Safety and Antiviral Activity of EGFR Inhibition by Erlotinib in Chronic Hepatitis C Patients: A Phase Ib Randomized Controlled Trial

Antonio Saviano, MD, PhD[1-3], François Habersetzer, MD, PhD[1-3], Joachim Lupberger, PhD[1,2], Pauline Simo-Noumbissie, PhD[3], Catherine Schuster, PhD[1,2], Michel Doffoël, MD, PhD[3], Catherine Schmidt-Mutter, MD, PhD[4] and Thomas F. Baumert, MD[1-3,5]

INTRODUCTION: **Significant hepatocellular carcinoma (HCC) risk persists after chronic hepatitis C (CHC) cure. Preclinical studies have shown that erlotinib, an oral epidermal growth factor receptor (EGFR) inhibitor, has an antiviral activity and HCC chemopreventive effect. Erlotinib is metabolized in the liver, and its safety in patients with CHC is unknown. This study aimed to assess the safety and antiviral activity of erlotinib in patients with CHC.**

METHODS: **In this investigator-initiated dose-escalation phase Ib prospective randomized double-blind placebo-controlled study, noncirrhotic hepatitis C virus (HCV) patients received placebo or erlotinib (50 or 100 mg/d) for 14 days with a placebo-erlotinib ratio of 1:3. Primary end points were safety and viral load reduction at the end of treatment (EOT). The secondary end point was viral load reduction 14 days after EOT.**

RESULTS: **This study analyzed data of 3 patients receiving placebo, 3 patients receiving erlotinib 50 mg/d, and 3 patients receiving erlotinib 100 mg/d. One grade 3 adverse event was reported in the placebo group (liver enzymes elevation), leading to treatment discontinuation and patient replacement, and 1 in the erlotinib 100 mg/d group (pericarditis), which was not considered to be treatment-related. Grade 2 skin rash was observed in 1 erlotinib 100 mg/d patient. No significant HCV-RNA level reduction was noted during treatment, but 2 of the 3 patients in the erlotinib 100 mg/d group showed a decrease of >0.5 log HCV-RNA 14 days after EOT.**

DISCUSSION: **Erlotinib demonstrated to be safe in noncirrhotic CHC patients. An antiviral activity at 100 mg/d confirms a functional role of EGFR as an HCV host factor in patients. These results provide perspectives to further study erlotinib as an HCC chemopreventive agent in patients with CHC.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A796

Clinical and Translational Gastroenterology 2022;13:e00492. https://doi.org/10.14309/ctg.0000000000000492

INTRODUCTION

Hepatitis C virus (HCV) is a major cause of hepatocellular carcinoma (HCC) and liver cirrhosis (1). HCV infection treatment has been for a long time a clinical challenge with few therapeutic options with limited efficacy and considerable side effects (2). Direct antiviral agents (DAAs) are now available to effectively treat HCV infection with very few side effects (3–5). However, DAAs have no direct effect on liver fibrosis and cancer pathways (6). Moreover, a large series of studies in several US, Asian, and European cohorts have elegantly demonstrated that HCC risk persists after HCV cure, especially in patients with advanced liver fibrosis and concomitant risk factors (7–12).

Epidermal growth factor receptor (EGFR) is a host cell factor for HCV cell entry and infection, promoting the coreceptor association of CD81 and claudin-1 and viral glycoprotein-dependent membrane fusion (13,14). HCV modulates EGFR phosphorylation and downstream signaling by prolonging EGFR activity and increasing hepatocyte proliferation and stellate cell activation, impairing antiviral response and activating the Nuclear factor-κB signaling pathway (15–17). Moreover,

[1]Inserm, U1110, Strasbourg, France; [2]Institut de Recherche sur les Maladies Virales et Hépatiques (IVH), Université de Strasbourg, Strasbourg, France; [3]Institut Hospitalo-Universitaire, Service d'hépato-gastroentérologie, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; [4]Centre d'Investigation Clinique, Inserm 1434, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; [5]Institut Universitaire de France (IUF), Paris, France. **Correspondence:** Antonio Saviano, MD, PhD. E-mail: saviano@unistra.fr. Thomas F. Baumert, MD. E-mail: thomas.baumert@unistra.fr.

Received January 7, 2022; accepted March 21, 2022; published online April 1, 2022

EGFR signaling activates the STAT3 pathway and mitogen-activated protein kinase pathways, which are key players in liver fibrogenesis and carcinogenesis (15,18–21). A functional polymorphism in the epidermal growth factor gene is associated with risk for HCC (22–24) in HCV and nonviral liver disease in US and Asian cohorts, and a score including epidermal growth factor polymorphism has been shown to predict clinical deterioration in HCV cirrhosis (25). The EGFR signaling pathway is also relevant in HCC progression, and the activation of EGFR has been shown to reduce HCC treatment response (26).

The discovery of EGFR and STAT3 signaling pathways in the pathogenesis of chronic HCV infection and carcinogenesis has resulted in performing clinical trials investigating erlotinib for HCC chemoprevention (Clinical Trial NCT02273362). Erlotinib is an oral EGFR tyrosine kinase inhibitor that is used as an antineoplastic agent in non-small cell lung cancer, pancreatic cancer, and renal cell carcinoma (27). In animal models for liver disease, erlotinib has been shown to impair, significantly and efficiently, the progression of chronic liver disease to HCC (15). Furthermore, studies in HCV-infected human liver chimeric mice have suggested that a short-term course of erlotinib reduces HCV viral load by around 0.5–1 $\log_{10}$ (13). These data suggest that EGFR has a dual role in HCV-induced liver disease: first, as an HCV host dependency factor, because it is a crucial proviral factor in the HCV life cycle, and second, as a therapeutic target, because EGFR is a mediator of HCC risk validated by robust genetic association studies in patients and preclinical proof of concept. Collectively, a large body of preclinical evidence suggests that erlotinib may serve as an HCC chemopreventive agent in chronic hepatitis C (CHC). However, no data are available on the safety and antiviral efficacy of erlotinib in patients with chronic HCV infection.

