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Evaluation of Elexacaftor/Tezacaftor/Ivacaftor-Mediated Drug-Induced Liver Injury Using a Liver-On-Chip Model

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ABSTRACT

Elexacaftor/tezacaftor/ivacaftor (ETI), a cystic fibrosis transmembrane conductance regulator (CFTR) modulator therapy, has provided great improvements in lung function and well-being for people with CF. The use of ETI has been complicated by reports of rare but significant liver function test elevations in clinical trials and drug-induced liver injury (DILI) in real-world use. Previous research by our group revealed that oxidative stress is the major driver of ETI-mediated DILI, and in silico ETI DILI simulations resulted in elevations of the emerging biomarker glutamine dehydrogenase (GLDH) which match the time course of predicted transaminase elevations. The assessment of emerging biomarkers and therapeutic strategies in a clinically relevant model will spur the development of more effective treatments for ETI-mediated DILI. This study used the human-relevant liver-on-chip model to investigate GLDH as a biomarker and ETI dose reduction and silymarin antioxidant administration as therapeutic strategies for ETI-mediated DILI. We found that GLDH was not as sensitive as ALT and albumin for detecting DILI due to ETI. Dose reduction was a more effective treatment strategy for ETI DILI than silymarin, which did not significantly lower ALT levels.

1 | Introduction

Cystic fibrosis (CF) is a genetic disorder characterized by defective chloride ion transport due to mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, leading to a progressive decline in lung function coupled with chronic respiratory infections and organ dysfunction [1]. The introduction of elexacaftor/tezacaftor/ivacaftor (ETI), a triple-combination corrector-potentiator therapy, has revolutionized CF treatment by providing significant clinical benefits, including improved lung function, reduced pulmonary exacerbations, and enhanced quality of life, for patients with at least one F508del mutation, encompassing approximately 90% of the CF population [2]. However, despite its transformative impact, evidence highlights a rare but serious risk of drug-induced liver injury (DILI) associated with ETI use.

Clinical trials and post-marketing surveillance have reported elevated liver enzymes, jaundice, and, in rare cases, severe hepatotoxicity necessitating treatment discontinuation [3]. For instance, phase III trials of ETI noted alanine aminotransferase (ALT) and aspartate aminotransferase (AST) elevations in up to 10% of patients, with severe cases (<1%) requiring close monitoring or withdrawal [2, 4]. Case reports have further documented idiosyncratic DILI, including instances of acute liver failure and delayed hepatic necrosis [5–8]. Mechanistic studies from our group suggest that ETI's components, particularly ivacaftor, induce significant

Study Highlights

- What is the current knowledge on the topic?
  - Elexacaftor/tezacaftor/ivacaftor (ETI), a triple-combination therapy for cystic fibrosis (CF), provides significant quality of life improvements for people with CF, but its use has led to drug-induced liver injury (DILI) in clinical trials and case reports. Research from our group has demonstrated that oxidative stress is the main driver of ETI DILI, and in silico simulations predicted elevations in glutamate dehydrogenase (GLDH), an emerging DILI biomarker, and that antioxidant treatment and ETI dose reduction resulted in lower frequencies of ALT elevations. However, neither GLDH, nor ETI dose reduction, nor antioxidant administration has been assessed using a clinically relevant platform.
- What question did this study address?
  - This study addresses the need to identify biomarkers of ETI-mediated DILI and evaluate therapeutic approaches to treat DILI. Due to its greater similarity to the human liver and validated accuracy in identifying hepatotoxic compounds, the liver-on-chip system (liver chip) was used to assess DILI biomarkers, including GLDH, and therapeutic strategies such as silymarin (antioxidant) administration and dose reduction.
- What does this study add to our knowledge?
  - Elevations in ALT and urea as well as decreases in albumin levels are indicative of ETI-mediated DILI, while GLDH is less predictive as a biomarker. Silymarin administered with ETI did not significantly lower alanine aminotransferase (ALT) levels, while reduced-dose ETI resulted in significantly lower ALT levels.
- How might this change clinical pharmacology or translational science?
  - The liver-on-chip platform has been demonstrated by this study to be effective in post-marketing DILI investigations for ETI. Similar experiments to assess biomarkers and treatment modalities may be performed for other drugs on the market known to cause DILI.

oxidative stress in hepatocytes, contributing to liver injury [9]. Currently, there are no known early-stage biomarkers and therapeutics for ETI-induced DILI. In silico modeling of ETI DILI by our group predicted elevations in glutamate dehydrogenase (GLDH), an emerging biomarker for DILI, following a similar time course as ALT and AST. The model also predicted that ETI dose reduction and hypothetical antioxidant administration significantly reduced the frequency of transaminase elevations. Further investigations into this novel biomarker, GLDH, and potential therapeutic strategies for ETI-mediated DILI in a clinically relevant model are needed to improve the safety of highly effective modulator therapies for people with CF.

