PROTOCOL TITLE:
Correlation of blood gene expression (TruGraf Liver) with liver biopsy in pediatric liver transplant recipients

PRINCIPAL INVESTIGATOR:

Name: Ryan Fischer
Department: Pediatrics, Division of Gastroenterology
Phone: Office (816) 302-3410 | Cell (402) 680-8354
Email Address: rtfischer@cmh.edu

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REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?

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STUDY INFORMATION

1.0 Study Summary*

This study is an investigator-initiated, prospective, single-cohort trial to assess the ability of the TruGraf® Liver gene expression panel (GEP) to predict rejection in pediatric liver transplant recipients undergoing surveillance and for-cause liver biopsy.

2.0 Objectives*

2.1 Purpose, specific aims or objectives: To evaluate the performance of the blood-based TruGraf® Liver GEP to accurately diagnose rejection, confirmed by surveillance or for-cause liver biopsy. Additional objectives include comparison of GEP to non-rejection injury (e.g., infection) found on biopsy as well as changes in the GEP during rejection treatment and follow-up.

2.2 Hypothesis: TruGraf® Liver GEP will correlate with histologic evidence of quiescence or rejection in liver biopsies from a pediatric liver transplant population.

3.0 Background*

While liver transplantation is a life-saving measure for both children and adults with end-stage liver disease, the transplant recipient is at lifelong risk of liver transplant rejection and possible loss of the transplanted organ. Immunosuppressive medications are used to prevent unwanted immune activity against the transplanted organ. However, the use of immunosuppression (IS) is a constant balancing act. Too much IS risks infection, side effects of medication (e.g., kidney injury) or the development of some cancers. Too little, and the patient's immune system can reject the transplanted organ. Unfortunately, available serum liver enzyme tests (e.g., alanine aminotransferase or ALT, aspartate aminotransferase or AST, gamma-glutamyl transferase or GGT) are not a reliable indicator of rejection in the pediatric liver transplant recipient (1). Elevated enzymes are often associated with rejection, but they may also result from other liver stressors including infection and medication effect. Equally concerning, patients may have active rejection with otherwise normal enzyme levels.

Anti-allograft immune activity or quiescence can only be defined by histopathologic evaluation of a liver biopsy specimen. The Rejection Activity Index (RAI) measures—on a scale of 0-3 for each category—the degree of portal inflammation, bile duct injury, and venous endothelial damage seen on liver biopsy (2). Thus, the RAI score can range from 0 (no signs of rejection) to 9 (the most severe rejection). In general, scores of 1-2 are considered "indeterminate" with lower risk of consequential rejection. Scores of 3 or greater are typical of rejection, and programs usually increase or adjust IS strategies in these patients.

Treatment can include high-dose steroids or biologic agents that predispose patients to infection and medication side effects. While liver biopsy is often obtained in patients with elevated AST, ALT, GGT, etc. to rule out rejection, these enzymes may be elevated in other conditions described above. Conversely, transplant recipients may have rejection without elevated liver enzymes, and programs (including ours) often obtain "surveillance" biopsies at regular intervals to ensure immune quiescence.

Surveillance biopsies in the pediatric population have shown an association with reduced IS requirements, significantly fewer rejection episodes, and an association with superior allograft and patient survival (3). Yet, these invasive procedures are costly and carry risk to the patient and allograft. The Liver Care Center at Children's Mercy performs surveillance biopsies on all pediatric liver transplant recipients bi-annually until they are transitioned into an adult program. The estimated cost of surveillance biopsy ranges from $1,500 to $3,000 (4), not including additional hospital charges and miscellaneous fees. While the biopsies are performed with the utmost care using ultrasound guidance, significant bleeding (and even death) can complicate the procedure.

Utilizing the TruGraf® Liver GEP (Transplant Genomics, Inc. (TGI); Lenexa, KS) as a "liquid biopsy" may offer a non-invasive alternative to the surveillance biopsy and/or for-cause liver biopsy. The test does not require liver tissue, and instead is run on a routinely obtained blood sample. This may provide opportunity for more frequent monitoring of liver injury and may aid in IS management. If the GEP correlates with and discriminates between biopsy results associated with rejection and those associated with quiescence, there is opportunity to reduce risk to liver transplant recipients, carefully personalize medication regimens, and produce a cost-savings.