The aim of this phase Ib prospective randomized double-blind placebo-controlled study was to evaluate the safety and antiviral activity of erlotinib in patients with HCV infection.

METHODS

Patients and study design

This phase Ib randomized, double-blind, placebo-controlled trial was conducted at University Hospitals of Strasbourg, Strasbourg, France. Patients aged between 18 and 65 years were eligible, if they had a chronic HCV infection genotype 1 with HCV viral load higher than 10^4 UI/mL and did not have cirrhosis at liver biopsy or transient elastography performed at the latest 1 year before inclusion. Additional inclusion criteria were (i) a weight of minimum 45 kg, (ii) body mass index between 18 and 25 kg/m^2, and (iii) nonsmoker or a smoker of less than 3 cigarettes/d. Patients were excluded if they had one of the following conditions: (i) active hepatitis B and/or human immunodeficiency virus infection; (ii) cirrhosis proved by liver biopsy (METAVIR F4) or transient elastography (≥12 kPa); (iii) liver decompensation; (iv) other chronic liver disease than HCV infection; (v) total bilirubin higher than 21 μmol/L or alanine aminotransferase (ALT) 3-fold higher the normal values; (vi) history of gastrointestinal bleeding, diverticulosis, keratitis, or corneal ulcer; (vii) any clinically significant cardiovascular, neurological, psychiatric, or metabolic comorbidity that could affect the study compliance; (viii) glucose, lactose, or galactose intolerance or malabsorption; and (ix) any other known contraindication to erlotinib, as per manufacturer's guidance.

All patients provided an informed written consent, and the study protocol was approved by the institution's human ethical committee (CPP Est IV, Strasbourg, France) and conformed to the ethical guidelines of the 1975 Declaration of Helsinki. The clinical trial protocol has been publicly registered (No. EudraCT 2012-002069-36, Clinical Trial NCT01835938).

This was a single ascending dose trial consisting of 3 cohorts testing a different dose of erlotinib (50, 100, or 150 mg/d) or placebo. Each cohort enrolled 4 patients, 3 treated with erlotinib and 1 placebo. A simple randomization method was used. Eligible patients were randomized first in the lowest dose cohort (50 mg) and then in the following higher doses after treatment completion of the last patient in the lower cohort and revision of safety data by an independent safety committee. Patients who were not able to complete this study for reasons other than treatment toxicity were replaced.

The experimental drug and placebo were formulated as encapsulated powder. Dose administration was planned as follows: for the 50 mg cohort, patients received 2 capsules of erlotinib 25 mg or placebo once a day and for the 100 and 150 mg cohorts, they received 1 capsule of the corresponding erlotinib dose or placebo once a day.

Erlotinib and placebo capsules were identical; the treatment was prepared in anonymized containers with no differences between the placebo and the active drug. Patients and all the personnel involved in the clinical care of the patients were unaware of the assigned treatment. The hospital pharmacy dispensed the treatment at 1 time using an anonymized code. The randomization sequence and anonymization codes were concealed by the Chief Research and Innovation Office of the hospital and were revealed to the investigators only after termination of the trial.

HCV-RNA levels and laboratory tests were performed initially 14 days before the treatment. The treatment was administered once per day for 14 days, and patients were followed up by clinical examination and laboratory tests at days 2, 4, 7, 10, and 14 during the treatment and at 7 and 14 days after the end of the treatment.

Laboratory tests performed at each time point were the following: complete blood count, prothrombin time, activated partial thromboplastin time, fibrinogen, glucose, sodium, potassium, urea, creatinine, aspartate aminotransferase, ALT, total and conjugated bilirubin, alkaline phosphatase, gamma-glutamyl transferase, HCV-RNA level (Abbott real-time polymerase chain reaction HCV assay $m2000_{sp}$-$m2000_{rt}$, Abbott Molecular Diagnostics, Rungis, France) with a lower limit of detection of 12 UI/mL, and urinary human chorionic gonadotropin tests for women in childbearing age.

End points

The primary end point of this study was the evaluation of erlotinib's safety in patients with HCV infection for the maximum tolerated dose and toxicity according to the Common Terminology Criteria for Adverse Events, version 4.0. The coprimary end point was the antiviral efficacy defined as 1 $\log_{10}$ reduction of HCV RNA at day 14 of the treatment. The secondary end point was the study of HCV-RNA changes at 14 days after the end of the treatment.

Statistical analysis

Randomized patients who received at least 1 dose and have detectable baseline HCV RNA were included in the analysis. Data

from patients treated with placebo were pooled. Categorical variables were described as absolute values and percentages; continuous variables were described as median and range or median absolute deviation. The data were analyzed by R v4.0.1 (R Core Team 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/). No formal sample size calculation was required for this type of trial.