Glutamate dehydrogenase (GLDH) is a liver-specific mitochondrial enzyme and an emerging biomarker for DILI. GLDH is involved in amino acid oxidation and urea production and is expressed in the mitochondrial matrix of hepatocytes [10, 11]. GLDH is highly liver-specific: unlike ALT and AST, the levels of GLDH are not elevated in response to muscle injury [11]. Therefore, GLDH is less likely to be a false positive marker for liver injury than transaminases. Elevated serum GLDH indicates a loss of mitochondrial membrane integrity, which could result from the DILI mechanism of mitochondrial dysfunction [12]. Accordingly, for liver injury induced in rats by acetaminophen, which is known to cause mitochondrial damage, serum GLDH levels became significantly elevated ($p < 0.001$) in 24 h and were higher than those of ALT [13]. In human subjects following acetaminophen administration, GLDH and ALT levels were highly correlated ($r = 0.88$) [14].

Silymarin, historically used to treat liver disorders, is the extract of the milk thistle plant (Silybum marinum) containing the major active component silibinin (aka silybin) [15]. Silymarin exhibits antioxidant effects that prevent lipid peroxidation from reactive oxygen species, helping maintain the fluidity of the liver cell membrane and improving its resilience against injury [16]. A prospective study comparing silymarin and ursodeoxycholic acid (UCDA, an anticholestatic agent) on anticonvulsive drug-induced hypertransaminasemia showed superiority for the effect of silymarin on DILI, as the reduction in ALT was significantly greater ($p = 0.017$) in the silymarin group than in the UCDA group after 1 month of treatment, with minimal side effects [17]. In addition, a multicenter, retrospective, non-intervention cohort study found that the administration of silibinin significantly improved liver function tests in patients with DILI [18]. A meta-analysis of randomized controlled trials (RCT) of silymarin as a prophylactic therapy for antituberculosis drug-induced liver injury revealed that silymarin significantly reduced ALT ($p < 0.001$) and AST ($p = 0.001$) levels vs. placebo among a total of 1198 (585 in silymarin groups and 613 in placebo groups) patients across five RCTs [19].

Conventional animal models, while widely used, often fail to predict human-specific DILI due to differences in hepatic metabolism, immune responses, and drug pharmacokinetics [20, 21]. The human quad-culture liver-on-chip system (aka liver chip) addresses these limitations by recapitulating the human liver microenvironment in vitro. This microfluidic platform integrates primary human hepatocytes, liver sinusoidal endothelial cells, stellate cells, and Kupffer cells under dynamic flow conditions, mimicking physiological blood perfusion and bile canalicular function. Validated for DILI assessment, the liver chip accurately detects clinical biomarkers of liver injury in response to known hepatotoxic compounds with high sensitivity (87%) and specificity (100%), surpassing the predictive capacity of traditional 2D hepatocyte cultures and animal models [22, 23].

This study leverages the liver chip to investigate ETI-induced DILI. The objectives of our study are (1) to identify biomarkers of ETI liver injury in response to daily ETI exposure; and (2) to evaluate the therapeutic strategies of dose reduction and a candidate antioxidant therapeutic, silymarin, aimed at attenuating hepatotoxicity. By investigating the biomarkers underlying ETI-mediated DILI and testing potential interventions, this project seeks to reduce the risk of this rare but life-threatening

complication, thereby enhancing the safety profile of this transformative therapy for CF patients.

