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Lumacaftor-ivacaftor effects on cystic fibrosis-related liver involvement in adolescents with homozygous F508 del-CFTR

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Short title: Orkambi and liver

Highlights

The effects of lumacaftor-ivacaftor on CFTR associated liver disease remain unclear.

We investigated the effect of lumacaftor-ivacaftor in Cystic Fibrosis patients with liver involvement.

Gammaglutamyl transferase level improved significantly during 1 year follow up.

This was not correlated with any change in liver imaging.

These findings may suggest a potential benefit of CFTR modulators on CF liver disease.

Abstract

Background. The effects of lumacaftor-ivacaftor on cystic fibrosis transmembrane conductance regulator (CFTR)-associated liver disease remain unclear. The objective of the study was to describe the effect of this treatment on features of liver involvement in a cystic fibrosis (CF) adolescent population homozygous for F508del.

Methods. Clinical characteristics, liver blood tests, abdominal ultrasonography (US), and pancreas and liver proton density fat fraction (PDFF) by magnetic resonance imaging, were obtained at treatment initiation and at 12 months for all patients. Biomarkers of CFTR activity (sweat chloride test, nasal potential difference, and intestinal current measurement) were assessed at initiation and at 6 months therapy.

Results. Of the 37 patients who started ivacaftor/lumacaftor treatment, 28 were eligible for analysis. In this group, before treatment initiation, 4 patients were diagnosed with multinodular liver and portal hypertension, 19 with other forms of CF liver involvement, and 5 with no signs of liver involvement. During treatment, no hepatic adverse reactions were documented, and no patient developed liver failure. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gammaglutamyl transferase (GGT) decreased significantly following initiation of lumacaftor-ivacaftor, and remained so after 12 months treatment. This was not correlated with changes in clinical status, liver and pancreas US and PDFF, fecal elastase, or lumacaftor-ivacaftor serum levels. The most “responsive” patients demonstrated a significant increase in biomarkers of CFTR activity.

Conclusions. These results may suggest a potential beneficial effect of CFTR modulators on CF liver disease and warrant further investigation in larger, prospective studies.

Keywords: CFTR modulator; Cystic-fibrosis liver disease; hepatobiliary; cirrhosis; liver enzymes; pediatrics

Abbreviations:

AST: aspartate aminotransferase

ALT: alanine aminotransferase

BMI: body mass index

CF: cystic fibrosis

CFLD: cystic fibrosis associated liver disease

ppFEV₁: percent predicted forced expiratory volume in one second

GGT: gamma-glutamyl transferase

ICM: intestinal current measurement

MRI: magnetic resonance imaging

NPD: nasal potential difference

Pdff: proton density fat fraction

SCT: Sweat Chloride Test

US: ultrasonography

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Introduction

Cystic fibrosis (CF) is a progressive genetic disease affecting around 100,000 children and adults worldwide [1]. This disease is due to mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) whose most common mutation is p.Phe508del (F508del). This mutation causes abnormal folding of the *CFTR* protein and its early degradation by the proteasome [2,3]. Correction of the F508del processing defect in F508del homozygous patients by the therapeutic association of lumacaftor, a *CFTR* corrector, and ivacaftor, a *CFTR* potentiator, improves lung function and nutritional status and reduces incidence of pulmonary exacerbations [4–7]. Determining the impact of *CFTR* modulators on extrapulmonary disease is of critical importance, particularly on liver disease, the third cause of death after respiratory failure and transplantation-related complications in patients with CF [1]. Liver involvement in CF patients shows considerable variability in expression and severity, including hepatobiliary involvement (such as focal biliary fibrosis, multilobular cirrhosis), obliterative portal venopathy, steatosis and other liver abnormalities resulting from infections, long-term antibiotic therapy, diabetes, nutritional deficiencies, hypoxemia, or hepatic congestion from right-side heart failure [8]. However, the pathogenesis of hepatobiliary disease in CF and the reason why focal biliary fibrosis progresses to multilobular cirrhosis in a minority of 5–10% patients remain poorly understood [9,10]. A sustained increased level of gammaglutamyl transferase (GGT) is the unique predictive biological marker of progression towards cirrhosis that has been identified to date [11,12]. Importantly, no therapy (including ursodeoxycholic acid) has proven effective to prevent or halt the progression of liver disease towards cirrhosis and portal hypertension [13].

Paradoxically, very few data documenting liver outcomes whilst on *CFTR* modulators have been reported, probably because severe liver disease was an exclusion criterion in *CFTR* modulator trials. A recent study including 39 homozygous F508del patients reported a decrease in serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gammaglutamyl transferase (GGT) and bilirubin a year after lumacaftor-ivacaftor initiation and a second study including 20 F508del homozygous patients suggested an improvement in hepatic steatosis [14]. There has been no study reported to date, focusing on the evolution of liver outcomes in patients treated by lumacaftor-ivacaftor therapy.

The objective of the study was to describe the effect of lumacaftor-ivacaftor treatment in a homozygous F508del adolescent population, in particular on features of liver involvement in CF. Changes in respiratory and pancreatic status and their correlation to *CFTR* epithelial function restoration, liver biochemistry, abdominal Ultra Sound(US) and proton density fat

fraction (PDFF) of the liver measured by magnetic resonance imaging (MRI), were also evaluated.

Patients and Methods

Study population

This study was conducted in the pediatric CF-center of the University Hospital Necker-Enfants Malades (Paris, France). All F508del homozygous patients aged 12 years and older, with a sweat test above 60 mmol/L who started lumacaftor-ivacaftor therapy between August 1, 2013 and December 31, 2017 were included in the study. Patients who received lumacaftor-ivacaftor in our center were part of the TRAFFIC Vertex and the BIO-CFTR trial (ClinicalTrials.gov NCT 02965326), approved by the Ile de France 2 Ethics Committee. Informed consent was obtained from legal guardians for each child participating in the studies, and included the possibility to conduct post-hoc analysis on the data collected during the studies. Patients who could not complete the evaluation at 12 months were excluded from the analysis.

Investigations

Patients were evaluated at initiation of lumacaftor-ivacaftor (M0) and at 12 months (M12). Main clinical features including body mass index (BMI), percent predicted expiratory volume in 1 second (ppFEV₁) and number of days on antibiotics were recorded. Liver tests, abdominal US, liver and pancreas MRI were performed at M0 and M12. For the purpose of the study, liver tests were also recorded at 12 months preceding the initiation of lumacaftor-ivacaftor (M-12) and 6 months after initiation (M6). We also considered the AST to Platelet Ratio Index (APRI) and Fibrosis-4 index (FIB-4), commonly used as surrogates for advanced liver fibrosis [15–17]. The upper limits of normal (ULN) for ALT were set at 32 IU/L and 26 IU/L, for AST at 42 IU/L and 37 IU/L, for GGT at 25 IU/L and 17 IU/L, in males and females, respectively [18].

