THE LANCET Infectious Diseases

Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Kohli A, Kapoor R, Sims Z, et al. Ledipasvir and sofosbuvir for hepatitis C genotype 4: a proof-of-concept, single-centre, open-label phase 2a cohort study. *Lancet Infect Dis* 2015; published online July 15. http://dx.doi.org/10.1016/S1473-3099(15)00157-7.

SUPPLEMENTARY APPENDIX

Contents

Supplementary Figure and Table Legends

Figure S1: Rapid Decline and Normalization in ALT and AST on Treatment

Alanine aminotransferase (ALT) levels declined to normal by week 4 in 76% of patients treated with ledipasvir + sofosbuvir. A similar pattern was observed with aspartate aminotransferase (AST) levels.

Table S1: Patients With Hepatitis C Virus RNA Lower than the Level of Quantification Using Assay with Lower Limit of Quantification of 12 IU/mL (Abbott)

Table S2: Decline in HCV Viral Load at various times of treatment and follow-up

Table S3: Patients with HCV RNA levels below the level of detection at various times of treatment and follow-up

Supplemental Appendix 1: Inclusion and Exclusion Criteria

Figure S1: Decline and Normalization in ALT and AST on Treatment

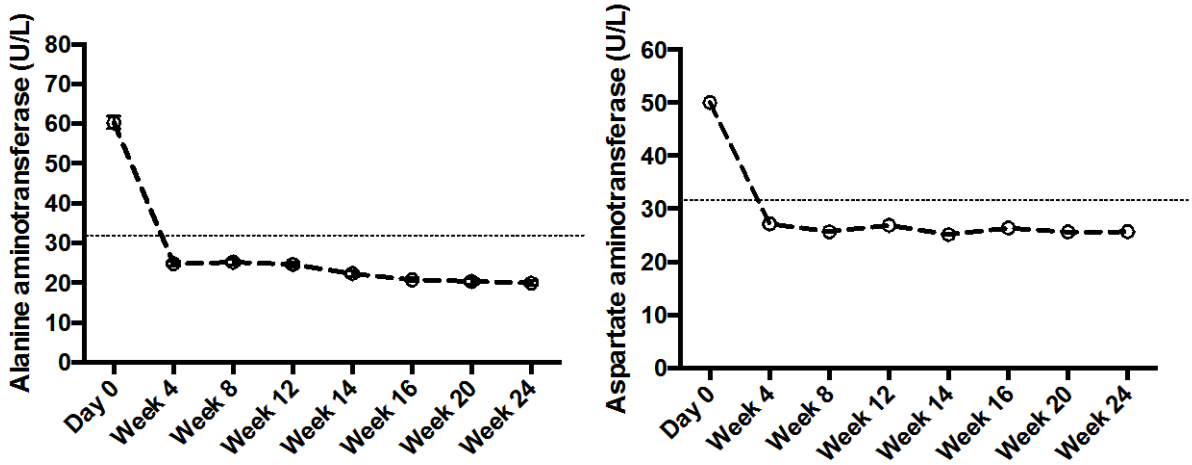

Table S1: Patients with Hepatitis C Virus RNA Lower than the Level of Quantification Using Assay with Lower Limit of Quantification of 12 IU/mL

Time Point	Ledipasvir + Sofosbuvir *n*=21 12 wk
During Treatment - *–n (% [95% CI])*	
Week 4	15 (71[51-91])
Week 8	19 (90[70-99])
Week 12	20 (95[76-100])
Post Treatment Period *–n (%[95% CI])*	
Week 4	20 (95[76-100])
Week 12	20 (95[76-100])

Table S2: Patients with HCV RNA Levels Below the Level of Detection at Various Times of Treatment and Follow-up

Time Point	Ledipasvir + Sofosbuvir N=21 12 wk	
	Abbott	Roche
During Treatment		
Week 4	2 (9)	13 (68)
Week 8	13 (62)	18 (90)**
Week 12	18 (86)	20 (95)
Post Treatment Period		
Week 4	20 (95)	--
Week 12	20 (95)	20 (95)

