-free DAA use is unlikely to have a significant impact on HCV disease population burden. Modelling data in the Irish setting of the improved efficacy with DAA therapy predicts a reduction of only 1.7% in viraemic HCV cases if there is no corresponding increase in treatment uptake [28]. A strategy where treatment numbers are gradually increased to 4,700 patients annually by 2020, while prioritising treatment for those with advanced fibrosis up to 2018 has the potential to reduce HCV prevalence by 94% by 2025 in Ireland [28]. Scaling up treatment to achieve these targets require specific strategies to screen and treat marginalised groups including migrants from countries of high prevalence (COHP) and difficult to access people who inject drugs and expansion of treatment into the community setting.

1.2 Pharmacokinetics

1.2.1 Pharmacokinetic parameters and drug metabolism

Orally administered drugs are absorbed from the gastrointestinal tract and delivered to the liver by the portal circulation. Some drugs are readily extracted and metabolised on their first pass through the liver, resulting in only small amounts of the parent drugs reaching the systemic circulation – known as first pass metabolism.

Following oral administration, the plasma concentrations of a drug reaches a maximal concentration (C_{max}) over a defined time (T_{max}). The area under the concentration-time curve (AUC) is a function of plasma drug concentrations over time and is a measure of systemic drug exposure. A blood sample drawn before the next dose is due is the trough plasma concentration (C_{trough}) and is not always synonymous with the minimal plasma concentration (C_{min}) – i.e. the lowest plasma concentration of a drug during a dosing interval.

The half-life of a drug is defined as the time taken for the plasma concentrations to decrease by half and provides an indication of drug elimination. Repeated drug administration results in the accumulation of the drug in the body. Steady state concentrations during a dosing interval are reached when the amount of drug administered equals the amount of drug eliminated from the body. The vast majority of drugs achieve steady state concentrations after 5 half-lives.

The bioavailability (F%) of a drug is a measure of the proportion of the administered drug which reaches the systemic circulation intact. Bioavailability of a drug may be directly quantified from the ration of AUC following intravenous and oral administration.

The apparent volume of distribution of a drug (V_D) is the hypothetical volume in which the total amount of drug in the body would be required to be dissolved in order to reflect the drug concentration attained in plasma. It has no direct physiological meaning and is not a "real volume". Drug clearance (CL) is defined as the volume of plasma in the vascular compartment cleared of drug per unit time by the processes of metabolism and excretion.

The liver is the major site for drug metabolism through the cytochrome P450 enzyme family. CPY450 enzymes play a central role in drug metabolism, with CPY3A isoenzymes involved in the metabolism of the majority of drugs [29]. CYP enzymes can be induced or inhibited by a number of drugs, resulting in increased or decreased metabolism of CYP substrates. Drug transporters such as P-gp (P-glycoprotein), the BCRP (Breast Cancer Resistance Protein) and the organic anion transporters OATP1B1 and OATP1B3 – are increasingly recognised for their role in drug metabolism and clearance, as well as drug-drug interactions.

1.2.2 Drug disposition in liver disease

Direct acting antivirals for HCV are administered to patients with underlying liver disease with varying degrees of fibrosis. Treating patients with advanced fibrosis and cirrhosis with DAA therefore requires an understanding of altered drug disposition in liver disease as liver dysfunction can affect drug absorption, protein binding and distribution, metabolism and elimination.

The pharmacokinetics of drugs with an intermediate to high first pass effect will be altered in patients with cirrhosis, with increased systemic exposure resulting from portosystemic shunting and decreased activity of a number of important drug metabolizing enzymes.

For most drugs, hepatic metabolism is the major elimination pathway. Hepatic drug clearance (CL_H), defined as the volume of plasma from which a drug is removed completed by the liver per unit time, is a function of hepatic blood flow (Q_H) and the hepatic extraction ratio (E_H) of the drug:

$$CL_H = Q_H \times E_H$$

Since E_H depends on liver blood flow, the intrinsic clearance of unbound drug (CL_{int}), and the fraction of unbound drug in blood (fu), the following fundamental equation for hepatic clearance has been derived:

$$CL_H = Q_H \times \frac{fu \times CL_{int}}{Q_H + fu \times CL_{int}}$$

This equation is based on the "venous equilibrium model", a kinetic model used to describe the relationship between hepatic drug clearance and the three determinants of hepatic drug elimination. Drugs can be categorised according to the efficacy of the liver in removing the substance from the circulation as having a high ($E_H > 0.7$), low ($E_H < 0.3$) or intermediate ($0.3<E_H<0.7$) hepatic extraction ratio. The hepatic clearance of highly extracted drugs is rate limited by liver blood flow, and clearance of these drugs is said to be blood flow-limited and relatively insensitive to changes in binding of drugs to proteins or enzyme/transporter activity. Disease states associated with alteration in liver blood flow and porto-systemic shunting will have a significant impact in the hepatic clearance of these drugs when administered orally. Drugs with low hepatic extraction ratios are considered to be limited by enzyme/transporter capacity. The distribution of highly protein bound drugs may be substantially altered in patients with cirrhosis [30].

1.2.3 Population pharmacokinetic modelling

Pharmacokinetic models are hypothetical structures that are used to describe the fate of a drug in a biological system following its administration. Population pharmacokinetic modelling (PopPK) is an invaluable tool in PK studies. PopPK aims at quantitative assessment of typical pharmacological parameters, and the inter-individual and residual variability in drug absorption, distribution, metabolism and excretion [31-32]. It allows for the use and analysis of sparse PK sampling in characterising interindividual variability in PK parameters as well as examining co-variates for drug exposure. Three steps are involved in developing a PopPK model: (i) defining the structural model and number of compartments (e.g. single vs multiple compartment models), (ii) identifying statistical parameter estimates to define the population Clearance, Volume of distribution and relative bioavailability, and (iii) examining the effect of covariates on the model fit and parameter estimates [31-32]

1.3 Telaprevir

Telaprevir (formerly VX-450) is a small molecule NS3/4A serine protease inhibitor with specific activity against HCV genotype 1, approved for the treatment of HCV genotype 1 infection in combination with peginterferon and ribavirin. The recommended dosing regimen was 750mg tds for 12 weeks, taken orally with food containing at least 20g of fat. Alternatively, telaprevir could be given at a dose of 1125mg bd for 12 weeks with food containing at least 20g of fat. Peginterferon and ribavirin (dual therapy) were continued for an additional 12 or 36 weeks based on previous treatment experience, IL-28B genotype and on-treatment viral response in non-cirrhotic patients. In cirrhotic patients, dual therapy was continued for an additional 36 weeks [33].

1.3.1 Pharmacokinetics

Telaprevir is orally bioavailable with a significant food effect on absorption. The area under the concentration-time curve (AUC) has been shown to increase by 237% with a standard fat meal (249 kcal, 21g of fat) compared to fasting conditions in healthy volunteers [34]. Telaprevir is a P-glycoprotein (P-gp) substrate, and it's absorption can be altered by other substrates that induce or inhibit intestinal P-gp [35]. It is moderately protein bound to alpha-1-acid glycoprotein (AAG) and albumin (59-76%) in a concentration dependent manner [35]. As it is moderately protein bound, a potential for increased free fraction exists as a result of protein-binding displacement. Telaprevir has a large apparent volume of distribution (V/F) of 252L, consistent with penetration of telaprevir into tissues beyond the systemic circulation. The interindividual variability of V/F was estimated to be about 72% in population pharmacokinetic analyses [35].

Telaprevir undergoes extensive hepatic biotransformation, involving oxidation, hydrolysis and reduction reaction producing 10 metabolites. *In vitro* studies suggest that telaprevir is both a substrate and moderate inhibitor of the CYP3A4 isoenzyme [Vertex 2010]. Data on phase II or III metabolism are limited. Approximately 1% of radiolabelled ^{14}C telaprevir is recovered in urine following oral administration, suggesting renal excretion is clinically insignificant [35].

Pharmacokinetic (PK) data in HCV infected patients are almost exclusively limited to three times daily telaprevir dosing, with only two studies to date providing PK data in patients treated with twice daily telaprevir dosing [36-37]. For HCV genotype-1 infected patients with combination dual therapy, steady state concentrations were reached after 3-7 days on telaprevir when dosed at 750mg tds; an effective half-life of 9-11 hrs. Average ($\pm$ SD) values of C_{max} , C_{min} and $AUC_{0\text{-}8h}$ based on population pharmacokinetic analyses from

phase 3 trials were 3260 (± 946) ng/ml, 2690 (± 827) ng/ml, and 24,400 (± 7180) ng.h/ml respectively [35]. Hepatic impairment has an impact on telaprevir PK. Telaprevir exposure ($AUC_{0\text{-}8h}$) was reduced by 15% with mild hepatic impairment, and by 46% in moderate hepatic impairment compared to healthy volunteers in a multiple-dose study with tid dosing [38].

1.3.2 Clinical data

In a clinical proof of concept study, 2250mg/day of Telaprevir resulted in an average reduction in plasma HCV viral load of 3.0 $\log_{10}$ and 4.65 $\log_{10}$ after 2-day and 14-day dosing respectively [39]. The combination of Telaprevir (750mg q8h) with Peginterferon alfa-2a (180mcg weekly sc) for 15 days produced a greater HCV plasma RNA decline compared to Telaprevir mono-therapy (-5.49 $\log_{10}$ vs -3.99 $\log_{10}$ respectively) [40]. The addition of Ribavirin to Telaprevir and Peginterferon Alfa-2a produced a mean HCV viral load decline of 6.45 $\log_{10}$, with 12/12 (100%) of patients having undetectable HCV plasma RNA after 28 days of treatment in a proof of concept phase 1b study [41].

The safety and efficacy of Telaprevir based triple therapy (combined with peginterferon alfa and ribavirin) was assessed in three phase 3 registration trials – ADVANCE, REALIZE and ILLUMINATE [26-27, 42].

The ADVANCE study was a phase 3, randomised, double-blind, placebo-controlled trial of 1088 patient treatment naive patients with HCV genotype 1 infection. Patients received either (i) Placebo, peginterferon Alfa-2a and ribavirin for 48 weeks, (ii) 8 weeks of Telaprevir (750mg q8h) followed by 4 weeks of placebo, with 48 weeks of Peginterferon Alfa-2a and Ribavirin, or (iii) 12 weeks of Telaprevir (750mg q8h) with 48 weeks of Peginterferon Alfa-2a and Ribavirin. Patients who received Telaprevir and had

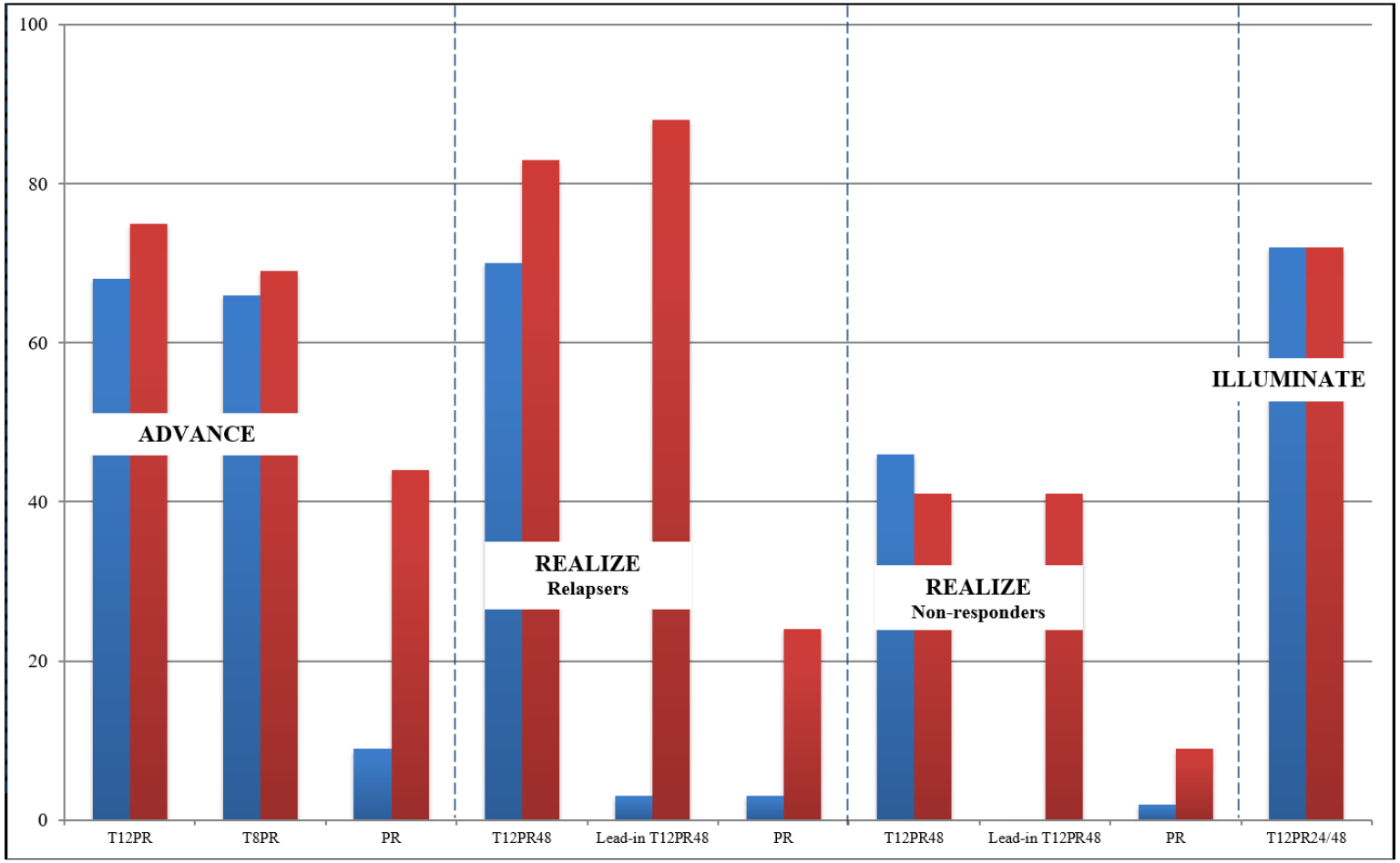
undetectable HCV RNA after 4 weeks of treatment qualified for response guided therapy and discontinued Peginterferon and Ribavirin after 24 weeks.

The REALIZE study was a phase 3, randomised control trial of 663 patients with HCV genotype 1 infection who had failed a course of Peginterferon-Alfa and Ribavirin despite receiving 80% of the intended dose. Patients were randomly assigned to one of two groups of Telaprevir (750mg q8h) with or without a lead-in period of therapy with Peginterferon Alfa-2a and Ribavirin, or a control group that received placebo with 48 weeks of Peginterferon Alfa-2a and Ribavirin.

The ILLUMINATE study was an open label phase 3 randomised trial which enrolled 540 treatment naive patients with HCV genotype 1 infection. Patients received 12 weeks of Telaprevir (750mg q8h), Peginterferon Alfa-2a and Ribavirin followed by 12 or 24 weeks of Peginterferon Alfa-2a and Ribavirin – patients who had undetectable HCV RNA at week 4 and 12 only received 24 weeks treatment in total.

The rates of RVR and SVR for the phase three trials are outlined in Figure 1.2. Across the phase 3 trials, triple therapy with telaprevir plus peginterferon and ribavirin in patients which chronic HCV genotype 1 (treatment-patients and relapsers to dual therapy) was more effective than dual therapy with higher SVR rates, regardless of IFNλ4 genotype, HCV sub-genotype, baseline HCV viral load and liver fibrosis. However, triple therapy was associated with an expanded side-effect profile and higher treatment discontinuation, demonstrated by the clear disparity between reported intention to treat and per-protocol (where non-virologic failures were excluded) SVR rates. Anaemia, gastrointestinal side-effects and skin rashes were commonly observed with triple therapy [Jacobson IM et al 2011]. The discontinuation rates ranged from 10-18% across the phase 3 studies [26-27, 42].

Figure 1.2 – Rates of RVR (blue) and SVR (red) in the ADVANCE, REALIZE and ILLUMINATE trials (T-Telaprevir, PR - peg-IFN/ribavirin)



A major limitation of the telaprevir clinical trial portfolio was the small proportion of patients with advanced fibrosis and cirrhosis, with approximately 12% of enrolled patients having cirrhosis at baseline, as the proportion of patients with cirrhosis was capped The high overall SVR rates observed in clinical trials did not translate into the real-world setting. In a multicentre observational study in academic and community centres, where 38% of patients had underling cirrhosis, early treatment discontinuation was observed in 39% of patients [43]. The real-world SVR rate was only 52%. In the CUPIC compassionate access programme of treatment experienced patients with cirrhosis, SVR_{12} was achieved in 72.4% of prior relapsers, 40% of previous partial responders and 19.4% of prior null responders [44]. Serious adverse events occurred in 49.9% of patients, including hepatic decompensation, severe infections in 10.4% and death in 2.2%.

1.4 Ledipasvir-Sofosbuvir

The fixed dose combination of sofosbuvir and ledipasvir (proprietary name Harvoni, Gilead Sciences Ltd) was the first oral interferon-free combination licenced for hepatitis C. It was approved for the treatment of HCV genotypes 1, 3 and 4.

1.4.1 Clinical Pharmacology and Pharmacodynamic properties

Ledipasvir ($C_{49}H_{54}F_2N_8O_6$ – molecular weight 889 g/mol) is an NS5A replication complex inhibitor that exhibits subnanomolar antiviral activity against HCV genotype 1a (EC_{50} 0.031 nM) and 1b (EC_{50} 0.004 nM) [45]. Sofosbuvir ($C_{22}H_{29}FN_3O_9P$ – molecular weight 529.45 g/mol) is a pangenotypic NS5B polymerase inhibitor with micromolar antiviral activity against HCV genotypes 1-6 with EC_{50} values of 0.014-0.11 µM [45]. The fixed dose combination of ledipasvir-sofosbuvir has an additive antiviral effect in G1a and 1b replicons.

Following oral administration, maximum plasma concentrations for ledipasvir are observed after 4-4.5h post dose. For sofosbuvir and its major circulating metabolite GS-331007, C_{max} concentrations are achieved between 0.8-1hr and 3.5-4hr post dose respectively. Ledipasvir is a both a substrate and weak inhibitor of P-gp and BCRP. Sofosbuvir is a substrate of P-gp and BCRP. Ledipasvir exhibits low pH dependent solubility and is practically insoluble across the pH range of 3.0-7.5; it is weakly soluble below pH 2.3. Acid reducing agents alter the absorption of ledipasvir. Ledipasvir C_{max} concentrations were approximately 17-20% lower when co-administered with famotidine 40mg bd [46]. Ledipasvir absorption is substantially reduced when given 2 hours after omeprazole with C_{max} and AUC reduced by 48% and 42% respectively [45]. There was no significant food effect on the absorption of ledipasvir-sofosbuvir in a phase 1 study [46]. Ledipasvir is highly protein bound to plasma proteins [47-48]. Sofosbuvir was approximately 82-85% protein bound in HCV-uninfected subjects, while GS-331007 is minimally protein bound [49].

Ledipasvir undergoes slow oxidative metabolism and is primarily excreted in faeces (70%) as unchanged parent drug [50]. Sofosbuvir is readily converted by plasma esterases to GS-31007 and is subsequently converted to a pharmacologically active nucleoside analogue triphosphate in hepatocytes. It is metabolised to an active triphosphate through a series of intracellular reactions. The major plasma metabolite is GS-331007, which is excreted primarily in urine. Neither sofosbuvir nor GS331007 are substrates for, or induce CYP450. Sofosbuvir, but not GS-331007, is a substrate of P-glycoprotein and BCRP [49].

1.4.2 Clinical Data

The efficacy and safety of the fixed dose combination of ledipasvir-sofosbuvir were evaluated in several clinical trials involving more than 2000 genotype 1 patients. The overall SVR rate in treatment naïve and treatment experienced patients with a 12 week-regimen was > 95%. Three large phase 3 trials were conducted.

ION-1 and ION-3 enrolled treatment-naïve genotype 1 patients. In the ION-1 trial, 865 patients were randomised to receive 12 or 24 weeks of ledipasvir-sofosbuvir with or without ribavirin for 12 or 24 weeks [51]. The SVR rate was 97% without any significant difference between the 12 and 24 weeks treatment arms. Side effects were more common in the ribavirin groups. The presence of cirrhosis had no impact on the safety profile or response rates, although the study was not powered to compare SVR rates between patients with and without cirrhosis (15.7% of the patients had cirrhosis at baseline).

In the ION-3 trial, 647 treatment naïve genotype 1 patients without cirrhosis were randomised to receive 8 weeks of ledipasvir-sofosbuvir with or without ribavirin for 8 weeks or ledipasvir-sofosbuvir without ribavirin for 12 weeks. SVR rates were numerically lower in the 8 week treatment arm, although treatment efficacy in patients with low viral load (< 6 million IU/ml) treated for 8 weeks was comparable to patients who received 12 weeks of therapy [52].

The ION-2 trial investigated the safety and efficacy of ledipasvir-sofosbuvir in treatment experienced genotype 1 patients, including 231 patients who failed first generation protease inhibitor therapy. Patients were randomised to 12 or 24 weeks of ledipasvir-sofosbuvir with or without ribavirin. The SVR rates ranged from 94 – 99% [53]. An

integrated analysis of all cirrhotic patients treated in the clinical trial programme revealed that treatment experienced cirrhotics represented the one group of patients who benefited from the addition of ribavirin if treatment was limited to 12 weeks of ledipasvir-sofosbuvir [54]. Extending treatment to 24 weeks obviated the need for ribavirin.

1.5 DAA therapy in patients with decompensated cirrhosis

Patients with chronic hepatitis C virus (HCV) infection and decompensated cirrhosis had limited treatment options before the availability of interferon-free DAA therapy. Until recently, liver transplantation was the only intervention associated with an improved survival in this group. The SOLAR-1 and SOLAR-2 trials demonstrated the antiviral efficacy of the fixed dose combination of sofosbuvir and ledipasvir with ribavirin in patients with decompensated cirrhosis with SVR rates of up to 88% [55-56]. These landmark trials also provided evidence of the effect of viral eradication on liver function, with a significant improvement in MELD and Child-Pugh-Turcot (CPT) scores in many patients who achieved a sustained virologic response (SVR). High SVR rates were also observed with a ribavirin free regimen of sofosbuvir and velpatasvir in the ASTRAL-4 trial [57].

However, even when decompensated cirrhotic patients achieve SVR, it remains unclear which patients will improve and achieve a compensated liver status and which will remain in a decompensated state. On-treatment mortality rates in real world cohorts range from 4-8% [58-59]. In the English early access program, of 409 patients treated, a median reduction in MELD score of 0.85 was observed but 18% of patients developed a new decompensating event. In a historically untreated control arm, the median MELD score increased by 0.75 and 28% developed a new decompensating event [58].

1.6 Aims of this thesis

There has been remarkable scientific progress over the past 30 years with the initial description of non-A non-B hepatitis, the discovery of the virus in 1989, and to the potential for HCV elimination with DAA therapy. The HCV treatment landscape has changed beyond recognition in a few short years. The development of HCV DAA has transpired rapidly – from the approval of the first generation protease inhibitors that were combined with peginterferon and ribavirin to all-oral single tablet combinations in less than 4 years.

Despite the dramatic success of DAA development, challenges remain on the path to cure. In 2011, boceprevir and telaprevir became the first direct acting antivirals approved for the treatment of HCV genotype 1 infection in combination with peginterferon and ribavirin. These regimens were more complicated than the standard of care (dual therapy) at the time, and proved less effective and more toxic in the real world than in clinical trials. Patients enrolled in clinical trials were more homogenous than real world populations that had failed interferon-based therapy, had less severe disease, and fewer co-morbidities and concomitant medications. Many of the early pharmacokinetic and drug-drug interaction studies were performed in healthy volunteers without HCV infection or underlying liver disease. Additionally, following expedited drug development and initial licencing, telaprevir was approved for twice daily dosing, with limited pharmacokinetic data for this dosing regimen in patients with cirrhosis.

The first generation protease inhibitors disappeared from treatment guidelines as quickly as they appeared with the movement away from interferon and its associated side-effect profile. Two interferon-free DAA combinations were approved in late 2014 and early 2015

in the US and Europe. The combination of ledipasvir (90mg) and sofosbuvir (400mg) in a single pill with or without ribavirin was approved for genotype 1 in the US, and for genotypes 1, 2, and 3 in the European Union. This regimen provided the first opportunity for treatment in special populations with historically limited treatment options. Early results of DAA therapy in patients with decompensated cirrhosis have been very encouraging, suggesting that viral eradication is possible in most patients. However, patient selection in this cohort remains a challenge. Understanding which patients will benefit from viral eradication in late stage disease is a major research priority, as some patients are better served by deferring treatment until after liver transplantation.

Treatment access is another challenge. DAA therapy is costly, and there is an emphasis on delivering cost-effective national treatment programmes. Persons who inject drugs (PWID) remain a highly marginalised group with a disproportionate burden of HCV related liver disease with limited access to treatment. Viral elimination is impossible without engaging PWID in care.

This thesis examines two distinct research areas related to DAA therapy for HCV – (1) the pharmacokinetics of DAA therapy in real-world patients and (2) DAA treatment in special populations.

Chapter 2 is a study of treatment outcomes of HCV dual therapy with pegylated interferon and ribavirin in PWID. Chapter 3 is a study of the pharmacokinetics of telaprevir in treatment naïve and treatment experienced patients with and without cirrhosis. Chapter 4 describes the development and validation of a bioanalytical method for the quantification of sofosbuvir, GS-331007, ledipasvir, daclatasvir and simeprevir in human plasma.

Chapter 5 is an investigation of the pharmacokinetics of ledipasvir in decompensated cirrhosis. The rationale for these studies was to generate quantitative data to describe the random and systematic variability of DAA pharmacokinetics in the real world setting. The bioanalytical technique was developed for therapeutic drug monitoring and can be used to individualise therapy in HCV patients, based on disease status and/or drug-drug interactions. Chapter 6 is a study of the outcomes and predictors of treatment benefit and/or adverse events in patients with decompensated cirrhosis. The aim was to develop a model that may guide physicians in selecting decompensated patients requiring treatment from those better served by treatment after liver transplantation.

CHAPTER 2

HCV TREATMENT IN PEOPLE WHO INJECT DRUGS (PWID)

2.1 Introduction

People who inject drugs (PWID) represent the majority of the hepatitis C virus (HCV) epidemic in the developed world [60]. The majority of new infections develop in active PWID, with this group accounting for more than 80% of new infections in high-income countries [61]. Furthermore, an additional large reservoir of infection exists amongst former PWID who remain undiagnosed. Public health initiatives which aim to reduce the burden of HCV and its attendant complications, or more ambitious strategies to target HCV elimination will therefore be unsuccessful unless they include strategies for engagement and retention of PWID in treatment and follow-up.

PWID are a highly marginalised group and HCV treatment uptake remains poor in this group with studies reporting annual treatment rates of less than 1.6 per 100 person-years in a community-based inner city cohort in Canada, with similar rates reported in Australia [62-63]. Low rates of treatment uptake have also been observed in the United States; with one community based cohort study of inner city Baltimore reporting treatment initiation in 28/579 (4.8%) HCV-infected PWID [64]. Data from the UK demonstrates a geographical variation in PWID treatment rates, ranging from 5 to >25 per 1000 PWID, and highlights the opportunity for upscaling treatment in areas of low treatment uptake [65]. Barriers to treatment of PWID relate to both system and patient factors, but also to reluctance among providers who perceive this group as “difficult to treat”.

Historically, HCV treatment guidelines have excluded PWID from consideration for treatment because of concerns regarding compliance and re-infection [66]. These concerns have not been realised in several small studies assessing treatment for PWID, and

outcomes have been comparable to large randomised trials of dual therapy in non-drug users [67-69]. The American Association for the Study of the Liver (AASLD) guidelines recommend that HCV therapy be considered in persons actively using illicit drugs or on opiate substitution therapy provided they wish to be treated and are willing to maintain frequent monitoring [19]. The recently updated European Association for the Study of the Liver (EASL) recommendations on treatment of HCV advocate treatment for individuals with ongoing risk of infection including active PWID, provided they wish to receive treatment and are willing to maintain regular appointments [70].

Important factors that have limited treatment uptake in PWID are the contraindications to and adverse effects from Interferon (IFN) based therapy. A study of PWID in a UK prison outreach clinic reported only 15% of HCV RNA positive inmates were eligible for treatment, and only 50% of eligible patients received treatment [71]. The development and availability of IFN-free direct acting antiviral (DAA) regimes with high efficacy, improved tolerability and a limited side effect profile will significantly increase the proportion of patients who can be offered HCV therapy [51, 72-73]. However, adherence to therapy in "real world" population groups will remain paramount in the DAA era to realise the SVR rates observed in clinic trials, and additionally limit the emergence of antiviral resistance.

We performed a retrospective analysis of treatment outcomes and adherence to peginterferon and ribavirin therapy in former and recent PWID and non-drug users at our centre. St. James's Hospital is a university teaching hospital in Dublin that serves a hinterland for 350,000 people with large Hepatology and Infectious diseases units. The hepatology centre provides a regional hepatitis service to 1.2 million persons, and an

analysis in 2014 confirmed that approximately 2,200 HCV seropositive patients were attending the unit, more than 80% of whom had a history of injecting drug use. The GUIDE clinic provides a national service to over 3,000 HIV-infected individuals, including 450 patients with HIV-HCV co-infection. This study was undertaken prior to the routine availability of DAA to inform national policy and guidelines regarding the treatment of PWID and access to DAA therapy. I collected the data and performed the statistical analysis for the study described in this chapter. Dr Ciaran Bannan provided the data for patients treated through the Department of GU Medicine and Infectious Diseases, St. James's Hospital, and Dr Shay Keating provided the data for the recent PWID cohort treated in a community methadone clinic included in the analysis.

2.2 Patients and Methods

Differences between PWID and non-drug users were analysed for adherence to treatment and outcome in all patients treated for chronic HCV infection in a university teaching hospital from 2002-2012. Anonymised patient data was retrospectively reviewed for the treatment period and monitored for at least 6 months follow-up after treatment. The PWID group included former and recent drug users who were engaged in an opiate substitution therapy (OST) programme in a community based drug treatment centre. Former PWID was defined as having stopped drug use for 6 months prior to treatment, whereas recent PWID was defined as drug use in the 6 months leading up to treatment. Former PWID patients did not have routine urinary drug testing during treatment, and abstinence was assessed from patient self-reporting alone. Recent PWID provided weekly urine samples for drug testing on treatment.

2.2.1 Patients

All patients who received treatment had compensated chronic HCV infection and detectable HCV RNA pre-treatment. Patients with cirrhosis were screened for hepatocellular carcinoma prior to treatment with combined liver ultrasonography and alpha fetoprotein. All patients treated over the 10 year period were included in the final analysis. This retrospective audit of clinical outcomes was performed in accordance with the Royal College of Physicians of Ireland guidance on clinical audit. As data controller, the St. James's Hospital review board approved this retrospective analysis of anonymised patient data.

Patients co-infected with HIV were considered for HCV treatment once they were established on an effective antiretroviral regimen or they had evidence of a satisfactory CD4 count (>350 cells/mm^3) prior to initiation of treatment. Exclusion criteria for HCV treatment included active alcohol abuse at the time of screening, decompensated cirrhosis, untreated psychiatric conditions, a Haemoglobin of less than 12g/dl at baseline. Relative contraindications to treatment were a neutrophil count of less than 1500/mm^3, and a platelet count of less than 50 x 10^9/ mm^3, although ten patients with HIV co-infection with moderate neutropenia and/or a platelet count of 25-50x10^9 were treated at the discretion of the treating physician. Risk factors for HCV transmission were recorded from the patient history and referral source at the screening assessment visit. Information on illicit or non-prescription drug use was collected directly from patients. The non-PWID group's risk factors included receipt of infected blood products, sexual transmission, or birth in an area of high HCV prevalence. Alcohol abuse was defined as the consumption of more than 21 standard units per week for men and 14 units per week for women.

2.2.2 Treatment protocol

All patients received comprehensive psychological assessment by specialised treatment nurses prior to therapy. Patients received standard dose weekly Peginterferon-α subcutaneous injections and weight based Ribavirin for 24 or 48 weeks based on genotype and the presence of pre-existing cirrhosis. Individuals who were HCV RNA positive by polymerase chain reaction (PCR) at week 24 were defined as non-responders and treatment was discontinued. Anaemia was managed by Ribavirin dose reduction and/or the use of Erythropoietin at the discretion of the treating physician. HCV RNA was measured using a highly sensitive serum PCR test (HCV Versant 3.0 Assay, Roche COBAS Taqman or Abbott Real Ti*m*e II HCV assay). Patients treated after 2006 were routinely assessed for rapid virologic response (undetectable HCV RNA after 4 weeks of therapy).