2 | Methods

2.1 | Liver-On-Chip Culture and Experimental Setup

The liver-on-chip system (Emulate Inc., Boston, MA) was used to model human liver responses to ETI and evaluate potential therapeutic interventions. Each chip comprised two channels separated by a porous membrane: a top channel seeded with primary human hepatocytes (LifeNet Health, no catalog #, Lot 2214423-01) and a bottom channel seeded with non-parenchymal cells (NPCs), including primary human liver sinusoidal endothelial cells (LSECs) (Cell Systems, Cat. # ACBRI 566, Lot 566.04.05.05.0 M), primary human hepatic stellate cells (LifeNet Health, no catalog #, Lot 2222162p0), and primary human Kupffer cells (LifeNet Health, no catalog #, Lot 2118082). Chips were prepared and cells were cultured under dynamic flow conditions (100 μL/h) in hepatocyte maintenance medium and NPC medium supplemented with 2% fetal bovine serum according to Emulate protocols as described by Shi et al. [24]. The media were prepared following Emulate protocols and the flow rate was chosen to minimize absorption of ETI by the PDMS membrane of the liver chip by rapidly saturating the membrane with the drug so that further absorption would be minimal. In a preliminary assay using blank liver chips without cells, a flow rate of 100 μL/h achieved a recovered concentration (chip outflow concentration/dosing concentration) of greater than 95% for all ETI compounds within 48 h after the start of dosing. Chips were equilibrated for 48 h prior to dosing to ensure stable metabolic function.

2.2 | Experimental Design

The liver chip experiment encompassed an ETI exposure range of 1× to 100× unbound plasma C_{max} ETI to assess ETI-induced drug-induced liver injury (DILI) and evaluate mitigation strategies. Chips were assigned to the following groups: 0.1% DMSO (Sigma-Aldrich, Cat. # D2650) (negative control), 1×, 10×, 30×, and 100× unbound plasma C_{max} ETI, 100× plasma C_{max} ETI + 10× plasma C_{max} silymarin (Sigma-Aldrich, Cat. # S0417-1G) (experimental therapeutic), and 100× unbound plasma C_{max} trovafloxacin (MedChemExpress, Cat. # HY-103399) (positive control). ETI compounds were sourced from AChemblock (elexacaftor, Cat. # U102941; tezacaftor, Cat. # G-6563; ivacaftor, Cat. # 10237).

The experiment was performed in 2 cycles, with each cycle utilizing 12 chips divided into four experimental groups ($n = 3$ chips per group), dosed daily for 7 days via the top and bottom channel inlets. The first cycle included 0.1% DMSO, 1× C_{max} ETI, 10× C_{max} ETI, and 100× trovafloxacin, while the second cycle included 30× C_{max} ETI, 100× C_{max} ETI, 100× C_{max} ETI + silymarin, and trovafloxacin. Dosing solutions were prepared in hepatocyte maintenance medium with 0.1% dimethyl sulfoxide (DMSO).

Dosing was performed daily by replacing the top and bottom channel medium with fresh medium containing the respective compounds. Chips were maintained at 37°C and 5% CO_2 throughout the 7-day dosing period.

2.3 | Biochemical Assays

Top channel outlet effluent was collected daily (2.4 mL per chip) to quantify biomarkers of liver function and injury. Prior to each assay, frozen (−80°C) effluent samples were thawed overnight at 4°C. Effluent samples were analyzed for alanine aminotransferase (ALT) and albumin levels using sandwich ELISA kits (Abcam, ALT ab234578, albumin ab179887) following vendor protocols. Effluent samples were analyzed to quantify urea and glutamate dehydrogenase (GLDH) levels using a colorimetric urea assay kit (Abcam, ab83362) and colorimetric kinetic assay kit (BioAssay Systems, DGLDH-100), respectively, following the vendor protocols.

2.4 | Morphological Analysis

Hepatocyte and NPC morphology were assessed on days 1, 3, 5, and 7 using brightfield microscopy. Images were captured with a Keyence BZ-X800 microscope (Keyence, Osaka, Japan) at 10× magnification, and were analyzed for cellular features, including cell viability, nuclear integrity, and cytoplasmic vacuolation. Images were scored based on the severity of cell debris agglomeration from zero (least severe) to four (most severe) according to the scoring matrix provided by Ewart et al. [23].

3 | Results

3.1 | Liver Biomarker Assays

ALT levels were not elevated at 1× and 10× C_{max} ETI over the 7-day period, with levels at or near that of the negative control, DMSO, and much lower than that of the positive control, trovafloxacin (Figure 1A). However, ALT levels were significantly elevated in the 100× C_{max} ETI and 100× C_{max} ETI + silymarin treatment groups compared to trovafloxacin, peaking at Day 2–3 and declining afterward (Figure 1A). Aside from an increase on Day 7, ALT levels for 30× C_{max} ETI were much lower than that of both 100× C_{max} ETI and 100× C_{max} ETI + silymarin and within the range that of 1× and 10× ETI and DMSO (Figure 1A,B). ALT levels in the 100× trovafloxacin group reached a peak of 0.07–0.08 μg/day/10^6 hepatocytes at Day 4 and slowly declined afterward (Figure 1A).

