that immune-mediated reactions from the SARS-CoV-2 mRNA vaccines are very rare and during the COVID pandemic, the vaccination programme continues to be crucial. We report this case to encourage vigilance for drug-induced reactions and to raise awareness to vaccination centres to incorporate it into their routine checks before administering second doses. Long-term follow up of identified individuals will be essential in determining the prognosis of this immune-mediated liver injury.

Financial support
The authors received no financial support to produce this manuscript.

Conflict of interest
The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions
DG and AAJ conceptualised the work. GT wrote the initial draft and all authors contributed to and approved the final manuscript.

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2021.09.031.

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Comment on "Synthetic human *ABCB4* mRNA therapy rescues severe liver disease phenotype in a BALB/c.*Abcb4*$^{-/-}$ mouse model of PFIC3"

To the Editor:

We have read with interest the paper by Wei *et al.*[1] and we thank the authors for their reference to our 2019 study on adeno-associated virus (AAV)-mediated gene therapy correction of progressive familial intrahepatic cholestasis type 3 (PFIC3) in a clinically relevant mouse model.[2] Their results utilizing lipid nanoparticles (LNP) to deliver functional human *ABCB4* mRNA to hepatocytes of BALB/c.*Abcb4*$^{-/-}$ mice and the therapeutic effect achieved in this severe PFIC3 mouse model with a high degree of fibrosis were quite remarkable. However, they framed their conclusions with respect to our previous study based on improper interpretations of several key aspects of our results. First, they did not consider our results when claiming they identified for the first time a 'minimum' of clinically meaningful restoration of hepatic phosphatidylcholine (PC) output, which was 10-42% of normal levels, *i.e. de novo* phenotypic ABCB4 enzymatic activity that resulted in a therapeutic effect. They stated that our results showed that a bile PC restoration of 70–100% was necessary for a therapeutic effect. In reality, our data pinpointed a threshold of around 4,000 μM of PC concentration in bile, which corresponded to 12–13% of the levels we measured in healthy wild-type mice (Fig. 1), which was clearly shown in our paper.[2] This is substantially less than the 70-100% they claimed we reported and actually shows

Keywords: Genetic therapy; Cholestasis; progressive familial intrahepatic 3; Dependovirus; Liver Cirrhosis; Child; Bile; Phospholipids.
Received 20 September 2021; received in revised form 28 September 2021; accepted 28 September 2021; available online 6 October 2021
https://doi.org/10.1016/j.jhep.2021.09.033

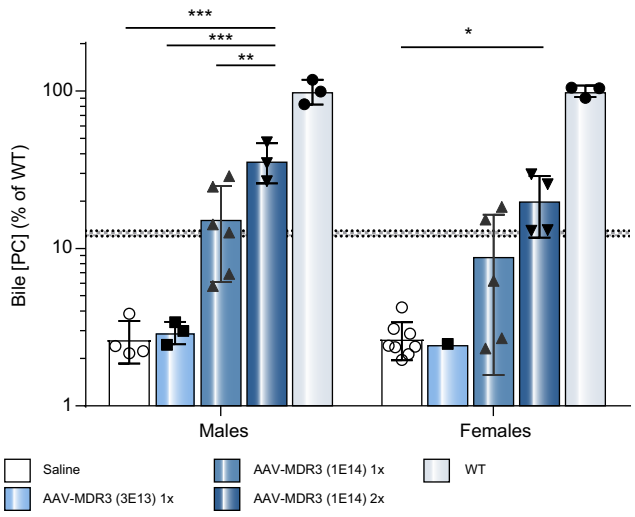

Fig. 1. Bile PC concentration in FVB.*Abcb4*$^{-/-}$ mice treated with AAV-MDR3. Two-week-old FVB.*Abcb4*$^{-/-}$ mice were treated intravenously with saline (white) or with AAV-MDR3 one time at 3E13 VG/kg (light blue), one time at 1E14 VG/kg (blue) or twice 3 weeks apart at 1E14 VG/kg (dark blue). WT mice were used for comparison (grey). Bile PC was quantified 12 weeks after treatment by fluorescent enzymatic assay (Phosphatidylcholine Assay Kit; Sigma). The threshold bile PC concentration above which a durable therapeutic effect was consistently observed is indicated with the grey horizontal bar. Statistics (one-way ANOVA/Tukey's multiple comparisons test): $^{*}p$ <0.05; $^{**}p$ <0.001; $^{***}p$ <0.0001, data are presented as mean ± standard deviation. *Adapted from*[2]. AAV, adeno-associated virus; PC, phosphatidylcholine; WT, wild-type.

that their results corroborated our findings much more closely than was indicated. The identification of a minimum level of phenotypic restoration needed to revert or prevent this disease is an important detail in the daunting task of translating minimum therapeutic doses from preclinical models to human patients with PFIC3. There are many confounding variables in this process, including different delivery efficiencies depending on the species or genetic background and inherent differences in disease phenotype between mice and humans. Thus, the more complete the understanding of the therapeutic process in the preclinical models, the better they will serve for transitioning therapies into the clinic.