2.2.3 Outcome measures

A sustained viral response (SVR) was defined as undetectable HCV RNA in serum at 24 weeks follow-up after completion of therapy. Treatment adherence was determined as patients who attended up to the pre-defined treatment completion date, and/or met virological stopping rules for non-response. Non-response was defined as $\leq$ 2 log decline in HCV RNA by treatment week 12, and/or detectable HCV RNA in serum after 24 weeks of therapy. Relapse was defined as patients with an end of treatment response (HCV PCR undetectable) who did not achieve an SVR. Re-infection was defined as any patient who achieved SVR_{24}, but had detectable HCV RNA during longer term follow-up. The classification of reinfection included patients who achieved an end of treatment response, but were diagnosed with a different HCV genotype during 24 weeks of follow-up.

2.2.4 Statistical Analysis

SPSS (Version 21, IBM, Chicago, Illinois, USA) was used for calculations. Chi-squared test for independence and the independent sample t-Test were used to compare baseline characteristics between the PWID and non-PWID groups. The proportions of non-adherence and treatment response between groups were compared and expressed as relative risks (RRs) with a calculated 95% confidence interval. The available number of patients was sufficient to significantly identify a RR of 1.5 between PWID and non-PWID groups with a power of 99% (α=0.05).

2.3 Results

From January 2002 to December 2012, one thousand patients were treated for chronic HCV infection. Of these, 608 were former PWID, and 85 were recent PWID. These groups were compared with 307 non-drug users who had other defined risk factors for HCV. Opiates accounted for the vast majority of injecting drug use in patients with a history of injecting drug use. These risk factors included receipt of infected blood or blood products (clotting factors, anti-D), sexual transmission and birth in an area of high HCV prevalence.

2.3.1 Baseline Patient Characteristics

Baseline characteristic for both groups are outlined in Table 2.1. The majority of former PWID and recent PWID were male, and were significantly younger than the non-PWID group. More than half of former PWID were infected with genotype 3 (52.5%), with genotype 1 infection being the second most common (41.8%). Only 5.6% had a genotype

other than 1 and 3. In all, 12% of former PWID had underlying cirrhosis, and 114 (16.5%) were co-infected with HIV.

Of the 85 recent PWID treated for HCV in a community based drug treatment centre during the study period, all received opiate substitution therapy. Genotype 1 infection was more common with lower degrees of fibrosis in the recent PWID group. None of the patients had established cirrhosis. In the non-PWID group, there were more women (36.2%), and the mean age was significantly higher at 43.0 years. Genotype 1 infection was the most common genotype (46.3%) followed by genotype 3 (42.7%). The proportion of patients with cirrhosis was similar to the former PWID group (14%), as was the percentage with HIV co-infection (14%).

Table 2.1 Baseline patient characteristics of former and recent PWID and non-PWIDs treated for chronic HCV infection

	Former PWID (n=608)	Recent PWID (n=85)	non-PWID (n=307)
Age (years) [mean $\pm$ SD]	36.2 $\pm$ 7.7	35.9 $\pm$ 6.6	43.0 $\pm$ 11.6
Sex (male) [n (%)]	547 (78.9)	67 (78.8)	196 (63.8)
Genotype [n (%)]			
1	290 (41.8)	42 (49.4)	142 (46.3)
2	29 (4.2)	5 (5.9)	15 (4.9)
3	364 (52.5)	38 (44.7)	131 (42.7)
4-6	10 (1.4)	0 (0)	19 (6.3)
Cirrhosis [n (%)]	83 (12)	0 (0)	43 (14)
HIV Co-infection	114 (16.5)	0 (0)	43 (14)
Log_{10} Viral Load – mean (range)	6.53 (2.10-7.84)	6.63 (4.30-7.33)	6.49 (4.06-7.64)

Pre-treatment HCV viral load was available in 557 (91.6%) of former PWID, 85 (100%) of recent PWID and 253 (82.4%) of non-PWID, and a higher percentage of PWID had a viral load of greater than 10^6 IU/ml. In all, 67% of patients with HIV co-infection were receiving anti-retroviral therapy prior to commencing HCV therapy. The majority (92%) of these were HIV virally suppressed.

2.3.2 Treatment completion/compliance

Six hundred and eight (608) former PWID, 85 recent PWID, and 307 non-PWIDs were commenced on HCV therapy. Treatment was discontinued in 71 former PWID (11.5%), 8 recent PWID (9.4%), and 46 non-PWIDs (15%) for virologic failure at week 24 of therapy. There was no significant difference in treatment non-completion (for reasons other than virologic non-response) between PWID and non-PWIDs [8.4% vs 6.8%, RR = 1.23, 95% CI 0.76-1.99]. Additionally, there was no significant difference in treatment non-completion between former and recent PWID [8.7% vs 5.9%, RR = 0.84, 95% CI 0.33-2.10].

Fifteen patients (17.6%) in the recent PWID group tested positive for opiates at least once during treatment, 11 (12.9%) tested positive for benzodiazepines, and 5 (5.8%) tested positive for cocaine. Seven patients tested positive for two of the drug classes, while 5 tested positive for all three classes. No patients reported injecting illicit drugs during treatment or in the 6 month post-treatment follow-up period.

2.3.3 Response to treatment

Treatment response rates are detailed in Table 2.2. The overall SVR rate in PWID (64.1%) was similar to non-PWIDs (60.9%) [RR = 1.05, 95% CI 0.95-1.17]. There was no significant difference in SVR rates between the groups when comparing genotype 1 and genotype 3 infections (Fig 2.1). As expected, genotype 1 infection was less responsive to Interferon therapy in both PWID and non-PWIDs (47.7% versus 48.4%, *p* = non-significant). PWID were more likely to be lost to follow-up after achieving a viral response at the end of treatment [RR = 2.73, 95% CI 1.16-6.43]. There was no significant difference in SVR between former and recent PWID for genotype 1 [47.2% vs 54.8%, RR = 0.82, 95% CI 0.61-1.10] and genotype 3 infection [73.9% vs 83.8%, RR = 0.91, 95% CI 0.77-1.07].

Follow up data on 219 former PWID who achieved SVR between 2002-2007 for a median of 57 months (range 6-168 months) indicated that 13 patients were re-infected with HCV, a reinfection rate of 10.5/1000 person-years of follow-up. All 13 patients had a relapse in injecting drug use. There was no significant difference in re-infection rate between former PWID with and without HIV co-infection. Follow-up data was available in 84/85 (99%) of recent PWID for a six month period post-therapy. There were no re-infections in the recent PWID group during this follow-up period.

Fig 2.1. Rates of sustained viral response (95% CI bars) with dual therapy in PWID (people who inject drugs) and non-PWID (a) for all genotypes – 64.1% ss 60.9% (b) genotype 1 – 48.6% vs 48.6% and (c) genotype 3 – 74.7% vs 73.3%.

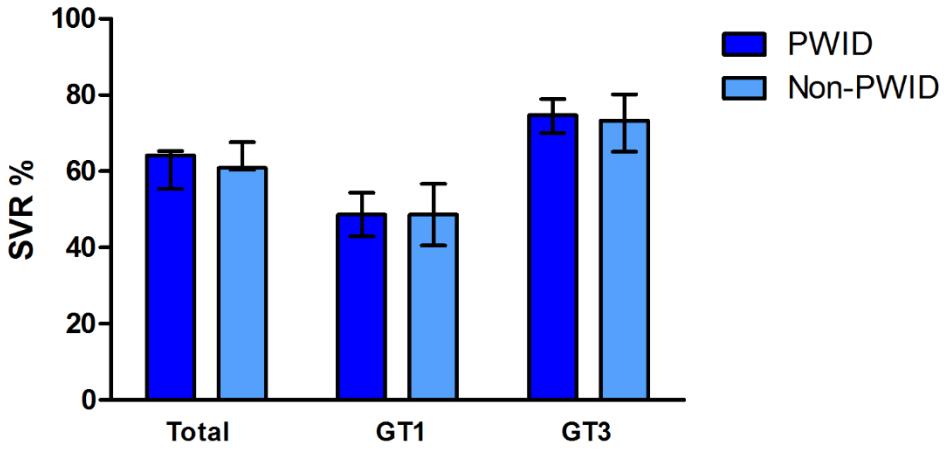


Table 2.2 Adherence and treatment responses in people who inject drugs (PWID) and non-PWIDs

	Former PWID n (%)	Recent PWID n (%)	Non-PWIDs n (%)	RR (95% CI) in Former versus recent PWID	RR (95% CI) in PWID versus non-PWID
Non-responders	71/608 (11.7)	8/85 (9.4)	46/307 (15)	1.24 (0.62-2.49)	0.74 (0.53-1.05)
Genotype 1 patients	52/248 (21)	8/40 (19)	32/142 (22.5)	1.02 (0.53-1.98)	0.92 (0.63-1.35)
Genotype 3 patients	14/326 (4.3)	0 (0)	7/131 (5.3)	-	0.72 (0.30-1.75)
End of treatment response	454/608 (74.7)	68/85 (82.9)	234/307 (76.2)	0.93 (0.83-1.05)	0.99 (0.92-1.07)
Genotype 1 patients	155/248 (62.5)	28/40 (70)	98/142 (69)	0.78 (0.68-0.90)	0.92 (0.80-1.05)
Genotype 3 patients	270/326 (82.8)	35/38 (94.6)	111/131 (84.7)	0.90 (0.81-0.99)	0.99 (0.91-1.08)
Sustained Viral Response	384/608 (63.2)	60/85 (70.6)	187/307 (60.9)	0.89 (0.77-1.04)	1.05 (0.95-1.17)
Genotype 1 patients	117/248 (47.2)	23/40 (54.8)	69/142 (48.6)	0.82 (0.61-1.10)	1.00 (0.81-1.23)
Genotype 3 patients	241/326 (73.9)	31/38 (83.8)	96/131 (73.3)	0.91 (0.77-1.07)	1.02 (0.91-1.15)
Non-adherence	53/608 (8.7)	5/85 (5.9)	21/307 (6.8)	0.84 (0.33-2.10)	1.23 (0.76-1.99)
Lost to Follow up	30/608 (4.9)	1/85 (1.2)	6/307 (2)	4.19 (0.58-30.4)	2.3 (0.97-5.45)

2.4 Discussion

This large retrospective study of a decade of HCV treatment outcomes demonstrates that PWID have similar treatment adherence to Peginterferon and Ribavirin as non-PWID patients with chronic HCV infection. Of the 693 recent or former PWID who commenced treatment, over 90% completed treatment at our centre. Additionally, PWID patients had a comparable response to treatment with an overall SVR rate of 62.4%. More than half of PWID patients were infected with genotypes 2 and 3, which have favourable response rates to interferon-based therapy. Allowing for this, there was no significant difference in SVR observed between the groups when controlling for genotype. PWID patients were younger but this had no effect on the response to therapy.

These data are in line with previously published cohort studies of treatment adherence and outcome in PWID. The Benelux study group reported no effect of a history of drug use on HCV treatment completion [68]. The SVR rate was approximately 40% and did not differ significantly between PWID and non-drug users having controlled for genotype. In the Benelux study, patients received non-pegylated Interferon, and consequently improved response rates were expected with the use of Peginterferon. Recent systematic reviews of HCV (dual therapy) have also illustrated that active PWID can respond favourably to treatment. A high-pooled SVR rate of 55.5% (95% CI 50.6-60.3%) with a high treatment completion rate of 83.4% was found in one systematic review of 11 studies among active PWID [74-75].

HIV co-infection is associated with accelerated progression of liver disease in patients with HCV resulting in increased morbidity and mortality [76-77]. Co-infected patients with a

history of injection drug use have an even higher risk for poorer clinical outcomes and consequently have a greater need for therapy [78]. Our co-infected PWID cohort achieved an SVR rate of 60.5%, indicating that this group at risk for unfavorable outcomes has comparable outcomes with the entire cohort.

The accepted consensus just over a decade ago was that PWID should not be offered therapy until they have been drug free for a period of six months [79-80]. Several small studies have since demonstrated that treatment outcomes even in active PWID are non-inferior, which resulted in updated guidelines [19, 70]. However, recommendations that active injection drug use should not preclude access to antiviral therapy have not been translated into enhanced treatment uptake among PWID. A study of physicians' attitudes in Canada found that whilst 90% would consider treating former PWID, only 20% of service providers were likely to treat active or recent drug users [81]. Community-based studies of PWID in Australia have shown only a modest improvement in HCV treatment uptake, ranging from 0.5-1.0% in 2004-05 (5-10 per 1000 infected) to 1.5-2.0% in 2009-10 (15-20 per 1000 infected) [63]. The biggest improvements in HCV treatment uptake have been observed in countries that have employed a comprehensive national strategy such as Scotland's HCV Action Plan [82-83]. Early results have been encouraging in terms of patient attendance at specialist centres following diagnosis and treatment uptake thereafter [84].

Another provider factor that may limit treatment access for PWID is the concern regarding re-infection risk in patients who continue to inject drugs. In our low-risk population of former PWID with HCV mono-infection, the re-infection rate was low at 10.5/1000 person

years of follow-up, with a median follow-up period of nearly 5 years. As viral homology sequencing was not performed in all patients with detectable HCV RNA after SVR, there was a risk of classifying some late relapse cases as re-infection. This potential classification error may be associated with an overestimation of the re-infection rate. All re-infection cases were associated with a relapse in injection drug use. Another limitation of the short-term follow-up and definition of relapse used was that mixed infection relapses could have been classified as reinfections.

Uncertainty about re-infection rates in published studies remain, due to high dropout rates in long-term follow up. Re-infection rates in a number of studies are consistently low, between 3-5% [85-87], with a slightly higher re-infection rate of 6.4% in PWID who reported ongoing injecting drug use post-SVR [88]. A meta-analysis of 43 studies which included 7,969 patients treated for HCV demonstrated that late-relapse or re-infection is higher in prisoners or those with on-going drug use (22.32/1,000 person years of follow-up). The highest risk was in patients with HIV/HCV co-infection (32.02/1,000 person years of follow-up). These rates are much higher than we observed in our cohort. Genotyping and viral sequencing was not universally used in classifying late relapse or re-infection in these studies, possibly resulting in an over-estimation of the re-infection rates [88].

A number of factors preclude some HCV-infected PWID from successful treatment with existing Interferon-based therapy. Patient-related factors which include chaotic lifestyles, psychiatric comorbidity and depression may influence patient adherence during treatment. Patient selection is therefore critical and may be optimised through careful pre-treatment

assessment management, with particular attention given to patient and provider factors known to impact on compliance. In our study, a combined clinician and nurse specialist assessment was used to select patients for treatment on a case-by-case basis. Patients with active psychiatric comorbidity were assessed by liaison psychiatry as part of the multi-disciplinary pre-treatment assessment. Whilst this assessment process introduces an element of selection bias, the high observed adherence and SVR rates in PWID result from treatment recruitment decisions made on an individual basis. A multidisciplinary approach to HCV treatment where treatment and counselling services are offered in a "one-stop-shop" has been shown to improve treatment uptake and adherence with therapy [89].

These data support the inclusion of recent and former PWID in any public health HCV treatment strategy aimed at reducing HCV prevalence as a first step towards elimination. The European guidelines advocate early DAA treatment for those with an on-going infection risk including PWID [70]. Interferon-free DAA regimens are now the standard of care, with treatment duration as short as 8 weeks, and observed SVR rates of > 95% [51, 72-73]. Co-morbid psychiatric conditions seen in up to 50% of PWIDs will no longer be a contraindication to therapy [90]. As treatment regimens become simpler and more tolerable, this should result in an expansion of the eligible treatment population. The model of care described in this study provided comparable outcomes for PWID and non-drug users with use of a more difficult to tolerate interferon-based therapy.

The C-EDGE CO-SRTAR was the first phase 3 trial to evaluate DAA therapy in individuals receiving OST including those with ongoing drug use [91]. Treatment naïve people with HCV genotypes 1, 4 or 6 were randomly assigned to receive 12 weeks of

therapy with grazoprevir-elbasvir or placebo (followed by 12 weeks of therapy). Overall, 96% of patients completed therapy with > 95% adherence. In an intent-to-treat analysis, SVR_{12} was achieved in 91%, with no impact of drug use at baseline or during treatment on SVR or adherence. Real-world data from the Irish ICORN registry have demonstrated high SVR rates comparable with the results of clinical trials in patients with compensated cirrhosis treated with interferon free DAA [92]. Injecting drug use was the major risk factor for HCV acquisition in patients evaluated in this study.

With simplification of therapy, it seems paradoxical that many payers have imposed even greater access restrictions to treatment, with some states in the US requiring 12 months abstinence from drug and alcohol use for re-imbursement [93]. These restrictions are difficult to justify in light of increasing evidence that HCV treatment is effective in PWID. The use of pre-treatment screening tools for illicit drug use do not help to indentify patients more likely to respond to therapy, and only serve as an added barrier to treatment, and are therefore discouraged by treatment guidelines [94]. Some concerns remain regarding the risk of re-infection, but this requires strategies to reduce the re-infection risk as part of treatment strategies rather than excluding PWID altogether. The nature of epidemic control is that re-infection risk will decline through successful treatment scale-up strategies in the populations with the highest prevalence as the reservoir for re-infection in PWID is reduced. There is a growing body of evidence supported by modelling data of such a "treatment as prevention" paradigm [95]. Additionally, prioritising PWID for treatment appears to be more a cost-effective initiative at reducing long-term health costs than treating non-PWID with comparable degrees of fibrosis [96]. HCV elimination is an ambitious target, but it will not be achieved by excluding PWID from treatment.

CHAPTER 3

PHARMACOKINETICS OF TELAPREVIR IN COMBINATION WITH PEGINTERFERON AND RIBAVIRIN IN THE REAL-WORLD SETTING

3.1 Introduction

Telaprevir is an orally bioavailable inhibitor of the non-structural 3/4A (NS3/4A) hepatitis C virus (HCV) protease, and was the first direct-acting antiviral licenced for the treatment of HCV. As previously discussed, telaprevir (750mg three times daily) in combination with pegylated interferon (Peg-IFN) and ribavirin (RBV) in patients with genotype 1 chronic hepatitis C infection was associated with significantly increased rates of sustained virologic response compared to dual therapy [26-27, 42, 97-99]. Based on phase 2 and 3 studies, telaprevir (750mg three times daily) in combination with Peg-IFN and RBV was approved in Canada and Japan in 2011, and in the United States and Europe in 2012 for the treatment of HCV genotype 1 infection.

Despite the extensive clinical trial portfolio required for FDA approval, there is a paucity of data on protease inhibitor pharmacokinetic variability, determinants of PK variability and the effects of these on virologic response and treatment outcomes. Telaprevir was initially approved at a dose of 750mg three times daily. The OPTIMIZE study demonstrated non-inferiority of twice daily telaprevir (1125mg) [37]. The licence and summary of product characteristics were amended in 2013 to reflect the option of twice daily dosing.

Extensively metabolised via hydrolysis, oxidation, and reduction, *in vitro* studies indicated that CYP3A is the major Cytochrome P-450 (CYP) isoenzyme involved in the metabolism of telaprevir. *In vivo* drug-drug interaction studies in healthy volunteers demonstrated that CYP3A inducers reduce telaprevir exposure to varying degrees based on their relative induction potency [100]. Telaprevir exposure may also be increased when co-administered with strong CYP3A inhibitors. As telaprevir undergoes extensive hepatic metabolism,

chronic liver disease would also be expected to impact on systemic drug exposure. As outlined in chapter 1, real-world studies demonstrated inferior treatment outcomes compared to those observed in phase 3 trials, especially in patients with cirrhosis.

The primary aim of this chapter was to determine real-world inter-individual variability of telaprevir pharmacokinetic parameters with twice-daily dosing. Secondary outcomes included determinants of interindividual variability (pharmacokinetic), and the relationship between telaprevir concentrations, viral kinetics, anaemia, and treatment outcome (pharmacokinetic-pharmacodynamic). The data from the pharmacokinetic studies in this chapter was used to develop a real-world population pharmacokinetic model to examine the relationship of co-variates on telaprevir concentrations. These studies were conducted in compliance with good clinical practice (GCP) and received ethics approval from the joint SJH/AMNCH research ethics committee and all patients provided informed consent.

Funding for these studies was obtained through a Health Research Board of Ireland research training fellowship for healthcare workers. This grant was awarded to me, as principal investigator, following a competitive application process which involved an international peer review process and interview by a multidisciplinary panel. I directly recruited the patients for all the studies described in this chapter, collected and analysed all the patient data and samples (including LC-MS/MS) and performed the statistical analysis. Henry Pertinez assisted with the development of the population pharmacokinetic model; I coded all of the data for the model and we developed the model together.

3.2 Patients

3.2.1 Study 1 – pilot study

As part of a pilot study, plasma samples were collected from 7 patients treated with telaprevir based triple therapy. All patients had HCV genotype 1 infection and were receiving telaprevir 750mg three times daily, pegylated interferon-α 2a 180mcg weekly by subcutaneous injection, and weight based ribavirin (≥75kg – 1200mg/daily; <75kg – 1000mg/daily). Median age was 55 years, and the 4/7 patients were dual therapy treatment experienced (2 non-responders and 2 relapsers). The majority of patients were non-CC for the IFNλ4 polymorphism (5 CT, 1 TT). None of the patients had clinical, radiological, biochemical or Fibroscan® evidence of cirrhosis.

3.2.2 Study 2 – Telaprevir sparse PK study

Trough plasma telaprevir concentrations were determined in 36 patients treated with telaprevir based triple therapy attending the clinic. All patients were prescribed telaprevir 1125mg twice daily, pegylated interferon-α 2a 180mcg weekly by subcutaneous injection, and weight based ribavirin. Telaprevir was administered for 12 weeks. Pegylated interferon-α and ribavirin were continued for a further 12 or 36 weeks based on virologic response on treatment day 28. Baseline patient characteristics are reported in table 3.1. Mean patient age was 40.8 years, and mean weight was 78.85 kg (range 51.6 – 113kg). The majority of patients were treatment naïve (86%), IFNλ4 non-CC (75%), and non-cirrhotic (89%). Patients with cirrhosis were treated for 48 weeks irrespective of the day 28 virologic response. Medications known to interfere with the metabolism of telaprevir were discontinued at least 1 week prior to the commencement of therapy. Patients were also directly questioned about the use of complementary and alternative medicines, herbal therapies and recreational drugs.

Table 3.1 Telaprevir sparse PK study (study 2) baseline patient characteristics

Baseline patient characteristics (n=36)	
Age (yrs) – mean ± SD	40.8 ± 7.1
Weight (Kg) – mean ± SD	78.8 ± 15.3
IL28B genotype – n (%)	
CC	9 (25)
CT	21 (58)
TT	6 (17)
Treatment experience – n (%)	
Naïve	31 (86)
Relapser	1 (3)
Non-Responder	4 (11)
HCV viral load – mean ± SD	Log_{10} 5.71 ± 0.98
Cirrhosis – n (%)	4 (11)
Liver stiffness (KPa) – mean (range)	8.15 (3.8 – 22.8)

3.2.3 Study 3 – Telaprevir intensive PK study

Twenty-one patients with HCV genotype 1 infection treated with telaprevir-based triple therapy participated in this study. Patients received telaprevir at a dose of 1125mg twice daily. Baseline patient characteristics are outlined in table 3.2. Intensive sampling was undertaken at steady-state at least 4 weeks after the initiation of therapy at the HRB clinical research facility (CRF). The CRF, a partnership between Trinity College Dublin and St James's Hospital is funded by the Wellcome Trust and the HRB, and provides specifically-designed state-of-the-art research facilities, including a day ward and an on-site laboratory for sample analysis. Medications known to interfere with the metabolism of telaprevir were discontinued at least 1 week prior to the commencement of treatment. Medication reconciliation was performed by our Hepatitis C specialist pharmacist, which included enquiring about the use of complementary and alternative medicines, herbal therapies and recreational drugs.

Table 3.2 Telaprevir intensive PK study (Study 3) baseline patient characteristics

Baseline patient characteristics (n=21)	
Age (yrs) – mean ± SD	41.9 ± 7.9
Male sex – n (%)	15 (71)
IL28B genotype – n (%)	
CC	3 (15)
non-CC	18 (85)
Treatment experience – n (%)	
Naïve	17 (81)
Non-Responder	4 (19)
HCV viral load – mean ± SD	Log_{10} 5.66 ± 1.12
Fibrosis stage – n (%)	
F1-2	17 (81)
F3-4	4 (19)

3.3 Materials & Methods

3.3.1 Study 1 – Pilot study

Patients attended the clinic after an overnight fast. A 5ml sample of blood was drawn from each patient at the end of a telaprevir dosing interval at steady-state (telaprevir C_{trough}). Telaprevir 750mg was then ingested with a full breakfast containing at least 20g of fat. A 5ml samples of blood was collected 3 hours post dose (telaprevir C3h). The samples were centrifuged at 1500 revolutions per minute (rpm) without delay and 0.5 mls of plasma was collected. Formic acid (25µL of 25% formic acid) was added to the plasma to stabilise the interconversion between telaprevir and its R-diastereomer (VRT-127394). The acidified plasma was stored at -80^{o}C until subsequent telaprevir analysis using High Performance Liquid Chromatography (HPLC).

HCV viral load was quantified using the Abbott Realtime HCV assay, with a lower limit of quantification of 12 iu/ml and analytical range of 12 – 10^8 iu/ml. HCV viral load was assessed on day 0 and day 28 of treatment. Rapid virological response (RVR) was defined as undetectable HCV RNA on treatment day 28.

3.3.2 Study 2 – Telaprevir sparse PK study

A 5ml sample of blood was drawn from each patient at the end of a telaprevir dosing interval. Trough samples were collected on day 3, and at the end of weeks 1, 2, 4, 8 and 12 of treatment. The samples were centrifuged at 1,500 revolutions per minute (rpm) without delay and 0.5 mls of plasma was collected. Formic acid (25µL of 25% formic acid) was added to the plasma. The acidified plasma was stored at -80ºC until subsequent telaprevir analysis using High Performance Liquid Chromatography (HPLC).

Study 3.3.3 Study 3 – Telaprevir intensive PK study

Patients attended the HRB clinical research facility at 08:00 following an overnight fast. A twenty-two gauge cannula was inserted into the antecubital fossa to facilitate blood sampling. At 08:30 hours, 5 millilitres of blood was taken through the cannula using a syringe. The first 2.5 mls of blood drawn was discarded as dead space. The blood drawn was placed into a bottle containing EDTA (time 0h). Patients then ingested their medications with telaprevir with food. Telaprevir 1125mg was ingested with a full breakfast containing at 20g of fat. Blood samples were then taken at 1, 2, 4, 6, 8, 10 and 12 hours post dosing. All samples were centrifuged without delay for five minutes at 1500 rpm. The samples were then transferred to a class II biologic safety cabinet for further processing. The separated plasma was pipetted into a sterile plastic container using a 1000µL pipette. Formic acid (25µL of 25% formic acid) was added to the separated

plasma and vortexed for 10 seconds. The acidified plasma was stored at -80^{o}C until telaprevir analysis using HPLC.

3.3.4 Analysis of plasma telaprevir concentrations

Plasma samples were pipetted (100µL) into 7mL glass tubes, and internal standard was added. The tube contents were thoroughly mixed by vortexing. Standard curves were prepared containing blank plasma, telaprevir, and VRT-127394 at concentrations of 10-5000 ng/mL. Quality control samples for the assessment of the precision and accuracy of the assay were prepared by adding known quantities of telaprevir and VRT-127394 to blank plasma samples. A deuterated stable internal standard (telaprevir-d11) was added to all samples to correct for any variation in extraction efficiency between individual samples. A liquid-liquid extraction technique was used. Test samples (unknown concentrations), standards and quality controls were extracted with Tert-Butyl Methy Ether (TBME) as an extraction solvent. After centrifuging for 5 minutes at 3,000 rpm, the organic phase was transferred to clean test tubes and evaporated to dryness. Dried samples were reconstituted in HPLC mobile phase and transferred to glass vials for injection into the HPLC. Telaprevir, VRT-127394 and telaprevir-d11 were separated on a reversed-phase Accucore C18 column (2.6µm 150mm x 2.1mm) with a gradient consisting of water:ammonia (25%), 100:0.01 (v/v) (mobile phase A) and ACN:MeOH:ammonia (25%), 15:85:0.01 (v/v/v) (mobile phase B). Tandem mass spectrometry (MS/MS) was then used to detect the telaprevir and VRT-127394 parent and daughter ions. The MS acquisition was performed with selective reaction monitoring mode using the respective $[M + H]^+$ ions, *m/z* 680.59 → 322.42 for telaprevir and VRT-127394, and 691.15 → 110.13 for telaprevir d-11. Concentrations were determined by comparison with the relevant calibrators of known concentration. The lower limit of quantification was 5ng/ml. The intra and inter-day

accuracies ranged from 96.5 to 106.2% and 95.9 to 108.8% for telaprevir, and 94.2 to 105.3% and 95.0 to 107.9% for VRT-127394. Intra and inter-day precision was less than 6.5% for both the analytes. The accuracy and intra-day precision at the LLQ was 1.4% and 5.0% respectively.

3.3.5 Pharmacokinetic and Statistical Analysis

Study 1 and 2: Telaprevir trough plasma (C_{trough}) and post dose (C3h) concentrations were reported as mean (range). *Study 3:* Telaprevir concentrations were evaluated for maximum plasma concentrations C_{max}, minimal plasma concentrations C_{min}, time to C_{max} (t_{max}), half-life ($t_{1/2}$) and AUC to 12 hours ($AUC_{0\text{-}12h}$). C_{max} and t_{max} were obtained by visual inspection of the data. $AUC_{0\text{-}12h}$, and $t_{1/2}$ values were determined using non-compartmental analysis using Win non-lin. Values reported are geometric mean (range). The geometric mean ratio was determined for comparison between groups. Differences in mean pharmacokinetic parameters were compared using the independent 2-sample student t-test. Linear regression analysis was used to investigate the relationship between HCV viral load decline and telaprevir exposure.

3.3.6 Telaprevir population pharmacokinetic model development

Data generated from study 3.2.2 and 3.2.3 were used to develop a population pharmacokinetic model for telaprevir. Sparse and intensive pharmacokinetic samples were used to describe the population PK profile for S-telaprevir. Non-linear mixed effects modelling (NONMEM version VI 2.0, level 1.1; ICON Development Solutions) was utilised to define a structural model which best describes the rich and sparse PK dataset using the first order conditional estimation with interaction (FOCE-I) algorithm. Several structural models (e.g. single/multiple/transit compartments, different absorption and

elimination kinetics, with and without lag time) were explored. Estimates of inter- and intra-occasion variability and residual error (incorporating additional and/or proportional error models) were then derived and the model was validated both internally (through simulations and visual predictive check) and externally (using sparse data from patients sampled in the clinical study, as well as consistency with published parameter estimates).

Covariate analysis: Once validated, clinical data was used to refine model parameter estimates and test for important covariates of drug exposure. The minimal objective function value (OFV) was used as a goodness-of-fit diagnostic with a decrease of 3.84 points corresponding to a statistically significant difference between models (p=0.05, chi-squared distribution with one degree of freedom). Once the appropriate structural model was established, the following covariates were explored: age, liver elasticity, fibrosis stage, gender, weight and co-medications. Graphical methods were used to explore the relationship of covariates versus individual predicted pharmacokinetic parameters. Each covariate was introduced separately into the model and only retained if inclusion in the model produced a statistically significant decrease in OFV of 3.84 ($p < 0.05$) and was biologically plausible. A backwards elimination step was then carried out once all relevant covariates were incorporated and covariates were retained if their removal from the model produced a significant increase in OFV (>6.63 points; P 0.01, χ^2 distribution, one degree of freedom).

3.4 Results

3.4.1 Study 1 – pilot study

Telaprevir trough concentrations ranged from 480 to 3231 ng/ml with dosing at 750mg three times daily. HCV viral kinetics are outlined in figure 3.1. Six out of seven (86%) patients achieved RVR, and mean C_{trough} and C3h telaprevir concentrations in this group were 1981.8 and 2970.1 ng/ml respectively. One patient did not achieve RVR and met day 28 stopping rules (HCV RNA >1000iu/ml). C_{trough} and C3h concentrations were 480 and 1244.9 ng/ml respectively.

3.4.2 Study 2 – telaprevir sparse PK study

The results of study 2 are displayed in figure 3.2. There was a marked variability in trough plasma telaprevir concentrations ranging from 568 to 5592 ng/ml. Trough concentrations were significantly lower on treatment day 3 compared to trough concentrations after end of week 1.