Albumin levels for 1× and 10× C_{max} ETI stayed relatively constant near the levels of the negative control (DMSO), while albumin levels for trovafloxacin decreased steadily over time (Figure 1B). Albumin levels in the 100× C_{max} ETI and 100× C_{max} ETI + Silymarin ETI groups rapidly declined over the first 2 days until reaching undetectable levels from Day 3 onward (Figure 1B). In contrast, 30× C_{max} ETI recorded the highest overall albumin levels but exhibited a slow but shallow decline in albumin levels over time (Figure 1B).

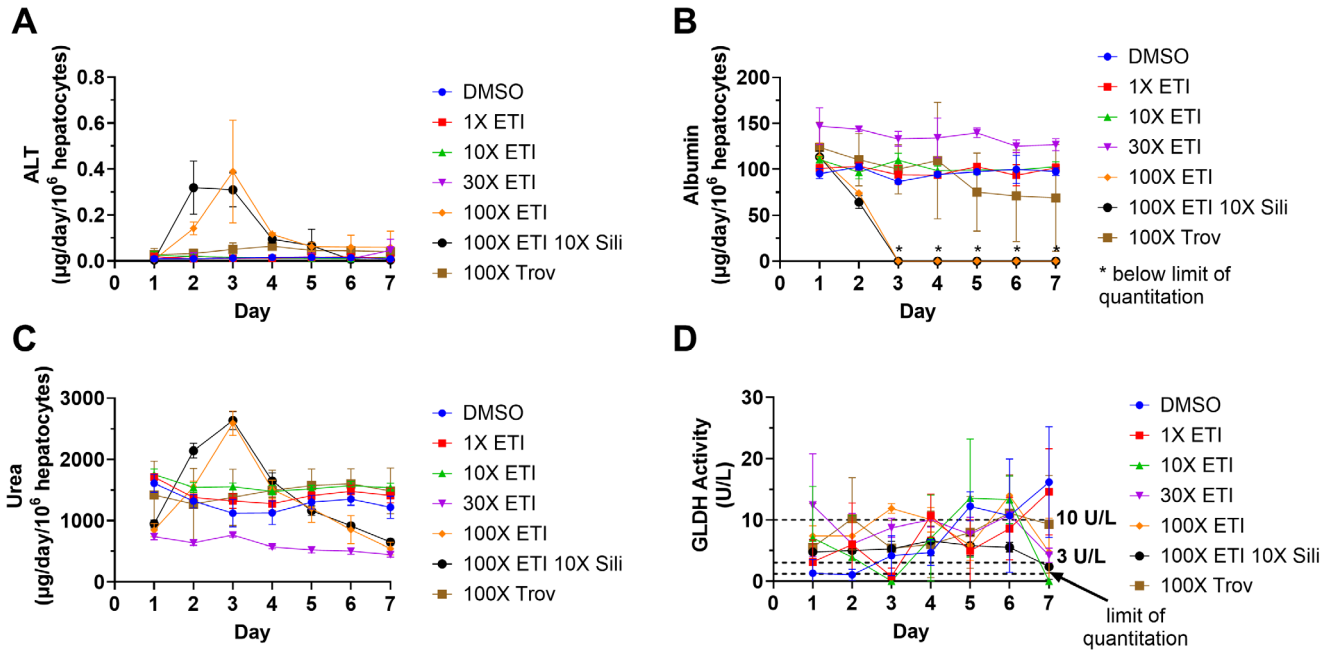

FIGURE 1 | Top (hepatocyte) channel biomarker results for the liver chip experiment. (A) ALT levels, (B) albumin levels, (C) urea levels, and (D) GLDH levels. Error bars represent standard deviation.

The urea levels for 1× and 10× C_{max} ETI, trovafloxacin, and DMSO remained steady within the range of 1000–2000 µg/day/10^6 hepatocytes (Figure 1C). The 100× C_{max} ETI and 100× C_{max} ETI + silymarin groups yielded notable elevations in urea compared to trovafloxacin and other groups, peaking at 2700 µg/day/10^6 hepatocytes on Day 3 and declining afterward (Figure 1C). Urea levels for 30× C_{max} ETI were consistently lower than those of trovafloxacin and other experimental groups throughout the 7-day period (Figure 1C).

