Project Update Report to Cystic Fibrosis Australia

Project Grants Scheme: CONQUER CYSTIC FIBROSIS (GI) INNOVATION GRANT 2023

Project Title: Establishing the utility of GCTM-5 as a Biomarker for Liver Pathology in Cystic Fibrosis Patients

Period Covered: July to December 2023

Overview

Following the grant approval and receipt of funding, significant progress has been made with establishing the utility of GCTM-5 as a biomarker for liver pathology in cystic fibrosis (CF) patients. Previously, we demonstrated the presence of GCTM-5 positive liver progenitor/stem cells (LPSCs) in CF patients. We also detected the presence of the GCTM-5 epitope in sera of CF patients. This was the basis of our successful application to CFA for support. We now report that the quantification of GCTM-5 positive cells in biopsies by immunohistochemistry (IHC) and suggests their numbers correlate with liver disease severity in patients with severe comorbidity. In addition, we have completed analysis of sera matched with the biopsies by ELISA. Together these findings suggest the GCTM-5 biomarker reflects the number of GCTM-5 positive cells in severely affected liver. While these findings are based on a small sample size of four control and three affected patients, they support our hypothesis that significant numbers of GCTM-5 positive LPSCs in liver biopsies and correspondingly high levels of its epitope in serum is an indicator of severe liver co-morbidity in CF patients.

Data generated during the funding period

1. Correlating GCTM-5 positive cells in biopsies with degree of liver co-morbidity

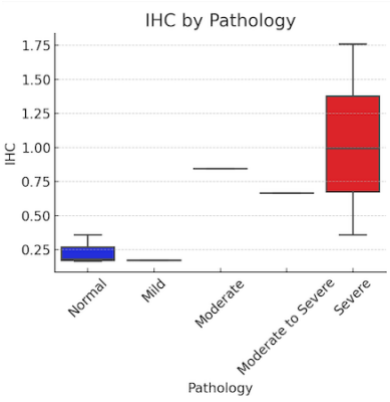

Figure 1. Quantification of GCTM-5-Positive Cells in Biopsies from CF patients with Varying Liver Pathology. Ten biopsies (blinded) were immunohistochemically (IHC) stained for GCTM-5 and positive cells quantified using the Zeiss Axioscan System. The severity of liver pathology is based on clinical records. Each pathology is represented by a value (n=1) or the mean of duplicate measurements for each patient sample. The error bar for Normal (n=4) and Severe patients (n=3) is the standard error.

The severity of liver disease in CF patients is based on clinical records and graded by a histopathologists. Liver biopsies from these patients were stained immunohistochemically with GCTM-5 Mab and slides digitised with an Axioscan.Z1 scanner (Carl Zeiss Microscopy GmbH). Quantification of GCTM-5 positive LPSCs was undertaken using the Zen lite module of the Zeiss Zen 3.9 Software package. While the small sample size precludes meaningful application of statistical

tests, the data suggests that the number of LPSCs identified is indicative of liver comorbidity in severely affected CF patients (Fig 1).

2. Correlating the GCTM-5 biomarker by ELISA with degree of liver co-morbidity

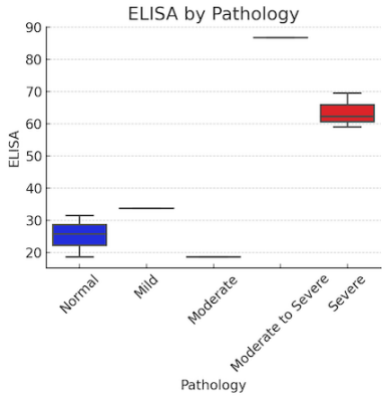

Figure 2. GCTM-5 levels by ELISA in Biopsies from CF patients with Varying Liver Pathology. Ten serum samples (blinded) that were matched to liver biopsies were quantified in duplicate by ELISA to determine level of the GCTM-5 epitope. The quantity of epitope is expressed as ng/mL. The severity of liver pathology is based on clinical records. Mild, Moderate and Moderate to Severe (n=1) data are represented by a line, and Normal and Severe (n=3) data by a rectangle. Each patient sample was measured in duplicate . The error bar for Normal and Severe patients is the standard error. One sample (Normal) was excluded due to extensive hemolysis resulting in an abnormally high reading (>120).