Figure 3.1 - Viral kinetics in patients receiving 750mg three times daily (LLQ – lower limit or quantification; LLD – lower limit of detection)

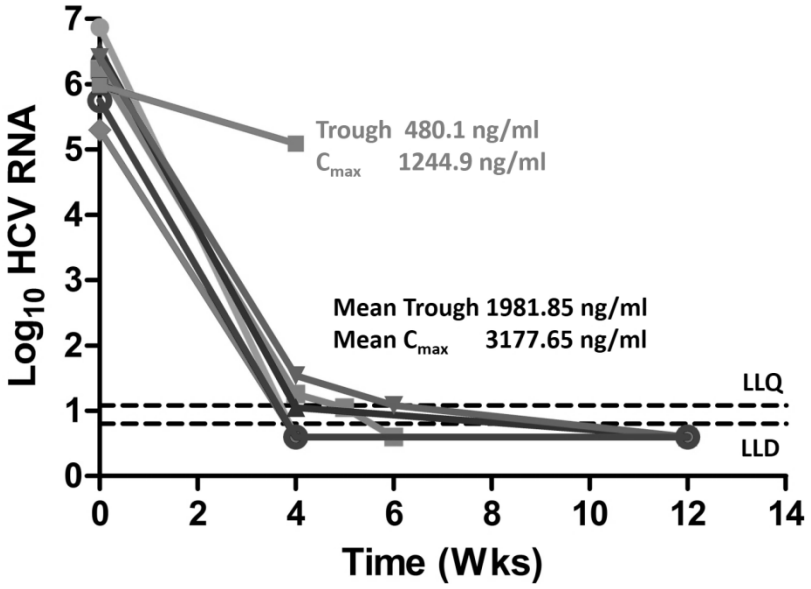
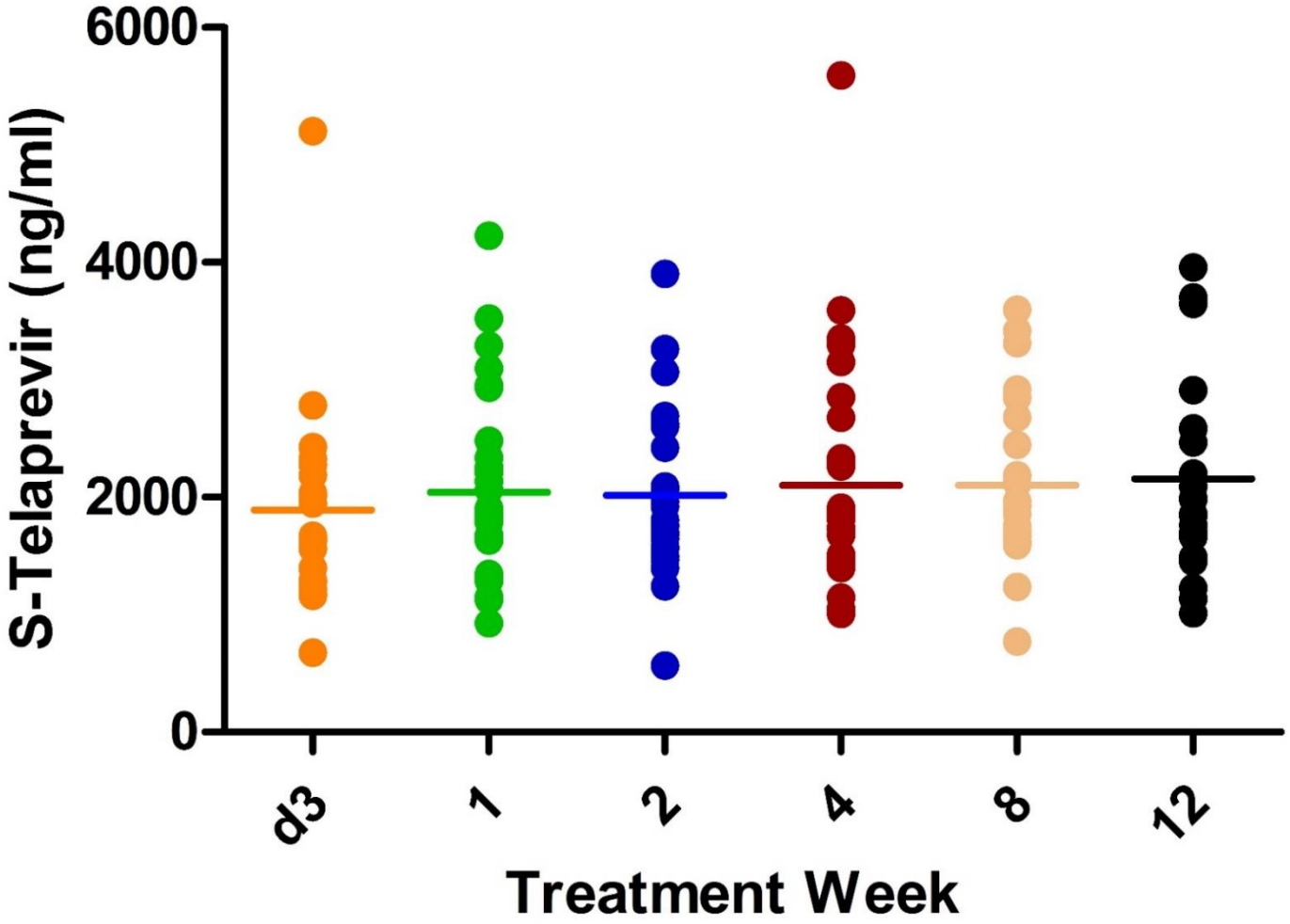


Figure 3.2 – Trough telaprevir concentrations during 12 weeks of triple therapy (n=36)



In all, 31/36 (86.1%) of patients achieved SVR_{12} by intention to treat analysis. A total of three patients discontinued therapy because of treatment related side-effects, and two patients had virologic failure. SVR_{12} rate by modified intention to treat analysis (excluding non-virological failure) was 92%.

3.4.3 Study 3 – Telaprevir intensive PK study

Telaprevir concentrations

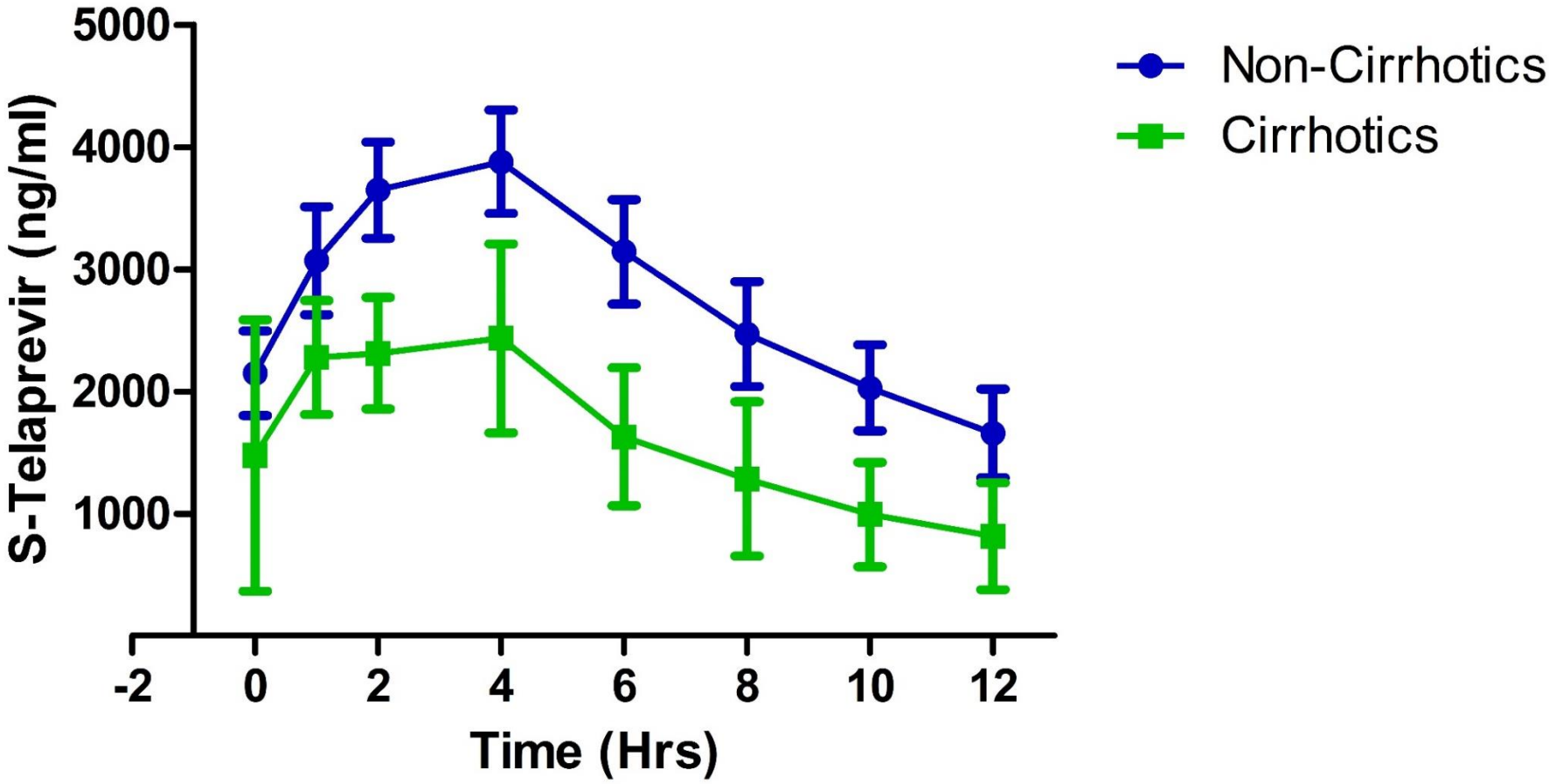
The results of study 3 are reported in table 3.3 and figure 3.3. Telaprevir AUC_{0-12h} ranged from 17,960 – 56,009 ng*hr/ml, and geometric mean telaprevir AUC_{0-12h} concentrations were significantly lower in patients with cirrhosis compared to non-cirrhotics (p=0.046). Telaprevir C_{max} was also significantly lower in patients with cirrhosis. There was no difference is median T_{max} between patients with and without cirrhosis, although plasma half-life was shorter in patients in cirrhotics.

Table 3.3 Summary of S-Telaprevir pharmacokinetic parameters (study 3)

PK Parameters	Metavir Fibrosis Stage		Total (n=21)	
	F1-F3 (n=19)	F4 (n=19)		
$t_{1/2}$ (h) – mean ± SD	7.5 ± 2.46	6.2 ± 0.59	7.4 ± 2.37	
T_{max} - median	4	4	4	
				CV%
C_{max} ($ng.ml^{-1}$)*	4082 (3691-4484)	2704 (2407- 3002)	3925 (3516-4335)	24
AUC_{0-12} ($ng^{*}hr.ml^{-1}$)*	34631 (30235-39027)	20185 (15554-24817)	32896 (28443-37349)	30

* Geometric mean (95% confidence interval); $t_{1/2}$ – plasma half-life; T_{max} – time to maximal plasma concentrations;
C_{max} – maximal plasma concentrations; AUC_{0-12} – area under the concentration curve from time 0-12 hours; CV – co-efficient of variance

Figure 3.3 – Pharmacokinetic curve of S-Telaprevir concentrations in patients with (n=2) and without cirrhosis (n=19)



Virologic response (PK-PD)

Seven out of twenty-one patients (33%) had a rapid virologic response. SVR_{12} was achieved in eighteen out of twenty-one (86%), and one patient experienced virologic failure.

The relationship between telaprevir exposure and viral load decline in the first 7 days of therapy is shown in figure 3.4. There was no significant relationship between week 1 viral kinetics and telaprevir AUC_{0-12}. However, in IL28-B non-CC patients this relationship was significant ($p = 0.0165$). There was no significant differences in telaprevir exposure in patients achieving a rapid virologic response compared to those with detectable HCV RNA at treatment day 28 (figure 3.5). There was no significant difference in telaprevir exposure between patients who achieved SVR and those with treatment failure.

Anaemia

There was a trend towards higher mean TVR exposure in patients who developed anaemia during the first 12 weeks of therapy, and this was significant in non-cirrhotics (figure 3.6).

3.4.4 Telaprevir Population pharmacokinetic model

A classic one-compartment model with first-order absorption, and linear elimination best described the data. Reasonable good fit was obtained with the base model. Ka estimates from published data were used to develop the model, and Ka was fixed at 0.23. A combined error model did not result in a better model fit. The base model with inter-individual variability was set-up as follows:

$$CL = \theta_1^{\eta 1}$$

$$V = \theta_2$$

$$Ka = 0.23$$

$$CL = \frac{F*D}{AUC}$$

Where CL = clearance, V = volume of distribution, Ka = absorbance rate constant, F = relative bioavailability, D = dose, θ estimates fixed effects and η is an estimate of between subject variability.

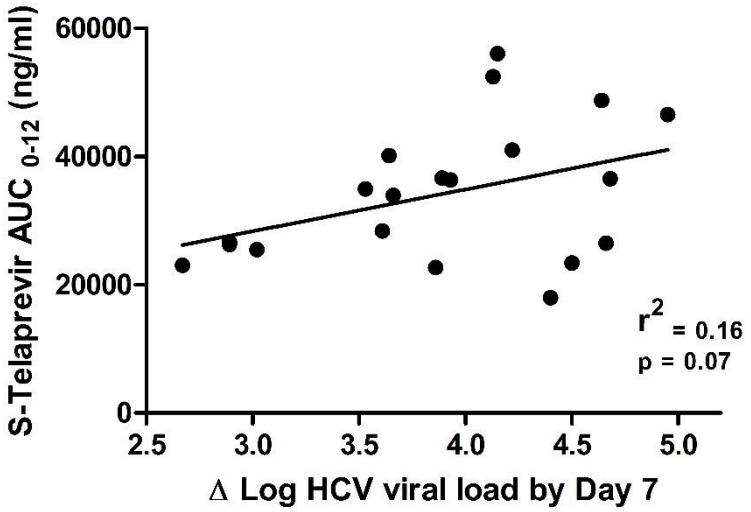
In the basic model, population clearance CL was 34.2 L/hr (CV ~20%), and population volume of distribution V was 257 L. Ka was fixed at 0.23 based on reported model estimates [35]. The model fit between observed and predicted telaprevir concentrations for individual patients and the population predicted values are illustrated in figure 3.7.

Covariate model

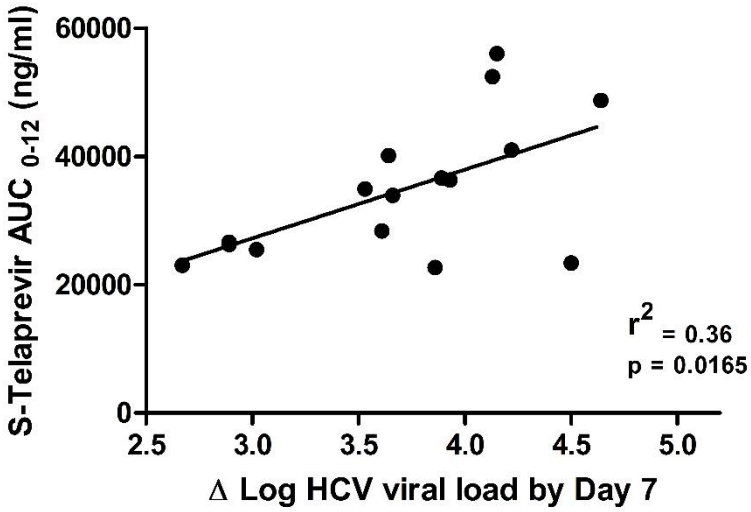
Baseline patient weight was added manually as a covariate before implementation of the stepwise covariate modelling process (SCM), and showed a significant improvement in fit ($p < 0.05$). In the forward step of the SCM, fibrosis stage was identified as having a statistically significant effect on clearance (and resulted in a reduction in the objective function by 5.01). Sex and IL28B genotype did not result in a better model fit and did not have a statistically significant effect on telaprevir clearance. Patient weight had the biggest effect on telaprevir clearance in the covariate model (with a reduction in the objective function of 15.06).

Figure 3.4 – Relationship between telaprevir concentrations and viral load decline during week 1 of therapy

(a) all patients



(b) IL28B non-cc patients



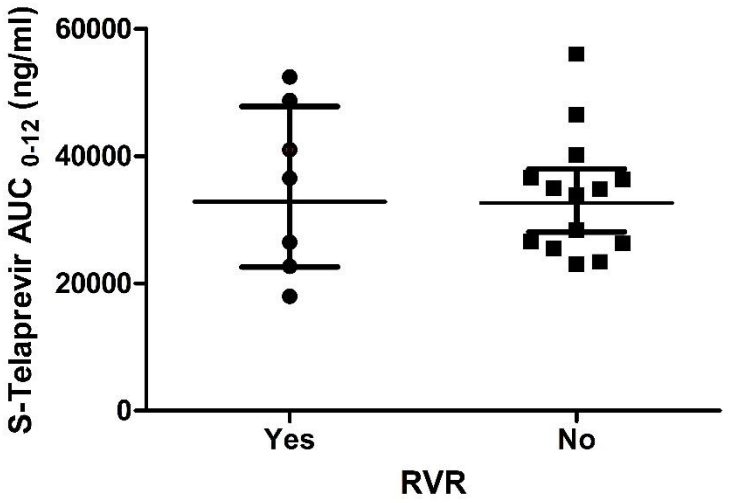


Figure 3.5 – TVR concentrations based on RVR status

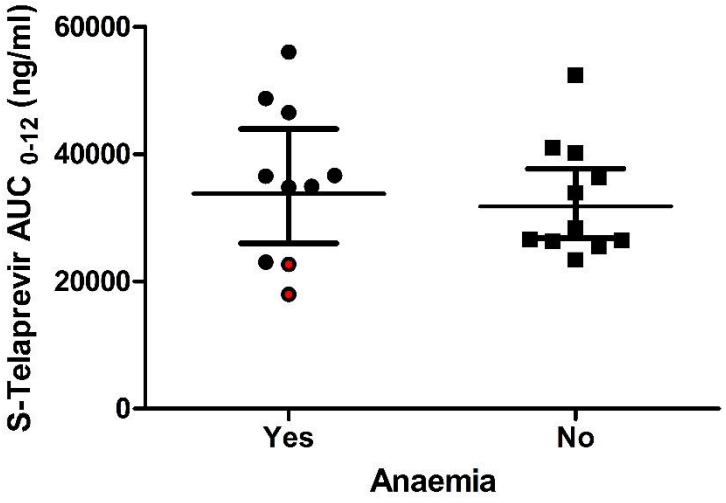


Figure 3.6 – TVR concentrations in patients with anaemia

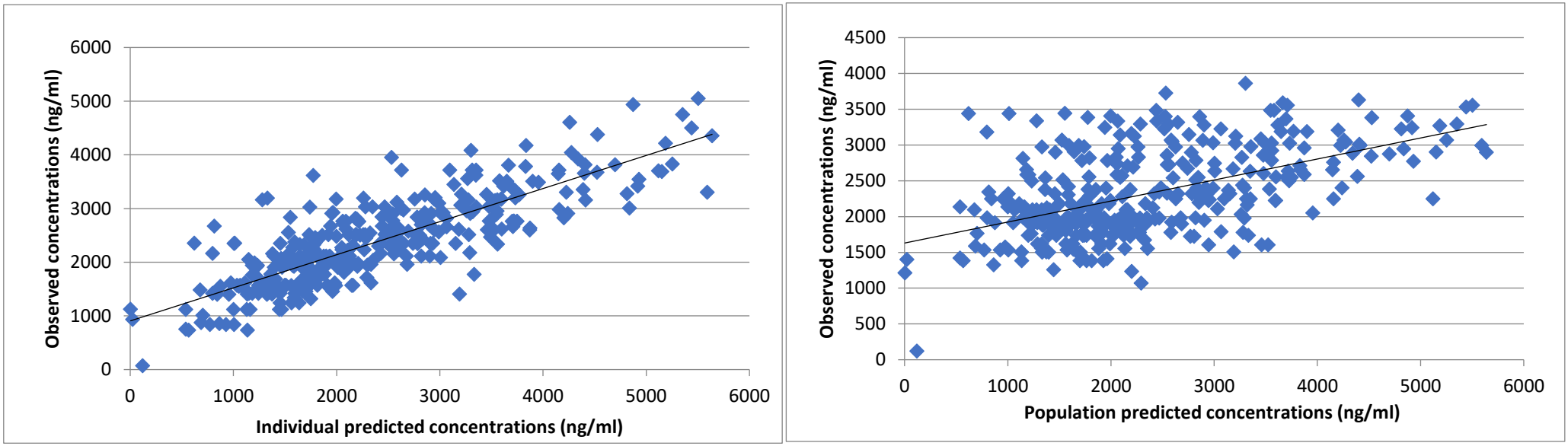


Figure 3.7 - Model fit for observed versus individual predicted telaprevir concentrations (IPRED) and (b) Model fit for observed versus population predicted concentrations (PRED)

3.5 Discussion

In study 2, there was a ten-fold variability noted in trough plasma telaprevir levels. This level of variability is characteristic of drugs metabolised by cytochrome P450 3A. However, other factors also play a role in the interindividual variability. Telaprevir is a substrate of p-glycoprotein, and individual variability in gut p-gp expression is another source of telaprevir PK variability. P-glycoprotein, a member of the ABC family of efflux transporters, is predominantly expressed in the proximal GI tract (duodenum and jejunum), with considerable interindividual variability demonstrated in healthy volunteers [101-103].

A single patient in study 2 had a trough telaprevir level of 5119 ng/ml on treatment day 3, and 5592 ng/ml on treatment day 28. This patient had inadvertently taken double the recommended dose of telaprevir (2250mg twice daily), and achieved a sustained virologic response with only 12 weeks of triple therapy. This patient had marked anaemia during the course of treatment, necessitating ribavirin discontinuation for one week followed by a dose reduction and the administration of granulocyte colony stimulation factor (g-CSF).

In our analysis, weight and fibrosis stage were the most significant predictors of telaprevir PK variability. In phase 2 and phase 3 studies, weight was found be a significant covariate for telaprevir exposure in popPK analysis, although differences in weight did not appear to be the major cause of variability [35]. The utilisation of popPK analysis allowed for the combined interpretation of data from both sparse (study 2) and intensive (study 3) studies, given the difficulty and cost with recruiting large numbers of patients for intensive PK studies.

Telaprevir exposure was significantly lower in patients with compensated CPT A cirrhosis. Previous studies demonstrated no *substantial* effect of mild hepatic impairment (CPT A cirrhosis) on the pharmacokinetics of telaprevir [35]. A phase 1 study showed that patients with moderate hepatic impairment (CPT B cirrhosis) had significantly *lower* exposure to telaprevir, with telaprevir AUC and C_{max} reduced by 46% and 49% respectively with q8h dosing in patients with CPT B cirrhosis compared to healthy volunteers [104]. It is also important to note that in this study HCV negative patients with hepatic impairment were given telaprevir at a dose of 750mg three times daily. HCV has biologically inhibitory effects on mitochondrial cytochromes [105]. As a result, PK studies in HCV negative patients are not directly comparable to patients with active HCV viraemia.

The reduced exposure of telaprevir in patients with hepatic impairment is unique. In contrast, the exposure of all other HCV protease inhibitors is *increased* in hepatic impairment [106-107]. As discussed in chapter 1, hepatic drug clearance (CL_H) is a function of liver blood flow and the hepatic extraction ratio of a drug (E_H). Compensatory mechanisms in patients with portal hypertension secondary to cirrhosis include dilatation of the portal vein resulting in increased liver blood flow [108]. As a consequence, the hepatic clearance of drugs with high hepatic extraction ratios may be increased in the setting of increased liver blood. Another potential explanation is reduced absorption of telaprevir in patients with cirrhosis. The shorter half-life, similar T_{max} and higher Clearance (CL) values observed in cirrhotics in our cohort suggest the former over the latter. Altered protein binding in liver disease may also explain the reduced concentrations. Optimisation of dosing regimen with twice daily telaprevir (1125mg bd) was investigated in a phase 2 study and a phase 3 post-licencing study. In the phase 2 study, mean telaprevir C_{min} and $AUC_{0\text{-}24h}$ were 21% and 6% lower respectively with twice daily dosing compared to tds

dosing [36]. This study included 2 patients with cirrhosis. In the OPTIMIZE study, steady state C_{max} was 18% higher (4307 $\pm$ 1233 ng/ml), while steady state C_{min} was 13% lower (3732 $\pm$ 1133 ng/ml) with bid dosing [37]. Total exposure was comparable, with mean $AUC_{0\text{-}24h}$ 8% higher with bid dosing. Another study showed no significant effect of moderate hepatic impairment in patients administered at a dose of 750mg three times daily [109]. Our study is the first to report intensive PK data in patients with cirrhosis to receive telaprevir at a dose of 1125mg twice daily. On the basis of these results, telaprevir administration three times daily appears to be the optimal dosing regimen to improve protease inhibitor exposure in patients with cirrhosis. There is a risk for development of protease inhibitor resistance with lower Ctrough levels in patients with cirrhosis if telaprevir is administered twice daily. This lesson echoes an early finding with the HIV protease inhibitor indinavir, which was associated with the development of resistance when twice daily dosing (compared to a three times daily regimen) was investigated [110]

A limitation of our study is the small number of patients with cirrhosis treated with telaprevir. This reflected the evolving treatment landscape at the time, with the emergence of data suggesting a high rate of serious adverse events in patients with cirrhosis and portal hypertension treated with telaprevir and boceprevir [111], and trial data reporting the safety of highly effective interferon-free direct acting antiviral therapy in HCV cirrhosis [54, 112].

There was no clear relationship between telaprevir concentrations and SVR in our study, and this is likely related to the high SVR rate observed in our cohort. In phase 3 studies, the relationship between telaprevir and SVR was weak and statistically non-significant [35]. The one patient in study 1 who failed to achieve SVR met the day 28 stopping rule

and had significantly lower telaprevir trough and C_{max} concentrations compared to patients who achieved SVR. The viral load decline observed during triple therapy is typically *biphasic* (figure 2.1) with (1) rapid phase 1 decline during the first 4 weeks of therapy, attributable to the effect of the direct-acting antiviral; and (2) a slower phase 2 viral decline between weeks 4 and 24, attributable to interferon. The rapid decline in the first 4 weeks of therapy results in a higher rate of RVR in patients treated with triple therapy compared to that historically seen with dual therapy. However, it is difficult to separate and study the effects of the protease inhibitor alone from the synergistic effect of the protease inhibitor with interferon and ribavirin on viral decline after week 1. The response to interferon, as predicted by the IL-28B genotype is the most significant predictor of SVR with telaprevir based triple therapy. In a meta-analysis of telaprevir phase 3 clinical trials, the odds ratio for achieving SVR in IL-28B cc patients was 7.84 compared to non-cc patients [113]. Previous treatment experience did not alter the significance of the IL-28B genotype in predicting SVR. In study 3, there was a clear relationship between viral load decline in the first week of therapy in IL-28B non-cc patients, with more rapid viral decay seen in patients with higher protease inhibitor concentrations.

There was a correlation between telaprevir concentrations and the development of anaemia in non-cirrhotics in our study. Anaemia was a commonly reported side effect in phase 3 trials with telaprevir. Ribavirin concentrations are increased by 50% when co-administered with telaprevir, which would result in increased risk for haemolysis and anaemia [114]. The relationship was not significant when patients with cirrhosis were included in the analysis. The reason for this is likely two-fold. Firstly, there was only a small number of patients with cirrhosis in the study who had lower baseline haemoglobin levels. Secondly, all patients with cirrhosis developed anaemia (necessitating ribavirin dose reduction), and

as a result there was no significant relationship between the telaprevir concentrations and the development of anaemia in this group.

In study 2 and study 3, there was a high treatment discontinuation rate secondary to side-effects compared to the rate of virologic failure. This high rate of treatment discontinuation with triple therapy observed in real world studies limited the applicability of triple therapy to large groups of patients in practice [43].

As a result, better tolerated direct acting antiviral (DAA) interferon free regimens have superseded interferon based triple therapy as the standard of care for hepatitis C. Chapters 4, 5 and 6 of this thesis examine and address specific questions related to DAA therapy

CHAPTER 4

SIMULTANEOUS DETERMINATION OF LEDIPASVIR, SIMEPREVIR, DACLATASVIR, SOFOSBUVIR AND IT'S METABOLITE IN HUMAN PLASMA BY HPLC-MS/MS TANDEM MASS SPECTROMETRY

4.1 Introduction

Bioanalysis is an essential part of pharmacokinetic and pharmacodynamics studies during drug development and in post-licencing phase 4 clinical studies. This process involves the identification and quantification of analytes (drug or metabolites) in biological samples (e.g. blood, plasma, serum, saliva, urine). The bioanalysis pathway encompasses sampling, sample preparation, analysis, calibration and data evaluation and reporting (figure 4.1).

The development of a bioanalytical method requires planning guided by specific properties of the respective analytes. These include the analyte chemical structure, partition co-efficient (pKa value), solubility properties, and stability and absorption properties. The standard method development process and validation strategies are outlined in table 4.1. In the development of a method for the simultaneous bioanalysis of more than one analyte, the respective analyte properties must be considered together in selecting an appropriate method. This is particularly relevant when selecting an appropriate extraction technique. Protein precipitation is more suited to hydrophilic analytes, whereas liquid-liquid extraction is more suitable for lipophilic analytes [115].

The FDA and EMA outline regulatory requirements and recommendations for bioanalytical development. Good clinical practice (GCP) laboratories are required to comply with these requirements with respect to bioanalytical methods developed and employed in such laboratories.

This chapter describes the development and validation of an LC/MS-MS method for the simultaneous quantification of (1) sofosbuvir, (2) GS-331007, (3) ledipasvir, (4)

daclatasvir and (5) simeprevir in human plasma. The rationale for developing this bioanalytical method is to facilitate pharmacokinetic (PK) studies in real world patients treated for HCV to answer specific questions related to DAA PK and drug interactions. There are no published methods describing the simultaneous quantification of these analytes. Furthermore, this method describing the analysis of 4 DAAs allows for high throughput measurement of drug concentrations in patients receiving several DAA combinations in single batch runs. It also provides a tool for therapeutic drug monitoring (TDM) in patients at risk for potential drug-drug interactions

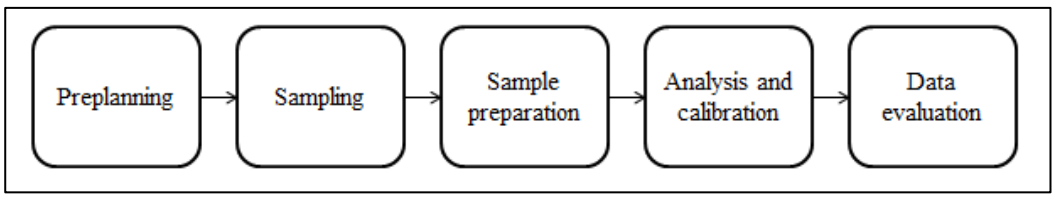
The analytical range of this bioassay reflects the range of concentrations that are expected to be observed in clinical practice, and is guided by the concentrations observed in various patient populations during the phase 2 and 3 development phases of the respective direct-acting antivirals. The successful clinical application of this method to human subjects is also described. Sujan Dilly-Penchala provided expertise and guidance for the initial method development at the University of Liverpool but I performed all the development laboratory work, validation studies and statistical analysis described in this chapter.

Table 4.1 Bioanalytical method development and validation strategies

Subject	Requirements
Analyte	Chemical Structure
	Chemical properties (solubility, pKa, stability, absorption)
	Analyte Purity
Sample Preparation	Nature and property of the matrix
	Matrix stability
	Choice of extraction method
	Extraction recovery
Method Validation	Calibration curve with QC samples
	Accuracy
	Precision
	Selectivity
	Matrix effect
	Carry-over
	Stability (short and long-term)

Adapted from [**115]**

Figure 4.1. The bioanalysis pathway



4.2 Analytes

4.2.1 Sofosbuvir and GS-331007

Sofosbuvir ($C_{22}H_{29}FN_3O_9P$ – *m/w 529.45 g.mol^{-1}*) is an NS5B uridine nucleotide polymerase inhibitor, which forms the backbone of a number of HCV DAA regimens. Sofosbuvir a pro-drug that is converted to an active triphosphate through a series of intracellular enzymatic phosphorylation reactions. The parent drug has a very short terminal half-life of 0.4 hours, and is soluble in dimethyl sulfoxide or methanol. Following oral administration of sofosbuvir, the majority of systemic drug exposure is in the form of GS-331007 (figure 4.2). GS-331007 ($C_{10}H_{13}FN_2O_5$ – *m/w 260.22 g.mol^{-1}*) is therefore considered the primary analyte of interest for the purposes of pharmacokinetic studies, and is minimally protein bound. The half-life of GS-331007 is 27 hours [116].