RESULTS

A total of 10 patients were randomized between July 2013 and February 2017, 5 in the 50 mg cohort and 5 in the 100 mg cohort. This study was prematurely terminated after the completion of the second cohort (100 mg) as per investigators' choice because effective DAA regimens became available for non cirrhotic HCV patients in France. One patient in the 100 mg cohort assigned to placebo had an undetectable baseline HCV-RNA level and therefore was excluded from this study and replaced. One patient in the 50 mg cohort terminated this study at day 10 of the treatment because of a significant increase in liver enzymes. Because the patient was assigned to the placebo group, a replacement was allowed, as per the protocol, and included in the analysis. Eight patients completed this study, 4 in each cohort, 50 and 100 mg. Nine randomized patients with detectable baseline HCV RNA were included in the analysis. Study design and patients' enrollment are shown in Figure 1. Patient's clinical characteristics and main laboratory data are presented in Table 1.

Safety analysis

All adverse events, severity, and association with the treatment, as per blind investigator assessment, are presented in Table 2. A

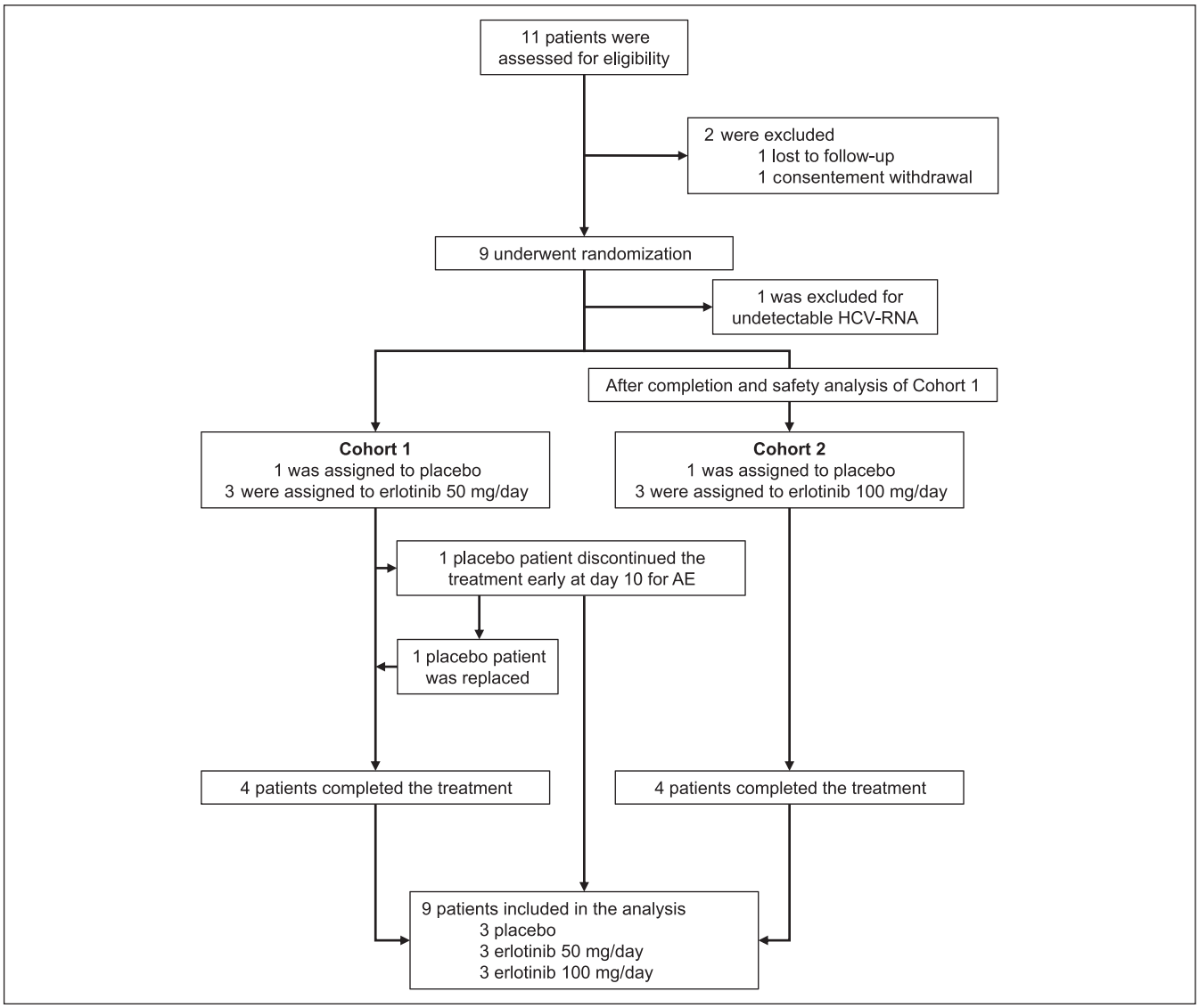

Figure 1. Overview of the study design. The diagram represents enrolled, randomized, and treated patients. All patients, who were HCV-RNA positive and received at least 1 dose of erlotinib or placebo, were included in the analysis. Patients were first enrolled and treated in the cohort 1 (erlotinib 50 mg/d or placebo), and safety data were reviewed by an independent committee before enrolling and treating patients in cohort 2 (erlotinib 100 mg/d or placebo). AE, adverse event; HCV, hepatitis C virus.