Sweat Chloride Test (SCT), fecal elastase and blood concentrations of lumacaftor and ivacaftor (peak and residual measured by Liquid Chromatography/Mass Spectrometry) were assessed at M0 and M6 as previously described [7,19–21]. Additionally, patients completed a Nasal Potential Difference test (NPD) and rectal biopsy for Intestinal Short-Circuit (Isc) current measurement (ICM) at initiation and 6 months treatment to assess CFTR activity [7].

All US examinations were reviewed by a single radiologist (JD) to minimize inter-observer variability. Hepatomegaly and splenomegaly were defined according to previously published ranges [22,23].

All MRI scans were reviewed by 2 radiologists in consensus (JD and LB). MRI was performed on a 1.5T scanner (Optima MR450, General Electric Healthcare, Milwaukee, USA), including axial Fat-Suppressed T2-weighted sequences with Periodically Rotated Overlapping Parallel Lines with Enhanced Reconstruction (PROPELLER) and quantification of PDFFF, using freehand whole-liver or whole-pancreas Region Of Interest (ROI) (region of interest) on Iterative Decomposition of water and fat with Echo Asymmetry and Least Squares estimation). IDEAL-IQ sequences (IDEAL-IQ: Cut-off values for hepatic steatosis grades 1, 2 and 3 were 4.1, 15.7 and 20.9, respectively[24].

Patients were assigned to one of the three following groups according to the phenotypic classification adapted from Debray et al presented in **Table S1** : multinodular liver (based on US definition), liver involvement without multinodular liver, or no evidence of liver involvement [25]. Portal hypertension was considered in any patient with splenomegaly at physical exam and/or imaging, with signs of hypersplenism such as thrombocytopenia (platelets < 150 G/L) and/or a progressive decline overtime in the platelet count, evidence of portosystemic collaterals at ultrasound or MRI, reversal of the portal flow at US, and/or the presence of gastrointestinal varices or hypertensive gastropathy whenever an eosogastroduodenoscopy was performed [25, 26]. Liver involvement without multinodular liver was defined by 1 or more of the following features: hepatomegaly, abnormal ALT or GGT (> 1.5 x ULN), imaging abnormalities (US increased homogeneous or heterogeneous echogenicity pattern), hepatic steatosis or fibrosis biopsy proven, and/or isolated portal hypertension. Patients with normal examination, imaging, ALT and GGT were considered to have no liver involvement.

Statistical analysis

Changes in the mean AST, ALT, GGT, and bilirubin levels at the four endpoints (M-12, M0, M6, M12) from the 28 patients included in the analysis are presented as median and interquartile (Q1-Q3). They were analyzed with repeated measures ANOVA, and the Bonferroni's multiple comparison test was used to compare all pairs of endpoints. Wilcoxon matched-pairs signed rank test was used to compare the differences in weight, height, BMI Z-score, ppFEV₁, and CFTR activity biomarkers (SCT, NPD and ICM measurements) before and after the introduction of lumacaftor-ivacaftor. A Spearman Rank correlation test was

used to evaluate the correlations between measures of CFTR biomarkers and changes in GGT, ALT, and PDFF across time.

All the statistical tests were performed on SAS version 9.3 software (SAS Institute, Inc, Cary, NC). They were two-sided with a significant threshold of 5%. Graphics were performed on GraphPad prism software v5.03 (La Jolia, California).

Results

1. Main characteristics of the study population

Of the 37 patients who started on lumacaftor-ivacaftor treatment in our center, 28 were eligible for analysis. The nine patients excluded from the analysis included five patients who stopped the treatment prematurely between 6 and 11 months because of lack of efficacy (n=3), chest tightness (n=1), interaction with anti-mycobacterial treatment (n=1), and 4 patients who moved to another center during the study period (**Figure 1**). The characteristics of all patients, including the 9 patients excluded from the analysis, are presented in **Table S2**.

Among the 28 patients eligible for analysis (13 males, 15 females), 4 patients were diagnosed with multinodular liver and portal hypertension, 19 with other forms of CF liver involvement, and 5 with no signs of liver involvement. Their characteristics and the results of their liver assessment at M0 and M12 are presented in **Tables 1** and **2**, respectively. Patients were on average 14.4 years old at inclusion. One patient had CF-related diabetes.

There was no significant improvement for any clinical or respiratory function parameter following lumacaftor-ivacaftor therapy, apart for BMI. Fecal elastase was undetectable at the initiation of the treatment and did not vary over time. Fourteen patients had quantification of pancreas PDFF by MRI at both M0 and M12. The median (Q1-Q3) pancreas PDFF did not change significantly between M0 (64% (42-74)) and M12 (72% (51-77)).

The values of the SCT (n=26, p<0.01), and the response to forskolin at ICM (n=12, p=0.04) were significantly improved at M6, whereas changes in $\Delta\text{lowCl}^-/\text{Iso}$ values did not reach statistical significance (n=17, p=0.06) (**Table 1**). Peak and residual blood concentrations of lumacaftor and ivacaftor were obtained in 14 patients at M6, and were similar across groups (**Table S3**).

2. Liver outcomes under lumacaftor-ivacaftor therapy

No hepatic adverse events occurred during treatment, therefore neither drug withdrawal nor dose reduction were necessary. Follow up included observation for any possible significant

rise in transaminases or in serum bilirubin levels (up to 3 times the ULN), or progression to liver failure or liver decompensation with ascites. None of these were observed during the follow up period.

GGT levels had significantly decreased at 12 months following introduction of treatment (**Table 2, Figure 2**). The same profile was observed for ALT, AST and bilirubin in the whole cohort and in the large group of patients with liver involvement without multinodular liver.

Twenty-three patients underwent abdominal US at both M0 and M12. US findings remained unchanged in 19 patients. In the 4 remaining patients, US examinations showed: resolution of the pre-treatment abnormalities of the liver pattern in 3 (i.e homogenous increased echogenicity (n=1) or heterogeneous pattern (n=2), and appearance of homogeneous increased echogenicity of the liver in 1 (**Figure S1**).