ELISA was used to measure the level of the GCTM-5 epitope (ng/mL) in sera for which we had matched liver biopsies and numbers of GCTM-5 positive LPSCs were previously determined (Fig 1). This indicates the level of the GCTM-5 biomarker is considerably elevated in CF patients with a severe disease comorbidity. The limitation of a small sample size notwithstanding, a *t* test reveals a significantly raised level of the biomarker (2.5-fold) in severely affected compared with non-affected CF patients (P<0.01).

3. Correlating the GCTM-5 biomarker by Luminex Assay with degree of liver co-morbidity

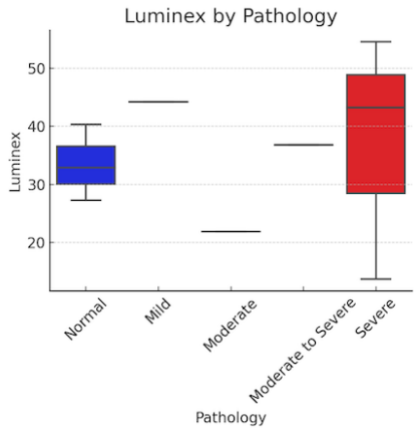

Figure 3. GCTM-5 levels by Luminex Assay in Biopsies from CF patients with Varying Liver Pathology. Ten serum samples (blinded) that were matched to liver biopsies were quantified in duplicate by Luminex Assay to determine level of the GCTM-5 epitope. The quantity of epitope is expressed as ng/mL. The severity of liver pathology is based on clinical records. Mild, Moderate and Moderate to Severe data (n=1) are represented by a line, and Normal (n=4) and Severe (n=3) data represented by a rectangle. Each patient sample was measured in duplicate. The error bar for Normal and Severe patients is the standard error.

Luminex assays were performed on sera with matched biopsies to compare this method against ELISA. This showed higher levels of the biomarker in Normal samples in CF patients with no evidence of liver comorbidity and a lower level of biomarker in CF patients with severe liver comorbidity. Hence there is no correlation between biomarker levels and liver comorbidity. We conclude that the current Luminex assay needs further optimisation. A positive outcome was the Normal sample that

was extensively hemolysed showed low levels of the biomarker, justifying its omission from the ELISA test reported above.

Conclusions and Future Directions

We conclude that IHC utilising the GCTM-5 Mab identifies CF patients with a severe liver comorbidity. This conclusion needs to be validated by increasing the sample size. Its reliability compared against serum transaminases can then be ascertained. Whether IHC identifies those with a mild to moderate condition requires larger sample sizes for each condition to be evaluated.

Regarding using the abundance of the GCTM-5 epitope in blood as a biomarker to assess the severity of liver comorbidity in CF patients, we have promising results based on ELISA of matched sera. This correctly identifies three out of ten samples with a severe comorbidity with statistically significant data.

At this time, the Luminex Assay appears inferior as Normal samples provide a higher reading and Severe samples a lower reading compared to ELISA, so no correlation is established between the biomarker and disease severity.

Future directions include increasing the sample size for all conditions with patient biopsies, all with matched sera. We are pleased to report that our collaborators at the QIMR-Berghofer have identified an additional 30 samples. There are also twenty biopsies and sera that are unmatched, with comprehensive patient information that will be useful.

We will continue to optimise the Luminex assay using an alternate monoclonal antibody ENPRO for which we have the hybridoma. We will also evaluate the ELISA with an aim to improve its sensitivity and reproducibility by refining the current protocol using a reference standard. This is obtained by concentrating serum-free medium in which xx cells that secrete the GCTM-5 biomarker are grown.