Second, the studies by Wei *et al.* were performed in 4-week-old mice, which showed that their approach was distinct in its ability to successfully revert fibrosis in juvenile mice. To substantiate their claims, they incorrectly cited our paper as having used older 7-week-old mice. In fact, our paper reported data showing an AAV-mediated therapeutic effect (including prevention of fibrosis) in both 5-week-old and 2-week-old mice. The misreporting of our results allowed them to imply that mRNA gene therapy holds an advantage over AAV treatment, which lies in being able to exert a durable therapeutic effect in very young study animals. Indeed, very young animals, like paediatric patients, represent a difficult challenge for AAV-mediated gene therapy because of a dilution effect due to the predominantly non-integrative nature of AAV genomes and the growing liver of immature recipients of treatment. One approach to circumvent the problem of the growing liver, as proposed by Wei *et al.*, would be the continual periodic readministration of mRNA-mediated therapy. This, due to the high volume of treatments (potentially, up to 10 or 20 a year) would exert a high burden on patients both financially and in terms of quality of life, as well as require safety evaluations for long-term treatments with LNP-mRNA.

However, under appropriate conditions, the feasibility of readministering AAV is well established,[3–5] and our most recent research has examined this hypothesis by testing the immunotolerogenic properties of rapamycin-loaded nanoparticles (ImmTOR) when coadministered with AAV and its ability to allow for subsequent AAV readministration to treat PFIC3 (Weber *et al.* manuscript in preparation). Indeed, the ability to readminister a gene therapy, regardless of its modality, will be of high importance for treating inborn errors of metabolism, most of which present and require treatment in early childhood while the liver is still growing.[6] To this end, any advancement in developing novel treatment strategies, such as coadministering immunomodulators with gene therapy, in order to improve long-term treatment outcomes in paediatric patients are of utmost importance.

Financial support
This study was entirely funded by Vivet Therapeutics.

Conflict of interest
NDW and GGA are employees and stockholders of Vivet Therapeutics. All other authors declare no conflict of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions
Drafting of the manuscript: NDW, JMG and GGA.

Data availability statement
All data needed to evaluate the conclusions in the paper are present in the paper. Additional data related to this paper may be requested from the authors.

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2021.09.033.

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Potential novel approaches to prevent the risk of infection in patients with variceal bleeding

To the Editor:

We read with great interest the paper by Martinez *et al.*[1] published recently in the Journal of Hepatology. The article made the important observation that the prevalence of infections is high (almost 20%) in patients with acute variceal bleeding (AVB) despite antibiotic prophylaxis and pointed to severe hepatic encephalopathy (HE grade III & IV) as independently associated with bacterial infections. Although several previous studies have described the risk of infection as being related to the severity of liver disease, we would like to highlight the possible mechanisms underlying the development of infection following a variceal bleed and point to potential novel approaches to reduce the risk of infection.

Isoleucine hypothesis: Haemoglobin is the only protein known to man that is deficient in a single essential amino acid, isoleucine, making it a very poor-quality protein. Ingestion of haemoglobin, as occurs during AVB causes severe hypoisoleucinemia due to a mechanism referred to as branched-chain amino acid antagonism as it is also very rich in leucine and valine.[2,3] This hypoisoleucinemia eventually results in diminished protein synthesis, DNA synthesis, cell proliferation, and subsequently impaired immune function.[2,3] In cirrhosis, administration of an amino acid mixture mimicking haemoglobin results in impairment of neutrophil function. It was shown that infusion of isoleucine during a simulated gastrointestinal bleed restores impaired protein synthesis by the liver, which is critical for immunity.[3] Taken together, the existing data suggest that isoleucine administration during an AVB may reduce the risk of bacterial infection and its concentrations may be a potential biomarker to guide intervention.

Role of ammonia: Given the peculiar amino-acid composition of haemoglobin, it is also one of the most ammoniagenic substances known.[4] In addition to the known neurotoxic role of ammonia, it also impairs neutrophil function – with the production of excess reactive oxygen species, systemic inflammation, oxidative stress, high spontaneous oxidative burst, and decreased phagocytosis – leading to a significantly greater risk of infection.[5] Data regarding ammonia concentrations from patients had who developed an infection in the Martinez *et al.* study would be interesting. Aggressive attempts to reduce ammonia concentrations rapidly may reduce the risk of infection.

Hepatic encephalopathy and sympathetic nervous system: There is an increasing literature base showing that brain dysfunction due to wide-ranging causes such as traumatic brain injury, stroke, and spinal cord injury[6–8] even with silent infarcts leads to immune dysfunction, a condition described as CNS injury-induced immunodepression (CIDS).[9] The proposed underlying mechanism is that the pro-inflammatory cytokines produced by injured brain tissue can directly activate the hypothalamic-pituitary-adrenal axis, activate the sympathetic nervous system, leading to immune dysfunction and thereby increasing the risk of infection. Therefore, the observations of Martinez *et al.* that HE is an independent predictor of infection is important and it is possible that the mechanisms underlying CIDS also apply to HE. To reduce the risk of infection, HE should be treated with a high degree of urgency and antibiotics initiated or modified if it is present.

In summary, targeting known mechanisms, as well as improving our understanding of the interaction between variceal bleeding, hyperammonaemia, HE, and the risk of infection, will further the development of novel therapeutic approaches.

Received 21 September 2021; accepted 22 September 2021; available online 1 October 2021
https://doi.org/10.1016/j.jhep.2021.09.027

Financial support

The authors received no financial support to produce this manuscript.

Conflicts of interest

Rajiv Jalan has research collaborations with Takeda, and Yaqrit, and consults for Yaqrit. Rajiv Jalan is the founder of Yaqrit Limited, which is developing UCL inventions for treatment of patients with cirrhosis. Rajiv Jalan is an inventor of ornithine phenylacetate, which was licensed by UCL to Mallinckrodt. He is also the inventor of Yaq-001, DIALIVE and Yaq-005, the patents for which have been licensed by his University into a UCL spinout company, Yaqrit Ltd.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

RJ, EA and DrS contributed equally to this letter and all have provided intelellectual input into the drafting and writing of this manuscript.