Table 1. Patients' clinical and laboratory characteristics

	Placebo (n = 3, pooled data)	Erlotinib 50 mg (n = 3)	Erlotinib 100 mg (n = 3)
Male/female	3/0	3/0	2/1
White/others	3/0	3/0	0/3
Age (yr)	48 (41–56)	48 (47–55)	53 (40–54)
HCV-RNA level log_{10} IU/mL	6.20 (6.07–6.73)	5.39 (5.17–5.70)	5.88 (5.41–6.18)
Genotype 1a/1b	0/3	1/2	0/3
Transient elastography (kPa)	6.6 (5.5–8.8)	4.8 (4.7–5.1)	5.8 (4.0–7.9)
Liver fibrosis F0-F1/F1-F2/F2	2/0/1	3/0/0	2/1/0
ALT (IU/L)	89 (31–140)	62 (45–92)	42 (27–47)
AST (IU/L)	67 (26–78)	38 (20–40)	30 (25–31)
γGT (IU/L)	31 (13–61)	36 (33–163)	19 (11–103)
ALP (IU/L)	104 (63–145)	61 (44–113)	104 (81–109)
Total bilirubin (μmol/L)	9.50 (5.40–10.30)	8.50 (7.00–9.80)	9.90 (6.60–10.90)
Hemoglobin (g/dL)	13.8 (13.5–15.4)	15.6 (14.9–16.9)	14.7 (11.4–15.0)
Platelet (10^9/L)	266 (187–292)	231 (205–241)	256 (205–290)
Creatinine (μmol/L)	58.9 (58.3–67.2)	72.3 (58.9–85.2)	75.6 (54.8–89.0)

ALP, alkaline phosphatase; ALT, alanine aminotransaminase; AST, aspartate aminotransferase; HCV, hepatitis C virus; γGT, gamma-glutamyl transferase.

summary of the safety data and maximum laboratory changes during the trial are summarized in Table 3. One serious (grade 3) adverse event was reported in a patient treated with placebo (liver enzyme elevation), leading to treatment discontinuation. One serious adverse event was reported in a patient treated with erlotinib 100 mg (pericarditis), which was considered not to be treatment-related. No patient receiving erlotinib discontinued the treatment. No major changes in laboratory values were observed. Adverse events were generally mild, and treatments were well tolerated.

Table 2. Reported adverse events according to the treatment group

	Placebo			Erlotinib 50 mg			Erlotinib 100 mg		
Adverse event	**Any grade**	**Drug-related[a]**	**Grade**	**Any grade**	**Drug-related[a]**	**Grade**	**Any grade**	**Drug-related[a]**	**Grade**
Headache	3[b]	No	2[b]	1	No	1	0	No	0
Liver enzymes elevation	1	Yes	3	0			0		
Insomnia	1	No	2	0			0		
Skin rash	1	Yes	1	1	Yes	1	1	Yes	2
Diarrhea	1	Yes	1	0			0		
Flu-like syndrome	1	No	1	0			0		
Dyspepsia	0			1	No	1	0		
Acute pericarditis	0			0			1	No	3[c]
Irritability	0			0			1	No	2[c]
Memory impairment	0			0			1	No	2[c]
Dyspnea	0			0			1	No	1[c]
Fever	0			0			1	No	1[c]
Conjunctivitis	0			0			1	No	1[d]
Tachycardia	0			0			1	No	1[d]
Loss of appetite	0			0			1	No	1[d]

[a]Based on blind investigator assessment.
[b,c,d] Same patient.

Table 3. Summary of adverse events and maximum changes in laboratory tests

	Placebo (n = 3, pooled data)	Erlotinib 50 mg (n = 3)	Erlotinib 100 mg (n = 3)
Treatment discontinuation for AEs	1	0	0
Serious AEs (grade ≥3)	1	0	1
Deaths	0	0	0
AEs	8	3	9
Patients with at least 1 AE	3	2	3
Treatment-emergent AEs (≥2 patients)			
Headache	3	1	0
Skin rash	1	1	1
Diarrhea or dyspepsia	1	1	1
Fever or flu-like syndrome	1	0	1
Mean ± SD of maximum change in laboratory parameters			
ALT (IU/L)	140 ± 200	9 ± 2	11 ± 9
AST (IU/L)	73 ± 88	7 ± 6	7 ± 4
γGT (IU/L)	23 ± 10	−10 ± 18	9.7 ± 8
ALP (IU/L)	−10 ± 8	−6 ± 6	13 ± 4
Bilirubin (μmol/L)	4 ± 2	5 ± 2	4 ± 2
Creatinine (μmol/L)	43 ± 27	20 ± 10	51 ± 20
Hemoglobin (g/dL)	−1.4 ± 1.1	1.1 ± 0.1	−1.4 ± 1.2
Platelet (10^9/L)	−33 ± 18	−23 ± 14	112 ± 176

AEs, adverse events; ALT, alanine aminotransaminase; AST, aspartate aminotransferase; γGT, gamma-glutamyl transferase.

Individual patients' characteristics, baseline laboratory data, adverse events, and maximum changes of safety laboratory data are presented in **Supplementary Table 1** (http://links.lww.com/CTG/A796).

HCV-RNA levels

No significant (≥1 $\log_{10}$) reduction of viral load was observed during the 14-day treatment course in any group. No patient in the placebo group decreased HCV RNA at day 28 (14 days after the end of the treatment). In the group of erlotinib 50 mg, 1 of 3 patients showed an HCV-RNA reduction of more than 0.5 log at day 28, while in the 100 mg group, 2 of 3 patients obtained an HCV-RNA reduction of more than 0.5 log, with 1 patient more than 1 log. Median HCV-RNA $\log_{10}$ fold changes at day 28 were 0.06 in the placebo group, 0.27 in the erlotinib 50 mg/d group, and −0.76 in the erlotinib 100 mg/d group. Virological response data are summarized in Table 4. Individual and median HCV-RNA levels are presented in Figure 2a,b. Individual and median $\log_{10}$ fold changes are shown in Figure 2c,d.