Thirteen patients underwent quantification of liver PDFF at both M0 and M12. The median (Q1-Q3) liver PDFF values did not change significantly between M0 (7% (5-10)) and M12 (6% (5-8)) (**Figure 3**). However, interestingly, 2 patients demonstrated a marked decrease in PDFF values from grade 3 to grade 1 (from 28% to 7% and from 21% to 7% respectively), despite unchanged US findings (normal echogenicity in one and increased homogeneous echogenicity in the other) between M0 and M12. These 2 patients also normalized GGT and ALT levels after initiation of therapy and were considered as “responders” based on a marked increase in ppFEV₁ (+17% and +19% respectively) and a significant improvement in $\Delta\text{LowCl}^-/\text{Iso}$ values (-13.8 mV and -3.8 mV respectively). By contrast, a 13-year-old male showed an increased PDFF value from 6% to 13% in association with the appearance of increased homogeneous echogenicity on US at M12. He was considered as a non-responder based on minimal improvement in lung function.

Changes in ALT and GGT were not correlated with modifications in liver imaging (PDFF, US echogenicity), nutrition (BMI z-score), respiratory disease (number of antibiotic days, ppFEV₁), pancreatic status (fecal elastase, pancreas PDFF) or CFTR biomarkers (chloride sweat concentration, $\Delta\text{LowCl}^-/\text{Iso}$ and $\Delta\text{Forskoline}$) (**Table S4**).

Discussion

This study is the first to provide a comprehensive assessment of liver involvement over a 12 month period following the initiation of lumacaftor-ivacaftor. Serum levels of GGT, AST, ALT, Bb and APRI scores improved significantly from initiation of therapy and remained lower until last follow-up at 12 months. Those changes were not correlated with changes in liver or pancreas PDFF, lumacaftor-ivacaftor serum levels, fecal elastase, antibiotic use and

biomarkers of CFTR activity. Importantly, in the patients with cirrhosis no hepatic adverse reactions were documented, and no patient developed liver failure at full dose.

Results of liver tests have previously been reported in randomized controlled studies of CFTR modulators to assess hepatic adverse events [4,27]. These studies, which reported increased serum ALT/AST levels in 5% of treated patients, excluded patients with cirrhosis. We did not observe any liver-related adverse event in the 4 adolescents with multinodular liver and portal hypertension who received lumacaftor-ivacaftor at full dose. Two had serum levels of lumacaftor-ivacaftor monitored, which remained within previously reported ranges. This observation is in line with a recent report of 4 pediatric F508del homozygous patients suffering from severe liver disease who were initiated with half a dose of ivacaftor-lumacaftor. Liver parameters remained stable during the initial study period and patients are currently being investigated on a full-dose regimen [28].

Overall, we observed a significant decrease in AST, ALT, GGT and Bb levels during the first year following initiation of lumacaftor-ivacaftor therapy, whereas they were persistently higher during the 12 months preceding the initiation of therapy. This profile was observed at the statistical level in the most important group of patients, with liver involvement without multinodular liver (n=19). Interestingly enough, the decrease was more pronounced in the group of patients with multinodular liver and portal hypertension, than in the groups of patients with other forms of CF liver involvement, or no liver involvement. Indeed, one of the most relevant findings in this study is the reduction in GGT in multinodular liver group from a mean of 50 to 23IU/L. The limited number of patients does not allow to analyse the improvement of liver tests at 12 months follow up in the different groups. These results warrant further investigation with larger sample size in each group to confirm improvement of liver tests in patients with liver disease.

Interestingly, a similar decrease in ALT and GGT was reported by Paluck and Linnane who studied the evolution of 39 homozygous F508del patients with repeated measurements of liver tests before and after initiation at 3, 6, 9 and 12 months [29]. Interestingly, the APRI and FIB-4 indexes which are surrogates of advanced liver fibrosis also decreased significantly [30,31].

We investigated the potential mechanisms underlying this decrease in liver tests. It is unlikely to be related to a decrease in hepatic steatosis as there was no correlation either with liver PDFF changes or evidence of steatosis by US. Thus, we do not confirm the observation by Kutney et al of a reduction in hepatic steatosis based on MRI-PDFF in patients treated with

lumacaftor-ivacaftor [14]. However, as only 2 of our patients had a liver PDFF at grade 2 or more, this might limit our ability to show significant decreases in steatosis.

Given that increased GGT levels are a feature of cholangiopathy, we propose that our observed decrease in GGT levels might reflect reduced biliary injury. Indeed, CFTR expression in the hepatobiliary system is restricted to the apical membrane of cholangiocytes lining the intra- and extrahepatic bile ducts. Studies, recently reviewed by Debray et al [10] and Fiorotto et al [32] support the hypothesis that the loss of CFTR in the biliary epithelium not only alters the Cl⁻ driving force necessary for ductular biliary secretion but also affects innate immune response to endotoxins coming from the intestine. This in turn triggers a pro-inflammatory response, leading to peribiliary inflammation and fibrosis. There is also evidence that the loss of CFTR in the intestine promotes gut dysbiosis, and therefore intestinal inflammation, endotoxemia, and cholangiopathy [33]. We thus hypothesize that the decrease in GGT levels with lumacaftor-ivacaftor therapy results, at least partially, from correction of CFTR in the biliary epithelium and in the intestinal mucosa. Indeed, reduced intestinal inflammation with lumacaftor-ivacaftor was recently reported in CF patients [34]. Strikingly, the most “responsive” patients in our study also demonstrated a significant increase in CFTR activity following initiation of lumacaftor-ivacaftor, in sweat glands, rectal mucosa and at a nearly significant level in the nasal mucosa. Moreover NPD and ICM changes provided the highest correlation coefficient with GGT and ALT changes but this did not reach statistical significance, possibly due to the limited number of patients. An additional benefit of lumacaftor-ivacaftor might also be the prevention of obliterative portal venopathy, often associated with chronic inflammation [35]. Further studies assessing serum or intestinal bile acids, FGF-19 or C4 levels may provide mechanistic insights to explain the decrease in GGT levels with CFTR modulator therapy.

The main limitations of this study are its retrospective design and the absence of a control group which precludes conclusions regarding causality between lumacaftor-ivacaftor therapy and the observed results. However, the evolution of liver enzymes over time, from stable levels during the year before treatment, to lower levels following treatment, would strongly suggest a positive effect of lumacaftor-ivacaftor therapy on CFTR-related cholangiopathy. Furthermore, outcome measures were not statistically adjusted for multiple comparisons. Thus, the results of the present study should be interpreted as hypothesis-generating only, and further investigation in dedicated trials are warranted. Finally, although the decrease in liver tests was most pronounced in the 4 patients with multinodular liver and portal hypertension, their values at initiation of treatment were lower than those reported in other patients with

more advanced disease [16]. It currently remains unclear whether the lumacaftor-ivacaftor combination may also have a beneficial effect in patients with more advanced disease.

In conclusion, this study shows that lumacaftor-ivacaftor therapy in F508del homozygous adolescents is associated with a sustained decrease of liver blood tests, especially GGT. These results are suggestive of a potential beneficial effect of CFTR modulators on CF liver disease and larger prospective studies are warranted to confirm these findings and evaluate if CFTR modulators can indeed change the course of CF liver disease for the better.