In HCV-infected subjects without hepatic impairment (n=8), the mean (CV%) C_{max} for sofosbuvir and GS-331007 were 603 (47) ng/ml and 1378 (19) ng/ml respectively. In HCV-infected patients with severe hepatic impairment, the mean (CV%) C_{max} for sofosbuvir and GS-331007 were 1125 (49) ng/ml and 1439 (59) ng/ml [45].

4.2.2 Ledipasvir

Ledipasvir ($C_{49}H_{54}F_2N_8O_6$ – *m/w 889 g.mol^{-1}*) is an NS5A inhibitor that is co-formulated with sofosbuvir as part of a fixed dose combination for HCV genotype 1 and 4. It has a long terminal half-life of 47 hours, and is highly protein bound (> 99.8%).

Based on population pharmacokinetic analyses, the mean C_{max} of ledipasvir is 323 ng/ml with an AUC of 7290 ng.h.ml^{-1} [45].

4.2.3 Daclatasvir

Daclatasvir ($C_{40}H_{50}N_8O_6$ – *m/w 738.88 g.mol^{-1}*) is a pangenotypic NS5A inhibitor that is licenced for co-administration with sofosbuvir. It has a plasma half-life of 12 – 15 hours, and is soluble in dimethyl sulfoxide and methanol. In HCV-infected treatment naïve patients without cirrhosis, daclatasvir C_{max} (CV%), C_{min} (CV%) and AUC (CV%) were 1534 (58) ng/ml, 232 (83) ng/ml, and 14122 (70) ng.h.ml^{-1} [117].

4.2.4 Simeprevir

Simeprevir ($C_{38}H_{47}N_5O_7S_2$ – *m/w 749.94 g.mol^{-1}*) is an NS3/NS4A serine protease inhibitor that is licenced for co-administration with sofosbuvir for the treatment of HCV genotype 1 and 4 infection. It has a half-life of 41 hours, and is soluble in dimethyl sufoxide and acetonitrile. The C_{max} of simeprevir is ~ 3000 ng/ml in healthy volunteers. Simeprevir geometric mean C_{min} (CV%) concentration in HCV-infected patients without cirrhosis at steady-state is 1936 (136) ng/ml [118].

The chemical structures and properties of the respective analytes are outlined in figure 4.3

Figure 4.2 - Sofosbuvir Metabolism

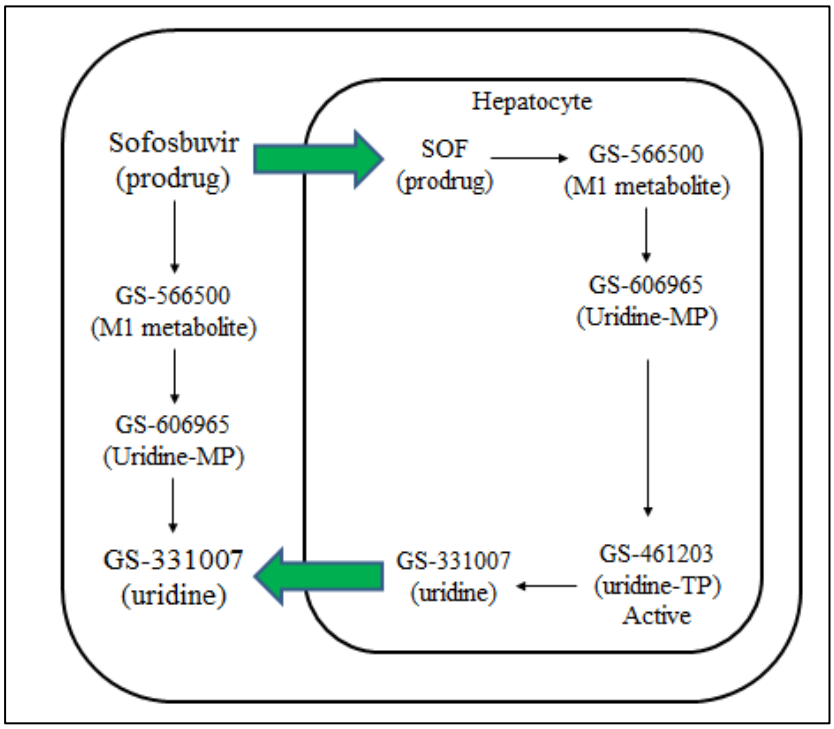
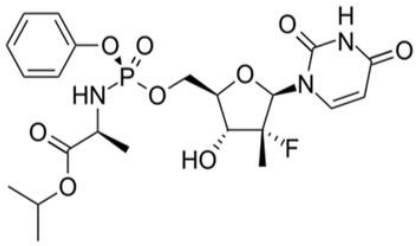
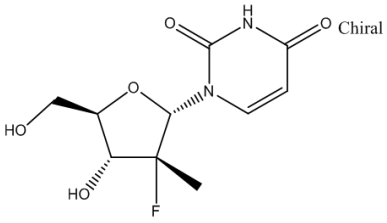


Figure 4.3 – Chemical structures and properties of sofosbuvir, GS-331007, ledipasvir, daclatasvir and simeprevir



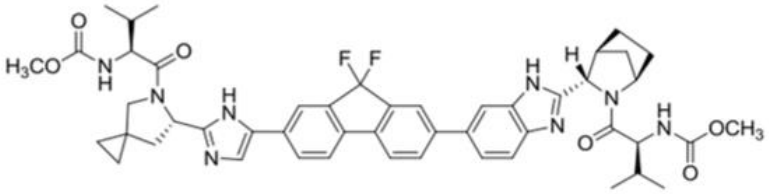
(a) Sofosbuvir [$C_{22}H_{29}FN_3O_9P$]

logP 1.62
pKa 9.3



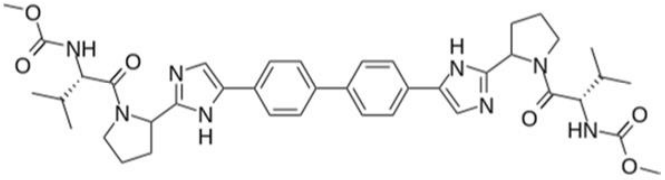
(b) GS-331007 [$C_{10}H_{13}FN_2O_5$]

log P -1.24
pKa 9.85

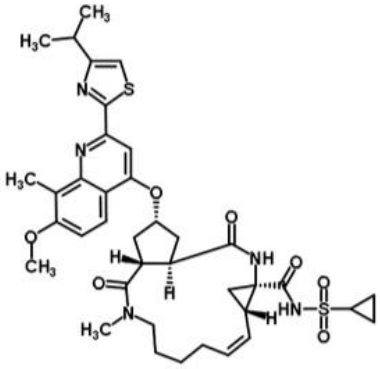


(c) Ledipasvir [$C_{49}H_{54}F_2N_8O_6$]

logP 3.8
pKa 3.76



(d) Daclatasvir [$C_{40}H_{50}N_8O_6$]



logP 4.67
pKa 11.15

(e) Simeprevir

logP 4.82
pKa 5.87

4.3 Materials and Methods

4.3.1 Chemicals materials

Reference standards of sofosbuvir, sofosbuvir-d6 (stable isotope labelled internal standard;IS), GS-331007, and ledipasvir were donated by Gilead Sciences, Inc. (Foster City, CA). Daclatasvir was donated by Bristol-Myers Squibb (Princeton, NJ), while Simeprevir and Simeprevir-d3 were donated by Janssen Pharmaceutica (Belgium). GS-331007-d3 IS, daclatasvir-d6 IS, simeprevir-d3 IS and ledipasvir-d6 IS were obtained from Alsachim (France).

LC-MS grade acetonitrile (ACN), methanol (MeOH), formic acid (minimum 95% pure) and tetra-butyl-methyl-ether (TBME) were obtained from Sigma-Aldrich (UK). Water was purified using a purelab classic UVF (Elga Lab water; High Wycombe, UK). Drug free (K_2EDTA) plasma was obtained from the National Blood Service (Liverpool, UK).

4.3.2 Equipment

The HPLC system consisted of a variable loop Accela autosampler and an Accela LC-pump (Thermo Electron Corporation, Hemel Hempstead, UK). A reverse phase Atlantis C_{18} column (100mm x 2.1mm x 3.0 µm, Waters Ltd, UK) was used to elute the analytes. The HPLC was connected to a TSQ Quantum Access triple quadrupole mass spectrometer (Thermo Electron Corporation, Hemel Hempstead, UK) equipped with a heated electrospray ionisation source. A E2M30 rotary vacuum pump (Edwards High Vacuum International, West Sussex, UK), a NM30LA nitrogen generator (Peak-scientific, Renfrewshire, Scotland, UK) and 99% pure argon gas (10L size, BOC gases, Worsely, Manchester, UK) were used. TSQ tune software (Thermo Electron

Corporation, Hemel Hempstead, UK) was used to optimise the tuning parameters. LCQuan software (Thermo Electron Corporation, Hemel Hempstead, UK) was used for data acquisition and processing purposes.

4.3.3 Liquid chromatography conditions

A gradient elution programme was used for chromatographic separation of the five analytes (and their respective internal standards) with mobile phase A (0.1% formic acid) and mobile phase B (Methanol – 0.1% formic acid) as follows: 0-1.0 min (95-5% A:B), 1.0-4.2 min (60-40% A:B), 4.2-8.8 min (5-95% A:B), and 8.8-11.0min (95-5% A:B). The flow rate was 400µL/min and the overall run time was 11.0 minutes. An outline of the mobile phase programme is detailed in table 4.2. The column oven temperature was maintained at 40^{o}C and the wash solution was methanol and water [50:50 (v/v)].

Table 4.2
Step-wise mobile phase gradient program consisting of A:water (0.1% formic acid) and B:methanol (0.1% formic acid)

Time	Mobile Phase		
	% A	% B	Flow rate (µL/min)
0.0	95	5	400
0.8	95	5	400
1.0	60	40	400
4.0	60	40	400
4.2	5	95	400
8.5	5	95	400
8.8	95	5	400
11.0	95	5	400

4.3.4 Mass spectrometry conditions

The mass spectrometer was operated in positive ion electro-spray ionisation mode using selection reaction monitoring (SRM). The electrospray voltage was 4.5 kV, the capillary temperature was 300^{o}C and the vaporiser temperature was set at 350^{o}C. The sheath pressure was set at 45 and the auxiliary gas pressure was set at 20. Argon was used as the collision gas at a pressure of 1.5mTorr. The parent-to-fragment [*m/z*] transitions, tube lens and relative collision energies are summarised in table 4.3.

Table 4.3

Parent-to-fragment (daughter) mass transitions, tube lens, relative collision energy (RCE) and retention time for all analytes using HPLC-MS/MS

Drug	Drug class	*Parent ion (m/z)*	Fragment ions *(m/z)*	Tube lens (V)	RCE (V)	RT (min)
Sofosbuvir	NS5B inhibitor	530.1	167	114	42	5.47
GS-331007	Sofosbuvir metabolite	261.1	96.1, 113.1	85	33,12	2.23
Ledipasvir	NS5A inhibitor	889.4	637.3, 732.4	165	44,39	5.44
Daclatasvir	NS5A inhibitor	739.3	338.9, 565.2	132	57,40	5.19
Simeprevir	Protease inhibitor	750.2	287.0, 314.9	140	55,43	7.09

4.3.5 Preparation of standard stock calibration standards and quality control samples

The standard stock solutions (1mg/ml) of sofosbuvir, GS-331007, daclatasvir, and simeprevir were prepared in methanol, and stored at 4^{o}C. A stock solution of (1mg/ml) of ledipasvir was prepared in acetonitrile. Stock solutions were then further diluted with drug free plasma to yield working solutions which were stored at -20^{o}C until use. On the day of analysis, working solutions were diluted in duplicate with appropriate volumes of drug free plasma (100 µl per calibrator level) to yield concentrations ranging from 2.5-1250 ng/mL (SOF), 10-2500 ng/mL (GS-331007, LDV), and 10-5000 ng/mL (DCV, SIM).

Internal quality control samples (QC) were prepared separately by the dilution of drug-free plasma to yield three QC levels, an LQC (SOF 6 ng/mL; GS-331007, LDV, DCV and SIM 24 ng/mL), MQC (SOF 100 ng/mL; GS-331007 and LDV 200 ng/mL; DCV and SIM 400 ng/mL), and HQC (SOF 1000 ng/mL; GS-331007 and LDV 200 ng/mL; DCV and SIM 400 ng/mL). Internal standard stock solutions (1mg/ml) of sofosbuvir-SIL, GS-331007-$13C_2D_3$, Daclatasvir-SIL and Simeprevir-D_3 were prepared separately in 100% Methanol. An internal standard stock solution (1mg/ml) of Ledipasvir-SIL was prepared in 100% ACN. A working stock internal standard solution (SOF/GS-331007/LDV/DCV/SIM - 1/2.5/2.5/5/2.5 ng/mL) was prepared through dilution of the respective 1mg/ml stock solutions in MeOH:water (50:50 v/v).

4.3.6 Sample extraction

A 100µL volume of spiked plasma calibration curve standards and QC samples were transferred to a set of 7 mL glass tubes. 20 µL of the working stock IS was added to the tubes and vortexed for a few seconds. An extraction solvent of TBME (2ml) was added to each tube. The tubes were kept on a rotator for 30 minutes at a rotation speed of 3 rpm (STR4, Stuart, UK) and then were centrifuged at 4500 rpm for 10 minutes at 4^{o}C (Thermo Scientific Haraeus® Multifuge 3SR). The samples were flash frozen using dry ice/methanol, with the supernatant layer being then transferred to a new set of pre-labelled glass tubes and the contents of the tubes evaporated in a stream of nitrogen at room temperature. Subsequently, the remaining residues were reconstituted with 200 µL of mobile phase (50:50% (v/v) mobile phase A and mobile phase B). Aliquots of 120 µL were transferred to auto-sampler vials, and 10 µL was injected onto the HPLC column.

4.4 Method Validation

Validation of the method was performed in accordance with FDA guidelines (Bioanalytic Method Validation, May 2001). The following method parameters were validated: selectivity, sensitivity, matrix effects, linearity, precision, accuracy, recovery, dilution integrity and stability.

4.4.1 Selectivity, sensitivity and linearity

Selectivity was assessed by analysing eight different lots of blank human plasma as per FDA and EMEA requirements. The area response of interfering substances or noise at the retention times of the individual analytes was acceptable if the percentage interference was less than 20% of the mean response of the lowest standard in the calibration curve or lower limit of quantification (LLQ; n=6). Equally, the area response of any interfering substances or noise at the retention rime of internal standard was acceptable if the percentage interference was less than 5% of the mean response of the internal standard areas in 6 LLQ samples.

The sensitivity of the assay was assessed by monitoring the response of six spiked LLQ samples. The six replicates should have a precision of $\leq$ 20% and with an accuracy of $\pm$ 20% of the nominal value of the LLQ. The linearity of the method was determined for the concentration range specified using five different calibration curves.

4.4.2 Precision and accuracy

Intra-day precision and accuracy were determined by analysing six replicates at each QC level read off a calibration curve in a single day. Inter-day precision and accuracy were determined by analysing six replicates at three different QC levels on five different analytical batch runs over a minimum of 5 days. The acceptance criteria for

accuracy were within $\pm$ 15% and precision was within $\leq$ 15% relative standard deviation (RSD).

4.4.3 Recovery and matrix effects

The matrix effect was examined quantitatively by the simultaneous post-column infusion of sofosbuvir, GS-331007, ledipasvir, daclatasvir, simeprevir and their respective internal standards into the MS/MS detector during the chromatographic analysis of six blank plasma extracts. The chromatographic signals of each transition were examined to ascertain that no major shift in MS/MS signal occurred during the analyte's retention time. The percentage recovery and matrix effect were quantitatively determined further by preparing QC samples of all five analytes as follows: (a) pure standard solutions of all analytes and their internal standards, in mobile phase, directly injected onto the HPLC column; (b) blank plasma extract samples from six different sources, spiked with the analytes and internal standards after extraction; (c) plasma samples extracted from six different sources spiked with analytes and internal standards before extraction. The process efficiency (overall recovery) was calculated as the ratio of the absolute peak-area response of the analytes in processed plasma samples spiked with drugs before extraction (c) to the peak area response of analytes in mobile phase (a) (c/a ratio in %). The matrix effect was assessed as the ratio of the peak area response of analytes added into blank plasma after the extraction procedure (b) to the peak areas of pure analytes in mobile phase (a) (b/a ratio in %). The acceptance for the recovery and matrix effect was that the % RSD of the areas obtained for the pre and post-extracted samples, and mobile phase equivalents and should be $\leq$ 15% individually at each level of QC samples and the %RSD of the percentage recoveries for all of QC levels should be < 115% at any level of concentration.

4.4.4 Dilution integrity and stability

Dilution integrity tests were performed during validation to ensure that clinical samples with concentrations above the assay upper limit of quantification (ULQ) could be successfully diluted to determine an accurate result. Dilution integrity samples (n=6) were prepared at two times the assay ULQ concentrations. The six replicates were then diluted by a factor of two and four times to give a result within the calibration range. The final concentrations were then derived by back-calculation using the relevant dilution factor.

Stability experiments were conducted to evaluate the stability of all five analytes under various conditions. Stability on the bench-top was evaluated over 16 hours. Freeze-thaw stability was determined over three freeze-thaw cycles over a period of three days. In each freeze-thaw cycle, the spiked plasma samples were frozen for 24 hours at -40^{o}C and then thawed unassisted at room temperature. After the samples had completely thawed, they were re-frozen for 12-24 hours at -40^{o}C. Processed sample stability (auto-sampler stability) was assessed by keeping extracted/processed samples in the auto-sampler at 10^{o}C for 72 hours. The freeze-thaw and processed sample stability was assessed by reading off freshly prepared calibration curves and comparing with the nominal concentrations. Finally, re-injection stability was tested by re-injecting an accepted precision and accuracy batch 72 hours later. Samples were considered stable if values were within the acceptance limits of accuracy ($\pm$ 15% of their respective nominal concentrations) and precision ($\leq$15% RSD).

4.5 Application to clinical samples

Blood samples were collected from three patients with HCV infection receiving daclatasvir 60mg once daily in combination with sofosbuvir 400mg once daily (two cirrhotic and one non-cirrhotic). Intensive pharmacokinetic sampling was performed at steady state. After the collection of a trough sample, blood samples were drawn at 0.25, 0.5, 1, 2, 4, 10 and 24 hours post dose. These timepoints were chosen based on the reported Tmax and half-lives of sofosbuvir, GS-331007 and daclatasvir described in the SmPC. Whole blood was collected in K_2EDTA tubes. Samples were centrifuged at 1500 rpm for 10 mins at 4^{O}C within 1 hour of sample collection. The plasma was transferred promptly to polypropylene tubes, and was stored at – 40^{O}C until sample analysis.

4.6 Results

4.6.1. Liquid chromatography and mass spectrometry conditions

The tandem mass ESI conditions for SOF, GS-331007, LDV, DCV and SIM and their respective internal standards were optimised in positive mode. Chromatographic separation was attempted using numerous combinations of acetonitrile, methanol, and buffers (formic acid, ammonium acetate) and over a range of pH values. A number of C_{18} HPCL columns were evaluated, including Hyrdo-RP (Phenomenex, Chesire, UK), and Atlantis C_{18} (Waters Ltd, Herefordshire, UK). The best resolution and peak shape for the analytes was achieved using an Atlantis C_{18} column (3.0 µm 100mm x 2.1 mm) [Figure 4.4 and 4.5].

The mobile phase and buffers were optimised to achieve the best peak shape and intensity for GS-331007. GS-331007 is weakly polar/hydrophilic with a partition co-efficient (log *P*) of -1.1. The use of acidified mobile phase and buffers (formic acid, ammonium acetate) produced the most optimal chromatographic results for GS-331007. A final gradient of water:methanol and 0.1% formic acid was ultimately found to be suitable, in which all analytes were protonated and well separated. Carryover was not observed for any of the analytes after injection of a high (ULQ) concentration.

Figure 4.4 – Chromatograms of (a) GS-331007, (b) GS-331007 internal standard, (c) sofosbuvir, (d) sofosbuvir internal standard, (e) daclatasvir and (f) daclatasvir internal standard

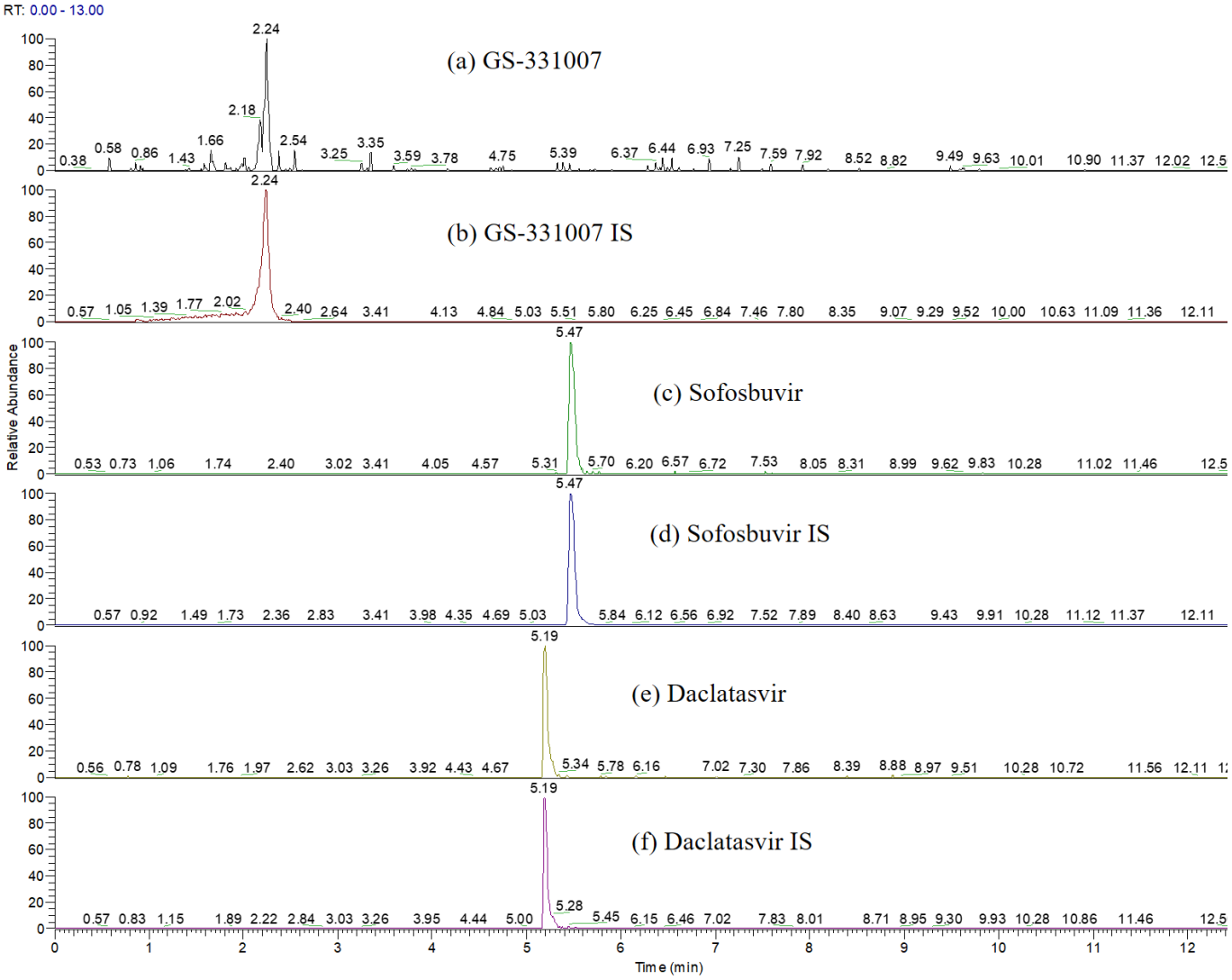
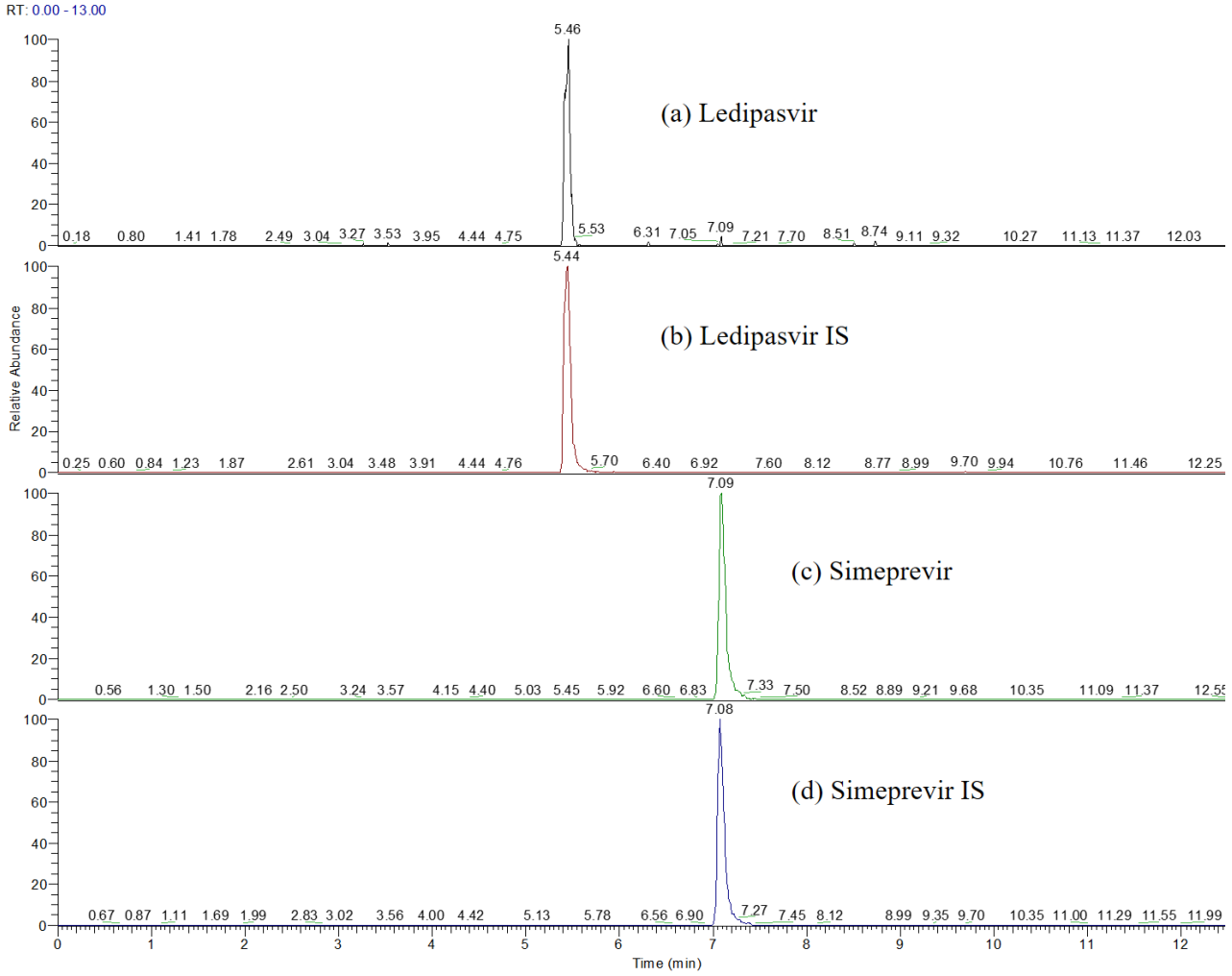


Figure 4.5 – Chromatograms of (a) ledipasvir, (b) ledipasvir internal standard, (c) simeprevir, (d) simeprevir internal standard



4.6.2 Sample extraction

Several sample extraction methods were evaluated including protein precipitation, solid phase extraction and liquid-liquid extraction. Hexane, ethylacetate and TBME were all assessed as potential solvents for extraction. Liquid-liquid extraction using TBME was the optimal extraction technique, giving the best recovery for the five analytes with negligible matrix effects. The method employed also produced a clean sample, reducing the amount of interfering constituents entering the electrospray source, thus minimising the potential for ion suppression.

4.6.3 Method Validation

4.6.3.1 Selectively, sensitivity and linearity

There was no significant interference observed in the processed blank plasma samples at the respective retention times for the analytes and their internal standards. The LLQ for sofosbuvir was 2.5ng/mL, while the LLQ for GS-331007, ledipasvir, daclatasvir and simeprevir was 10ng/mL. The signal-to-noise ratio at the LLQ for each analyte was within acceptance criteria (< 20% of the mean response at the LLQ). The standard calibration curves were linear over the concentration ranges specified, with a mean correlation co-efficient (r) of > 99.6%.

4.6.3.2 Recovery and matrix effects

The percentage recovery and matrix effects for SOF, GS-331007, LDV, DCV and SIM are outlined in table 4.4. Matrix effects, which are a result of ion suppression or enhancement of the analyte of interest was evaluated during method development because the assay precision and accuracy of the LC-MS/MS method can be significantly affected. There was no evidence of a matrix effect for any of the analytes.

Table 4.4 - Matrix effect, extraction yield, recovery analysis and process efficiency of sofosbuvir, GS-331007, ledipasvir, daclatasvir, simeprevir and their respective internal standards.

Analyte	Quality control (QC)	Nominal concentration (ng/mL)	Mean Peak area			ME (%) B/A	PE (%) C/A
			A (n=6)	B (n=6)	C (n=6)		
Sofosbuvir	LQC	6	111,106	111,972	107,311	100.8	96.6
	MQC	100	2,068,036	2,001,353	1,788,384	96.8	86.5
	HQC	1000	19,526,304	20,399,039	18,833,976	104.5	96.5
GS-331007	LQC	24	95,526	87,025	27,684	91.1	29.0
	MQC	200	809,199	751,915	262,520	92.9	32.4
	HQC	2000	6,026,916	6,572,535	2,801,027	109.0	46.5
Ledipasvir	LQC	24	13,721	20,400	23,912	101.4	126.5
	MQC	200	198,028	270,542	238,921	113.8	100.5
	HQC	2000	2,679,241	2,950,418	3,149,855	91.8	100.0
Daclatasvir	LQC	24	65,402	78,854	67,996	120.6	103.9
	MQC	400	1,284,842	1,279,950	1,142,548	99.7	88.9
	HQC	4000	10,656,835	11,243,163	10,793,085	105.5	101.3
Simeprevir	LQC	24	74,305	57,205	18,149	77.0	24.4
	MQC	400	1,576,789	1,290,832	304,853	81.8	19.3
	HQC	4000	15,111,726	15,682,416	2,681,606	103.8	17.8
Sofosbuvir IS		1000	3,906,460	3,817,115	3,742,214	97.7	95.8
GS-331007 IS		2500	1,031,622	995,255	325,439	96.5	31.5
Ledipasvir IS		2500	126,252	173,455	167,426	137.4	132.6
Daclatasvir IS		5000	776,004	801,789	785,802	103.3	101.3
Simeprevir IS		2500	9,573,342	6,371,263	2,327,544	66.6	24.3

A = peak area of standard solutions without matrix and without extraction; B = peak area of analytes spiked after extraction; C = peak area of analytes spiked before extraction. ME – Matrix effect; PE – process efficiency

4.6.3.3 Precision and accuracy

The developed method was both reliable and reproducible for the simultaneous quantification of sofosbuvir, GS-331007, ledipasvir, daclatasvir and simeprevir in human plasma. Inter and intra-day precision and accuracy for the assay are detailed in table 4.5. The accuracies (RE%) for the low, medium, and high QC levels of all analytes were all within ± 11.1%. The inter and intra-day precisions (RSD%) of the analytes were within acceptable limits, and ranged from 2.1% to 11.6%.

4.6.3.4 Dilution integrity and stability

Dilution integrity samples were prepared at a concentration of 2000ng/mL for Sofosbuvir, 4000ng/mL for GS-331007, and 8000ng/mL for ledipasvir, daclatasvir, and simeprevir. The samples were then diluted by a factor of two times and four times with blank human plasma. Mean back calculated concentrations were within 85.4-104.1% of their nominal value, with a %RSD of <5% for all analytes.

The results of the stability experiments are outlined in table 4.6. The data demonstrate that SOF, GS-331007, ledipasvir, daclatasvir, and simeprevir were stable under the various storage and processing conditions described.