DISCUSSION

DAAs have been shown to be safe and effective in treating CHC and are now the standard of care for patients with HCV infection (3). HCC is a major complication of chronic HCV infection and a main cause of liver-related mortality (1). Although HCV cure after

Table 4. Summary of virological response

	Median HCV-RNA $\log_{10}$ FC at day 14 (end of treatment)	HCV-RNA reduction ≥1 $\log_{10}$ at day 14 (end of treatment)	Median HCV-RNA $\log_{10}$ FC at day 28 (end of follow-up)	HCV-RNA reduction ≥0.5 $\log_{10}$ at day 28 (end of follow-up)	HCV-RNA reduction ≥1 $\log_{10}$ at day 28 (end of follow-up)
Placebo (n = 3, pooled data)	0.08	0	0.06	0	0
Erlotinib 50 mg (n = 3)	−0.03	0	0.27	1	0
Erlotinib 100 mg (n = 3)	−0.12	0	−0.76	2	1

FC, fold changes; HCV, hepatitis C virus.

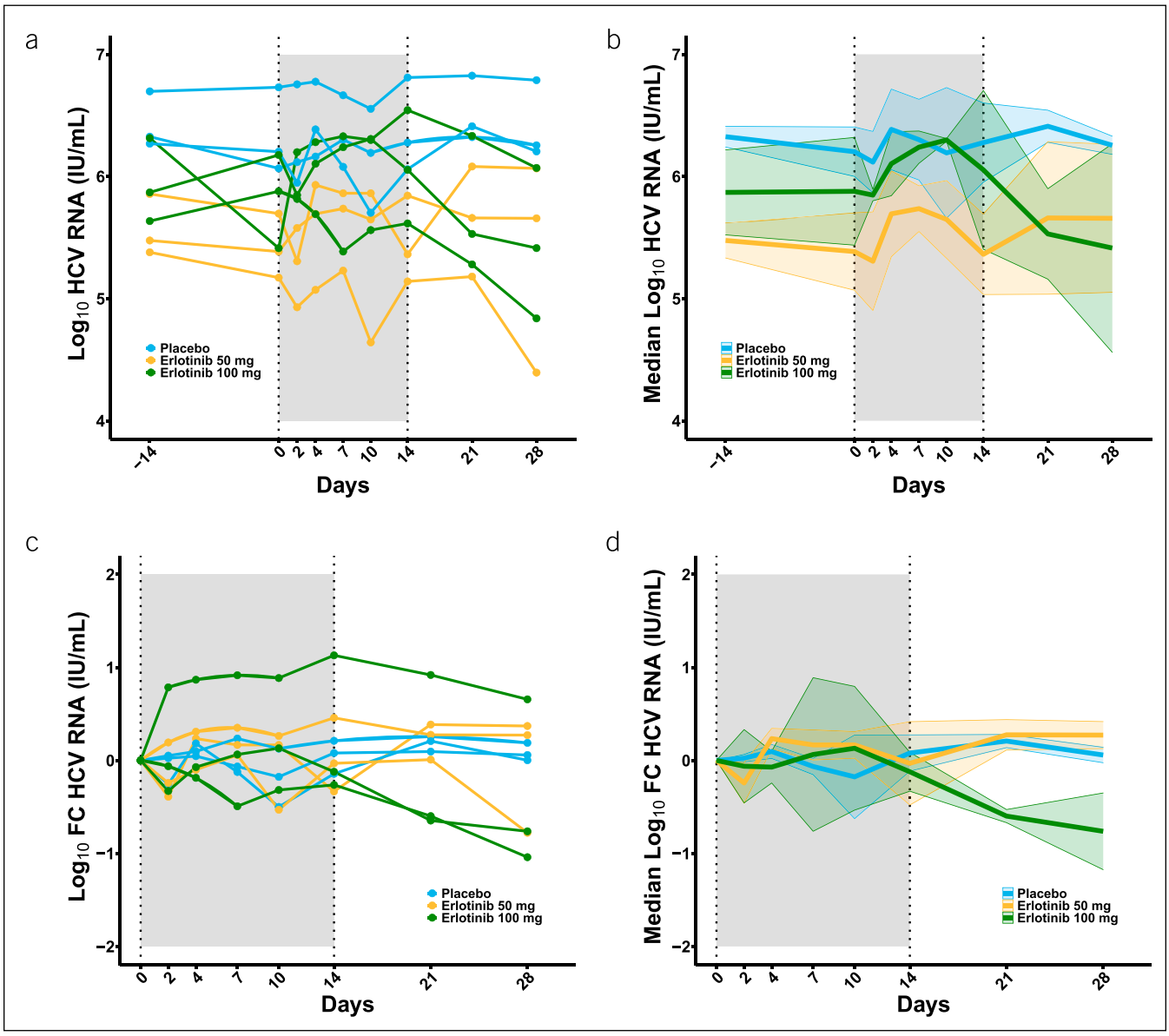

Figure 2. Log_{10} HCV RNA levels and fold changes in patients treated with erlotinib or placebo. (**a**) Individual HCV RNA levels. (**b**) Median HCV RNA levels and median absolute deviation (shaded area) according to the treatment group. (**c**) Individual fold changes in HCV RNA levels. (**d**) Median HCV RNA fold-change levels and median absolute deviation (shaded area) according to the treatment group. The gray area represents the treatment administration period (day 0–14). FC, fold changes; HCV, hepatitis C virus.