GLDH levels for all treatment groups across both experiments largely stayed within or slightly exceeded the normal reference range of 3–10 U/L by less than twofold (Figure 1D). Five of 56 measurements fell below the limit of quantification (0.4 U/L) and were set to the lower limit of quantification in the analysis. To evaluate the relative GLDH levels between treatment groups for the 7-day dosing period, the area under the curve (AUC) for GLDH was calculated using the trapezoidal method in GraphPad Prism (Dotmatics, Boston, MA). The GLDH AUC of DMSO, 1× C_{max} ETI, and 10× C_{max} ETI fell within a narrow range of 39.87–41.30 days * U/L, while the GLDH AUC of trovafloxacin was greater at 48.05 days * U/L (Figure 2). The GLDH AUC of 30× and 100× C_{max} ETI was the highest at 51.61 days * U/L and 55.18 days * U/L, respectively, while the GLDH AUC of 100× C_{max} ETI + silymarin was the lowest at 31.81 days * U/L (Figure 2). No significant differences between groups were detected by a one-way ANOVA with multiple comparison tests.

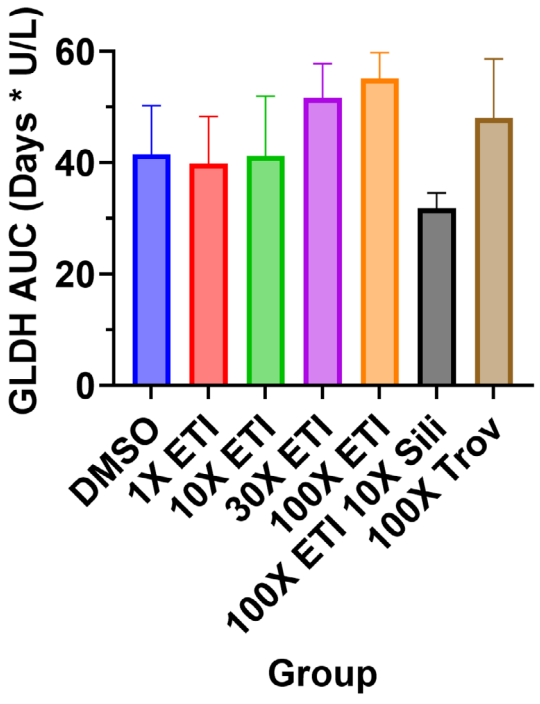

FIGURE 2 | Area under the curve (AUC) for GLDH across all experimental groups. Significant differences between treatment conditions were not detected by a one-way ANOVA. Error bars represent standard error of the mean.

3.2 | Hepatocyte and NPC Morphology

1×, 10×, and 30× C_{max} ETI showed minor damage to hepatocytes and NPCs, with average morphology scores of less than 2 across 7 days (Figure 3A,B). DMSO showed little to no cellular damage to hepatocytes and NPCs throughout the experiment (Figure 3A,B). The 100× C_{max} ETI and 100× C_{max} ETI + silymarin groups exhibited increasing signs of hepatocyte agglomeration and cell debris over time, which became more pronounced at Day 5–7 for hepatocytes and Days 3–7 for NPCs. At Day 7, these groups showed severe morphological damage, with extensive aggregation of cell debris and compromised cell viability, scoring 3–4 on the severity scale. Compared to the 100× C_{max} ETI and 100× C_{max} ETI + silymarin groups, 100× C_{max} trovafloxacin exhibited a similar rise in