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Contributors

David Drummond participated in data collection, carried out the analysis, drafted the initial manuscript, and approved the final manuscript as submitted; Jérémy Dana and Laureline Berteloot coordinated the imaging data collection, participated in data collection and interpretation, revised the manuscript critically, and approved the final manuscript as submitted; Matthieu Cornet performed the imputation data analysis. Elena K. Schneider-Futschik performed the lumacaftor-ivacaftor concentration measures; Frédérique Chedevergne, Céline Bailly-Botuha, Thao Nguyen-Khoa, Muriel Le Bourgeois, participated to data collection and revised the manuscript. Muriel Girard participated in the conception of the study, in data interpretation, revised the manuscript critically, and approved the final manuscript as submitted; Dominique Debray participated in the conception of the study, participated in data interpretation, revised the manuscript critically, and approved the final manuscript as submitted; Isabelle Sermet conceived the study, enrolled the patients, performed the study and collected the data as principal investigator, participated in data analysis and interpretation, revised the manuscript critically, and approved the final manuscript as submitted;

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All authors have approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Figure legends

Figure 1. Flow-chart of the patients included in the study.

ITT: intention to treat.

Figure 2. Evolution of mean AST, ALT, GGT, and bilirubin levels before and after introduction of lumacaftor-ivacaftor for patients included in the per protocol analysis.

AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT gamma-glutamyl transferase; Mo: months. Only patients with no missing value at any endpoint are represented on the graphs. The number of patients was n=25 for AST; n=26 for ALT; n=26 for GGT, and n=25 for bilirubin. Dotted lines represent the upper limit of normal values. $p < 0.001$ for all intergroup comparisons; * $p < 0.05$ for Bonferroni's multiple comparison test.

Figure 3. Evolution of the liver proton density fat fraction (PDFF) of the liver before (M0) and 12 months after (M12) initiation of lumacaftor-ivacaftor therapy.

Table 1. Characteristics of patients before and 12 months after initiation of lumacaftor-ivacaftor therapy.

Characteristics Median (Q1;Q3)	All patients n=28			Multinodular liver n=4		Liver involvement without multinodular liver n=19		No liver involvement n=5	
	M0	M12	p	M0	M12	M0	M12	M0	M12
Sex M/F	13/15			2/2		10/9		1/4	
Age	14.4 (13.0;16.2)	15.4 (14.8;17.5)		14.3 (13.0;17.0)	15.2 (14.0;18.3)	14.3 (12.8;14.8)	15.4 (14.7;16.2)	15.4 (12.2;18.5)	17.2 (13.6;19.5)
Weight (Z-score)	-0.6 (-0.9;-0.1)	-0.4 (-0.7;0.2)	<0.01	-0.4 (-0.8;-0.1)	0.0 (-0.5;0.2)	-0.5 (-0.9;0.1)	-0.2 (-0.7;0.3)	-0.7 (-2.0;-0.5)	-0.7 (-1.3;-0.4)
Height (Z-score)	-0.5 (-1.0;0.2)	-0.7 (-1.3;0.2)	0.58	-0.3 (-2.1;1.1)	-0.3 (-1.9;0.8)	-0.6 (-0.9;0.2)	-0.7 (-1.3;0.1)	-0.2 (-0.8;0.1)	-0.2 (-0.9;0.3)
Body mass index (Z-score)	-0.6 (-1.2;0.1)	-0.3 (-0.9;0.1)	<0.01	-0.7 (-1.3;0.7)	-0.4 (-0.4;0.7)	-0.5 (-1.1;0.1)	-0.1 (-0.9;0.5)	-1.2 (-2.0;-0.6)	-1.0 (-1.5;-0.3)
Days of antibiotics (12 months before/after)	60 (30;110)	41 (15;75)	0.07	67 (30;155)	45 (4;154)	75 (30;135)	45 (15;75)	45 (4;45)	30 (8;87)
ppFEV ₁ (%)	69 (51;88)	73 (48;95)	0.12	64 (56;83)	55 (44;89)	71 (51;89)	86 (51;95)	76 (41;89)	67 (46;92)
SCT* (mmol/L)	104 (94;110) (n=26)	78 (66;99) (n=26)	<0.01	104 (98;108) (n=4)	56 (31;72) (n=3)	104 (34;110) (n=17)	82 (66;102) (n=18)	104 (92;113) (n=5)	87 (67;100) (n=5)
ΔNPD Low Cl ⁻ /Iso* (mV)	1.0 (-4.9;2.9) (n=17)	-4.5 (-7.6;0.2) (n=17)	0.06	2.1 (0.1;3.1) (n=3)	0.5 (-7.0;5.0) (n=3)	1.4 (-5.8;4.7) (n=12)	-6.2 (-7.9;-2.4) (n=11)	-2.3 (-5.7;1.2) (n=2)	1.7 (-4.6;6) (n=3)
Δ Forskolin* (μA/cm ²)	2.3 (-3.6;4.3) (n=12)	10.8 (1.6;14.2) (n=12)	0.04	3.6 (n=1)	-4.0 (n=1)	2.3 (-4.5;4.5) (n=10)	12.8 (7.2;18.1) (n=9)	-3.1 (n=1)	4.5 (-0.8;9.7) (n=2)

Mo: months. ppFEV₁: percent predicted forced expiratory volume in one second; SCT: Sweat Chloride Test; ΔNPD Low Cl⁻/Iso: sum of the response to perfusion of low Cl⁻ solution and 10 μM isoproterenol during the nasal potential difference test (NPD); Δ Forskolin: response to forskolin at the Intestinal Current Measurement (ICM). * The SCT, NPD and ICM were performed at M6.

Table 2. Liver assessment of patients before and 12 months after initiation of lumacaftor-ivacaftor therapy.