Table 4.5
Precision (%) and accuracy (%) for quantification of sofosbuvir, GS-331007, ledipasvir, daclatasvir and simeprevir at all QC concentrations

Analyte	Quality control (QC)	Nominal concentration (ng/ml)	Intra-day			Inter-day		
			Mean	Precision (%RSD)	Accuracy (%)	Mean	Precision (%RSD)	Accuracy (%)
Sofosbuvir	LQC	6	6.59	6.62	109.9	6.55	4.65	109.1
	MQC	100	101.26	4.00	101.2	101.60	2.52	101.6
	HQC	1000	1072.62	4.65	107.2	1061.68	3.59	106.1
GS-331007	LQC	24	23.85	10.19	99.4	24.16	9.27	100.7
	MQC	200	203.05	11.16	101.5	211.97	8.56	106.0
	HQC	2000	2047.60	7.04	102.4	2029.17	8.41	101.5
Ledipasvir	LQC	24	24.84	11.57	103.5	26.15	10.08	109.0
	MQC	200	191.28	7.42	95.6	195.91	7.86	98.0
	HQC	2000	2058.72	8.48	102.9	2018.7	8.22	101.0
Daclatasvir	LQC	24	26.42	8.51	110.1	25.84	10.14	107.6
	MQC	400	399.65	3.78	99.9	402.33	2.08	100.6
	HQC	4000	4251.42	4.56	106.3	4174.47	2.37	104.4
Simeprevir	LQC	24	26.66	10.42	111.1	26.27	11.43	109.4
	MQC	400	402.56	4.18	100.6	403.67	5.02	100.9
	HQC	4000	4158.80	5.24	104.0	4101.47	3.78	102.5

Table 4.6 - Short-term stability data of sofosbuvir, GS-331007, ledipasvir, daclatasvir and simeprevir at all QC concentrations

Stability	Analyte	Nominal concentration (ng/ml)	Mean (n=6)	SD	Precision (%)	Accuracy (%)
Benchtop	Sofosbuvir	6	6.5	0.42	6.4	109.0
		100	97.8	4.43	4.5	97.8
		1000	1022.3	37.68	3.7	102.2
	GS-331007	24	23.7	2.26	9.5	98.7
		200	192.4	10.58	5.5	96.2
		2000	2001.5	88.4	4.4	100.0
	Ledipasvir	24	23.2	1.65	7.1	96.8
		200	183.0	6.68	3.6	91.5
		2000	2012.1	125.0	6.2	100.6
	Daclatasvir	24	26.2	25.3	3.7	109.2
		400	413.5	13.8	3.3	103.4
		4000	4286.3	75.5	1.8	107.2
	Simeprevir	24	25.2	0.97	3.8	105.1
		400	386.7	22.04	5.7	96.7
		4000	4148.3	252.0	6.1	103.7
Autosampler (72h)	Sofosbuvir	6	5.8	0.46	7.9	96.9
		100	94.0	2.86	3.0	94.0
		1000	1048.5	40.09	3.8	104.8
	GS-331007	24	24.0	1.84	7.7	100.0
		200	195.3	4.46	2.3	97.6

		2000	1984.2	57.2	2.9	99.2
	Ledipasvir	24	24.1	1.98	8.2	100.5
		200	182.4	4.25	2.3	91.2
		2000	1969.9	78.25	4.0	98.5
	Daclatasvir	24	23.7	1.95	8.3	98.6
		400	368.4	14.64	4.0	92.1
		4000	3942.8	83.93	2.1	98.6
	Simeprevir	24	23.5	2.14	9.1	97.7
		400	355.4	11.09	3.1	88.9
		4000	3745.5	141.08	3.8	93.6
Re-injection	Sofosbuvir	6	6.2	0.52	8.4	103.1
		100	99.3	3.26	3.3	99.3
		1000	1074.0	62.45	5.8	107.4
	GS-331007	24	23.7	1.32	5.6	98.9
		200	190.7	4.19	2.2	95.5
		2000	2092.5	105.42	5.0	104.6
	Ledipasvir	24	23.2	1.75	7.5	96.8
		200	186.4	5.75	3.1	93.2
		2000	2106.2	60.75	2.9	105.3
	Daclatasvir	24	24.4	2.36	9.7	101.8
		400	385.3	17.37	4.5	96.3
		4000	4236.7	216.31	5.1	105.9
	Simeprevir	24	25.0	1.62	6.5	104.4
		400	387.0	15.59	4.0	96.8

		4000	4210.9	229.54	5.5	105.3
Freeze-thaw (3 cycles)	Sofosbuvir	6	6.1	0.59	9.7	101.6
		100	93.6	3.70	4.0	93.6
		1000	1000.8	67.97	6.8	100.1
	GS-331007	24	25.1	2.05	8.2	104.2
		200	199.0	13.3	6.7	99.5
		2000	1910.1	121.2	6.3	95.5
	Ledipasvir	24	22.5	1.01	4.5	89.9
		200	179.6	6.79	3.8	88.0
		2000	1832.0	107.02	5.9	89.4
	Daclatasvir	24	26.0	1.03	4.0	108.2
		400	414.6	26.35	6.4	103.6
		4000	4081.3	159.69	3.9	102.0
	Simeprevir	24	25.4	1.25	4.9	105.7
		400	369.6	17.93	4.9	92.4
		4000	3811.2	170.18	4.5	95.3
Heat Stability	Sofosbuvir	6	6.4	0.24	3.7	105.9
		100	101.4	4.35	4.3	101.4
		1000	1055.7	57.3	5.4	105.6
	GS-331007	24	24.1	1.37	5.7	104.4
		200	198.0	14.62	7.4	102.6
		2000	2110.5	93.83	4.4	105.5
	Ledipasvir	24	22.8	1.68	7.4	94.8
		200	196.6	13.8	7.0	98.3

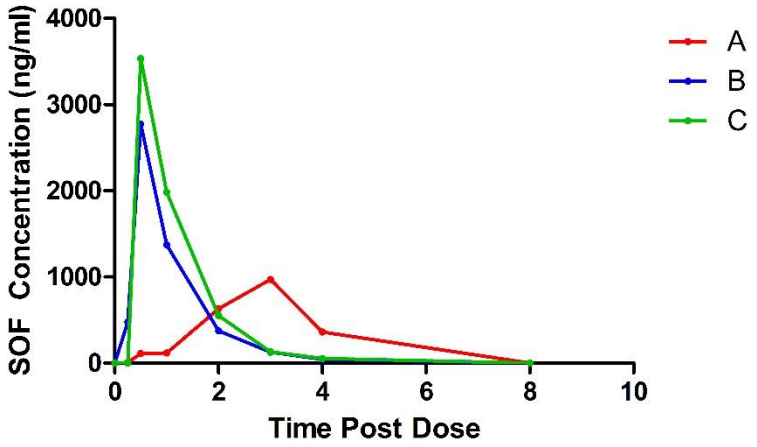
		2000	2079.3	157.3	7.6	104.0
	Daclatasvir	24	26.6	0.57	2.2	110.7
		400	430.7	15.7	3.7	107.7
		4000	4302.3	223.7	5.2	107.6
	Simeprevir	24	24.3	1.44	5.9	101.4
		400	403.3	24.12	6.0	100.8
		4000	4065.2	337.3	8.3	101.6

4.6.4 Application to clinical samples

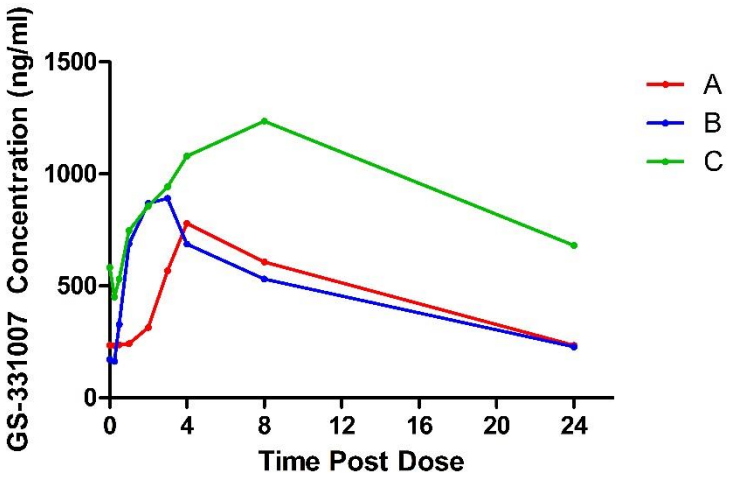
The pharmacokinetic profiles of patients (n=3) receiving combination therapy with sofosbuvir-daclatasvir therapy for HCV genotype 3 are depicted in figure 4.6 (a) – (c). Sofosbuvir geometric mean for C_{max} (CV%) was 2122 (62) ng/ml. The Geometric mean (CV%) for GS-331007 C_{max} and C_{min} were 863 (10) ng/ml and 331 (78) ng/ml respectively. Daclatasvir mean (CV%) C_{max} and C_{min} were 1392 (26) ng/ml and 237 (67) ng/ml respectively. Patient A T_{max} was prolonged for all 3 analytes compared to patients B and C [figure 4.6 (a) – (c)]. This patient had Type 2 Diabetes Mellitus with macrovascular complications, suggestive of delayed drug absorption secondary to impaired gastric emptying. This phenomenon has been described in diabetic patients treated for tuberculosis [119].

Figure 4.6 – Pharmacokinetic profiles of sofosbuvir, GS-331007 and daclatasvir

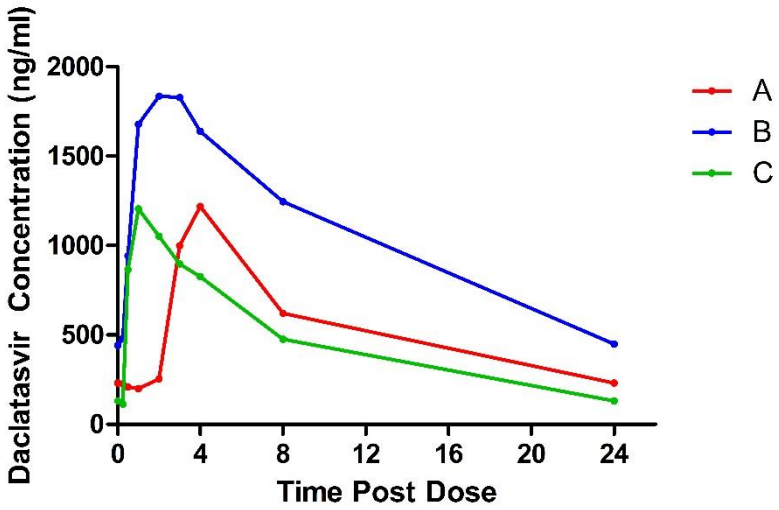
(a) Pharmacokinetic profile of Sofosbuvir (n=3)



(b) Pharmacokinetic profile of GS-331007 (n=3)



(C) Pharmacokinetic profile of daclatasvir (n=3)



4.7 Discussion

This chapter describes the development and validation of an LC-MS/MS bioassay of the simultaneous quantification of ledipasvir, daclatasvir, simeprevir, and sofosbuvir and its metabolite GS-331007. This is the first reported HCV multi-drug bioassay across such a wide analytical range. The described method was both reliable and reproducible for the simultaneous quantification of all analytes. There were no issues with carry-over, and short and long-term stability (> 18 months) of spiked samples and stock concentrations were demonstrated.

The five analytes exhibit different acid dissociation constants (pKa) and lipophilicity (Log D or Log P), and behave differently in chromatographic separation and extraction. Based on these chemical properties, the extraction technique and chromatographic conditions were optimised for sofosbuvir and its metabolite. Sofosbuvir is a weak acid and has a logP value 1.6, and its nucleoside metabolite GS-331007 is relatively polar in nature. Retention of the metabolite GS-331007 was difficult using a conventional C18 column, as the metabolite eluted during the void volume time. The remaining analytes were all relatively non-polar and were separated based on polarity. The use of an Atlantis dc18 reverse phase column allowed for the simultaneous retention and elution of the polar (sofosbuvir, GS-331007 and simeprevir) and non-polar (ledipasvir, daclatasvir) analytes. Atlantis dc18 columns are universal, silica-based, reversed phase C18 columns that are used for polar compound retention. The Atlantis dc18 columns feature di-functionally bonded C18 ligands that have been optimised for use with highly aqueous mobile phases, including 100% water, and exhibit strong retention of polar compounds without excessive retention of non-polar compounds.

Solubility was another challenge for the simultaneous quantification of these analytes. Ledipasvir was poorly soluble in methanol at room temperature. Heating the stock solutions increased solubility in methanol, but ledipasvir precipitated out of solution when the stock solution cooled to room temperature. Ledipasvir was highly soluble in acetonitrile (ACN), and stock solutions and QC samples were prepared in ACN.

The wide analytical range for the analytes in this bioanalysis method covers the expected concentrations that would be observed in clinical studies, including patients with decompensated cirrhosis and renal impairment. The use of dilution techniques increases the upper limit of quantification for ledipasvir, daclatasvir and simeprevir to 8000 ng/ml. This is particularly useful for simeprevir in quantifying outliers in pharmacokinetic studies, as simeprevir is a substrate of CYP3A4, which exhibits high variability in pharmacokinetics. The high sensitivity of this bioassay with a lower limit of quantification of 2.5 ng/ml for sofosbuvir, and 10ng/ml for GS-331007, ledipasvir, daclatasvir and simeprevir is ideal for use in pharmacokinetic tail studies or therapeutic drug monitoring for adherence/compliance.

A laboratory standard operating procedure (SOP) for this bioassay was developed in accordance with GCLP requirements. This assay may be of use in the real-world clinical setting as part of therapeutic drug monitoring of dose optimisation in patients with complex drug-drug interactions such as those receiving potent enzyme inducers for epilepsy. In Chapter 4, this bioanalysis method was used in the assessment of DAA concentrations in diverse real-world cohorts of patients with advanced compensated and decompensated HCV-related liver disease.

CHAPTER 5

PHARMACOKINETICS OF LEDIPASVIR IN DECOMPENSATED CIRRHOSIS

5.1 Introduction

Ledipasvir was the first orally bioavailable NS5A inhibitor licenced for the treatment of HCV genotypes 1 and 4. Although originally approved for the treatment of genotype 3 infection, it is no longer recommended for this indication because of limited antiviral efficacy in genotype 3 patients [70]. As previously discussed, the safety and efficacy of ledipasvir as part of a fixed dose combination with sofosbuvir has been demonstrated in three large phase 3 studies in compensated cirrhotic and non-cirrhotic patients [51-53]. Ledipasvir-sofosbuvir was also the first DAA combination approved for patients with decompensated cirrhosis. The SOLAR-1 and 2 trials reported on the safety and efficacy of this regimen is this cohort of patients with unmet medical need and historically no other treatment options.

There is limited real world data on the pharmacokinetic variability of ledipasvir, and determinants of this PK variability, especially in special populations such as those with decompensated cirrhosis. The absorption of ledipasvir has been characterised in healthy volunteers and HCV-infected patients. Following oral administration, maximal plasma concentrations are achieved after 4-4.5 hours post dose [49]. This absorption is pH dependent, and is expected to be altered by drugs that modulate gastric pH [49]. There are no reported pharmacokinetic-pharmacodynamic data on the clinical significance of any alteration in absorption by acid-reducing agents. Ledipasvir undergoes hepatic oxidative metabolism and is not a substrate or inhibitor of CYP-450 enzymes, with 70% of the oral dose excreted unchanged in faeces. The pharmacokinetics of ledipasvir are not affected by renal or hepatic impairment [49, 108].

An understanding of PK variability can help aid clinicians in individually modifying treatment to maximise the probability of achieving SVR.

The fixed dose combination of ledipasvir/sofosbuvir was the first interferon-free therapy licensed for hepatitis C. There was emerging data on the effect of acid suppression on the absorption of ledipasvir and this represented an area of uncertainty with a pressing need for further data to guide management. The primary aim of this chapter was to characterise the pharmacokinetics of ledipasvir in patients with advanced and decompensated cirrhosis, including individual variability, determinants of PK variability, covariates for ledipasvir exposure and the effect of PPI therapy on ledipasvir PK and treatment outcome. A secondary aim was to develop and validate a population pharmacokinetic model for ledipasvir in patients with decompensated cirrhosis.

These studies were conducted in compliance with good clinical practice (GCP). Study 5.2.1 received ethics approval from the joint SJH/AMNCH research ethics committee and all patients provided informed consent. Ethical approval for study 5.2.2 was received from the HCV research UK tissue and data access committee which is constituted as a research ethics committee.

I was responsible for recruiting all patients treated as part of the Irish early access programme (study 5.2.1) including sample collection and analysis. Patients were recruited across four treatment sites. Samples and anonymised patient data for study 5.2.2 were provided by HCV research UK but I analysed all the patient samples, and performed the statistical analysis. Henry Pertinez (University of Liverpool) provided expertise for the population pharmacokinetic model development and assisted with this.

5.2 Patients

Study 5.2.1 – Pharmacokinetics of ledipasvir-sofosbuvir in Patients with Decompensated Cirrhosis in the Irish Early Access Programme

Trough plasma samples were prospectively collected from 32 patients with decompensated cirrhosis undergoing DAA treatment with ledipasvir-sofosbuvir as part of the Irish early access programme. Patients had either Child-Pugh-Turcot class B or C cirrhosis, or a history of decompensation with ascites and/or encephalopathy (with subsequent recompensation to CPT class A). Baseline patient characteristics are outlined in table 5.1. Mean age was 52.8 and the median MELD score was 10. One quarter had HIV co-infection and all patients had HCV genotype 1 infection. Patients received 12 weeks of therapy with the fixed-dose combination of ledipasvir-sofosbuvir with ribavirin. In line with the SOLAR-1 and 2 clinical trials, ribavirin was initially administered at a starting dose of 600mg in a divided daily dose. Dose escalation of ribavirin to a maximum of 1000 or 1200 mg/day based on body weight was allowed if the starting dose was well tolerated, and the haemoglobin levels remained higher than 10 g/dL without haematological growth factor support. Ribavirin dose reductions were instituted in patients who could not tolerate the starting dose of 600mg/day based on haematological parameters or other ribavirin side effects.

At baseline, 15/32 (46.8%) of patients were receiving concomitant PPI therapy. In keeping with the manufacturer's recommendation, the dose was reduced to the equivalent of 20mg omeprazole and patients were advised to take the PPI dose at the same time as ledipasvir-sofosbuvir. There was no significant difference in age, median MELD score, median HCV

viral load or albumin between patients receiving and not receiving PPI therapy at baseline. There was a slightly higher proportion of patients with CPT class A (with a history of decompensation) in the non-PPI group. The majority of patients were reduced to 20mg omeprazole (80%) and three patients were changed to 15mg lansoprazole.

Table 5.1 Study 5.2.1 baseline patient characteristics

Baseline patient characteristics	n=32
Age – mean $\pm$ SD	52.8 $\pm$ 10.5
MELD – median $\pm$ SD	10 $\pm$ 3.2
MELD categories – n (%)	
< 10	13 (40)
10-15	15 (47)
> 15	4 (13)
HIV co-infection – n (%)	8 (25)
Child-Pugh-Turcot – n (%)	
A	6 (18.8)
B	21 (65.6)
C	5 (15.6)
Median Platelets – x 10^9 (range)	85 (32-287)
Median Albumin – g/dL (range)	32 (14-42)

Study 5.2.2 – Pharmacokinetics of ledipasvir-sofosbuvir in patients with advanced cirrhosis in the NHS England expanded access programme

This cohort was comprised of 314 patients with advanced or decompensated cirrhosis treated with ledipasvir-sofosbuvir as part of the NHS England expanded access programme (EAP). The EAP treated patients with severe liver disease of all genotypes, who were "at significant risk of death or irreversible damage within 12 months due to hepatic or extrahepatic manifestations" [58]. Criteria for inclusion were decompensated cirrhosis – ascites, variceal bleeding or encephalopathy (past or present), CPT score $\geq$ 7, or non-hepatic manifestation of HCV likely to lead to irreversible damage in 12 months and intolerant to, or failed peg-IFN and ribavirin therapy, or exceptional circumstances as determined by a review panel. The treatment cohort included patients with a prior history of liver transplantation. All patients received a maximum of 12 weeks of therapy with ledipasvir-sofosbuvir (90/400mg), with or without ribavirin. Inclusion and dosage of ribavirin was at the discretion of the treating physician. Baseline patient characteristics are outlined in table 5.2. The majority of patients (67%) had CPT class B cirrhosis at baseline. Median platelet count and albumin were lower in patients with CPT B and C cirrhosis compared to those with CPT A. PPI use was common in the treatment cohort, with 46.8% of patients receiving PPI therapy at baseline.

Table 5.2 Study 5.2.2 baseline patient characteristics

		Child-Pugh-Turcot A (n=81)	Child-Pugh-Turcot B (n=211)	Child-Pugh-Turcot C (n=21)	Total (n=314*)
Age (yrs) – median (range)		56	55	54	56 (28-79)
Weight (Kg) – median (range)		81.3	82.2	78.4	(47-150)
MELD – median		9	11.25	17	11
MELD score, n (%)	$\leq$ 10	51 (71.1)	71 (35.9)	0	122 (40.6)
	11- 15	30 (39.5)	107 (54)	8 (38)	145 (48.3)
	16-20	1 (1.3)	17 (8.6)	7 (33.3)	25 (8.3)
	> 20	0	2 (1)	6 (28.6)	8 (2.6)
Platelets – median		92 K/ml	78 K/ml	67 K/ml	79.5 K/ml
Albumin (g/dL) - median		3.6	3.1	2.6	3.2
Proton pump inhibitor use, n (%)					147 (46.8)
Genotype – n (%)	1				199 (63.4)
	2				12 (3.8)
	3				83 (26.4)
	4				16 (5.1)

5.3 Materials & Methods

5.3.1 Pharmacokinetics of ledipasvir-sofosbuvir in Patients with Decompensated Cirrhosis in the Irish Early Access Programme (Study 5.2.1)

A 5ml sample of blood was drawn from each patient at the end of a ledipasvir-sofosbuvir dosing interval (i.e. 24 hours after the previous dose). Trough samples were collected at the end of treatment weeks 1, 2, 4, 8, and 12 weeks. A day three trough sample was also collected from 10 patients. The samples were centrifuged at 1500 revolutions per minute (rpm) and 0.5mls of plasma was collected. The plasma was stored at -80^0C until subsequent ledipasvir-sofosbuvir analysis using High Performance Liquid Chromatography (HPLC). HCV Viral load was measured using the Abbott RealT*ime* at baseline, at the end of week 4 of treatment, at the end of treatment (week 12) and 12 weeks post treatment.

5.3.2 Pharmacokinetic of ledipasvir-sofosbuvir in patients with advanced cirrhosis in the NHS England expanded access programme (Study 5.2.2)

Plasma samples were prospectively collected from 20 centres through the HCV Research UK registry and biobank. Random plasma samples were collected at steady-state on 3-4 occasions across random time points during therapy. The time of sample collection and last dose administered were both recorded. A 5 ml sample of blood was drawn on each sampling occasion, and was transported overnight to the HCV Research UK biobank in Glasgow. Stability testing (see chapter 4) confirmed that samples were stable at room temperature overnight. At the biobank, samples were centrifuged at 1500 rpm and 0.5mls

of plasma was collected. The plasma was stored at -80^{0}C until subsequent HPLC analysis. Frozen plasma was transported using dry-ice to minimise the number of freeze thaw cycles allowing for repeated analyses. Anonymised patient demographic data and clinical and virologic outcomes were collected through the HCV research UK registry.

5.3.3 Analysis of ledipasvir-sofosbuvir concentrations

The bioanalytical method developed in chapter 4 was used to measure ledipasvir and sofosbuvir concentrations. Plasma samples (100µL) were pipetted into 7mL glass tubes, and internal standard was added. Standard curved were prepared containing blank plasma, ledipasvir, and sofosbuvir of known concentrations. Quality control samples for the assessment of the precision and accuracy of the assay were prepared by adding known quantities of ledipasvir and sofosbuvir to blank plasma. Working stock IS (20 µL) was added to the tubes and vortexed for a few seconds. An extraction solvent of TBME (2ml) was added to each tube. The tubes were kept on a rotator for 30 minutes at a rotation speed of 3 rpm (STR4, Stuart, UK) and then were centrifuged at 4500 rpm for 10 minutes at 4^{o}C (Thermo Scientific Haraeus® Multifuge 3SR). The samples were flash frozen using dry ice/methanol, with the supernatant layer then transferred to a new set of pre-labelled glass tubes and the contents of the tubes evaporated in a stream of nitrogen at room temperature. Subsequently, the remaining residues were reconstituted with 200 µL of mobile phase (50:50% (v/v) mobile phase A and mobile phase B). Aliquots of 120 µL were transferred to auto-sampler vials, and 10 µL was injected onto the HPLC column.

5.3.4 Pharmacokinetic and statistical analysis

Ledipasvir trough concentrations (C_{trough}) were reported as geometric mean (range). The geometric mean ratio for ledipasvir concentrations was calculated to compare C_{trough} patients with and without concomitant PPI therapy (95% CI). Differences in mean pharmacokinetic parameters between groups were compared using the independent sample t-test for normally distributed variables. Univariate regression was used with a prespecified significance level of 0.05 to examine the relationship between various baseline factors (age, weight, albumin, MELD score, CPT class and score, platelet count, PPI use and time post dose) and ledipasvir concentrations. Using forward selection, significant variables were carried into a multivariate regression model to define factors that predict ledipasvir concentrations. The development of a population pharmacokinetic model using NONMEM is outlined in section 5.4.

5.4 Ledipasvir population pharmacokinetic model development

Data generated from study 5.2.2 was used to develop a population pharmacokinetic model for ledipasvir. Sparse pharmacokinetic samples were used to describe the population PK profile for ledipasvir. Non-linear mixed effects modelling (NONMEM version VI 2.0, level 1.1; ICON Development Solutions) was then utilised to define a structural model which best describes the sparse PK dataset using the first order conditional estimation with interaction (FOCE-I) algorithm. Several structural models (e.g. single/multiple/transit compartments, different absorption and elimination kinetics, with and without lag time) were explored. Estimates of inter- and intra-occasion variability and residual error (incorporating additional and/or proportional error models) were then derived and the model was validated both internally (through simulations and visual predictive check) and

externally (using sparse data from patients sampled in the clinical study, as well as consistency with published parameter estimates).

Covariate analysis: Once validated, clinical data was used to refine model parameter estimates and test for important covariates of drug exposure. The minimal objective function value (OFV) was used as a goodness-of-fit diagnostic with a decrease of 3.84 points corresponding to a statistically significant difference between models (p=0.05, chi-squared distribution with one degree of freedom). Once the appropriate structural model was established, the following covariates were explored: age, weight, gender, PPI therapy, platelet count, albumin, MELD score and Child-Pugh-Turcot class/score. Graphical methods were used to explore the relationship of covariates versus individual predicted pharmacokinetic parameters. Each covariate was introduced separately into the model and only retained if inclusion in the model produced a statistically significant decrease in OFV of 3.84 ($p < 0.05$) and was biologically plausible. A backwards elimination step was then carried out once all relevant covariates were incorporated and covariates were retained if their removal from the model produced a significant increase in OFV (>6.63 points; P 0.01, χ^2 distribution, one degree of freedom). Comparison between predicted drug exposures from Monte Carlo simulations and observed data was performed to indicate any problems with model bias, shrinkage or over-fitting.

5.5 Results

5.5.1 – Pharmacokinetics of ledipasvir-sofosbuvir in Patients with Decompensated Cirrhosis in the Irish Early Access Programme (Study 5.2.1)

Steady state ledipasvir C_{trough} concentrations ranged from 34 to 431 ng/ml with dosing at 90mg once daily. Day 3 ledipasvir C_{trough} concentrations were significantly lower than week 1 trough concentrations (figure 5.1). Steady date trough GS-331007 concentrations ranged from 31 to 1320 ng/ml with sofosbuvir dosing at 400mg once daily. SVR_{12} was achieved in 26/32 (81.2%) of patients with 12 weeks of ledipasvir-sofosbuvir with ribavirin. Mean trough ledipasvir concentrations were significantly lower in relapsers (95 ng/ml) compared to patients achieving SVR_{12} (174 ng/ml) (p= 0.01).

Mean trough ledipasvir concentrations were numerically lower in patients receiving concomitant PPI therapy compared to patients not receiving PPI therapy (145.9ng/ml vs 175.4ng/ml). The geometric mean ratio of ledipasvir trough in patients receiving PPI therapy was 0.79 (95% CI GMR ratio 0.66 – 0.88) compared to no PPI.

5.5.2 - Pharmacokinetic of ledipasvir-sofosbuvir in patients with advanced cirrhosis in the NHS England expanded access programme (Study 5.2.2)

Ledipasvir and GS-331007 geometric mean concentrations were 254.1ng/ml (90% CI 199.4-226.8 ng/ml) and 558 ng/ml (90% CI 524.9-592 ng/ml) respectively. The range of ledipasvir and GS-331007 concentrations observed in the cohort are displayed in figure 5.2 and 5.3.