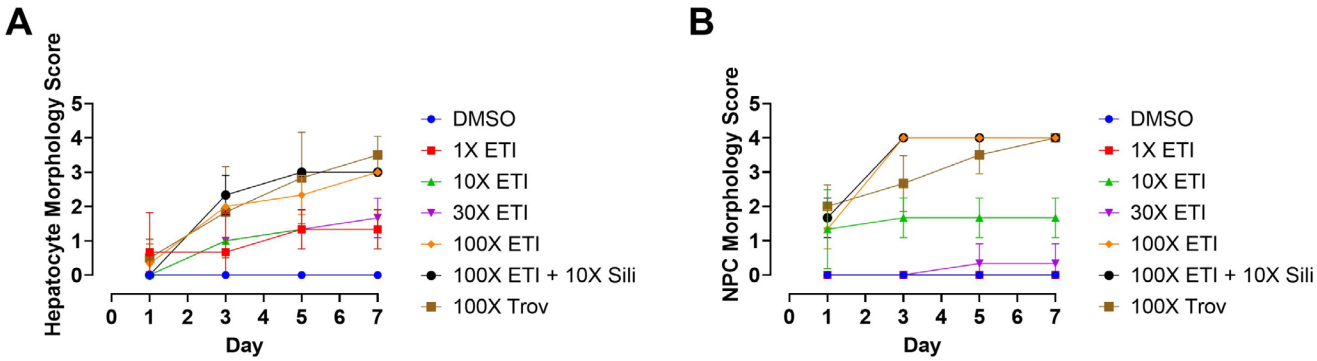

FIGURE 3 | Morphology scores for the hepatocyte channel (A) and NPC channel (B). Higher scores indicate greater cell death and more severe agglomeration of cell debris. Error bars represent standard deviation.

morphological damage to hepatocytes and a slower rise in damage to NPCs, but still yielded extensive hepatocyte agglomeration and NPC death by Day 7. Representative images for each experimental group at Days 1 and 7 are shown in Figure S1.

4 | Discussion

4.1 | Biomarkers of ETI-Mediated DILI

ALT was a clear biomarker for ETI-mediated DILI, as the peak ALT levels for 100× C_{max} ETI and 100× C_{max} ETI + silymarin surpassed that of the positive control, trovafloxacin, by greater than 5-fold. The dose–response relationship for ETI and ALT was similar to that of published data for trovafloxacin, which showed major increases in ALT at 100× C_{max} that were 10- to 20-fold greater than that of 1× to 30× C_{max} [23]. Reduction of albumin levels (indicative of a loss of liver synthetic function) in 100× C_{max} ETI ± silymarin also clearly reflected ETI toxicity, as there was a rapid decrease in albumin until undetectable levels at Day 3, a much greater decline than that of trovafloxacin. An increase in urea for 100× C_{max} ETI ± silymarin peaking at Day 3, possibly due to the release of urea from dying hepatocytes, appeared to be a biomarker for ETI DILI as well. The day of peak urea levels for 100× C_{max} ETI and 100× C_{max} ETI + silymarin coincided with the day of peak ALT levels and the day on which albumin declined to undetectable levels.

The severity of changes in ALT, albumin, and urea corresponded to the intensity of morphology score increases for the treatment groups, verifying that the biomarker levels were signals of actual liver toxicity. For example, the steep rise in ALT and urea for 100× C_{max} ETI ± silymarin as well as a drop in albumin from Day 1 to Day 3 coincided with the rapid increase in morphology scores during that same time period. Meanwhile, the ALT, urea, and albumin levels for 1×, 10×, and 30× C_{max} ETI changed much less dramatically over the course of the experiment, mirroring the mild and slow increase in morphology scores for the same groups. Taken together, the biomarker and morphology score data show that the lower doses of ETI resulted in minimal damage to the cells in the liver chip compared to the highest dose, supporting the therapeutic strategy of dose reduction for ETI DILI.

GLDH did not appear to be a predictive biomarker for ETI-mediated DILI, as its levels remained near the reference range for all ETI concentrations tested throughout the 7-day period. Analysis of the AUC of GLDH did reveal differences roughly matching the toxicity of treatment groups; however, the lack of statistically significant pairwise differences in AUC as determined by ANOVA and the absence of major GLDH elevations above the upper limit of normal suggest that GLDH may be a less sensitive translational biomarker when compared with ALT, albumin, or urea for detecting ETI-mediated DILI in the clinical setting.

A previous liver chip study found that elevations in GLDH were associated with elevations in ALT and AST for the hepatotoxic compound, JNJ-3 [22]. Unlike ETI, an approved drug, JNJ-3 was discontinued in preclinical development due to hepatocellular necrosis, portal fibrosis, and biliary hyperplasia [22]. This suggests that GLDH elevations may be drug-specific and dependent on the type and severity of damage to the liver, which may explain the differences in GLDH levels between JNJ-3 and ETI.