Characteristics Median (Q1;Q3)	All patients n=28			Multinodular liver n=4		Liver involvement without multinodular liver n=19			No liver involvement n=5	
	M0	M12	p	M0	M12	M0	M12	p	M0	M12
GGT (IU/L)	19 (13;42)	14 (11;18)	<0.01	50 (23;98)	23 (18;52)	17 (13;45)	14 (11;18)	0.009	13 (8;20)	11 (9;13)
ALT (IU/L)	30 (23;43)	21 (17;33)	0.02	45 (23;80)	36 (23;42)	31 (25;45)	21 (17;33)	0.04	27 (21;30)	19 (13;28)
AST (IU/L)	34 (27;43)	26 (22;34)	<0.01	48 (33;63)	37 (29;51)	34 (27;41)	26 (21;32)	0.02	31 (28;42)	26 (17;35)
Bilirubin (mmol/L)	6 (4;8)	5 (4;7)	0.03	7 (5;20)	7 (5;9)	6 (4;8)	5 (4;7)	0.04	5 (4;5)	4 (2;7)
APRI	0.35 (0.21;0.54)	0.24 (0.16;0.36)	<0.01	0.79 (0.60;1.72)	0.64 (0.45;1.34)	0.31 (0.17;0.47)	0.24 (0.15;0.29)	0.01	0.34 (0.25;0.41)	0.21 (0.14;0.34)
Fib-4	0.37 (0.25;0.50)	0.31 (0.18;0.53)	0.05	0.75 (0.44;1.65)	0.74 (0.57;1.15)	0.29 (0.19;0.44)	0.28 (0.18;0.35)	0.17	0.36 (0.31;0.52)	0.20 (0.18;0.52)
PDFF liver (%)	7 (5;10) (n=13)	6 (5;8) (n=13)	0.14	7 (5;8) (n=3)	5 (5;11) (n=3)	7 (6;16) (n=9)	6 (5;8) (n=9)	0.09	5 (n=1)	2 (n=1)
PDFF pancreas (%)	64 (42;74) (n=14)	72 (51;77) (n=14)	0.80	63 (28;76) (n=3)	70 (16;82) (n=3)	70 (47;74) (n=9)	73 (50;76) (n=9)	0.33	48 (36;60) (n=2)	67 (54;79) (n=2)

AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT gamma-glutamyl transferase; APRI: AST to Platelet Ratio; Fib-4: Fibrosis-4.

References

- [1] European Cystic Fibrosis Society. ECFS Patient Registry; Annual Data Report 2019.
- [2] Van Goor F, Straley KS, Cao D, González J, Hadida S, Hazlewood A, et al. Rescue of DeltaF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L1117-1130. <https://doi.org/10.1152/ajplung.00169.2005>.
- [3] Rowe SM, Miller S, Sorscher EJ. Cystic Fibrosis. *N Engl J Med* 2005;352:1992–2001. <https://doi.org/10.1056/NEJMra043184>.
- [4] Wainwright CE, Elborn JS, Ramsey BW, Marigowda G, Huang X, Cipolli M, et al. Lumacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del CFTR. *N Engl J Med* 2015;373:220–31. <https://doi.org/10.1056/NEJMoa1409547>.
- [5] Ratjen F, Hug C, Marigowda G, Tian S, Huang X, Stanojevic S, et al. Efficacy and safety of lumacaftor and ivacaftor in patients aged 6-11 years with cystic fibrosis homozygous for F508del-CFTR: a randomised, placebo-controlled phase 3 trial. *Lancet Respir Med* 2017;5:557–67. [https://doi.org/10.1016/S2213-2600\(17\)30215-1](https://doi.org/10.1016/S2213-2600(17)30215-1).
- [6] Burgel P-R, Munck A, Durieu I, Chiron R, Mely L, Prevotat A, et al. Real-Life Safety and Effectiveness of Lumacaftor-Ivacaftor in Patients with Cystic Fibrosis. *Am J Respir Crit Care Med* 2019. <https://doi.org/10.1164/rccm.201906-1227OC>.
- [7] Masson A, Schneider-Futschik EK, Baatallah N, Nguyen-Khoa T, Girodon E, Hatton A, et al. Predictive factors for lumacaftor/ivacaftor clinical response. *J Cyst Fibros Off J Eur Cyst Fibros Soc* 2019;18:368–74. <https://doi.org/10.1016/j.jcf.2018.12.011>.
- [8] Dana J, Girard M, Debray D. Hepatic manifestations of cystic fibrosis. *Curr Opin Gastroenterol* 2020;36:192–8. <https://doi.org/10.1097/MOG.0000000000000624>.
- [9] Boëlle P-Y, Debray D, Guillot L, Clement A, Corvol H. Cystic Fibrosis Liver Disease: Outcomes and Risk Factors in a Large Cohort of French Patients. *Hepatology* 2019;69:1648–56. <https://doi.org/10.1002/hep.30148>.
- [10] Debray D, Corvol H, Housset C. Modifier genes in cystic fibrosis-related liver disease. *Curr Opin Gastroenterol* 2019;35:88–92. <https://doi.org/10.1097/MOG.0000000000000508>.
- [11] Karnsakul W, Wasuwanich P, Ingviya T, Vasilescu A, Carson KA, Mogayzel PJ, et al. A longitudinal assessment of non-invasive biomarkers to diagnose and predict cystic fibrosis-associated liver disease. *J Cyst Fibros* 2020;19:546–52. <https://doi.org/10.1016/j.jcf.2020.05.002>.
- [12] Bodewes FAJA, van der Doef HPJ, Houwen RHJ, Verkade HJ. Increase of Serum γ -Glutamyltransferase Associated With Development of Cirrhotic Cystic Fibrosis Liver Disease. *J Pediatr Gastroenterol Nutr* 2015;61:113–8. <https://doi.org/10.1097/MPG.0000000000000758>.
- [13] Kutney K, Donnola SB, Flask CA, Gubitosi-Klug R, O’Riordan M, McBennett K, et al. Lumacaftor/ivacaftor therapy is associated with reduced hepatic steatosis in cystic fibrosis patients. *World J Hepatol* 2019;11:761–72. <https://doi.org/10.4254/wjh.v11.i12.761>.
- [14] Colombo C, Alicandro G, Oliver M, Lewindon PJ, Ramm GA, Ooi CY, et al. Ursodeoxycholic acid and liver disease associated with cystic fibrosis: A multicenter cohort study. *J Cyst Fibros* 2021;S1569-1993(21)00090-4. <https://doi.org/10.1016/j.jcf.2021.03.014>. Online ahead of print.
- [15] Leung DH, Khan M, Minard CG, Guffey D, Ramm LE, Clouston AD, et al. Aspartate aminotransferase to platelet ratio and fibrosis-4 as biomarkers in biopsy-validated pediatric cystic fibrosis liver disease. *Hepatol Baltim Md* 2015;62:1576–83. <https://doi.org/10.1002/hep.28016>.