** One patient specimen quantity not sufficient for processing, HCV RNA was <43 IU/mL at week 4 and <15 at week 12

Table S3: Change in HCV RNA from Day 0 (Mean Standard Deviation)

Time Point	**Ledipasvir + Sofosbuvir**	
	n=21	
	12 wk	
	Abbott	**Roche**
	log_{10} IU/mL	**log_{10} IU/mL**
During Treatment		
-mean ± SD		
(Range)		
Week 4	4.5 ± 1.2	4.1 ± 1.5
	(0.1-5.5)	(0.3 – 5.9)
Week 8	4.8± 0.6	4.2 ± 1.5
	(3.3-5.5)	(3.2 – 5.9)
Week 12	4.9 ± 0.6	4.4 ± 1.2
	(3.3-5.5)	(3.2 – 5.9)
Post Treatment Period		
Week 4	4.9 ± 0.6	--
	(3.3-5.5)	--
Week 12	4.9 ± 0.6	4.4 ± 1.2
	(3.3-5.5)	(3.2 – 5.9)

Supplemental Appendix 1: Inclusion and Exclusion Criteria

Main Inclusion Criteria

1. Eighteen years of age or older at screening.

2. Female study participants with childbearing potential (as defined below) and all males must be willing to practice either:
    - Abstinence from sexual intercourse or
    - One or more forms of effective barrier contraception throughout dosing and for 30 days following the last dose.This cannot include hormonal contraception for female subjects.

    Effective forms of barrier contraception include:
    - a male condom with spermicide
    - use by female sexual partner of a female condom with spermicide

    Non-childbearing potential (i.e., physiologically incapable of becoming pregnant) includes any female who:
    - Has had a hysterectomy or
    - Has had a bilateral oophorectomy (ovariectomy) or
    - Is post-menopausal (a demonstration of a total cessation of menses for ≥1 year)
    - Has had a bilateral tubal ligation or fallopian tube inserts

3. Chronic HCV GT-4 infection as documented by ≥1 measurement of serum HCV RNA ≥ 2,000 IU/mL during screening and at least one of the following:
    a. A positive anti-HCV antibody, HCV RNA, or HCV genotype test result ≥12

months prior to the baseline (day 0) visit together with current positive HCV RNA and anti-HCV antibody test results **or**

b. Positive HCV RNA test and anti-HCV antibody test results together with a liver biopsy consistent with chronic HCV infection or a liver biopsy performed before enrollment with evidence of chronic hepatitis C infection disease, such as the presence of fibrosis.

4. Patients may include subjects with compensated cirrhosis.

Cirrhosis is defined as any one of the following:

a. Any biopsy showing cirrhosis.

b. A FibroTest® score of ≥0.75 AND an AST: platelet ratio (APRI) of >2 performed within 12 months of screening.

Liver imaging within 6 months of Day 0 to exclude hepatocellular carcinoma (HCC) is required in patients with cirrhosis.

Absence of cirrhosis is defined as one of the following:

a. A liver biopsy performed within 36 calendar months of screening showing absence of cirrhosis.

b. A FibroTest® score of < 0.48 AND APRI of <1 performed within 6 months of screening.