DAA treatment reduces HCC incidence, the risk is not eliminated. Chemopreventive drugs would be an important therapeutic tool to reduce HCC occurrence in patients at high risk (28). Preclinical data suggest that EGFR pathway inhibition by erlotinib exerts both an antiviral effect (13) and an HCC chemopreventive effect (15), with the potential to treat HCV and reduce the residual HCC risk at the same time. Erlotinib's metabolism is primarily hepatic, and liver failure and hepatorenal syndrome have been previously reported (29). No pharmacokinetic nor safety data are available in patients with chronic HCV infection.

Our data show that erlotinib is generally well tolerated in patients with HCV infection, with only 1 serious adverse event in the group of erlotinib 100 mg as in the placebo group. No significant liver enzyme elevation was observed in erlotinib-treated patients, and the 1 event of pericarditis in the 100 mg/d group was not considered to be treatment-related and did not lead to treatment discontinuation. The adverse events were generally mild in all the cohorts, and known erlotinib side effects, such as, skin and gastrointestinal toxicity, were observed in all the groups, placebo included, suggesting no increased risk in patients with HCV infection.

This trial could not identify the maximum tolerated dose because it was prematurely terminated, and no dose limiting toxicity has been observed at the highest dose tested (100 mg/d).

According to the study design, patients with liver cirrhosis were excluded and no assumption can be made on this population. The highest fibrosis stage in the erlotinib 100 mg/d group was METAVIR F1-F2, which represents significant chronic liver

damage without liver function reduction or portal hypertension. Hence, further studies are needed to evaluate the safety of erlotinib in patients with chronic liver disease and advanced liver fibrosis.

Regarding the efficacy, a short course of 14 days of erlotinib at 50 or 100 mg/d did not significantly reduce HCV load during treatment, but a significant viral load reduction was observed off-therapy during the follow-up in the group of 100 mg/d. In contrast to the findings observed for erlotinib, a significant reduction in viral load in the first days of treatment is typically observed with the current DAA regimen used to treat HCV.

The time difference of viral activity between erlotinib and DAA is likely because of the different mechanism of action. While DAAs directly inhibit viral replication, erlotinib inhibits viral infection by reducing viral cell entry, which will inhibit cell spread and infection of uninfected cells (5,13,30,31). Therefore, compared with DAAs, the kinetics of viral decline is slower, and a longer treatment duration is likely needed as also shown for the hepatitis delta virus entry inhibitor bulevirtide (32,33). The inhibition of cell reinfection and cell spread and the later onset of the antiviral effect could explain the HCV RNA reduction in the erlonitib 100 mg group observed only after the end of the 14-day treatment course. Indeed, a similar kinetic of viral load changes has been reported for both HCV and hepatitis delta virus entry inhibitors in patients and animal models, with an antiviral effect starting around 2 weeks after the treatment initiation and lasting several days or weeks after the end of treatment (30,32–35).

Although we did not assess a long-term treatment course of erlotinib, these data suggest that erlotinib has a lower antiviral efficacy than the currently approved DAA in the first 2 weeks of treatment. This finding is consistent with data from other clinical trials of HCV entry inhibitors such as a small molecule inhibitor of scavenger receptor B1 (34,35), with no molecule approved in this class so far (5).

Collectively, data from this trial and previous trials suggest that the antiviral efficacy of HCV entry inhibitors in monotherapy is probably inferior to that of approved DAAs. At the same time, the dose-dependent antiviral activity of erlotinib confirms the role of EGFR as an HCV host dependency factor in patients and provides further evidence that EGFR also plays a relevant role in HCV-induced liver disease. It is of interest to note that the magnitude of viral load reduction was similar to the decrease observed in HCV-infected human liver chimeric mice, confirming the validity of this animal model for the study of HCV-host interactions and disease biology (13).

Regarding the efficacy on HCC chemoprevention, this trial was not designed to answer this question, and further studies are needed to determine the role of this drug in the HCC prevention strategy. Data from *in vivo* experiments suggest that the HCC chemopreventive dose of erlotinib is 2-fold to 4-fold lower than the antiviral one (13,15). The data from this trial show the antiviral effect of the 100 mg/d dose, which is a standard dose used in clinical practice, indicating that HCC chemopreventive trials could be designed with lower erlotinib doses (25 or 50 mg/d), which would reduce toxicity risk and increase patient tolerability and compliance.

Limitations of this study were the small sample size, the short treatment course of erlotinib, and the inclusion of mostly patients with genotype 1b CHC. Although the limitation to genotype 1b may reduce the generalization of the results, it is worth noting that HCV genotype 1b is most prevalent worldwide (36) and shows a high association with HCC risk (37).

In conclusion, in this phase Ib, randomized, double-blind, placebo-controlled trial, erlotinib demonstrated to be safe in non-cirrhotic HCV patients and showed an antiviral activity which was markedly lower compared with that of DAAs.

Although this trial was not designed to test the chemopreventive effect of this compound and further studies will need to be conducted to evaluate the value of erlotinib in HCC prevention, the safety data provide a perspective to further study erlotinib as a chemopreventive agent in patients with CHC.