Figure 5.1 - Ledipasvir C_{trough} concentrations (ng/ml) during ledipasvir-sofosbuvir therapy (box plot mean and range) (n=32)

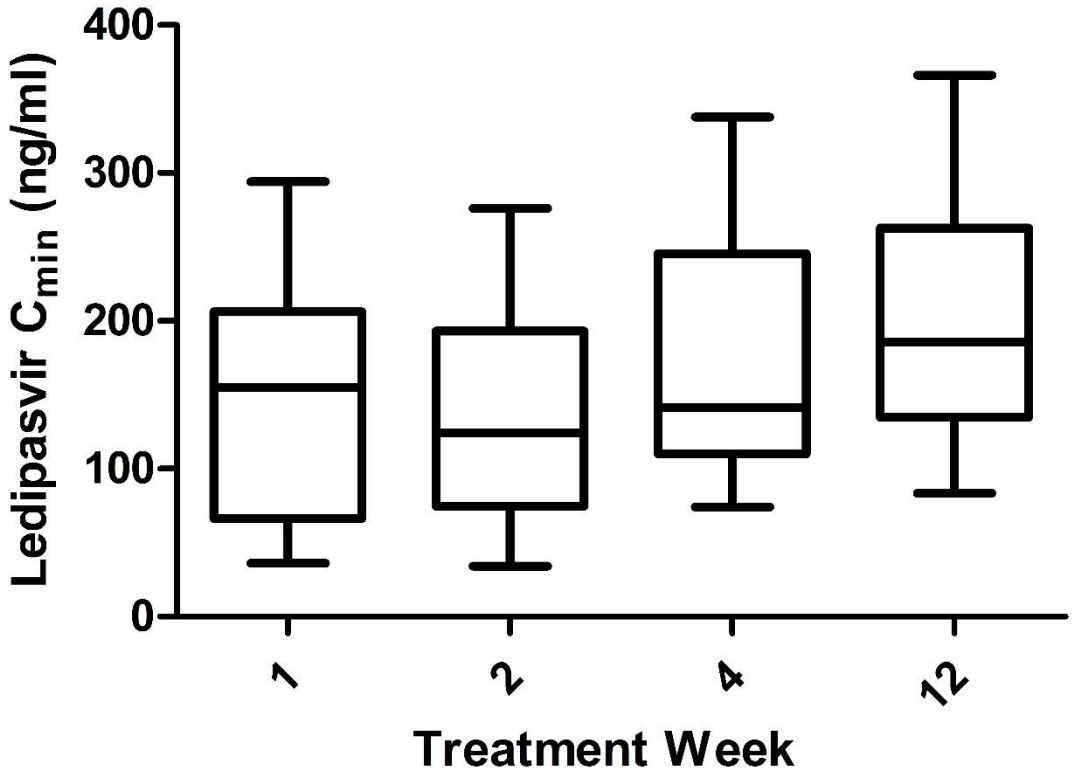


Figure 5.2 - Ledipasvir concentrations (ng/ml) in the NHS UK EAP versus time post-dose (hr) (n=314)

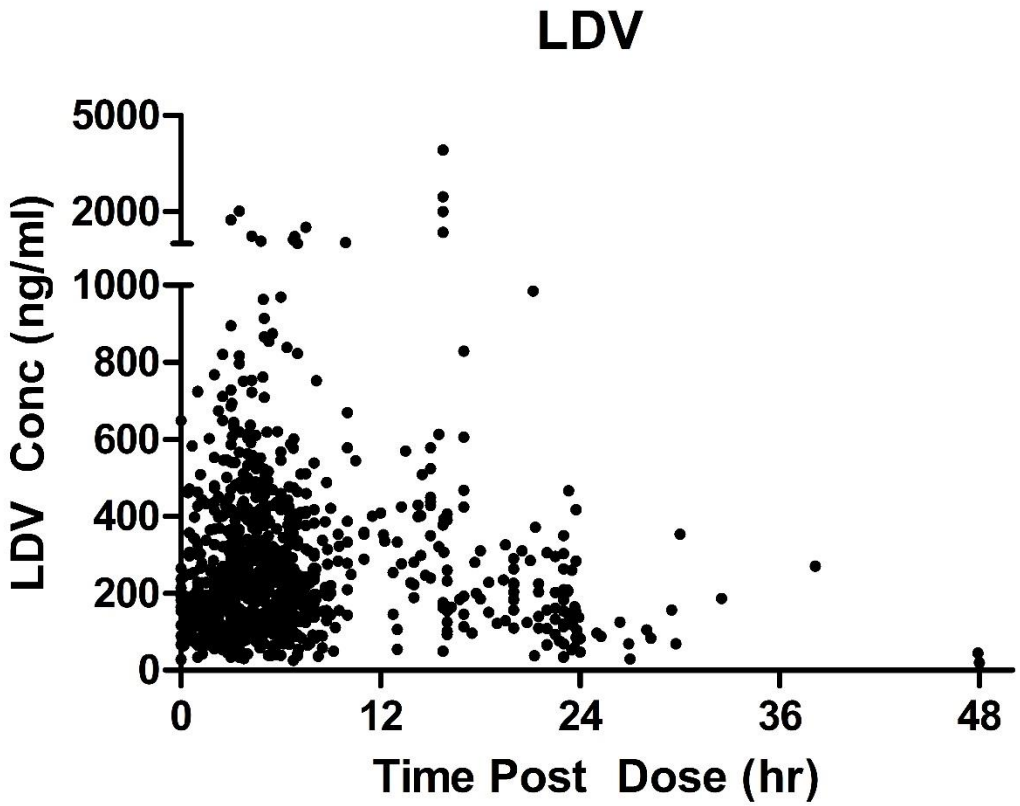
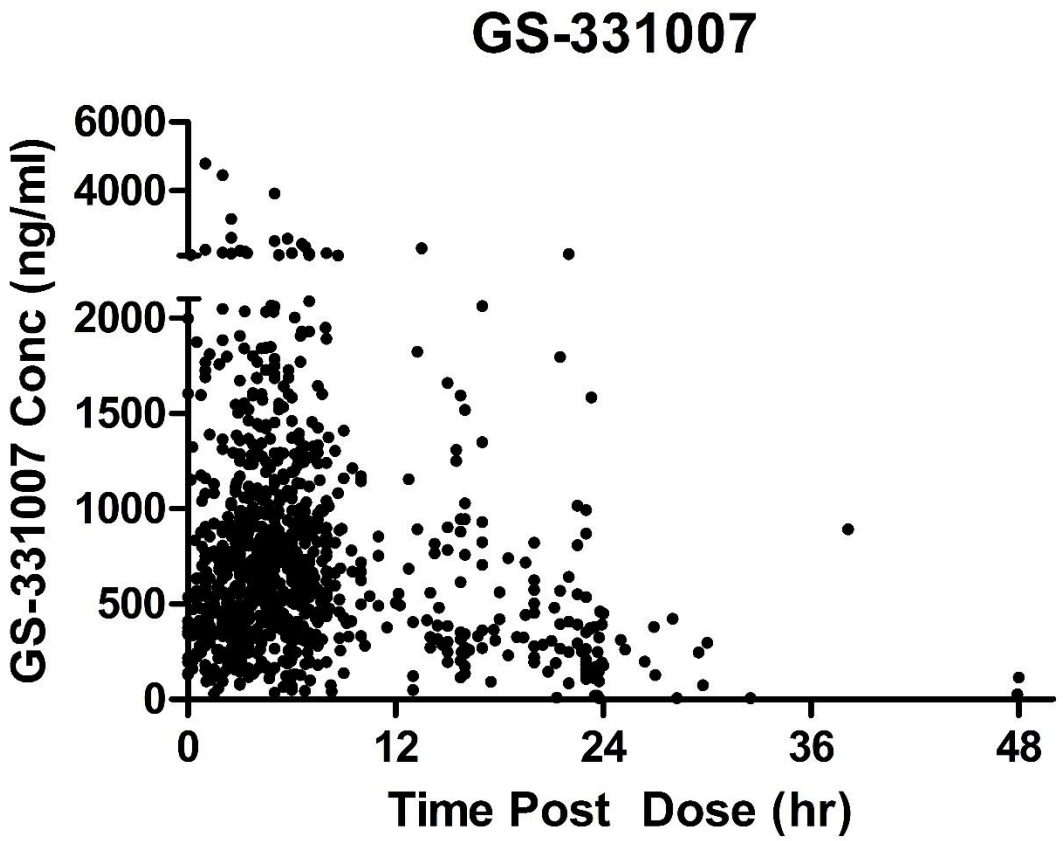


Figure 5.3 - GS-331007 concentrations (ng/ml) in the NHS UK EAP versus time post-dose (n=314)



In univariate analysis, weight, time post-dose, albumin, platelet count, PPI use and MELD were significant predictors of ledipasvir concentrations (table 5.3). There was no significant association between age, CPT score and ledipasvir concentrations. Using forward selection, a multivariate linear regression model was developed. In the final multivariate model, weight, time post-dose, albumin, platelet count and PPI use remained significant predictors of ledipasvir concentrations (table 5.4) . For every 10kg increase in weight, there was an associated decrease of 15.3 ng/ml (90% CI -21.8 – - 8.9 ng/ml) in ledipasvir concentrations. Baseline PPI use was the most significant predictor of ledipasvir concentrations, with a β-co-efficient of – 94.0 in patients receiving baseline PPI therapy (90% CI -115 – -72.9). There were four data points considered to be outliers – ledipasvir concentrations from a single patient were > 1200 ng/ml at 15 hours post-dose. The final model predictors remained significant with the inclusion or exclusion of the outliers.

Table 5.3 Predictors of ledipasvir concentrations (univariate analysis)

Variable	β-coefficient	90% CI	p-value
Weight – per 10kg increase	-18.6	-25.3, -11.9	< 0.0005
Time post-dose (hr)	-2.71	-4.68, -0.73	0.02
Albumin – per unit (g/dL)	3.9	2.3, 5.5	< 0.0005
Platelets – per unit (K/ml)	0.67	0.46, 0.87	< 0.0005
PPI therapy – **Yes** vs No	-92.6	-114.2, -71	<0.0005
Age	1.28	-0.1, 2.66	0.128
Child-Pugh-Turcot Score	-7.79	-15.6, 0.87	0.104
MELD score	-4.32	-7.31, -1.33	0.01

Table 5.4 Predictors of ledipasvir concentrations (multivariate analysis)

Variable	β-coefficient	90% CI	p-value
Weight – per 10kg increase	-15.3	-21.8, -8.9	<0.0005
Time post-dose (hr)	-2.18	-4.0, -0.31	0.05
Albumin – per unit (g/dL)	2.45	0.9, 4.0	0.009
Platelets – per unit (K/ml)	0.631	0.43, 0.83	< 0.0005
PPI therapy – **Yes** vs No	-94.0	-115, -72.9	<0.0005

The overall SVR_{12} rate in the entire cohort was 83.1%. The SVR rate was significantly higher with genotype 1 infection compared to genotype 3 infection (93.5% vs 56.6%). The SVR rate was numerically lower in genotype 1 patients receiving PPI therapy, but this failed to reach statistical significance (96% vs 90%, p = 0.056).

5.5.3 Population pharmacokinetic analysis of ledipasvir in patients with advanced and decompensated cirrhosis from the NHS England expanded access programme

Data from study 5.2.2 was used to develop a population pharmacokinetic model for ledipasvir. Ledipasvir pharmacokinetics were best described using a one-compartment model. The population clearance (CL) was estimated at 13.9 L/hr. Estimated for volume of distribution (V) was higher than expected at 1630L. Inter-individual variability for CL and V were 48% and 35% respectively. Weight was incorporated into the final model with a significant reduction in the objective function with the inclusion of weight as a covariate on CL and V. The effect of PPI therapy on CL, V and relative bioavailability (F) was explored. The relative bioavailability of ledipasvir was 34% lower in patients receiving PPI therapy. Monte Carlo plots of model fit are shown in figure 5.4. The model fit was good with no evidence of model bias, over-fitting or shrinkage. The final model parameters are outlined in table 5.5.

Figure 5.4. - Monte-Carlo Model Simulation of the NONMEM ledipasvir population pharmacokinetic model

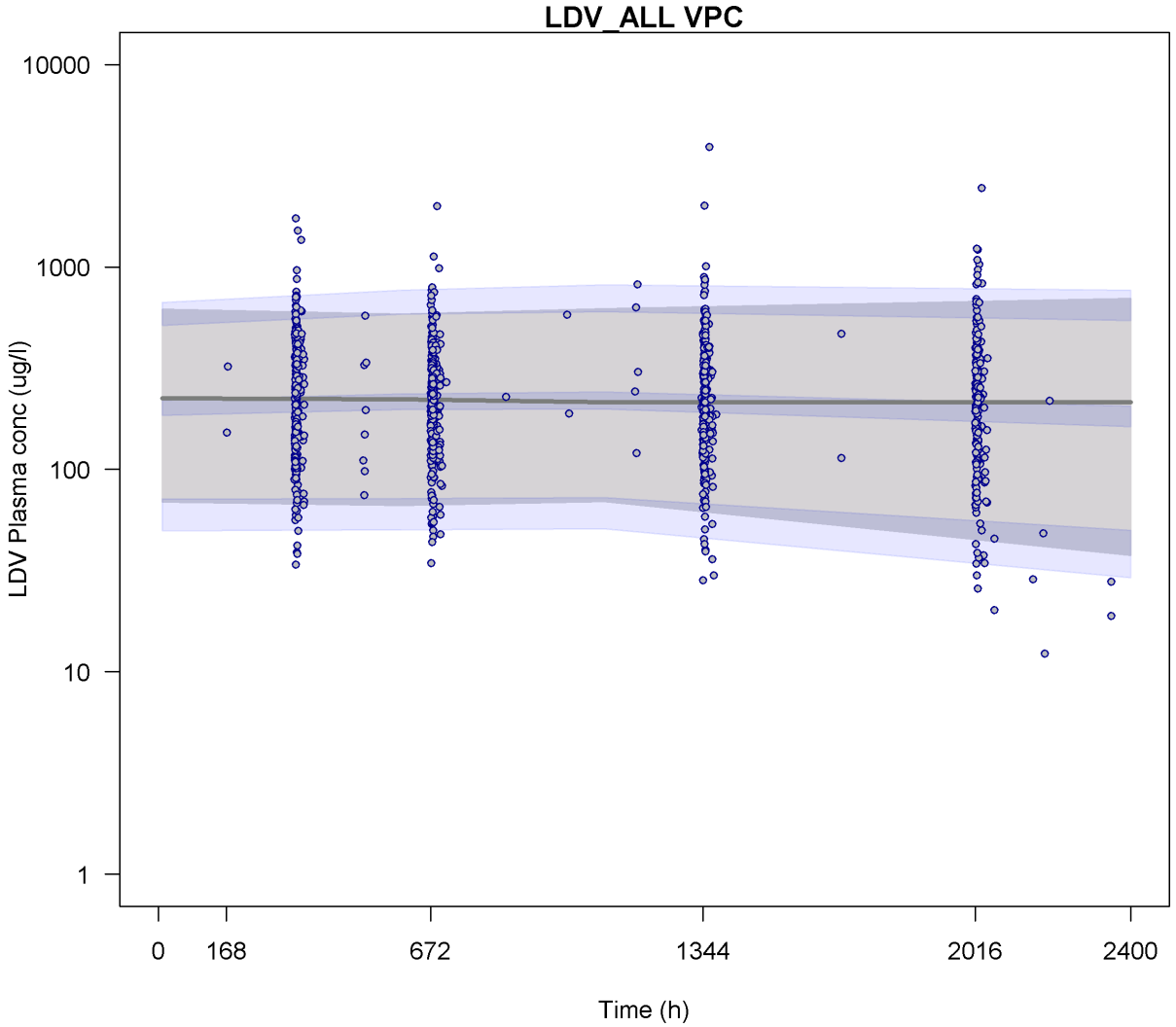


Table 5.5 Population pharmacokinetic model parameters for ledipasvir

Parameter	Estimated	%RSE	Parameter	Estimated	% RSE
CL/F	13.9 L/hr	5%	IIV CL	0.231	21%
V/F	1630	10%	IIV V	0.126	63%
Cov PPI effect	0.66	7%	IIV F	0.138	34%
Ka	0.326	(fixed)			
F (relative)	1	(fixed)	Prop Res Error	0.101	6%

CL = clearance; V = volume of distribution; F = bioavailability; IIV = inter-individual variability; Ka = absorbance rate constant

5.6 Discussion

In study 5.2.1 (Irish EAP study), ledipasvir trough concentrations were 20% lower in patients receiving concomitant PPI therapy. The range of concentrations observed was consistent with those observed in phase 1 studies of patients with moderate (CPT class B) and severe (CPT class C) hepatic impairment. This is the first study to report ledipasvir concentrations in patients with HCV infection receiving concomitant PPI therapy during treatment. PPI therapy accounted for the majority of the systemic variation in ledipasvir concentrations. The effect was modest, with a 20% reduction in mean ledipasvir C_{trough} in patients on low dose PPI therapy. Ledipasvir solubility decreases with increasing pH, and drugs that increase gastric pH are expected to decrease ledipasvir plasma concentrations. PPI therapy was contraindicated in all phase 2 and 3 studies with ledipasvir-sofosbuvir, including the SOLAR-1 and 2 studies of patients with decompensated cirrhosis. In keeping with the prescribing information, patients were advised to take their PPI dose at the same time as ledipasvir-sofosbuvir to minimise any potential pharmacokinetic interaction. The PPI dose was also reduced to the equivalent of 20mg of omeprazole (lansoprazole 15mg or esomeprazole 20mg). However this data does confirm a PK effect that can be mitigated by

discontinuation of PPI therapy during treatment, especially in the absence of a solid indication.

In study 5.2.1, ledipasvir concentrations were lower in patients who experienced viral relapse compared to those who achieved SVR_{12}. The SVR rate observed in our cohort was similar to the phase 2 SOLAR-1 and 2 trials. In the ION phase 3 trials, SVR rates were numerically lower in treatment-experienced patients (both compensated cirrhotic and non-cirrhotic) with ledipasvir concentrations in the lowest quartile relative to the highest quartile of observed exposures (88% vs 100%). Extending treatment to 24 weeks eliminated this differential in SVR rates [Gilead Sciences 2014]. There are no previously reported data on the exposure-response relationship of ledipasvir in patients with decompensated cirrhosis. Treatment extension to 24 weeks for patients with ledipasvir-sofosbuvir in patients with lower ledipasvir exposure or discontinuing concomitant PPI therapy may therefore result in improved treatment response rates in patients with decompensated cirrhosis.

Study 5.2.2 (NHS England EAP) reports on the pharmacokinetics of ledipasvir-sofosbuvir in a large cohort of 314 patients with advanced and decompensated cirrhosis. Weight, albumin, platelet count and PPI use were significant predictors of ledipasvir concentrations in this study. Although statistically significant, the relationship between albumin concentrations was weak and unlikely to be of clinical significance. Lower ledipasvir concentrations in patients with lower platelet counts is likely related to portosystemic shunting in patients with significant portal hypertension. Weight was a significant predictor of ledipasvir pharmacokinetics, with a reduction in ledipasvir concentrations for every 10Kg increase in weight. Weight has previously been shown to be a statistically significant

but not clinically meaningful covariate for ledipasvir exposure [120]. PPI use at baseline and during therapy was the most significant covariate on ledipasvir concentrations. There was no effect of CPT class, CPT score or MELD score on ledipasvir concentrations in multivariate analysis although there was an association between MELD score and ledipasvir concentrations in univariate analysis. These data confirm the lack of effect of hepatic impairment on ledipasvir metabolism as previously reported in HCV negative patients with decompensated cirrhosis [49].

A one-compartment population pharmacokinetic model was developed using the data from study 5.2.2 to describe the population profile of ledipasvir Clearance (CL), Volume of distribution (V) and Fractional bioavailability (F). The model fit was good, with no evidence of model bias, shrinkage or overfitting. The population CL of 13.9L/hr was consistent with previously reported data in non-cirrhotic and compensated cirrhotic patients. The volume of distribution was significantly higher than expected. This may relate to the advanced liver disease in this population, with ascites and severe portal hypertension compared the previously reported pop-PK model that did not include decompensated patients [120]. Volume of distribution may also have been over-estimated in the model because of the limited data sampling in the absorption phase. Ledipasvir is absorbed in the proximal GI tract with maximal concentration (T_{max}) observed at four hours. The majority of samples were collected more than four hours post-dose. As a result, the absorption constant Ka was fixed based on reported data from the literature. Fixing Ka was associated with a significantly higher estimate of V. Weight was again noted to be a statistically significant covariate on CL and V in the final model.

The relative bioavailability of ledipasvir was 34% lower in patients receiving concomitant PPI therapy. Hepatic and renal impairment have no significant effect on ledipasvir PK and variability in absorption appears to account for a large part of the inter-individual variability of ledipasvir PK [49]. This variability in absorption is further modulated by PPI use. Gastric pH is also higher in patients with cirrhosis compared to healthy control, especially during the night hours [121]. There has been conflicting data on the effect of PPI therapy on SVR rates with ledipasvir-sofosbuvir in HCV genotype 1 infection. This is probably a reflection of the heterogeneity of the patient populations in these real world datasets. In this study, SVR rates were numerically lower, with a trend towards statistical significance, in patients with HCV genotype 1 infection receiving concomitant PPI therapy. In the HCV-TARGET real-world study, the absence of PPI therapy at baseline was associated with an odds ratio of 3.02 (95% CI 1.51-6.05) for achieving SVR [122]. In the TRIO study, there was no effect of low dose PPI therapy on SVR [123]. However, twice daily PPI therapy was associated with a reduced odds ratio for SVR (OR 0.11, 95% CI 0.02 – 0.59). PPI therapy may be a modifiable factor to maximise SVR rates in patients undergoing therapy with ledipasvir-sofosbuvir for HCV genotype 1 infection.

CHAPTER 6

WHO GETS BETTER? PREDICTORS OF CLINICALLY MEANINGFUL IMPROVEMENTS IN LIVER FUNCTION IN PATIENTS WITH DECOMPENSATED HEPATITIS C (HCV) CIRRHOSIS RECEIVING DIRECT-ACTING ANTIVIRAL (DAA) THERAPY

6.1 Introduction

Hepatitis C treatment is now recommended for all patients, except those with a shortened life expectancy that cannot be altered by viral eradication [AASLD-IDSA 2016]. Historically, patients with chronic hepatitis C virus (HCV) infection with decompensated cirrhosis had limited treatment options. Until recently, liver transplantation was the only intervention associated with an improved survival in this group. However, with the development of sofosbuvir-based direct acting antiviral (DAA) therapy, treating patients with decompensated cirrhosis has become a reality. The SOLAR-1 and SOLAR-2 trials demonstrated antiviral efficacy of the fixed dose combination of sofosbuvir (SOF) and ledipasvir (LDV) with ribavirin (RBV) in patients with decompensated cirrhosis [55-56]. These trials also provided evidence of the effect of viral eradication on liver function, with a significant improvement in MELD and Child-Pugh-Turcotte (CPT) scores in a proportion of patients who achieved a sustained virologic response (SVR). High SVR rates and improvements in liver function were also observed with SOF and velpatasvir (VEL) with or without RBV in the ASTRAL-4 trial [57]. These data supported the approval of LDV/SOF+RBV and SOF/VEL+RBV for the treatment of HCV in patients with decompensated cirrhosis.

The management of patients with decompensated HCV cirrhosis has evolved radically with DAA therapy, resulting in a high likelihood of virologic cure. However, in contrast to patients with compensated liver disease, viral eradication in patients with decompensated cirrhosis may not be associated with an improvement in transplant-free-survival or the severity of liver decompensation in all patients. While the majority of patients are likely to have an improvement in MELD/CPT scores with therapy, the extent to which this represents a clinically meaningful improvement in liver function and patient reported

outcomes remains unclear. Data suggest that viral eradication in a subset of patients who remain decompensated post-treatment may result in an improvement in MELD score which subsequently precludes them from liver transplant consideration. The term "MELD purgatory" has been used to describe these patients who may paradoxically be disadvantaged by undergoing therapy [124-125].

There is an urgent need for data to refine our understanding of the reversibility of hepatic decompensation with viral eradication and ultimately define the "point of no return", the degree of liver dysfunction where HCV therapy does not yield any meaningful clinical benefit [126]. Pre-treatment predictors of benefit are needed to guide patient selection for therapy, and more importantly identify a group of patients for whom therapy is futile and liver transplantation and DAA therapy post-transplant should be recommended.

The aims of this chapter were to understand the outcomes of patients with decompensated cirrhosis undergoing DAA treatment and to identify factors to predict treatment benefit and/or adverse events of liver transplantation, death or MELD purgatory. This study was undertaken during a visiting research fellowship at Harvard Medical School. In collaboration with Dr Michael Curry, I drafted a study protocol and submitted a research proposal to Gilead Sciences. Gilead provided the clinical data and I conducted the statistical analysis with the assistance of Elliot Tapper and Gordon Zhiang. The BE3A score was developed in collaboration with Gordon Zhiang, who also performed the internal validation.

6.2 Patients and Methods

6.2.1 Patients

A detailed research proposal was submitted to Gilead Sciences in May 2016. Gilead Sciences approved the research protocol and provided anonymised data from four published clinical trials for this analysis. An integrated analysis of data from four clinical trials of sofosbuvir-based interferon-free DAA therapy in patients with advanced liver disease (SOLAR-1, SOLAR-2, ASTRAL-4 and GS-US-334-0125) was then performed. [55-57, 127].

SOLAR-1 and SOLAR-2 were open-label phase 2 randomised trials comparing 12 or 24 weeks of therapy with ledipasvir and sofosbuvir fixed-dose combination with ribavirin, and included patients with cirrhosis and moderate (CPT B) or severe (CPT C) hepatic decompensation. SOLAR-1 also included patients post-transplant and enrolled patients from the United States, whereas SOLAR-2 enrolled patients from Europe, Canada, Australia and New Zealand. ASTRAL-4 was an open-label phase 3 randomised trial comparing 12 weeks of the fixed dose combination of velpatasvir and sofosbuvir with or without ribavirin, with 24 weeks of velpatasvir/sofosbuvir. ASTRAL-4 enrolled patients with decompensated cirrhosis who had not undergone liver transplantation. GS-US-334-0125 was an open-label phase 2 study conducted at nine international centres. Consecutively enrolled patients received 48 weeks of sofosbuvir and ribavirin. Approximately 60% of the patients had a CPT score of 7-10 (Class B or C). In this study, we pooled patients in these four clinical trials with CPT B or C without a history of liver transplantation at the beginning of the trial.

6.2.2 Inclusion and exclusion criteria

All four studies enrolled patients aged at least 18 years old and had chronic hepatitis C infection, with detectable HCV plasma RNA, and decompensated cirrhosis (CPT B or C). SOLAR-1 and SOLAR-2 included patients with genotype 1 or 4 HCV infection, whereas ASTRAL-4 recruited patients with all HCV genotypes. GS-US-334-0125 enrolled patients with genotypes 1 – 4 HCV infection. The exclusion criteria of the 4 clinical trials were largely similar. Patients with hepatitis B or HIV co-infection or previous exposure to an NS5A inhibitor were excluded. Patients with any of the following baseline laboratory criteria were excluded: platelets $\leq$ 30,000/mm^3, alanine aminotransferase, aspartate aminotransferase or alkaline phosphatase $\geq$ 10 times the upper limit or normal. SOLAR-1 and SOLAR-2 excluded patients with a total bilirubin > 10 mg/dL, while patients with a total bilirubin > 5 mg/dL were excluded from ASTRAL-4 and GS-US-334-0125.

6.2.3 Definitions of end points

CPT score was calculated from the prothrombin time, serum albumin and bilirubin, and the presence and/or severity of ascites and encephalopathy. MELD score was calculated from the serum bilirubin, creatinine and INR. CPT and MELD scores were calculated at baseline and at follow-up visits.

The primary study end point for this analysis was the proportion of patients achieving a clinically meaningful treatment benefit following DAA therapy, which was defined as achieving sustained CPT class A compensated cirrhosis from the start of therapy (i.e. down-staging from CPT B $\rightarrow$ A or C $\rightarrow$ A). Two secondary end-points were also investigated: death or liver transplantation and MELD purgatory. MELD purgatory was defined as persistent CPT class B or C decompensation with a MELD score < 15.

6.2.4 Type and duration of antiviral therapy

All patients received sofosbuvir-based interferon-free DAA therapy. In the SOLAR-1 and SOLAR-2 trials, patients were randomised in a 1:1 ratio to receive either 12 or 24 weeks of treatment with ledipasvir 90mg and sofosbuvir 400mg as a fixed dose combination tablet (ledipasvir-sofosbuvir) once daily plus ribavirin. Ribavirin was initially administered at a starting dose of 600mg in a divided daily dose. Dose escalation of ribavirin to a maximum of 1000 or 1200 mg/day based on body weight was allowed if the starting dose was well tolerated, and the haemoglobin levels remained higher than 10 g/dL without haematological growth factor support. Ribavirin dose reductions were instituted in patients who could not tolerate the starting dose of 600mg/day based on haematological parameters or other ribavirin side effects.

In the ASTRAL-4 trial, patients were randomised in a 1:1:1 ratio to receive treatment with velpatasvir 100mg and sofosbuvir 400mg in a fixed dose combination tablet (velpatasvir-sofosbuvir) once daily for either 12 weeks with or without ribavirin or 24 weeks alone. Ribavirin was administered at a dose of 1000 or 1200mg (based on baseline weight) orally with food twice daily. Ribavirin dose modification or interruption and use of growth factors were permitted to manage anaemia. In the GS-US-334-0125 study, all patients received 48 weeks of sofosbuvir 400mg once daily and ribavirin 1000 or 1200mg daily.

6.2.5 Independent variables

Independent variables in the data analysis were chosen based on their clinical relevance to cirrhosis and liver decompensation. These variables included baseline characteristics: age, gender, ethnicity, body mass index (BMI); response to therapy: SVR_{12}; clinical status before treatment: the presence of ascites, encephalopathy, the levels of alanine

aminotransferase (ALT), albumin, total bilirubin, INR, glomerular filtration rate (GFR) and the MELD score. All variables were categorised to facilitate clinical interpretation. Binary variables for ascites and encephalopathy were adopted to capture their presence or absence. Cut-offs used in calculating CPT score were used for albumin, INR and total bilirubin. Age was treated as a binary variable of less or more than 65-years-old after evaluating its relationship to end-points in a linear relationship. Conventional cut-off points were used for BMI. GFR cut-off point of 60 mL/min per 1.73 m^2 was used to capture patients with renal impairment. Abnormal ALT was defined as more than 60 IU/dL, which approximates 1.5x upper limits of normal (ULN) in most reports from clinical laboratories. We did not use gender-specific cut-off as the gender difference in ALT levels in cirrhosis has not been defined. The MELD score was categorised into three groups: 6-11, 12-14, and ≥15, in relevance to the practical consideration during liver transplant evaluation.

6.2.6 Statistical analysis

The full analysis dataset included 622 subjects with CPT class B or C cirrhosis at baseline that were enrolled in the three phase-2 (SOLAR1, SOLAR 2 and GS-US-334-0125) and one phase-3 (ASTRAL 4) clinical trials, who received at least one dose of study drug. A total of 5,322 valid observations in follow-up were used in the analyses. Cox proportional hazard models were generated for three outcomes during the treatment: (i) achieving sustained CPT-A status, (ii) death or transplantation, and (iii) sustained MELD purgatory. All models were right-censored at 40 weeks to prevent the bias from a few subjects followed beyond that from one study. Exact marginal likelihood method was used to handle tied failures in calculating hazard ratios. Proportional hazard assumption was evaluated in each model based on Schoenfeld residuals. For models not satisfying proportional hazard assumption, we evaluated the survival curves in a case-by-case

situation and typically calculated hazard ratio using a shorter duration of follow-up. Univariate analyses were performed to estimate hazard ratios for age, gender, ethnicity, components of CPT score, BMI, albumin, ALT, AST, platelet count, GFR, MELD score as well as its components. Variables used in multivariate analyses were chosen based on the significance of each variable in the univariate analysis and their biological relevance. Sensitivity analyses were conducted to evaluate whether the severity in ascites and encephalopathy were associated with outcomes. In addition, we investigated the associations of MELD score in place of its components in multivariate analyses. A competing risk model was generated to calculate the sub-hazard ratio (SHR) for achieving sustained CPT A status while competing for the risk of death or liver transplantation. Kaplan-Meier failure functions were used to graph the accumulative events of achieving a sustained CPT A status or death or liver transplantation in relationship to SVR_{12}.

To construct a predictive score for DAA treatment outcomes, we identified five baseline factors that have the strongest associations with the primary outcome based on the HR and Z-value of each variable. We first compared the C-statistics of logistic regression models of these five parameters in either continuous variables (for BMI, ALT, and albumin), categorical variables (similar to those used in the CPT score), or a 0-5 simple point-based score. We compared both unweighted (1 point for each variable) and weighted approaches (two points for variables with more than four-fold changes in HR). The predictive values of these models were compared by Cox regression models and the area under the receiver operative curve (AUROC) using nonparametric ROC analysis after logistic regression. The final model included five variables: BMI, encephalopathy, ascites, ALT and albumin, which was named the BE3A score. The BE3A score can be calculated by simple numerical summation of its components, where one point is added for normal BMI (<25), absence of encephalopathy, absence of ascites, ALT more than 1.5 times the upper limits of normal

(>60 IU/L) and normal albumin (>3.5 g/L) respectively. The performance of this score in predicting reduction to CPT class A status or assessing risk for death or liver transplantation was tested using Cox proportional hazard analysis and Kaplan-Meier sensitivity analysis. The probability of reduction to CPT class A status within 36 weeks was also calculated using coefficients from a logistic regression of the BE3A components where BMI, ALT and albumin were used as continuous variables. The probability was calculated from the odds ratio using the following equation:

$$Probability = \frac{odds}{1 + odds}$$

, where the odds for achieving CPT A status was expressed as:

$$odds[Improvement] = e^{-0.06[BMI]-1.27[encephalopathy]-1.32[ascites]+1.13[albumin]+0.01[ALT]-2.17}$$

, and the odds for death or transplant was defined as:

$$odds[death\ or\ transplant] = e^{-0.04[BMI]+0.22[encephalopathy]+0.54[ascites]-1.43[albumin]-0.01[ALT]+2.51}$$

, in which 1 was used for the presence of encephalopathy or ascites, and 0 for its absence respectively, BMI was in the unit of kg/m^2, albumin in the unit of g/dL and ALT in the unit of IU/L. A web-based calculator for the point estimates can be found at http://www.be3ascore.com. The performance of the point-based BE3A score (0-5) and probability estimates using multivariate regression was further validated using parametric bootstrapping, where the mean and 95% CI of the AUROC was estimated from 100 simulated sample cohorts.

6.3 Results

6.3.1 Baseline patient characteristics

A total of 502 CPT B and 120 CPT C patients received at least one dose of study drug and were included in the final analysis. The baseline patient characteristics are outlined in Table 6.1. The breakdown of patients from each clinical trial is shown in Table 6.2. The majority of patients (71.9%) were male with a median age of 59 years. Approximately one-third had a baseline BMI $\geq$ 30 kg/m^2. Patients with CPT C cirrhosis had higher INR and bilirubin levels, and a lower baseline albumin and platelet counts compared to patients with CPT B cirrhosis. The majority of patients (77%) had ascites at baseline and at least mild encephalopathy (59%).