5. Ability to communicate effectively with the study investigator and other key personnel.

6. Willing to give written informed consent and comply with the study restrictions and requirements.

7. Opioid-dependent individuals must be participating in a supervised treatment program.

8. Subjects must have an external primary care doctor (outside of the CC and the NIH) for their medical management.

Main Exclusion Criteria

1. Current or prior history of any of the following:
    a. Clinically-significant illness (other than HCV) or any other major medical disorder that may interfere with subject treatment, assessment or compliance with the protocol; subjects currently under evaluation for a potentially clinically-significant illness (other than HCV) are also excluded.
    b. Gastrointestinal disorder or post-operative condition that could interfere with the absorption of the study drug.
    c. Poor venous access interfering with required study blood collection.
    d. Clinical hepatic decompensation (i.e., ascites, encephalopathy or variceal hemorrhage).
    e. Solid organ transplantation.
    f. Significant pulmonary disease, significant cardiac disease or porphyria.
    g. Unstable psychiatric disease (Subjects with psychiatric illness that is well-controlled on a stable treatment regimen or currently not requiring medication may be included).
    h. Any malignancy or its treatment that in the opinion of the PI may cause ongoing interference with host immunity; subjects under evaluation for malignancy are not

eligible.

i. Significant drug allergy (such as anaphylaxis or hepatotoxicity).

j. Substance abuse, which in the opinion of the investigator is likely to interfere with medication adherence or study compliance.

k. Lactose allergy, patients with lactose intolerance will be evaluated on a case-by-case basis.

2. Positive test results at screening for hepatitis B virus (HBV) surface antigen (HBsAg), HBV DNA (if medically indicated) or anti-HIV antibody.

3. Prior exposure to any direct-acting antivirals for HCV infection.

4. History of clinically significant chronic liver disease due to other etiology (e.g., hemochromatosis, autoimmune hepatitis, Wilson's disease, α1-antitrypsin deficiency, alcoholic liver disease, >moderate non-alcoholic steatohepatitis and toxin exposures).

5. Use of herbal/natural remedies for potential benefit to the liver within 21 Days of Day 0.

6. History of ascites, variceal hemorrhage, hepatic encephalopathy, or conditions consistent with decompensated liver disease.

7. Screening or baseline ECG with clinically significant ECG findings, or a personal/first degree relative history of Torsade de pointes.

8. Abnormal hematological and biochemical parameters at screening, including:
    a. Neutrophil count<750 cells/mm^3.
    b. Hemoglobin level <9 g/dL. If Hgb< 11g/dL in women and < 12 g/dL in men other causes of anemia should be excluded as medically indicated.
    c. Plateletcount≤50,000 cells/mm^3.
    d. Estimated glomerular filtration rate <50 mL/min/1.73 m^2.
    e. ALT or AST level ≥10 times upper limit of normal (ULN).
    f. Serum lipase level ≥1.5 times ULN at screening or during the screening period in a patient with symptoms consistent with pancreatitis.
    g. Total bilirubin level ≥2.0 times ULN, except in subjects with Gilbert's syndrome.
    h. Albumin level ≤3.0 g/dL in patients without cirrhosis, albumin ≤ 2.8 g/dL in cirrhotic patients.

9. Poorly controlled diabetes mellitus indicated by hemoglobin A1C >9% at screening.

10. Donation or loss of blood of >400 mL within 8 weeks prior to the first dose of the study drugs.

11. Known hypersensitivity to GS-5885, GS-7977 or formulation excipients.

12. Pregnant/Breastfeeding women.

13. Need for use of the following medications from 21 days prior to the start of study drugs through the end of treatment (unless otherwise specified in Table 8-10):

a. Hematologic stimulating agents (e.g. erythropoiesis-stimulating agents (ESAs); granulocyte colony stimulating factor (GCSF); thrombopoietin (TPO) mimetics).
b. Chronic systemic immunosuppressants including, but not limited to, corticosteroids (prednisone equivalent of >10 mg/day for > 2 weeks), azathioprine, or monoclonal antibodies (e.g., infliximab).
c. Investigational agents or devices for any indication.
d. Medications for disease conditions **excluded** from the protocol (e.g., active cancer, transplantation) are not listed under this Concomitant Medication section and are disallowed in the study.
e. Concomitant use of certain medications or herbal/natural supplements per PI discretion expected to result in pharmacokinetic interactions resulting in increases or decreases in exposure of study drug(s) as listed in Table 8-10 of this protocol.