- [16] Lewindon PJ, Puertolas-Lopez MV, Ramm LE, Noble C, Pereira TN, Wixey JA, et al. Accuracy of Transient Elastography Data Combined With APRI in Detection and Staging of Liver Disease in Pediatric Patients With Cystic Fibrosis. *Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc* 2019;17:2561-2569.e5. <https://doi.org/10.1016/j.cgh.2019.03.015>.
- [17] Stonebraker JR, Ooi CY, Pace RG, Corvol H, Knowles MR, Durie PR, et al. Features of Severe Liver Disease With Portal Hypertension in Patients With Cystic Fibrosis. *Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc* 2016;14:1207-1215.e3. <https://doi.org/10.1016/j.cgh.2016.03.041>.
- [18] Bussler S, Vogel M, Pietzner D, Harms K, Buzek T, Penke M, et al. New pediatric percentiles of liver enzyme serum levels (alanine aminotransferase, aspartate aminotransferase, γ -glutamyltransferase): Effects of age, sex, body mass index, and pubertal stage. *Hepatol Baltim Md* 2018;68:1319–30. <https://doi.org/10.1002/hep.29542>.
- [19] Schneider EK, Reyes-Ortega F, Li J, Velkov T. Optimized LC-MS/MS Method for the High-throughput Analysis of Clinical Samples of Ivacaftor, Its Major Metabolites, and Lumacaftor in Biological Fluids of Cystic Fibrosis Patients. *J Vis Exp JoVE* 2017. <https://doi.org/10.3791/56084>.
- [20] Schneider EK, Reyes-Ortega F, Wilson JW, Kotsimbos T, Keating D, Li J, et al. Development of HPLC and LC-MS/MS methods for the analysis of ivacaftor, its major metabolites and lumacaftor in plasma and sputum of cystic fibrosis patients treated with ORKAMBI or KALYDECO. *J Chromatogr B Analyt Technol Biomed Life Sci* 2016;1038:57–62. <https://doi.org/10.1016/j.jchromb.2016.10.026>.
- [21] Reyes-Ortega F, Qiu F, Schneider-Futschik EK. Multiple Reaction Monitoring Mass Spectrometry for the Drug Monitoring of Ivacaftor, Tezacaftor, and Elexacaftor Treatment Response in Cystic Fibrosis: A High-Throughput Method. *ACS Pharmacol Transl Sci* 2020;3:987–96. <https://doi.org/10.1021/acspsci.0c00103>.
- [22] Konuş OL, Ozdemir A, Akkaya A, Erbaş G, Celik H, Işik S. Normal liver, spleen, and kidney dimensions in neonates, infants, and children: evaluation with sonography. *AJR Am J Roentgenol* 1998;171:1693–8. <https://doi.org/10.2214/ajr.171.6.9843315>.
- [23] Megremis SD, Vlachonikolis IG, Tsilimigaki AM. Spleen length in childhood with US: normal values based on age, sex, and somatometric parameters. *Radiology* 2004;231:129–34. <https://doi.org/10.1148/radiol.2311020963>.
- [24] Runge JH, Smits LP, Verheij J, Depla A, Kuiken SD, Baak BC, et al. MR Spectroscopy-derived Proton Density Fat Fraction Is Superior to Controlled Attenuation Parameter for Detecting and Grading Hepatic Steatosis. *Radiology* 2018;286:547–56. <https://doi.org/10.1148/radiol.2017162931>.
- [25] Debray D, Narkewicz MR, Bodewes FAJA, Colombo C, Housset C, de Jonge HR, et al. Cystic Fibrosis-related Liver Disease: Research Challenges and Future Perspectives. *J Pediatr Gastroenterol Nutr* 2017;65:443–8. <https://doi.org/10.1097/MPG.0000000000001676>.
- [26] Procopet B, Berzigotti A. Diagnosis of cirrhosis and portal hypertension: imaging, non-invasive markers of fibrosis and liver biopsy. *Gastroenterol Rep* 2017;5:79–89. <https://doi.org/10.1093/gastro/gox012>.
- [27] McNamara JJ, McColley SA, Marigowda G, Liu F, Tian S, Owen CA, et al. Safety, pharmacokinetics, and pharmacodynamics of lumacaftor and ivacaftor combination therapy in children aged 2-5 years with cystic fibrosis homozygous for F508del-CFTR: an open-label phase 3 study. *Lancet Respir Med* 2019;7:325–35. [https://doi.org/10.1016/S2213-2600\(18\)30460-0](https://doi.org/10.1016/S2213-2600(18)30460-0).

- [28] Lim A, Balouch F, Cheney J, Lewindon P, Roberts J, Schneider, et al. Orkambi in patients with cystic fibrosis and severe liver disease (Abstract TP92). *Respirology* 2020;25:111–230. <https://doi.org/10.1111/resp.13778>.
- [29] Paluck F, Linnane B. GP274 Liver tests in F508del homozygous cystic fibrosis patients on orkambi. *Arch Dis Child* 2019;104:A144–5. <https://doi.org/10.1136/archdischild-2019-epa.333>.
- [30] Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006;43:1317–25. <https://doi.org/10.1002/hep.21178>.
- [31] Lin Z-H, Xin Y-N, Dong Q-J, Wang Q, Jiang X-J, Zhan S-H, et al. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. *Hepatology* 2011;53:726–36. <https://doi.org/10.1002/hep.24105>.
- [32] Fiorotto R, Villani A, Kourtidis A, Scirpo R, Amenduni M, Geibel PJ, et al. The cystic fibrosis transmembrane conductance regulator controls biliary epithelial inflammation and permeability by regulating Src tyrosine kinase activity. *Hepatology* 2016;64:2118–34. <https://doi.org/10.1002/hep.28817>.
- [33] Debray D, El Mourabit H, Merabtene F, Brot L, Ulveling D, Chrétien Y, et al. Diet-Induced Dysbiosis and Genetic Background Synergize With Cystic Fibrosis Transmembrane Conductance Regulator Deficiency to Promote Cholangiopathy in Mice. *Hepatology* 2018;2:1533–49. <https://doi.org/10.1002/hep4.1266>.
- [34] Tétard C, Mittaine M, Bui S, Beaufile F, Maumus P, Fayon M, et al. Reduced Intestinal Inflammation with Lumacaftor/Ivacaftor in Adolescents with Cystic Fibrosis. *J Pediatr Gastroenterol Nutr* 2020. <https://doi.org/10.1097/MPG.0000000000002864>.
- [35] Wu H, Vu M, Dhingra S, Ackah R, Goss JA, Rana A, et al. Obliterative Portal Venopathy Without Cirrhosis Is Prevalent in Pediatric Cystic Fibrosis Liver Disease With Portal Hypertension. *Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc* 2019;17:2134–6. <https://doi.org/10.1016/j.cgh.2018.10.046>.