Table 6. 1 Baseline patient characteristics

	CPT B (n=502)	**CPT C (n=120)**	**Total (n=622)**
Age, median (IQR)	59 (54, 62)	58 (52, 62)	59 (54, 62)
Ethnicity, n (%)			
White	455 (90.6)	109 (90.8)	565 (90.7)
Non-White	47 (9.4)	11 (9.2)	37 (9.3)
Gender			
Male, n (%)	360 (71.7)	87 (72.5)	448 (71.9)
BMI, n (%)			
<20 kg/m2	10 (2.0)	2 (1.7)	12 (1.9)
20-25 kg/m2	125 (24.9)	26 (21.7)	151 (24.3)
25-30 kg/m2	200 (39.8)	48 (40)	248 (39.9)
$\geq$ 30 kg/m^2	167 (33.3)	44 (36.7)	211 (33.9))
MELD Score, n (%)			
6 - 11	269 (53.6)	10 (8.3)	279 (44.9)
12 - 14	154 (30.7)	38 (31.7)	192 (30.7)
$\geq$ 15	79 (15.7)	72 (60.0)	151 (24.3)
Bilirubin (mg/dL), median	1.6 (1.1, 2.2)	3.4 (2.5, 4.2)	1.8 (1.2, 2.8)
Albumin (g/dL), median	3.0 (2.8, 3.4)	2.5 (2.3, 2.8)	2.9 (2.6, 3.3)
INR, median (IQR)	1.2 (1.1, 1.3)	1.4 (1.3, 1.5)	1.2 (1.1, 1.4)
Platelet count (10^9/mm^3), median	82 (61, 116)	68 (51, 90)	79 (60, 112)
Ascites, n (%)			
None	137 (27.3)	6 (5.0)	143 (23.0)
Mild/Moderate	345 (68.7)	82 (68.3)	427 (68.6)
Severe	20 (4.0)	32 (26.7)	52 (8.4)
Encephalopathy, n (%)			
None	236 (47.0)	20 (16.7)	256 (41.2)
Mild/Moderate	266 (53.0)	93 (77.5)	359 (57.7)
Severe	0	7 (5.8)	7 (1.1)

Table 6.2 Summary of the four decompensated cirrhosis clinical trials

	GS-US-334-0125	SOLAR-1	SOLAR-2	ASTRAL-4
Number of patients	28	182	161	251
CPT class, n (%)				
B	26 (92.9)	130 (71.4)	106 (65.8)	240 (95.6)
C	2 (7.1)	52 (28.6)	55 (34.2)	11 (4.4)
HCV genotype, n (%)				
GT 1	20 (71.4)	179 (98.4)	146 (90.7)	193 (76.9)
GT 2	2 (7.1)			12 (4.8)
GT 3	4 (14.3)			37 (14.7)
GT 4	2 (7.1)	3 (1.6)	15 (9.3)	
Treatment duration, n (%)				
12 weeks		92 (50.5)	81 (50.3)	168 (66.9)
24 weeks		90 (49.5)	80 (49.7)	83 (33.1)
48 weeks	28 (100)			
SVR_{12}, n (%)	19 (74.7%)	155 (88.6)	134 (87.3)	220 (89.7)
Death	0	10 (5.5)	15 (9.3)	10 (4.0)
Liver transplant	0	7 (3.8)	10 (6.2)	0

6.3.2 Clinical outcomes

The overall sustained virological response rate at 12 weeks post treatment (SVR_{12}) was 85% (528/622). The median duration of follow-up was 255 days (IQR 251-336) from the beginning of treatment. During this follow-up period, a total of 17 patients underwent liver transplantation. There were a total of 35 deaths during the study and follow-up period. Follow-up data up to 36 weeks from the start of treatment was available in 82.5% of patients. Despite incomplete follow-up data at specified time points, in 36 weeks from the start of treatment, an incremental decline in the proportion of CPT class B and class C patients was noted accompanied by an increase in CPT class A patients (figure 6.1). Of the 528 patients who achieved SVR_{12} with follow up data available to week 36, 31.6% of patients with baseline CPT class B cirrhosis and 12.3% of patients with CPT class C cirrhosis experienced a "clinically meaningful treatment" benefit.

6.3.3 Factors associated with a clinically meaningful treatment benefit

In the Cox proportional hazard model, patients who achieved SVR_{12} had a significantly higher chance of achieving sustained CPT class A with a hazard ratio (HR) of 2.1 (95% CI 1.0 - 4.3, p = 0.04) (Table 6.3, Figure 6.2). A total of 169 out of 528 (32%) patients with SVR_{12} improved to CPT A at the end of the study, whereas only 10.6% of patients without SVR_{12} did so. In addition to SVR_{12}, African American race, an abnormal ALT more than 1.5x ULN, and a GFR less than 60 $mls.min^{-1}$ per $1.73m^2$ were associated with achieving a sustained CPT class A (Table 6.3). The presence of ascites, or encephalopathy, albumin less than 3.5g/dL, and an increased BMI were associated with an increased risk of not achieving

Figure 6.1 – Change in CPT grade from baseline to week 36 from the start of DAA therapy

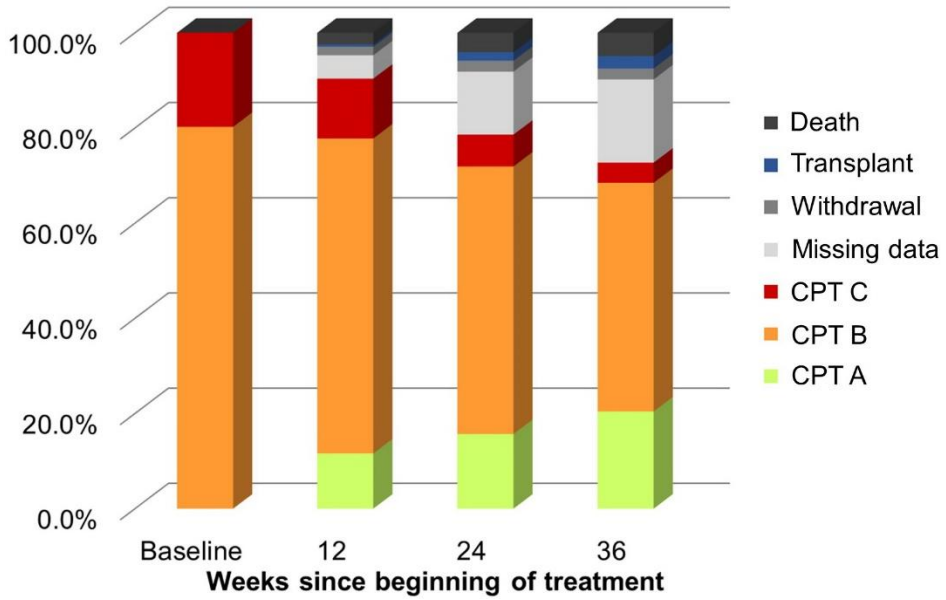
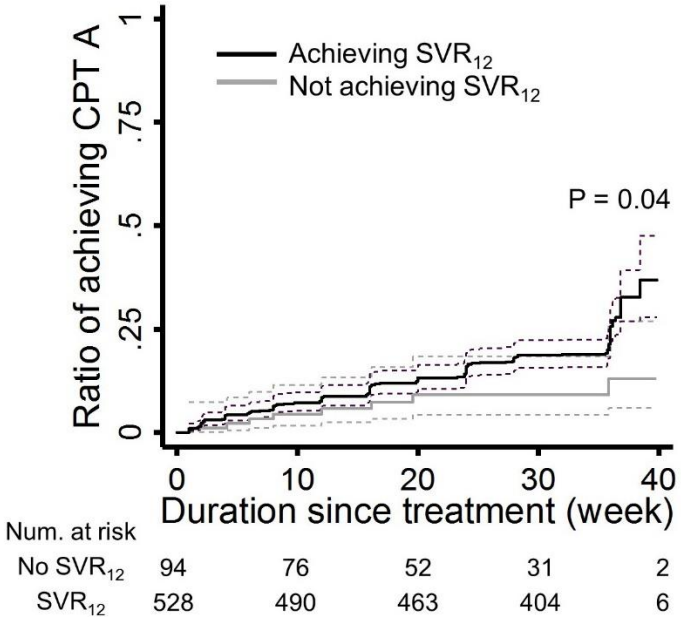


Figure 6.2 - Impact of SVR on achieving clinical improvement



Kaplan-Meier curves of achieving a CPT A status were plotted for those with or without SVR_{12} in black and gray solid lines respectively. Dashed lines represent 95% CI. P value calculated by Cox proportional hazard regression right-censored at 36 weeks.

Table 6.3 Predictors for achieving CTP A with DAA treatment

		Univariate model [1]			Multivariate model [2]			Competing risk model [3]		
		HR	**95% CI**	**p value**	**HR**	**95% CI**	**p value**	**SHR**	**95% CI**	**p value**
Age above 65	No	Ref			Ref			Ref		
	Yes	1.17	0.74 - 1.84	0.5	0.97	0.55 - 1.71	0.9	1.06	0.67 - 1.67	0.8
Gender	Female	Ref			Ref			Ref		
	Male	1.05	0.73 - 1.51	0.8	1.02	0.66 - 1.58	0.9	1.20	0.82 - 1.75	0.4
Ethnicity	Caucasian	Ref			Ref			Ref		
	African American	2.92	1.78 - 4.77	<0.001	2.33	1.25 - 4.36	0.008	2.89	1.71 - 4.88	<0.001
	Others	1.42	0.62 - 3.24	0.4	1.60	0.65 - 3.91	0.3	1.40	0.67 - 2.92	0.4
SVR_{12}	No	Ref			Ref			Ref		
	Yes	2.12	1.03 - 4.34	0.04	1.75	0.80 - 3.85	0.2	2.85	1.43 - 5.70	0.003
BMI	<25	Ref			Ref			Ref		
(kg/m^2)	25-30	0.68	0.47 - 1.00	0.05	0.51	0.32 - 0.82	0.005	0.63	0.43 - 0.91	0.02
	>30	0.50	0.32 - 0.76	0.001	0.61	0.37 - 1.00	0.05	0.60	0.39 - 0.93	0.02
Ascites	No	Ref			Ref			Ref		
	Yes	0.21	0.14 - 0.31	<0.001	0.15	0.10 - 0.24	<0.001	0.30	0.20 - 0.45	<0.001
Encephalopathy	No	Ref			Ref			Ref		

	Yes	0.29	0.21 - 0.42	<0.001	0.18	0.11 - 0.29	<0.001	0.29	0.20 - 0.41	<0.001
Albumin	> 3.5	Ref			Ref			Ref		
(g/dL)	2.8 - 3.5	0.67	0.42 - 1.06	0.08	0.29	0.16 - 0.51	<0.001	0.35	0.22 - 0.57	<0.001
	< 2.8	0.49	0.29 - 0.82	0.007	0.11	0.05 - 0.24	<0.001	0.23	0.12 - 0.42	<0.001
ALT	< 1.5	Ref			Ref			Ref		
(ULN)	> 1.5	2.16	1.52 - 3.07	<0.001	1.80	1.18 - 2.76	0.007	1.89	1.35 - 2.65	<0.001
Bilirubin	<2	Ref			Ref			Ref		
(mg/dL)	2-3	0.94	0.63 - 1.40	0.8	0.39	0.23 - 0.66	<0.001	0.63	0.43 - 0.93	0.02
	>3	0.80	0.51 - 1.26	0.3	0.36	0.03 - 0.77	0.001	0.46	0.27 - 0.77	0.003
INR	<1.7	Ref			Ref			Ref		
	1.7 - 2.2	0.44	0.16 - 1.20	0.1	0.48	0.21 - 1.10	0.08	0.76	0.43 - 1.34	0.3
	>2.2	0.8	0.11 - 5.74	0.8	0.16	0.03 - 0.77	0.02	0.19	0.03 - 1.33	0.09
GFR	< 60	Ref			Ref			Ref		
(mL/min/1.73 m^2)	> 60	1.52	1.04 - 2.22	0.03	1.29	0.80 - 2.10	0.3	1.22	0.84 - 1.78	0.3
MELD	6-11	Ref								
	12-14	1.04	0.72 - 1.50	0.8						
	≥ 15	0.66	1.42 – 1.04	0.08						

[1]All univariate Cox proportional hazard models are right-censored at 40 weeks, except for ascites and encephalopathy, which are censored at 36 weeks to satisfy the proportional hazard assumption.

[2] Multivariate Cox proportional hazard model include all factors except for MELD and are right-censored at 36 weeks.

3 Competing risk model was generated based on Cox proportional hazard model to calculate sub-hazard ratio (SHR) for achieving CPT A as primary outcome and death or liver transplantation as a competing risk.

CPT class A. In a multivariate Cox proportional hazard model, the presence of ascites or encephalopathy, the levels of bilirubin, albumin, and INR, the five components in the CPT score, all had decreased hazard ratio in association with lower chance of meaningful clinical improvement. Abnormal ALT remained significantly associated with achieving sustained CPT A, whereas SVR_{12} was not significant. In a sensitivity analysis, severe ascites did not have a lower chance of achieving clinical improvement in comparison to mild to moderate ascites. There were not enough patients with severe encephalopathy to evaluate the impact of the degree of encephalopathy. Interestingly, MELD categories were not significantly associated with a meaningful clinical improvement in univariate analysis (Table 6.3). When MELD score was used in place of its components (bilirubin, INR and GFR) in the multivariate model, higher MELD categories, MELD 12-14 and ≥15 in comparison to ≤11, were associated with lower chance of clinical improvement with hazard ratios of 0.69 and 0.34 respectively (95% CI 0.46 – 1.04, $p = 0.08$ and 0.20 - 0.58, $p < 0.001$).

6.3.4 Factors associated with liver transplant or death

Achieving SVR_{12} was associated with a significant reduction in the likelihood of transplant or death (HR 0.003, 95% CI 0.0005 – 0.02, $p < 0.001$) (Table 6.4, Figure 6.3A). As death could occur before the SVR_{12}, this association was potentially influenced by a confounding-by-indication. To address this, a sensitivity analysis was performed that examined only those who survived beyond the SVR_{12} time-point. In this sensitivity analysis, achieving SVR_{12} remained significantly protective against death or transplantation with a hazard ratio of 0.12 (95% CI 0.04 – 0.38, $p < 0.001$) (Figure 6.3B).

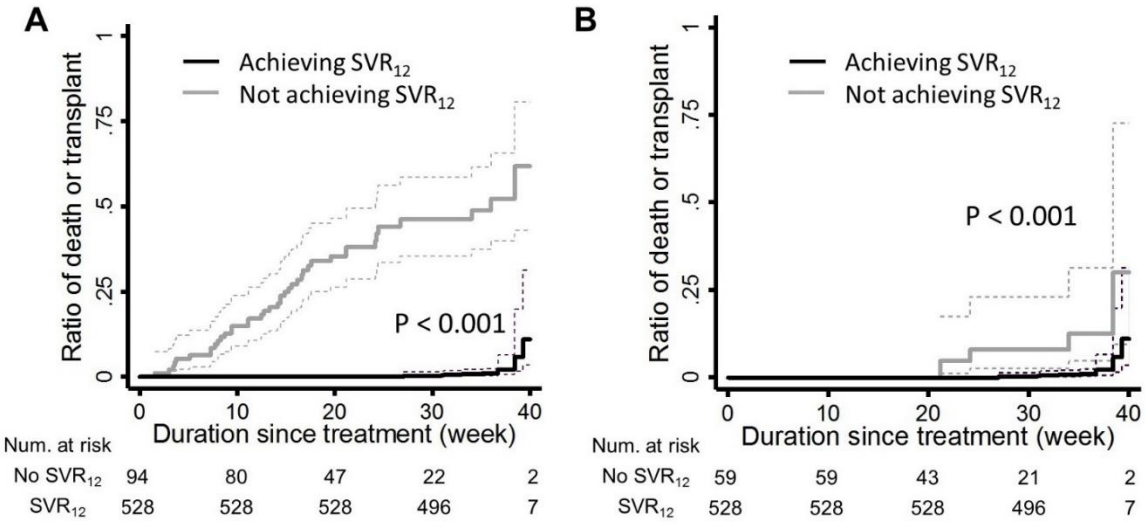
Table 6.4 Predictors for death or transplant with DAA treatment

		Univariate model [1]			Multivariate model [2]		
		HR	**95% CI**	**p value**	**HR**	**95% CI**	**p value**
Age above 65	No	Ref			Ref		
	Yes	1.28	0.60 - 2.75	0.5	1.01	0.44 - 2.31	1.0
Gender	Female	Ref			Ref		
	Male	1.12	0.59 - 2.12	0.7	1.36	0.68 - 2.72	0.4
BMI	< 25	Ref			Ref		
(kg/m^2)	25-30	1.00	0.52 - 1.92	1.0	0.89	0.45 - 1.75	0.7
	> 30	0.58	0.27 - 1.26	0.2	0.54	0.24 - 1.21	0.1
Ascites	No	Ref			Ref		
	Yes	1.85	0.83 - 4.13	0.1	2.07	0.86 - 4.97	0.1
Encephalopathy	No	Ref			Ref		
	Yes	1.43	0.79 - 2.62	0.2	1.40	0.74 - 2.66	0.3
Albumin	> 3.5	Ref			Ref		
(g/dL)	2.8 - 3.5	3.14	0.41 - 23.8	0.2	3.76	0.49 - 28.9	0.2
	< 2.8	12.08	1.65 – 88.5	0.01	11.4	1.51 - 86.2	0.02
ALT	< 1.5	Ref			Ref		
(ULN)	> 1.5	0.38	0.20 - 0.70	0.002	0.45	0.24 - 0.87	0.02
Bilirubin	< 2	Ref			Ref		
(mg/dL)	2-3	2.12	0.99 - 4.51	0.05	2.08	0.94 - 4.58	0.07
	> 3	4.36	2.21 - 8.58	<0.001	3.97	1.89 - 8.33	<0.001
INR	< 1.7	Ref			Ref		
	1.7 - 2.2	1.31	0.59 - 2.94	0.5	0.68	0.30 - 1.56	0.4
	> 2.2	3.22	0.77 - 13.5	0.1	4.09	0.90 - 18.58	0.07
GFR	< 60	Ref			Ref		
(mL/min per 1.73 m^2)	> 60	1.43	0.74 - 2.75	0.3	1.63	0.80 - 3.32	0.2
MELD	6-11	Ref					
	12-14	2.43	1.05 – 5.61	0.04			
	≥ 15	5.52	2.57 – 11.8	<0.001			
SVR_{12}	No	Ref					
	Yes	0.003	0.0005- 0.02	<0.001			

[1] Univariate cox proportional hazard models are right censored at 40 weeks, with the exception of SVR_{12} censored at 30 weeks to satisfy the proportional hazard assumption.

[2] Multivariate cox proportional hazard models include all variables in univariate analysis except for MELD and SVR_{12} and right-censored at 40 weeks.

Figure 6.3 Impact of SVR on death and liver transplantation



Kaplan-Meier curves of death or liver transplant were plotted for those with or without SVR_{12} in black and grey solid lines respectively. Individuals that died or underwent liver transplantation before SVR_{12} are included in panel A, but excluded in panel B. Dashed lines represent 95% CI. P value calculated by Cox proportional hazard regression right-censored at 40 weeks.

We then examined baseline factors associated with liver transplant or death. Besides MELD score, albumin less than 2.8 g/dL and abnormal bilirubin were significantly associated with increased hazard ratios of death or liver transplant in univariate analysis. An abnormal ALT more than 1.5 times ULN was found to be protective with a hazard ratio of 0.38. In a multivariate Cox proportional hazard model, a low albumin and abnormal bilirubin remained significantly associated with higher hazard ratio of death or transplant, while abnormal ALT was associated with a lower risk.

6.3.5 The competing risk between liver transplant or death and meaningful clinical improvement

Patients with decompensated cirrhosis undergoing DAA treatment face competing risk between death or transplant and potential clinical improvement. We generated a multivariate model for achieving CPT A status while using the outcome of death or liver transplantation as a competing risk (Table 6.3). In this multivariate competing risk model, the sub-hazard ratio (SHR) for SVR_{12} increased to 2.85 (95% CI 1.43 – 5.70, p = 0.003) consistent with its reciprocal impact on these two outcomes. Ascites, encephalopathy, albumin, and bilirubin remained significantly associated with achieving a sustained CPT A status, although their SHRs trended closer toward 1.0, consistent with their associations with death or liver transplantation.

6.3.6 Factors associated with MELD purgatory

Among the 622 study subjects, 471 patients met the criteria of MELD purgatory (Zone 4) at the beginning of treatment (Figure 6.4). At the end of the treatment, among surviving patients without liver transplant, 330 patients were left in MELD purgatory while 179 patients achieved CPT A status (Zone 1 or 2). In Cox proportional hazard analyses of 417

patients that did not have sustained MELD purgatory throughout treatment, achieving SVR12 decreased the hazard ratio of MELD purgatory by half in both univariate and multivariate analyses (Table 6.5). The presence of ascites, encephalopathy, and low levels of serum albumin were associated with increased risks of MELD purgatory in both univariate and multivariate analyses. An ALT level more than 1.5 times ULN displayed a significant protective association against MELD purgatory in univariate analysis, but not significant in the multivariate analysis. Elevated MELD score was associated with higher hazard of MELD purgatory, although the hazard ratio was not significantly different between MELD of 12-14 and ≥15 in univariate analyses. The association between baseline MELD score and MELD purgatory was not statistically significant in multivariate analysis.

6.3.7 A simple model to predict treatment outcome in patients with decompensated HCV cirrhosis

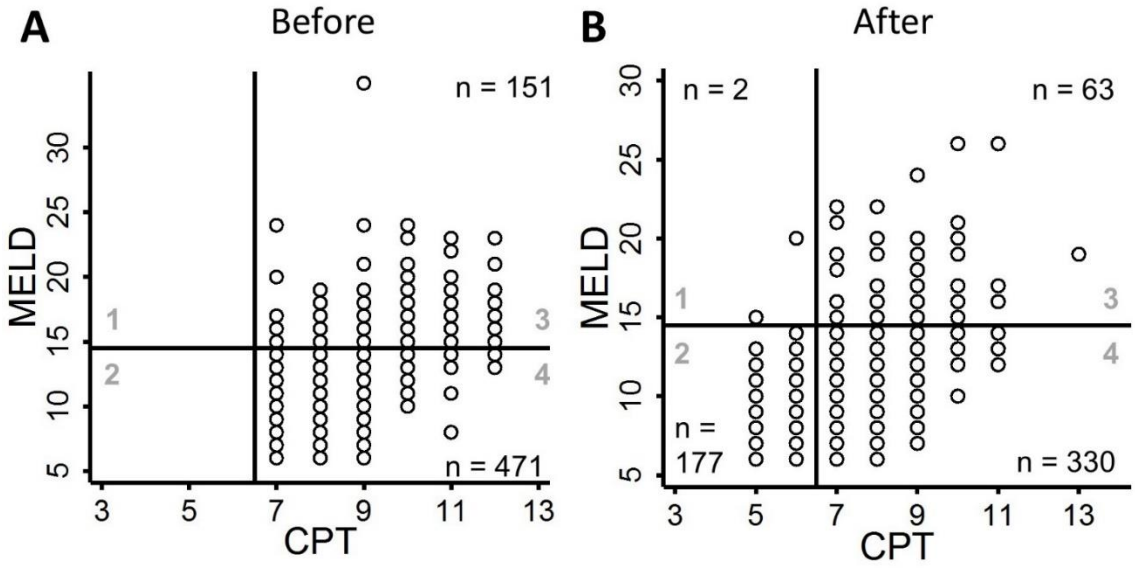
Several pre-treatment baseline factors had robust associations with a reduction to CPT class A after DAA therapy. We identified five factors with the highest association to devise a score to predict clinical improvement. This score is composed of BMI, encephalopathy, ascites, ALT and albumin, and hence named the BE3A score. The BE3A score can be calculated by the sum of its five components, each has one point when the criterion is met: normal BMI (<25), absence of encephalopathy, absence of ascites, ALT more than 1.5 times the upper limits of normal (>60 IU/L) and normal albumin (>3.5 g/L) (Table 6.6). In the Cox proportional hazard regression model, the five components of the BE3A score individually all significantly predicted achieving CPT A status (p values <0.001 to 0.01), with the absence of ascites and encephalopathy having the highest HRs of 3.1 and 3.0 respectively, normal albumin and increased ALT HRs of 1.9 and 2.7 respectively, and normal BMI the lowest HR of 1.6. As only one patient had a BE3A score of 5 in this

cohort, those with a BE3A score of 4 and 5 were combined as one group in this analysis. Each point increase in the BE3A score was associated with a progressive increase in the likelihood of reduction to CPT class A with DAA treatment. A BE3A score of 4-5 was associated with a 75% chance of achieving CPT A, while those with a score of 0 or 1 were associated with a <5% and 25% chance of achieving CPT A at 36 weeks respectively (Figure 6.5A). A BE3A score of 3 and 4+ are associated with 87.0% and 98.2% specificity of achieving a sustained CPT A status, whereas a BE3A score of 1 and 0 was associated with 92.3% and 99.6% specificity of not achieving a CPT A status. There were no significant differences in BE3A scores of 1-5 in predicting liver transplantation or death. However, a BE3A score of 0 was associated with a 25% probability of death or a need for liver transplantation by week 36, with a sensitivity of 62.8% and specificity of 75.1% (Figure 6.5B). A BE3A score of 2-5 was associated with 86.3-100% specificity of mortality or needing liver transplantation in 36 weeks.

In a sensitivity analysis, the 5-point BE3A score (model 3) was found to have an AUROC of 0.75 in predicting CPT status A at 36 weeks after the initation of DAA, in comparisons to an AUROC of 0.81 using continous variables (model 1) or 0.77 using point-based variables (model 2) in logistic regressions. All three models performed significantly better than baseline MELD score (AUROC 0.56) in predicting achieving a CPT A status. A web-based BE3A calculator was therefore created to provide a more accurate estimate of the probability to achieve CPT A status with DAA treatment using continuous parameters of BMI, ALT and albumin. This calculator can be accessed at http://www.be3ascore.com. The accuracy of the BE3A score and BE3A calculator was internally validated using parametric bootstrap ROC analysis. The 95% CI of ROC for the 5-point BE3A score to predict achieving CPT class A was 0.748 – 0.755 from 100 simulated cohorts, while that

for the BE3A calculator was 0.798 – 0.806. A regression model using variables in BE3A also moderately predicts death or transplant with an estimated AUROC of 0.747, with a 95% CI of 0.743 – 0.757 in the bootstrap simulation.

Figure 6.4 MELD purgatory before and after DAA treatment



The MELD and CPT scores of each patients in the clinical trials were plotted before the DAA treatment (A) and at the last follow-up (B). MELD purgatory was defined as CPT B or C (score ≥ 7) and MELD score ≤ 14 (Zone 4 on the MELD/CPT plot). Each open circle represents one or more patient with the corresponding MELD and CPT scores. The number of observations in each zone was indicated in each quadrant.

Table 6.5 Predictors for MELD purgatory with DAA treatment

		Univariate model [1]			Multivariate model [2]		
		HR	**95% CI**	**p value**	**HR**	**95% CI**	**p value**
Age above 65	No	Ref			Ref		
	Yes	0.85	0.51 - 1.42	0.5	0.86	0.50 - 1.47	0.6
Gender	Female	Ref			Ref		
	Male	0.86	0.59 - 1.25	0.4	0.85	0.58 - 1.25	0.4
SVR_{12}	No	Ref			Ref		
	Yes	0.45	0.29 - 0.70	<0.001	0.51	0.32 - 0.81	0.004
BMI	<25	Ref			Ref		
(kg/m^2)	25-30	0.97	0.64 - 1.47	0.9	0.98	0.64 - 1.49	0.9
	>30	1.25	0.81 - 1.91	0.3	1.06	0.69 - 1.64	0.8
Ascites	No	Ref			Ref		
	Yes	1.95	1.29 - 2.94	0.001	1.79	0.16 - 2.76	0.008
Encephalopathy	No	Ref			Ref		
	Yes	1.81	1.29 - 2.55	0.001	1.66	1.15 - 2.39	0.006
Albumin	> 3.5	Ref			Ref		
(g/dL)	2.8 - 3.5	2.30	1.06 - 5.01	0.04	2.63	1.19 - 5.79	0.02
	< 2.8	3.06	1.39 - 6.73	0.005	2.75	1.20 - 6.26	0.02
ALT	< 1.5	Ref			Ref		
(ULN)	> 1.5	0.60	0.43 - 0.84	0.003	0.79	0.56 - 1.13	0.2
Bilirubin	<2	Ref			Ref		
(mg/dL)	2-3	1.32	0.87 - 1.99	0.2	1.36	0.88 - 2.10	0.2
	>3	1.57	1.06 - 2.33	0.02	1.40	0.92 - 2.15	0.1
INR	<1.7	Ref			Ref		
	1.7 - 2.2	1.50	0.96 - 2.34	0.07	1.04	0.65 - 1.68	0.9
	>2.2	0.43	0.06 - 3.11	0.4	0.80	0.11 - 5.84	0.8
GFR	< 60	Ref			Ref		
(mL/min per 1.73 m^2)	> 60	1.05	0.71 - 1.55	0.8	1.15	0.76 - 1.73	0.5
MELD	6-11	Ref					
	12-14	1.62	1.05 – 2.51	0.03			
	≥ 15	1.76	1.16 – 2.68	0.008			

[1] Univariate Cox proportional hazard models were right-censored at 40 weeks.

[2] Multivariate cox proportional hazard model includes all variables in the univariate analysis except for MELD and were right-censored at 40 weeks.

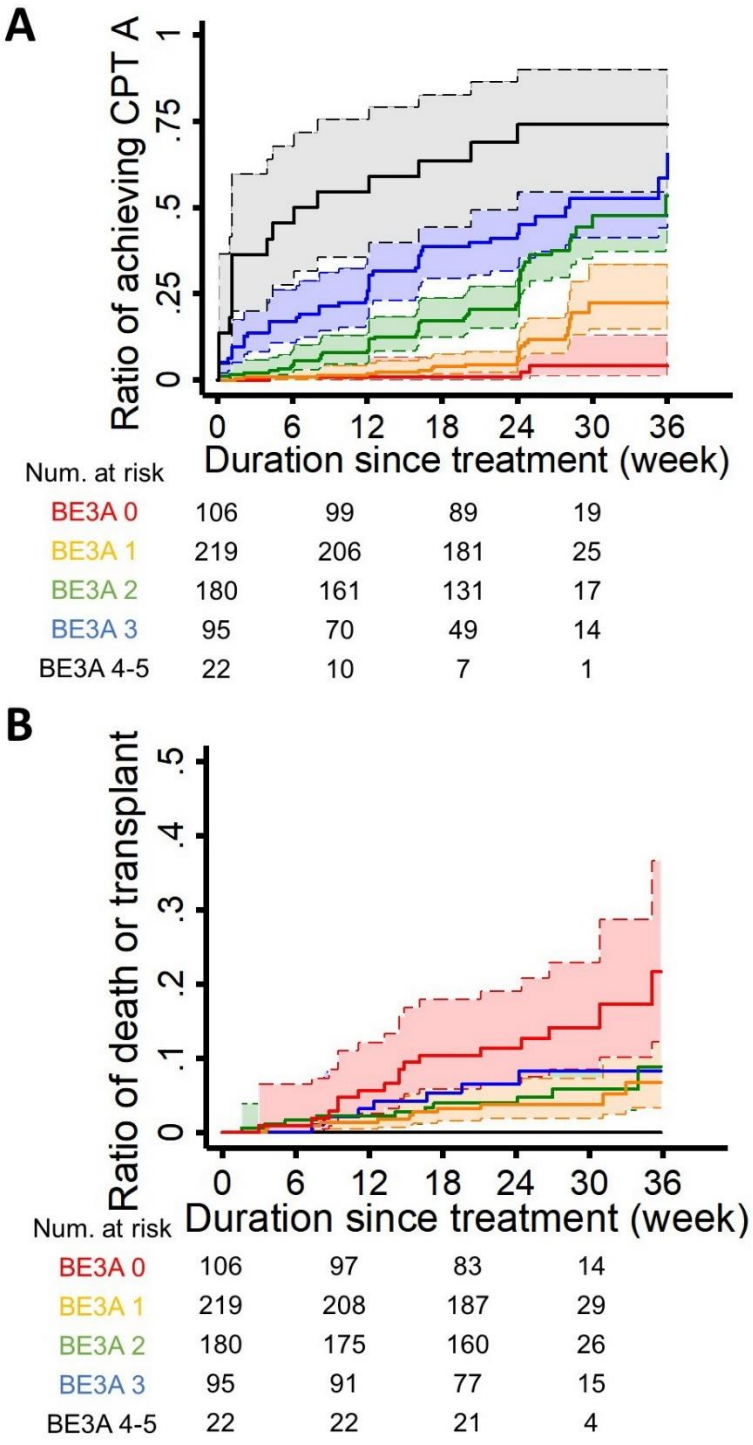
Table 6.6 BE3A score

Category	Findings	Score
BMI	< 25	1
Encephalopathy	No	1
Ascites	No	1
ALT	> 1.5 x ULN	1
ALB	> 3.5 g/dL	1

Table 6.7 Cox proportional hazard regression of BE3A score for achieving CTP class A

BE3A score	Num of obs	HR	95% CI	p value
0	106	Ref		
1	219	4.17	1.26 – 13.83	0.02
2	180	13.02	4.08 – 41.55	<0.001
3	94	21.16	6.55 – 68.29	<0.001
4-5	23	52.28	15.2 – 179.7	<0.001

Figure 6.5 - Kaplan Meier curves of achieving CPT A and death/transplant with BE3A score



Kaplan-Meier curves of different BE3A categories were plotted for achieving a CPT A status (A) and death or liver transplantation (B). Dashed lines represent 95% CI. Number of observations at 0, 10, 20, and 30 weeks were shown below each panel.