4.2 | Therapeutic Strategies: Dose Reduction and Silymarin

Silymarin did not have a significant observable impact on reducing the toxicity of ETI, as the results for ALT, albumin, urea, and morphology scores were very similar between the 100× C_{max} ETI and 100× C_{max} ETI + Silymarin groups. It is possible that the dose of silymarin used, 10× plasma C_{max}, was not high enough to counteract the reactive oxygen species generated by 100× C_{max} ETI. Alternatively, a secondary mechanism for ETI-mediated DILI, such as mitochondrial dysfunction due to electron transport chain inhibition, may have contributed to toxicity.

Unlike silymarin, reduced doses of ETI from 30× plasma C_{max} and lower did show significantly lower levels of ALT, higher albumin levels, and more moderate urea levels than 100× C_{max} ETI, suggesting that ETI dose reduction may be an effective therapeutic strategy for mitigating ETI-mediated DILI.

4.3 | Implications for Clinical Practice

In response to growing evidence of DILI due to ETI in post-marketing reports, the FDA-approved labeling of ETI has recently been updated (December 2024) to include revised monitoring (earlier and more frequent measurement) of liver function

tests (e.g., ALT, AST, alkaline phosphatase, and bilirubin) and guidance on treatment interruption for significant elevations until the abnormalities resolve. Data from the liver-on-chip experiments in this investigation reinforce that traditional liver function tests such as ALT are reliable as biomarkers for ETI-mediated DILI. In addition, significant reductions in albumin and spikes in levels of urea corresponding to peak ALT levels may provide additional means of detecting liver injury. GLDH showed minor but observable differences in toxicity of treatment groups but does not surpass ALT, as there were no elevations of GLDH significantly above the upper limit of normal.

While use of antioxidants to prevent DILI is actively being investigated, data from our liver-on-chip experiments did not demonstrate a therapeutic effect of silymarin in mitigating ETI-induced DILI. Additional studies evaluating other potential antioxidants are warranted.

Dose reduction is a highly promising therapeutic strategy for ETI-mediated DILI, as evidenced by the reduced ALT levels of $30\times C_{max}$ ETI compared to that of $100\times C_{max}$ ETI. This liver chip data corroborates findings from a previous clinical cohort study by our group, which demonstrated that ETI dose reduction resulted in the resolution or improvement of adverse effects (ALT elevation, mental health disruptions) in 13 out of 15 patients [25]. Future studies for ETI dose reduction can be conducted in a larger population to confirm these findings.

4.4 | Limitations and Future Directions

While this study provides valuable insights into the biomarkers of ETI DILI and potential therapeutic strategies, there are several limitations that should be considered. The liver-on-chip system offers a highly relevant model of human liver function, but it does not fully replicate the complexity of human physiology, including the influence of factors such as genetic variation, comorbidities, or concurrent drug use. These factors may be accounted for in future liver chip studies by pooling cell stocks from multiple donors, and by testing ETI with other medications for cystic fibrosis to determine the impact of drug interactions on DILI biomarkers. In addition, because the liver chip only allows for a short experimental window of up to 7 days, the long-term effects of both ETI and therapeutic interventions like dose reduction and silymarin should be investigated in a clinical setting to assess potential chronic toxicity or efficacy over extended periods of treatment. In addition, the hepatotoxic effects of some drugs have been shown to be augmented in the presence of inflammation. In particular, Rubiano et al. (2021) found that trovafloxacin induced higher levels of lactate dehydrogenase (LDH), indicative of tissue damage, in liver chips co-dosed with LPS compared to those not dosed with LPS [26]. Future experiments could be conducted in the liver chip assessing the effect of ETI and LPS coadministration on biomarker levels to investigate the contribution of inflammation to ETI hepatotoxicity.

5 | Conclusion

Our study demonstrates that the human liver chip model can be used to evaluate potential novel biomarkers of ETI-mediated DILI and therapeutic strategies for mitigating this risk. GLDH may not offer benefits over traditional liver function tests such as ALT, but dose reduction was shown to be a promising approach for reducing ETI-induced liver injury and may inform future clinical practices in the treatment of cystic fibrosis. Further studies are needed to explore additional emerging DILI biomarkers and optimize antioxidant therapeutic strategies for improving the safety profile of ETI in clinical settings.

Author Contributions

A.S. and P.M.B. wrote the manuscript. A.S., Z.-X.L., and P.M.B. designed the research. A.S., P.A., and Z.-X.L. performed the research. A.S. analyzed the data. Z.-X.L. and P.M.B. contributed analytical tools.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** cts70400-sup-0001-Supinfo.pdf.