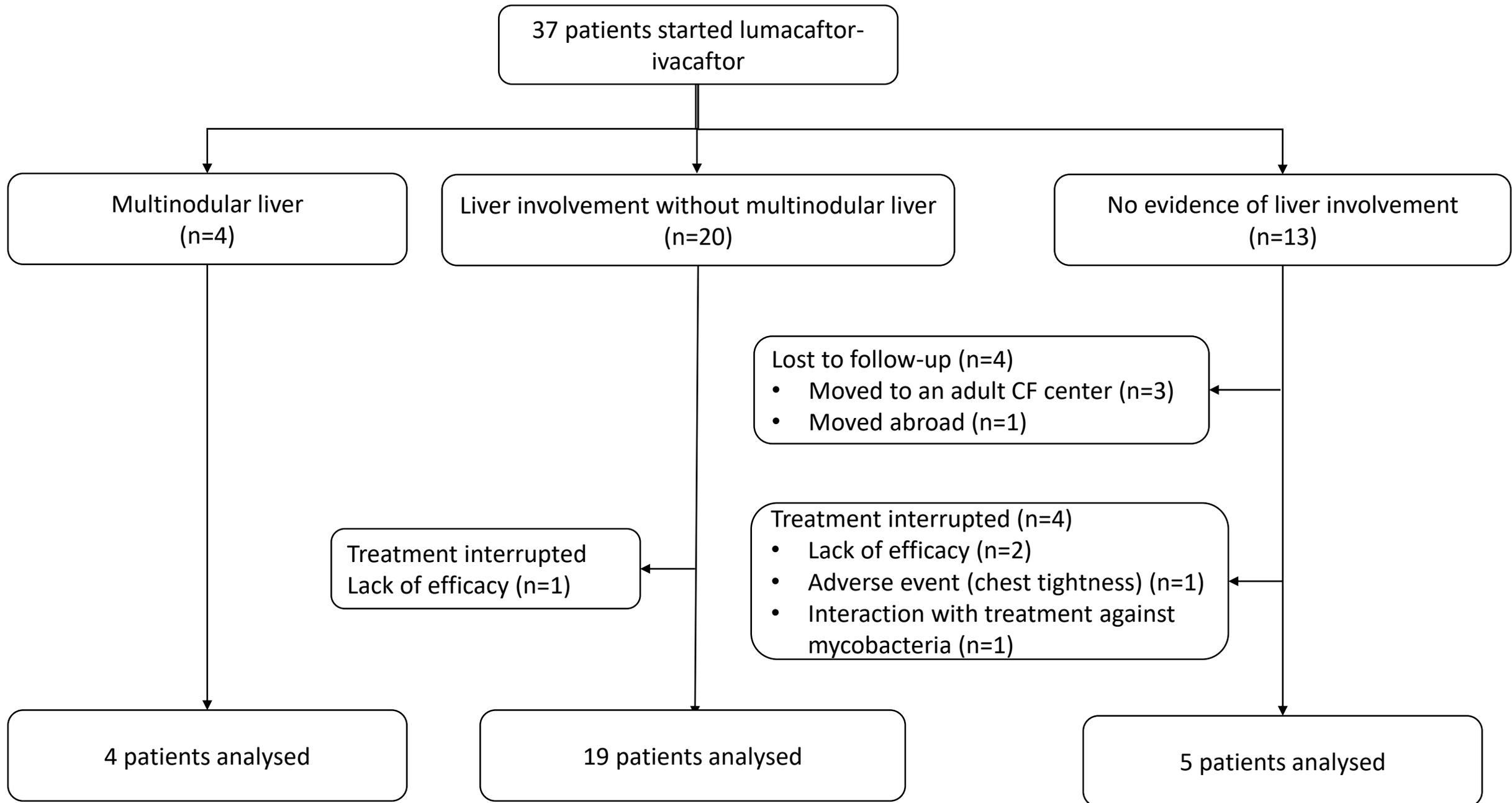


Figure 2

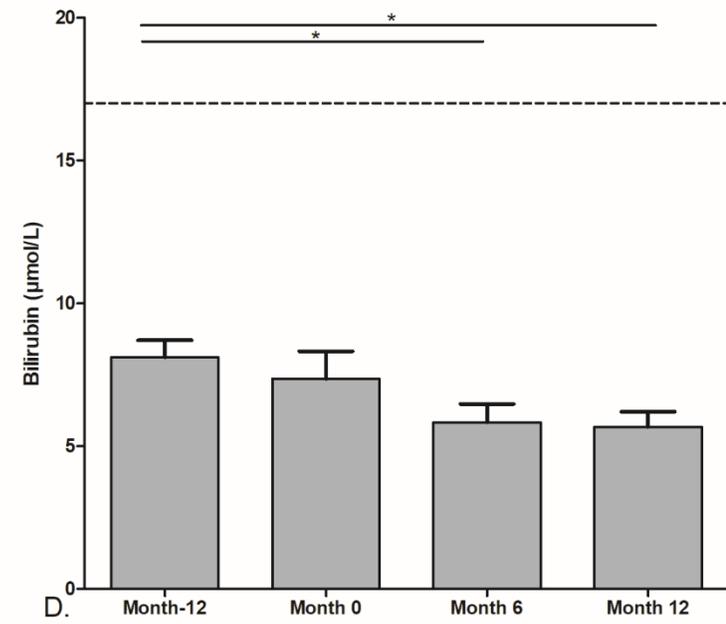
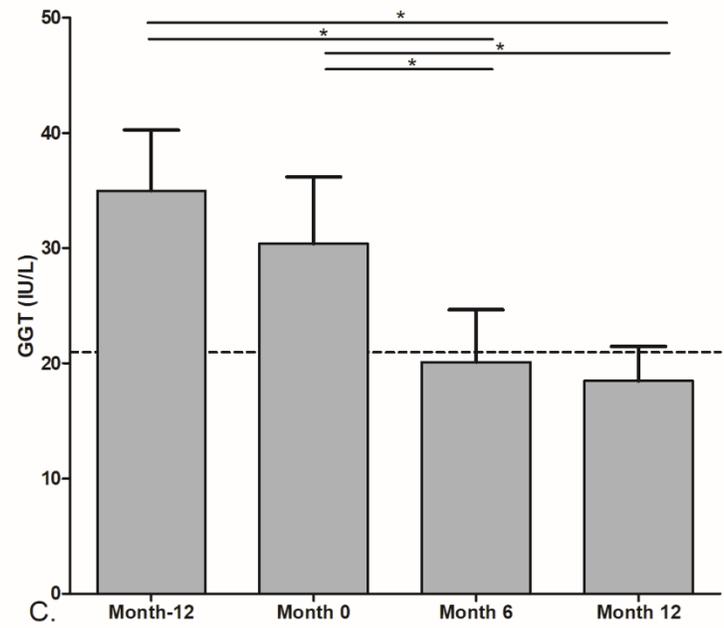
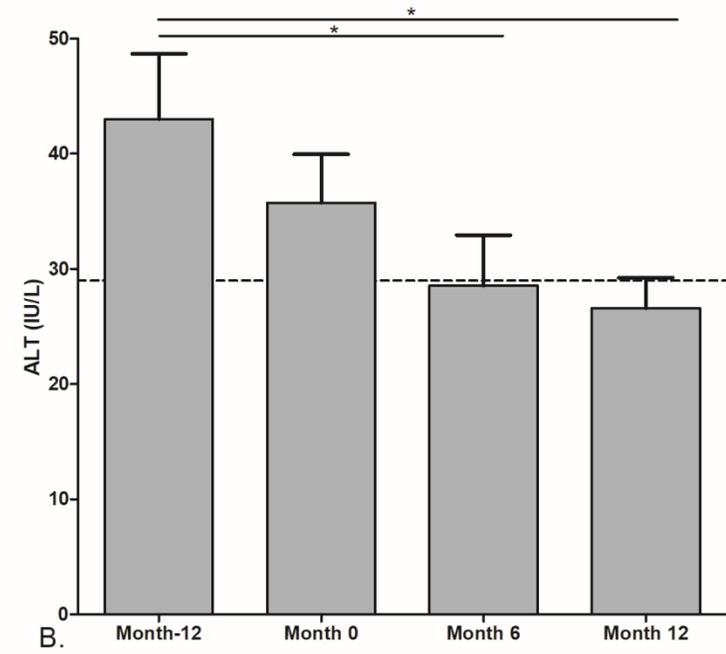
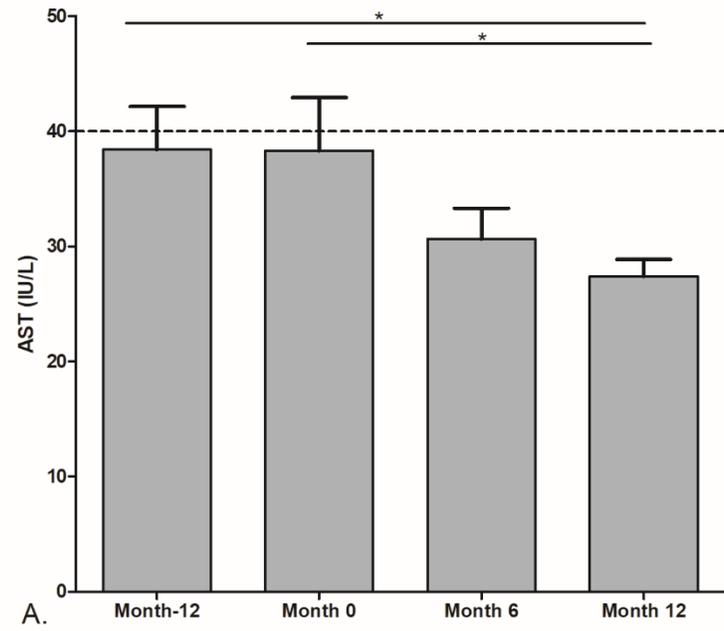