CONFLICTS OF INTEREST

Guarantor of the article: Thomas F. Baumert, MD.

Specific author contributions: A.S.: analyzed the data and wrote the MS. F.H., M.D., C.S.-M., and T.F.B.: designed and supervised the trial. P.S.-N. and C.S.-M.: collected the data. J.L.: uncovered the scientific rationale for the study and revised the MS. C.S.: revised the MS.

Financial support: This work was supported by DGOS-Inserm (AAP-RCT 2012, HUS 5189, A12027MS, ASE12018MSA to T.F.B.), the European Union (EU H2020-HEPCAR #667273 to J.L. and T.F.B. and ERC AdG ERC-AdG-2014-HEPCIR #671231 and ERC PoC-HEPCAN #862551 to T.F.B.), the French Cancer Agency (ARC) and Institut Hospitalo-Universitaire (IHU), Strasbourg (TheraHCC2.0 IHU201901299), the Agence Nationale de Recherche sur le Sida et les hépatites virales (ANRS ECTZ103701, ECTZ131760, ECTZ160436, and ECTZ171594 to J.L. and ANRS ECTZ104017 and ECTZ75178 to T.F.B.), the Fondation de l'Université de Strasbourg (HEPKIN) (TBA-DON-0002) and the Inserm Plan Cancer 2019–2023, the US National Institute of Health (R01CA233794 to T.F.B.), and the Institut Universitaire de France (IUF to T.F.B.). This work of the Interdisciplinary Thematic Institute IMCBio, as part of the ITI 2021–2028 program of the University of Strasbourg, CNRS, and Inserm, was supported by IdEx Unistra (ANR-10-IDEX-0002) and by the SFRI-STRAT'US project (ANR 20-SFRI-0012) and EUR IMCBio (ANR-17-EURE-0023) under the framework of the French Investments for the Future Program.

Potential competing interests: Inserm and the University of Strasbourg have filed a patent (WO2013024158A1) on host cell kinases as targets for antiviral therapy against HCV infection.

ACKNOWLEDGMENTS

We thank Jerome de Sèze, MD, PhD, Alexandre Bolle, PharmD, and the team of the CIC Strasbourg for their support; Samira Fafi-Kremer, PharmD, Ph.D. (Laboratoire de Virologie, CHU Strasbourg) for support and advice during the initiation of this study; and Sébastian Vuola, MD for his assistance in data collection. We also thank Anne Hutt-Clauss, PharmD, Service de Pharmacie, CHU Strasbourg for her support and help and the Laboratoire de Virologie, CHU Strasbourg, for performing viral load analyses.

LIVER

Study Highlights

WHAT IS KNOWN

- ✓ Current antiviral agents for hepatitis C do not eliminate hepatocellular carcinoma risk.
- ✓ EGFR is a host factor for HCV infection and replication.
- ✓ EGFR has been suggested to be a target for HCC chemoprevention.
- ✓ In preclinical *in vivo* studies, erlotinib demonstrated to have both antiviral and HCC chemopreventive activities.
- ✓ Erlotinib has hepatic metabolism and no safety data are available in patients with HCV infection.

WHAT IS NEW HERE

- ✓ In this phase Ib prospective double-blind randomized placebo-controlled study, erlotinib demonstrated to be safe in non-cirrhotic HCV patients.
- ✓ Antiviral activity confirms a functional role of EGFR as an HCV host factor in patients.
- ✓ The results support further studies to investigate erlotinib as an HCC chemopreventive drug in patients with liver disease due to chronic hepatitis C.