6.4 Discussion

The availability of highly effective DAA therapy provides new opportunities for HCV cure in patients who were previously ineligible for treatment [55-57]. However, the decision to offer antiviral therapy to decompensated patients must be guided by a clear understanding of the probability of deriving benefit against the risk of causing undue harm. This is especially important in patients who are liver transplant candidates, as DAA therapy is highly safe and effective post-transplant. The efficacy of these regimens in patients with decompensated cirrhosis has resulted in unanticipated challenges in the management of such patients who are liver transplant candidates. The goal of therapy in this setting must look beyond viral eradication alone, and the competing risks for harm or death in this population need to be considered.

This study utilises data from four clinical trials which includes only decompensated patients treated with SOF-based DAA therapy in phase 2 and 3 clinical trials, to identify the characteristics of patients with decompensation who derive a treatment benefit from HCV viral eradication. A clinically meaningful treatment benefit was defined as down-staging to CPT class A which would be synonymous with delisting from a transplant list. In this large cohort of 622 patients with CPT B and C cirrhosis, 31.1% of patients had compensated cirrhosis at 36 weeks from the start of treatment. The benefit in patients with baseline CPT class C cirrhosis was more modest with only 12% achieving a clinically meaningful treatment benefit. In a multicentre European study of decompensated patients listed for transplantation treated with DAA therapy, the cumulative incidence of delisted patients was 0% at 24 weeks, 10.3% at 48 weeks, and 19.2% at 60 weeks. There was a higher proportion of patients with a MELD score > 20 compared to our study indicating

possibly sicker populations that were less likely to be removed from the transplant list. [128].

The proportion of patients experiencing treatment benefit in our study is consistent with the benefit of antiviral therapy observed in patients with hepatitis B and hepatic decompensation. A prospective longitudinal study of 707 patients who presented with first-onset decompensation with chronic hepatitis B demonstrated a significant impact of anti-viral therapy on transplant free survival [129]. A significant improvement in liver function was observed, with 33.9% of treated patients delisted for liver transplantation [129]. Delisted patients maintained clinical improvement for up to 5 years of follow-up. In the study by Jang et al, transplant-free survival was dependent on viral suppression, being significantly higher in responders. Similarly, in our study, viral response was the most significant predictor of improvement in liver function, and ultimately a clinically meaningful treatment benefit. In a competing risk model, SVR was associated with a hazard ratio of 2.9 for significant treatment benefit, and a hazard ratio of 0.10 for death or liver transplantation. The benefits of SVR in patients with compensated cirrhosis are well established, with a reduction in all-cause mortality, liver-related mortality and need for liver transplantation in patients who achieved SVR with interferon based therapy [130]. Our data demonstrate the benefit of SVR with DAA in patients with decompensated cirrhosis.

There has been considerable interest in identifying a MELD score cut-off that predicts treatment benefit/risk with DAA therapy in decompensated cirrhosis. Guideline recommendations to date have largely been based on expert opinion with limited data to support a defined MELD cut-off. EASL recommends that liver transplant candidates

without HCC with a MELD score of ≥ 18-20 should be transplanted first, without antiviral therapy; HCV infection should be treated after liver transplantation [70]. Guidelines form the International Liver Transplant Society suggest that patients with MELD scores of ≥ 30 who are not expected to undergo liver transplantation within three months should not be treated with antiviral therapy [131]. An analysis of real world data from the Spanish Hepa-C registry found that MELD score alone (cut off < 18) was the best predictor of survival with DAA therapy [132]. However, there were only 24 patients with a baseline MELD score of ≥ 18 in the study and 13 patients with CPT class C, with the outcomes of these group compared to 564 patients with compensated CPT class A cirrhosis. Additionally, a significant number of decompensated patients were treated with a protease inhibitor, which are no longer recommended in patients with CPT class B/C cirrhosis because of serious adverse events related to altered protease inhibitor pharmacokinetics in this group. In our study, when MELD score was used instead of its components in multivariate analysis, higher MELD categories of 12-14 and ≥ 15, compared to < 12 were associated with lower ratio of clinical improvement.

In addition to MELD, we examined other baseline factors and individual components of the CPT score that predict treatment benefit. A number of other studies examined dynamic on-treatment factors to predict treatment benefit. In one study, older patients with low albumin (≤ 35 g/L), and hyponatraemia had the lowest odds of deriving functional treatment benefit [58]. Another study reported improvement in serum albumin concentrations on treatment as a predictor of treatment benefit [128]. We concentrated on baseline factors rather than dynamic on-treatment changes, as these predictors can be considered together in a decision algorithm of DAA therapy pre-transplant. Factors associated with clinically meaningful improvement were BMI < 25 Kg/m^2, normal albumin

and the absence of encephalopathy and/or ascites at baseline. Patients with a higher baseline ALT (> 1.5x ULN), suggesting the presence of active hepatocyte injury from HCV, were also more likely to benefit from treatment. Interestingly, African-Americans had a significantly high chance of achieving meaningful clinical improvement in this study. The mechanism of this differential benefit by ethnicity is not clear, although there was only a small number of African-American patients in the study.

Obesity was associated with reduced chance of achieving clinical treatment in our study, suggesting the potential presence of a competing secondary cause of progressive liver disease including hepatic steatosis and steatohepatitis in these patients. Obesity and diabetes have been shown to have a negative impact in patients with compensated cirrhosis post-SVR, with a two fold increased risk of HCC development in patients with diabetes and obesity post SVR [133-134]. Obesity has also been shown to be associated with persistently abnormal liver enzymes in patients who achieve SVR with DAA therapy, identifying a group of patients at risk of progressive liver disease despite viral eradication [135].

The presence of moderate or severe ascites was a negative predictive factor for treatment benefit. This would suggest that patients with refractory portal hypertension have progressed beyond “the point of no return” with DAA therapy. Reduction in portal hypertension may be delayed despite recovery in liver synthetic function. In a long-term study of treatment with sofosbuvir and ribavirin in patients with portal hypertension, significant reductions in portal hypertension were only observed at 96 weeks post treatment [127]. A recent study from Spain demonstrated continual improvements in portal hypertension for up to 5 years post-therapy, suggesting a longer time interval is required

for hepatic remodelling [136] The recent International Liver Transplantation Society guidelines suggest that refractory portal hypertension may determine the likelihood of DAA treatment benefit independently from MELD score [131]. The guidelines suggest that even patients with low or intermediate MELD scores who have refractory portal hypertensive complications should only be offered antiviral therapy selectively on a case by case basis.

In all, 330/572 (57.7%) patients remained in MELD purgatory at the end of follow-up, which decreased from 471/622 (75.7%) at the beginning of treatment. The definition of MELD purgatory in this study represents a group of patients who have persistent liver decompensation by CPT score with an improvement or stabilisation in MELD score following treatment. In MELD-based organ allocation regions, these patients retain an indication for liver transplantation but are disadvantaged by treatment. The presence of ascites, encephalopathy and hypalbuminaemia were significant predictors of MELD purgatory in this study, while SVR_{12} reduced the risk by half.

The BE3A score derived from this analysis may be of use to clinicians as a shared decision-making tool with patients in determining the potential benefit from DAA in decompensated cirrhosis in the pre-transplant setting. The score requires external validation, but also needs to be considered within regional transplant landscape and organ availability. Factors such as local wait list mortality, median MELD score at transplant and the utilisation of HCV positive and marginal donors all need to considered in parallel with the potential benefits and competing risks of DAA therapy pre-transplant. A limitation of the BE3A score is that it does not predict MELD purgatory or death for patients with lower BE3A scores.

These data must be interpreted in the context of the study design. First, the primary outcome of these trials was the safety and efficacy of DAA therapy in patients with decompensation, and follow-up data up to 36 weeks after the start of therapy was available in the majority of patients. However, longer-term follow-up studies are required to assess for improvements in portal hypertension parameters. Certain patient characteristics, such as low platelet count, significant jaundice and low creatinine clearance were excluded in these clinical trials, hence limiting the generalisability. Second, we lacked linkage to a transplant registry. Data on patients who underwent transplant during the follow-up period was available. However, data on patients that were inactivated or delisted during the study period was not available. Third, the rate of HCC development in patients who achieved a clinically meaningful treatment benefit requires a study with longer follow-up. Roll-over registry trials are likely to provide this valuable information. Fourth, the score requires external validation and must be considered within the regional transplant landscape and local organ availability. Fifth, factors such as local wait list mortality, median MELD score at transplant and the utilisation of HCV positive and marginal organs all need to be considered in parallel with the potential benefits and competing risks of DAA therapy pre-transplant. Treatment decisions in patients who are liver transplant candidates should be made by clinicians with expertise in liver transplantation, and should reflect these local factors. For example, MELD purgatory is less of a concern in centres with short wait list times, and lower median MELD scores at transplant. Local audit and review of treatment and transplant outcomes in patients with decompensated HCV cirrhosis are necessary to incorporate the BE3A score in the decision making process. Local country level registry data should provide some of the important data to explore longer term treatment benefit [59].

Real world data from the Scientific Registry of Transplant Recipients has demonstrated a 31.9% decrease in the liver transplant listing for decompensated cirrhosis related to HCV in the DAA era, suggesting an early beneficial effect of DAA therapy on outcomes at a population level [137]. Increasing DAA treatment uptake through screening and linkage to care is expected to further reduce the healthcare and societal burden of advanced liver disease secondary to HCV over the next decade. The new era of DAA therapy has expanded the eligible population for HCV therapy to include the sickest patients who are in need of liver transplantation. Our study identifies a group of such patients who are likely to derive benefit from treatment and who should be urgently considered for antiviral DAA therapy. Further longer-term studies are required to evaluate if this clinical improvement is sustained over time, with long term outcomes and survival rates.

CHAPTER 7

DISCUSSION

Discussion

This thesis addresses the personalisation of HCV DAA therapy, based on studies of (1) the clinical pharmacokinetics of HCV DAA therapy, and (2) the use of HCV DAA therapy in special populations. The clinical pharmacokinetics of the first generation protease inhibitor, telaprevir, and the second generation NS5A inhibitor, ledipasvir were examined in real world populations. The impact of viral clearance on liver function in patients with decompensated cirrhosis was investigated utilising data from the clinical trials registry of all patients with decompensated cirrhosis treated with sofosbuvir-based DAA therapy in phase 2 and 3 clinical trials.

Therapeutic drug monitoring for HCV DAA?

In our study, there was marked interindividual variability in plasma concentrations of telaprevir in a real-world mixed treatment-naïve and experienced population. Patients underwent a full assessment for potential drug-drug interactions prior to therapy, and were not taking any medications that induced or inhibit CYP3A. As discussed previously, the marked variability in our study likely reflects the normal distribution of CYP3A and P-gp activity in the population. Telaprevir exhibits non-linear pharmacokinetics, with increasing dose resulting in a less than proportional increase in exposure that is believed to be due to low solubility [35]. Telaprevir is also subject to a significant food effect, with the fat content of co-administered food having a significant influence on drug exposure. Patients in the intensive pharmacokinetic study were sampled following a breakfast containing 20g of fat, reducing the likelihood of any variability related to food effect.

The potential role for therapeutic drug monitoring (TDM) with the first generation HCV protease inhibitors has been proposed [138]. TDM in clinical practice requires four criteria

to be fulfilled: (1) an exposure-response relationship (2) wide interindividual variability in drug absorption, distribution, metabolism or excretion, (3) a narrow therapeutic index and (4) pharmacology response not accessible [139].

In phase 2b studies, increasing telaprevir C_{min} was associated with an increased rate of rapid virological response [140]. In our study, there was a significant relationship between telaprevir exposure and viral kinetics in the first week of therapy in IFN-λ4 non-cc patients. Another study demonstrated that day 3 telaprevir trough concentrations were associated with SVR, with trough day 3 concentrations > 1924ng/mL highly predictive of SVR [141]. In the study by Furusyo et al, patients received telaprevir at a dose of 750mg three times daily; all patients had HCV genotype 1b, and the rate of RVR was significantly higher at 71.4%. Our study had an RVR rate of 33%, and was the first real-world study to examine telaprevir PK with twice daily dosing in HCV genotype 1a and 1b infection. It demonstrated a wide interindividual variability in telaprevir C_{trough} and $AUC_{0\text{-}12h}$. There was no significant relationship between telaprevir exposure and SVR, and this may be attributable to the high SVR rate in our study. In non-cirrhotics, telaprevir exposure was significantly higher in patients that developed anaemia on treatment. Boceprevir, another 1st generation protease inhibitor also exhibits high PK variability with a range of inter-subject variability (percent coefficient of variation) in trough concentrations of 26-104% [142].

Although the pharmacological response (PD) to telaprevir is assessable by monitoring HCV viral load on treatment, it is clear that viral kinetics in the first few weeks of treatment impact on the likelihood of achieving RVR and SVR. Another important part of personalised care for HCV is the presence of resistant associated substitutions (RAS) at

baseline which can impact on viral kinetics early on in treatment. Stopping rules were developed during telaprevir therapy (i.e. day 28 viral load) to reduce risk of selecting resistant virus under antiviral pressure [33]. This personalisation of therapy enables the early identification of treatment futility and patients who are unlikely to benefit from ongoing prolonged therapy. Furthermore, the outcome of antiviral therapy (SVR) can only be assessed 12 weeks after the completion of therapy, and not all patients with a rapid virologic response achieve SVR. Therefore, dosage optimisation of telaprevir based on pharmacologic response is not feasible in practice. Telaprevir, as a CYP3A metabolite, is the potential victim of drug-drug interactions when co-administered with strong or moderate CYP3A inducers. Telaprevir is contraindicated with strong CYP3A inducers, including a number of HIV antiretrovirals. When co-administration is necessary with mild to moderate CYP3A inducers, telaprevir TDM may be useful in guiding dosage adjustments for patients in the lower quartiles of telaprevir exposure.

A role for TDM in HCV therapy was likely if telaprevir and boceprevir interferon-based therapy remained the standard of care. However, DAA monotherapy with peginterferon and ribavirin is no longer recommended in treatment guidelines [70, 94] and is no longer used in clinical practice. The PK, efficacy and safety issues associated with the first-generation DAAs have been largely overcome by newer agents. The second generation protease inhibitor paritaprevir is co-administered with ritonavir to modulate paritaprevir PK and allow for once daily dosing.

Is there a role for therapeutic drug monitoring with interferon free DAA therapy?

Pangenotypic interferon free DAA regimens are highly effective and virologic failure is rarely seen in treatment-naïve patients without advanced liver disease. These DAA have

long half-lives facilitating once-daily dosing and are not affected by hepatic impairment. Therefore, TDM is unlikely to improve treatment outcomes any further in the majority of patients treated for HCV.

However, there are a number of special populations where there is a role for TDM. Absorption of DAA may be impaired in patients with altered gastrointestinal anatomy (e.g. following gastric bypass surgery). The use of TDM to guide DAA dosing intervals in patients following gastric bypass has previously been described by our group [143].

Another group of patients who may benefit from TDM are patients receiving concomitant strong CYP3A inducers. This includes patients with epilepsy who are prescribed phenytoin, phenobarbitone or carbamazepine for seizure control that cannot be safely switched to alternative therapy. These older anti-epileptic drugs are contraindicated with all HCV DAA, and there are no DDI studies with these combinations to quantify the magnitude of the interaction. An adaptive dosing strategy using TDM for DAA to treat such patients has been described by our group [144]. TDM may also have a role in dosage optimisation of renally excreted DAA (sofosbuvir and ribavirin) in patients with severe renal impairment.

The clinical pharmacokinetics of DAA therapy in the real world setting

The combination of two or three DAAs from different classes without interferon provides for rapid viral suppression, allowing for shorter durations of therapy. In particular, the discovery of drug candidates that inhibit the viral non-structural protein 5A (NS5A) paved the way for interferon-free all oral DAA regimens. The NS5A protein plays a critical role

in HCV viral replication and assembly [145]. Inhibition of NS5A at picomolar concentrations is associated with significant reductions in HCV RNA levels in cell culture-based models, making these agents the most potent agents developed against the HCV virus [146].

The first generation NS5A inhibitors, ledipasvir and daclatasvir exhibit pH dependent solubility and both are co-administered with sofosbuvir. Ledipasvir is not a substrate or inhibitor of any cytochrome P450 isoenzymes, reducing the liability to drug-drug interactions. However, the absorption of ledipasvir is significantly reduced with increasing gastric pH. Ledipasvir C_{max} plasma concentrations are 48% lower when taken two hours after omeprazole [116]. Low dose PPI therapy (i.e. PPI at a dose equivalent to 20mg of omeprazole) is advised to be taken at the same time as ledipasvir-sofosbuvir to mitigate the PPI effect on absorption. Any effect of PPI therapy on potential HCV treatment outcomes is important, as PPI use is very common in HCV patients. In a study of primary care prescribing databases in the UK that included 4,622 HCV infected patients, 27.7% were receiving PPI therapy [147]. In our cohort of patients with decompensated cirrhosis, 46.8% were receiving PPI therapy.

Our study is the first reported to examine ledipasvir pharmacokinetics in patients receiving PPI therapy. Patients with decompensated cirrhosis were changed to once-daily low dose PPI and advised to take ledipasvir-sofosbuvir at the same time as their PPI. PPI therapy accounted for the majority of systematic variation in ledipasvir concentrations. In multivariate analysis, PPI use was the most significant predictor of ledipasvir concentrations. Ledipasvir C_{trough} plasma concentrations were approximately 20% lower in patients on low dose PPI therapy. This change is within the manufacturers no effect

boundary of 70 – 134% [148]. Population pharmacokinetic modelling was used to allow for random sampling from a large cohort of 314 patients with advanced liver disease. There was a 34% reduction in the relative bioavailability of ledipasvir in patients receiving concomitant PPI therapy. A limitation of our study was the lack of randomisation, as this was a prospective observational real-world cohort study. PPI therapy remained a significant predictor of ledipasvir concentrations when controlling for disease stage, MELD score and markers of portal hypertension.

In our study, SVR rates were numerically lower in genotype 1 patients receiving PPI, a finding that approached statistical significance. The effect of PPI therapy on treatment outcomes with ledipasvir-sofosbuvir have been examined in two large real-world cohorts with conflicting results. In the HCV-TARGET study, 30% were on PPI therapy at the start of treatment with ledipasvir-sofosbuvir. These patients had approximately two-fold lower odds of achieving SVR than those who were not [122]. The TRIO cohort study used a propensity matched scoring system to retrospectively assess the impact of PPI use on SVR with ledipasvir-sofosbuvir [123]. In contrast to the HCV-TARGET study, data on prescriptions filled including dose and type of PPI were available. PPI usage overall was not associated with SVR, although SVR rates were numerically lower in patients with cirrhosis receiving twice daily PPI therapy. There was a lower proportion of community-based treatment centres in the HCV-TARGET study which may in part account for the different findings. Patients in these centres tend to have a lower burden of advanced liver disease. The TRIO cohort had a higher rate of community based prescribing and treatment. Community based treatment is an important tool to increase treatment uptake in marginalised groups such as people who inject drugs and the high SVR rate in the TRIO cohort supports the use of the community scripts model of care.

Low-dose PPI use is unlikely to affect treatment outcome with ledipasvir-sofosbuvir in many patients. Despite the documented PPI effect on ledipasvir, 12 weeks of therapy with ledipasvir-sofosbuvir represents an over-treatment in most easy to treat treatment naïve patients without advanced liver disease. However, there may be a group of patients with multiple negative predictive factors for SVR in whom PPI therapy is best avoided where possible. In the early studies of sofosbuvir-based therapy the accumulation of a number of negative predictors resulted in lower SVR rates [149]. These included previous treatment experience, the presence of cirrhosis, high baseline HCV RNA levels, male sex, obesity, INFλ4 non-cc and baseline NS5A RASs. The HCV-TARGET study found that cirrhosis, low albumin, low platelet count, high total bilirubin, male sex and older age were factors independently associated with lower SVR rates with interferon-free DAA therapy in the real-world setting [150]. The use of PPI therapy in patients with many of these factors may result in lower SVR rates. A systematic review and meta-analysis of all published data is required going forward to fully characterise the effect of PPI therapy on easy and difficult to treat patients.

An understanding of the relationship between DAA exposure and SVR will be even more important with the move to shorter durations of therapy. The combination of ledipasvir-sofosbuvir with ribavirin was associated with an SVR rate of 68% in non-cirrhotics with just six weeks of therapy in a small phase 2 study [151]. There were no baseline patient factors that identified patients who were likely to respond with just six weeks of therapy. This raises the question whether patients who achieved SVR had higher drug exposure. Assessing drug concentrations in the first week of therapy may further identify this “super-rapid” responder sub-population. The SODAPI study looked at virologic response by day

two of treatment to predict patients who could be treated for as little as three weeks with an interferon-free combination of an NS5B inhibitor, an NS5A inhibitor and a protease inhibitor [152]. This is a potentially exciting area for future research.

The development and validation of a highly sensitive, robust and reproducible multi-drug HCV assay described in this thesis will be a valuable resource in addressing some of these outstanding questions including the management of complex drug-drug interactions in practice. One such area is epilepsy when patients are receiving strong enzyme inducers that are contraindicated with all HCV DAAs. There are no drug-drug interaction data with many of these agents. Dose optimisation with therapeutic drug monitoring in these patients may provide an opportunity for cure in a group of patients with unmet medical need.

Special populations

The interferon era of HCV therapy was characterised by the existence of special populations who were considered difficult to treat. These included patients with cirrhosis, chronic kidney disease, HIV-HCV co-infection and African Americans. Persons who inject drugs (PWID) were historically considered to be a special population, with perceived inferior treatment outcomes with treatment and concerns voiced regarding treatment compliance and risks of reinfection. We sought to investigate treatment outcomes, compliance and re-infection rates in a large historical cohort of 1,000 patients treated with the standard of care therapy at the time. There was no difference between recent or former PWID and non-drug users with respect to treatment outcome, SVR rates or compliance. In our low risk population of former PWID with HCV mono-infection, the re-infection rate was low at 10.5/1000 person years of follow-up, with a median follow-up period of nearly 5 years. No reinfections were seen in the recent PWID cohort in the six months following

SVR. Treatment discontinuation rates were high in keeping with interferon-based therapy, although discontinuation rates were lower in recent and former PWID compared to non-drug users.

The toxicity of interferon-based therapy has been a considerable barrier to treatment. The availability of all-oral interferon free DAAs provides an opportunity to achieve upscaling of therapy in PWID with the aim of HCV elimination in this marginalised cohort. Early results of DAA therapy with PWID have been very promising. The C-EDGE CO-STAR was the first phase 3 trial to evaluate DAA therapy in individuals receiving OST including those with ongoing drug use [91]. Treatment naïve people with HCV genotypes 1, 4 or 6 were randomised to receive 12 weeks of therapy with grazoprevir-elbasvir or placebo (followed by 12 weeks of therapy). Overall, 96% of patients completed therapy with > 95% adherence. In an intent-to treat analysis, SVR_{12} was achieved in 91%, with no impact of drug use at baseline or during treatment on SVR or adherence. The rate of re-infection in this study was 4.6 per 100 person-years, notably 50% of these re-infections were spontaneously cleared.

In the setting of a national HCV elimination strategy, it must be accepted that there will be incidences of re-infection if the population being treated includes current as well as former PWID patients. There are two important interventions required to minimise the rate of re-infection [153]. At an individual level, risk minimisation strategies such as needle exchange programmes, and interventions to prevent reinfection within injecting partnerships (such as the “bring a friend” strategy where injecting partners of an individual receiving treatment are recruited for treatment). At a population level, rapid-scale up of DAA therapy among PWID is required to minimise the re-infection rate. If more than 8%

of the total PWID population are treated every year, the pool of PWID susceptible to reinfection will decline within 10 years.

DAA therapy in decompensated cirrhosis. Who gets better?

Patients with HCV-related decompensated cirrhosis were considered a special population in the interferon-era as HCV therapy was contraindicated in this group. Liver transplantation was the only intervention associated with improved survival once hepatic decompensation has developed. The SOLAR-1 and SOLAR-2 studies represented landmark studies in this population of highly unmet need [55-56]. They demonstrated high SVR rates and improvement in MELD scores in many patients with just 12 weeks of therapy with ledipasvir-sofosbuvir with ribavirin. However, response rates were lower than those observed in patients with compensated cirrhosis [54]. High mortality rates were also reported in a real-world cohort of decompensated patients treated with DAA therapy [59]. The effect of "MELD purgatory", where patients MELD score is improved following viral eradication but retain an indication for transplantation, must also be considered in practice.

We defined a "clinically meaningful treatment benefit" as achieving CPT class A cirrhosis at the end of the follow-up period, an improvement that would be synonymous with transplant delisting in the absence of HCC in clinical practice. In keeping with data from patients with compensated cirrhosis [130] SVR was a significant predictor for treatment benefit in short-term follow-up. However, as SVR is not a baseline factor, it is not useful in terms of deciding whether or not to start treatment. We sought to identify pre-treatment baseline factors that predict who will improve or experience adverse events including transplantation and death during therapy.

In our analysis, factors associated with a "clinically meaningful" treatment benefit were baseline BMI < 25 Kg/m^2, albumin > 3.5 g/dL, ALT > 1.5 x the upper limit of normal, and the absence of ascites and/or encephalopathy at baseline. These factors were combined into an easy to calculate five point BE3A score (0-5). For every point increase in the BE3A score, there was a progressive increase in the likelihood of achieving CPT class A with DAA therapy. A BE3A score of 4-5 was associated with a HR of 52.28 (95% CI 15.2 – 179.7) of treatment benefit. The BE3A score was developed using a numeric approach with equal weight applied to all factors to allow it to be calculated easily by treating clinicians without the need for complex algorithms. The presence of ascites and encephalopathy in combination with a low serum albumin was associated with an increased risk of MELD purgatory.

One of the limitations of our study was the short-term follow-up. Median duration of follow-up in this study was 255 days (IQR 251-336) from the beginning of treatment. Cheung et al have reported outcomes of DAA therapy over 15 months after the start of treatment in 406 patients with decompensated cirrhosis treated as part of the NHS England EAP [154]. Of the 317 patients that achieved SVR, 17 (5%) developed new liver cancers, 39 (12%) underwent liver transplantation, 52 (16%) experienced further decompensation and 9 (3%) patients died from predominantly liver related causes. There was a reduction in the incidence of decompensations between 6-15 months (7%) compared to 0-6 months from the start of treatment (28%). Additionally there was no difference in the rate of HCC development between these two periods. The study by Cheung et al suggests that improvements in liver function following DAA therapy in decompensated cirrhosis are sustained over time.

The BE3A score requires external validation but will serve as a useful decision-making tool in selecting patients with decompensated cirrhosis for treatment with DAA therapy. Further data on the rate of development of *de novo* and recurrent HCC in this population in the five years after treatment will be required to guide full cost-benefit analyses on treatment of HCV pre-transplant. However, our data identifies a sub-set of patients who should be considered for treatment without delay to improve long term outcomes. A national HCV registry with longitudinal outcomes research will address the long-term benefits of DAA therapy in patients with compensated and decompensated cirrhosis.

The face of HCV care has changed dramatically in the last five years to the point that disease elimination is being discussed in many jurisdictions. The WHO has set a target of 2030 for disease elimination [1]. However, this target will require upscaling of treatment in marginalised populations and those with limited or little access to healthcare. For this aim to be successful in the absence of a vaccine, treatment programmes need to move towards a more personalised model of care to maximise treatment outcomes in all patients.

7.2 Outcomes from this thesis

Chapter 2
HCV treatment in people who inject drugs (PWID) • Data supporting inclusion of PWID in HCV-DAA national treatment programme targeting elimination in this cohort. • No effect of recent or former injecting drug use on treatment compliance or discontinuation rates with peg-interferon therapy. • Low HCV re-infection rates in low and medium risk cohorts.
Chapter 3
Pharmacokinetics of telaprevir as part of HCV triple therapy • Data on the variability for telaprevir pharmacokinetics in the real world setting with twice and three times daily dosing. • Guidance on optimal dosing of telaprevir in patients with cirrhosis.
Chapter 4
Development of a bio-analytical method for the measurement of DAA concentrations in human plasma • The development and validation of a bioassay for HCV DAA in human plasma • A bioanalytical tool for therapeutic drug monitoring in patients treated with HCV DAA and personalisation of therapy
Chapter 5
Pharmacokinetic of ledipasvir as part of interferon free DAA therapy • Data on ledipasvir pharmacokinetics and influences on ledipasvir drug concentrations in patients with cirrhosis • Guidance on the co-administration of proton pump inhibitors with ledipasvir
Chapter 6
Predictors of improvement in liver function following DAA therapy in patients with HCV and decompensation cirrhosis • Data on the outcomes following DAA therapy in decompensated cirrhosis • The BE3A score: a decision aid in selecting patients for therapy with DAA in decompensated cirrhosis that advances a patient-centre personalised model of care • The BE3A online calculator tool

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Appendix

The following publications are based on the work contained in this thesis:

Papers

Elsherif O, Bannan C, Keating S, McKiernan S, Bergin C, Norris S. Outcomes from a large 10 year hepatitis C treatment programme in people who inject drugs: No effect of recent or former injecting drug use on treatment adherence or therapeutic response. *PLoS One* 2017 Jun 21;12(6):e0178398

Elsherif O, Jiang ZG, Tapper EB, Huang KC, Osinusi A, Charlton M, Manns M, Afdhal N, Mukamal K, McHutchison J, Brainard DM, Terrault N, Curry MP. Who gets better? Predictors of clinically meaningful improvements in liver function in patients with decompensated Hepatitis C (HCV) cirrhosis receiving direct-acting antiviral (DAA) therapy. Submitted.

Oral presentations

18th International Workshop on Clinical Pharmacology of Antiviral Therapy
Chicago, IL 2017

Elsherif O, Irwing W, Dilly-Penchala S, McLaughlan J, Challanger E, Foster G, Norris S, Khoo S. Influences on pharmacokinetics of ledipasvir, sofosbuvir, and GS-331007 in patients with decompensated cirrhosis – results from the UK expanded access programme.

International Liver Transplant Society Meeting
Prague, Czech Republic 2017

Elsherif O, Tapper EB, Huang KC, Osinusi A, Charlton M, Manns M, Afdhal N, Brainard DM, Terrault N, Curry MP. Who gets better? Predictors or clinically meaningful improvements in liver function among patients with decompensated cirrhosis receiving DAA therapy.

Irish Society of Gastroenterology
Dublin 2014

Elsherif O, Dilly-Penchala S, Else LJ, McKiernan S, Khoo S, Norris S. Inter-individual variability in telaprevir exposure in patients undergoing anti-HCV therapy.

Abstract and poster presentations

The Liver Meeting 2017; 68th Annual Meeting of the American Association for the Study of Liver Diseases. Washington, DC

Elsherif O, Pertinez H, Irwing W, Dilly-Penchala S, McLaughlan J, Foster G, Norris S, Else L, Khoo S. Population pharmacokinetic analysis of ledipasvir in patients with decompensated cirrhosis – results from the UK Expanded Access Programme.

51st International Liver Congress of the European Association for the study of the Liver. Barcelona, Spain 2016

Elsherif O, Stewart S, Bergin C, McKiernan S, Crosbie O, Khoo S, Norris S. Determination of ledipasvir concentrations in patients with decompensated cirrhosis receiving low dose proton pump inhibitors during sofosbuvir-ledipasvir treatment for HCV genotype 1 infection.

The Liver Meeting 2015; 66th Annual Meeting of the American Association for the Study of Liver Diseases. San Francisco, CA.

Elsherif O, Houlihan D, Stewart S, Bergin C, Fanning L, Crosbie O, McKiernan S, Norris S, Khoo S. Determination of on-treatment pharmacokinetics of sofosbuvir and ledipasvir in patients with decompensated cirrhosis.

The Liver Meeting 2014; 65th Annual Meeting of the American Association for the Study of Liver Diseases. Boston, MA.

Elsherif O, Dilly-Penchala S, Else LJ, Khoo S, Norris S. Significant inter-individual variability in telaprevir exposure in patients undergoing anti-HCV therapy – need for "real world" pharmacokinetic data to identify vulnerable patients.

Elsherif O, Bannan C, Keating S, McKiernan S, Bergin C, Norris S. Outcomes of a HCV treatment programme in people who inject drugs over 10 years support a disease eradication strategy for all patients in the DAA era.

Irish Society of Gastroenterology 2013

Elsherif O, Quinn C, Hynes B, McGrath M, Irish H, McKiernan S, Norris S. The safety and tolerability of telaprevir and boceprevir based triple therapy for Hepatitis C genotype 1 in an Irish patient cohort.

Elsherif O, McKiernan S, Norris S. Late viral breakthrough and late relapse in the triple therapy era: a cautionary tale.