Figure 3

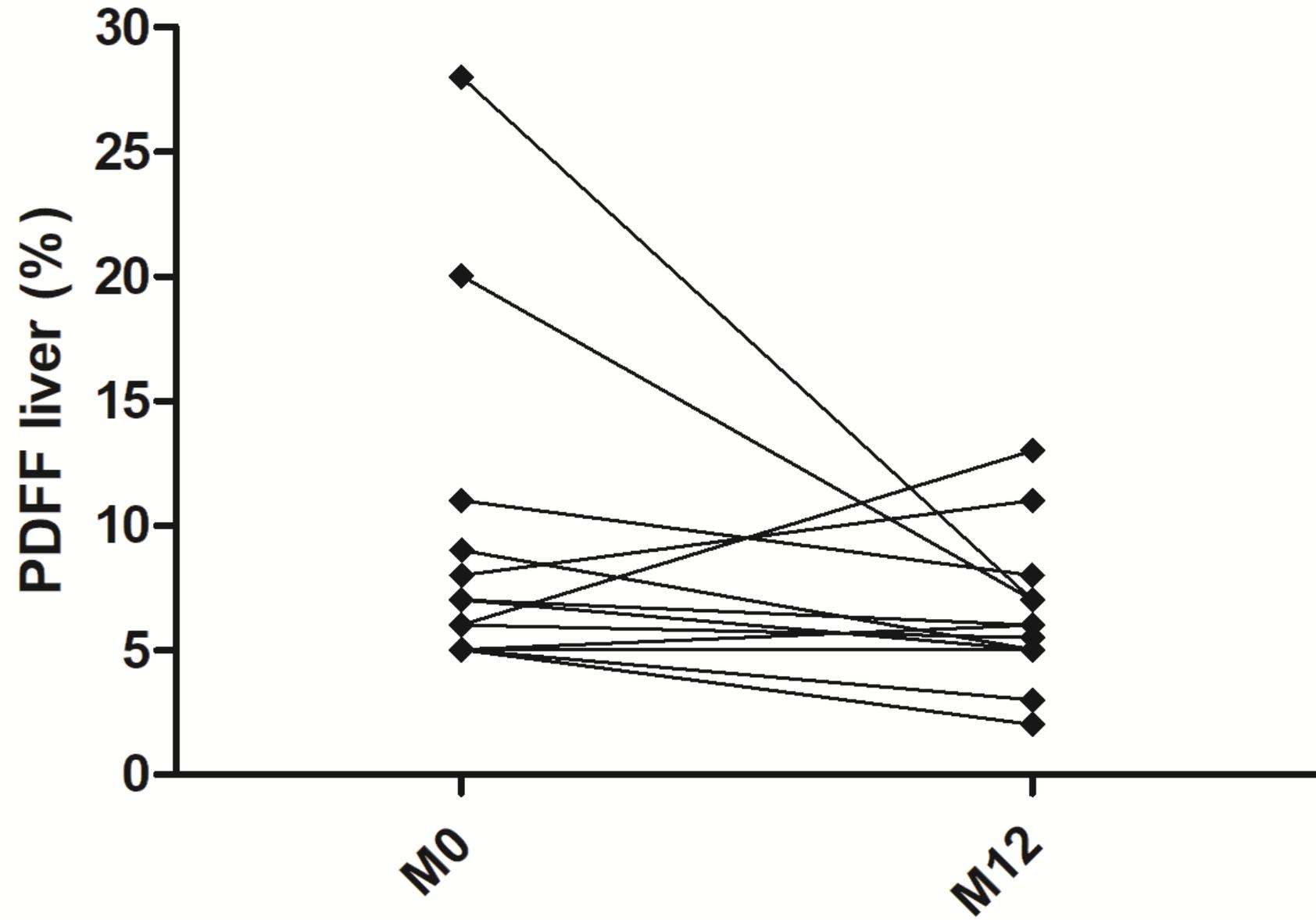


Figure S1