REFERENCES

1. Asrani SK, Devarbhavi H, Eaton J, et al. Burden of liver diseases in the world. J Hepatol 2019;70:151–71.
2. Zoulim F, Liang TJ, Gerbes AL, et al. Hepatitis C virus treatment in the real world: Optimising treatment and access to therapies. Gut 2015;64:1824–33.
3. European Association for the Study of the Liver, Clinical Practice Guidelines Panel, EASL Governing Board representative. EASL recommendations on treatment of hepatitis C: Final update of the series. J Hepatol 2020;73:1170–218.
4. Chung RT, Baumert TF. Curing chronic hepatitis C—the arc of a medical triumph. N Engl J Med 2014;370:1576–8.
5. Baumert TF, Berg T, Lim JK, et al. Status of direct-acting antiviral therapy for hepatitis C virus infection and remaining challenges. Gastroenterology 2019;156:431–45.
6. Hayes CN, Zhang P, Zhang Y, et al. Molecular mechanisms of hepatocarcinogenesis following sustained virological response in patients with chronic hepatitis C virus infection. Viruses 2018;10:531.
7. Hamdane N, Juhling F, Crouchet E, et al. HCV-induced epigenetic changes associated with liver cancer risk persist after sustained virologic response. Gastroenterology 2019;156:2313–29.e7.
8. Kanwal F, Kramer J, Asch SM, et al. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. Gastroenterology 2017;153:996–1005.e1.
9. Kanwal F, Kramer JR, Asch SM, et al. Long-term risk of hepatocellular carcinoma in HCV patients treated with direct acting antiviral agents. Hepatology 2020;71:44–55.
10. El-Serag HB, Kanwal F, Richardson P, et al. Risk of hepatocellular carcinoma after sustained virological response in Veterans with hepatitis C virus infection. Hepatology 2016;64:130–7.
11. Tanaka Y, Ogawa E, Huang CF, et al. HCC risk post-SVR with DAAs in east asians: Findings from the REAL-C cohort. Hepatol Int 2020;14:1023–33.
12. Owusu Sekyere S, Schlevogt B, Mettke F, et al. HCC immune surveillance and antiviral therapy of hepatitis C virus infection. Liver Cancer 2019;8:41–65.
13. Lupberger J, Zeisel MB, Xiao F, et al. EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy. Nat Med 2011;17:589–95.
14. Zona L, Lupberger J, Sidahmed-Adrar N, et al. HRas signal transduction promotes hepatitis C virus cell entry by triggering assembly of the host tetraspanin receptor complex. Cell Host Microbe 2013;13:302–13.
15. Fuchs BC, Hoshida Y, Fujii T, et al. Epidermal growth factor receptor inhibition attenuates liver fibrosis and development of hepatocellular carcinoma. Hepatology 2014;59:1577–90.
16. Brenndorfer ED, Karthe J, Frelin L, et al. Nonstructural 3/4A protease of hepatitis C virus activates epithelial growth factor-induced signal transduction by cleavage of the T-cell protein tyrosine phosphatase. Hepatology 2009;49:1810–20.
17. Roca Suarez AA, Baumert TF, Lupberger J. Beyond viral dependence: The pathological consequences of HCV-induced EGF signaling. J Hepatol 2018;69:564–6.
18. Hoshida Y, Villanueva A, Kobayashi M, et al. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. N Engl J Med 2008;359:1995–2004.
19. Lupberger J, Croonenborghs T, Roca Suarez AA, et al. Combined analysis of metabolomes, proteomes, and transcriptomes of hepatitis C virus-infected cells and liver to identify pathways associated with disease development. Gastroenterology 2019;157:537–51 e539.
20. Garcia-Lezana T, Lopez-Canovas JL, Villanueva A. Signaling pathways in hepatocellular carcinoma. Adv Cancer Res 2021;149:63–101.
21. Benkheil M, Paeshuyse J, Neyts J, et al. HCV-induced EGFR-ERK signaling promotes a pro-inflammatory and pro-angiogenic signature contributing to liver cancer pathogenesis. Biochem Pharmacol 2018;155:305–15.
22. Abu Dayyeh BK, Yang M, Fuchs BC, et al. A functional polymorphism in the epidermal growth factor gene is associated with risk for hepatocellular carcinoma. Gastroenterology 2011;141:141–9.
23. Tanabe KK, Lemoine A, Finkelstein DM, et al. Epidermal growth factor gene functional polymorphism and the risk of hepatocellular carcinoma in patients with cirrhosis. JAMA 2008;299:53–60.
24. Suenaga M, Yamada S, Fujii T, et al. A functional polymorphism in the epidermal growth factor gene predicts hepatocellular carcinoma risk in Japanese hepatitis C patients. Onco Targets Ther 2013;6:1805–12.
25. King LY, Johnson KB, Zheng H, et al. Host genetics predict clinical deterioration in HCV-related cirrhosis. PLoS One 2014;9:e114747.
26. Jin H, Shi Y, Lv Y, et al. EGFR activation limits the response of liver cancer to lenvatinib. Nature 2021;595:730–4.
27. Roskoski R Jr. Small molecule inhibitors targeting the EGFR/ErbB family of protein-tyrosine kinases in human cancers. Pharmacol Res 2019;139:395–411.
28. Li S, Saviano A, Erstad DJ, et al. Risk factors, pathogenesis, and strategies for hepatocellular carcinoma prevention: Emphasis on secondary prevention and its translational challenges. J Clin Med 2020;9:3817.
29. Saif MW. Hepatic failure and hepatorenal syndrome secondary to erlotinib. Safety reminder. JOP 2008;9:748–52.
30. Mailly L, Xiao F, Lupberger J, et al. Clearance of persistent hepatitis C virus infection in humanized mice using a claudin-1-targeting monoclonal antibody. Nat Biotechnol 2015;33:549–54.
31. Zeisel MB, Fofana I, Fafi-Kremer S, et al. Hepatitis C virus entry into hepatocytes: Molecular mechanisms and targets for antiviral therapies. J Hepatol 2011;54:566–76.
32. Bogomolov P, Alexandrov A, Voronkova N, et al. Treatment of chronic hepatitis D with the entry inhibitor myrcludex B: First results of a phase Ib/IIa study. J Hepatol 2016;65:490–8.
33. Loglio A, Ferenci P, Uceda Renteria SC, et al. Safety and effectiveness of up to 3 years' bulevirtide monotherapy in patients with HDV-related cirrhosis. J Hepatol 2021;76:464–9.
34. Rowe IA, Tully DC, Armstrong MJ, et al. Effect of scavenger receptor class B type I antagonist ITX5061 in patients with hepatitis C virus infection undergoing liver transplantation. Liver Transpl 2016;22:287–97.
35. Sulkowski MS, Kang M, Matining R, et al. Safety and antiviral activity of the HCV entry inhibitor ITX5061 in treatment-naive HCV-infected adults: A randomized, double-blind, phase 1b study. J Infect Dis 2014;209:658–67.
36. Gower E, Estes C, Blach S, et al. Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol 2014;61:S45–S57.
37. Bruno S, Crosignani A, Maisonneuve P, et al. Hepatitis C virus genotype 1b as a major risk factor associated with hepatocellular carcinoma in patients with cirrhosis: A seventeen-year prospective cohort study. Hepatology 2007;46:1350–6.