References

1. Rocque B, et al. "Clinical Value of Surveillance Biopsies in Pediatric Liver Transplantation." *Liver Transplant* (2022).

2. Demetris et al. "Banff Schema for Grading Liver Allograft Rejection: An International Consensus Document." Hepatology (1997).

3. Squires JE, Demetris AJ. "Surveillance Biopsies in Pediatric Liver Transplantation: Is the Juice Worth the Squeeze?" *Liver Transplantation* (2022).

4. Allen AM, et al. "Healthcare Cost and Utilization in Nonalcoholic Fatty Liver Disease: Real-World Data From a Large U.S. Claims Database." *Hepatology* (2018).

4.0 Study Endpoints

4.1 **Primary endpoint** will be the correlation of the TruGraf® Liver GEP with rejection activity index (RAI) on surveillance and for-cause biopsy.

4.2 **Secondary endpoints** will include

A) a comparison of the GEP to non-rejection injury (e.g., infection) found on biopsy

B) utility of the GEP for monitoring liver injury (i.e., as treatment is weaned, does the GEP return to a non-rejection correlate).

5.0 Study Design*

5.1 **Study Design:** This will be a prospective cohort study.

5.2 All pediatric liver transplant recipients undergoing surveillance or for-cause liver biopsy will be approached for inclusion in the study. For those consented, study staff will collect medical history, demographics, height and weight, laboratory results, liver-related medical information, and up to 4 blood samples. The initial blood sample will be obtained at standard of care labs performed the week prior to liver biopsy or at the time of biopsy. Additional blood samples will be collected at standard of care blood draws at weeks 8-12, 20-24, and 30-36 and stored for potential GEP analysis. If subjects do not get a SOC blood draw at these time points we will get a sample at their nearest SOC draw. We will not have a blood draw done just for research. The PI will review blood draw amounts to ensure that they do not exceed NIH blood draw limitations. If they do, the blood used for research will not be drawn. Blood samples will be used to evaluate the TruGraf® Liver GEP (see section 6.0 for more info on TruGraf® GEP).

5.3 **Table of Events:**

The only event on this study that is not standard of care are informed consent at start of study and the collection of additional blood at weeks 8-12, 20-24, and 30-36 for GEP analysis that are done at the same time as standard of care blood draws.

6.0 Study Interventions/Investigational Agent*

6.1 **Description:** This study will evaluate the TruGraf® Liver GEP, a panel that measures messenger RNA levels of 120 genes that are differentially expressed in transplant quiescence and subclinical rejection. Blood samples will be used to run the GEP and data as outlined in section 26.1 obtained from subjects chart will be used with GEP results to evaluate the GEP.

6.2 **Drugs or Biologics:** *N/A*

6.3 Medical Devices. *The blood test being studied is not of substantial importance in diagnosing, curing, mitigating or treating disease, or otherwise preventing impairment of human health and doesn't present a potential form serious risk to the health safety or welfare of the participant. The gold standard for diagnosing liver transplant rejection is biopsy, which is being done as part of standard of care outside of the study and the results of this blood test will not be used to direct care. As it is a blood test and not an implant it does not meet any of the other criteria in the regulations so does not require an IDE/HDE. There is no risk to the subject outside of the general risk of phlebotomy which is being done for standard of care. The de-identified blood samples will be sent to TGI and the TruGraf Liver GEP test will be done at their laboratory. None of the investigators will store, handle or administer the blood test.*

Device Name	FDA Approval Status and Use in this Study	IDE/HDE applicability
TruGraf® Liver GEP	Other	Exempt from IDE requirements

6.4 Behavioral Intervention*: N/A*

PARTICIPANT MANAGEMENT

7.0 Inclusion and Exclusion Criteria*

7.1 Eligibility Criteria:

Inclusion Criteria

- All liver transplant patients at least 1 year of age and less than 18 years of age undergoing surveillance or for-cause liver biopsy

Exclusion Criteria

- Non-English, non-Spanish Speaking

7.2 Equitable Selection*:* This study will promote diversity by offering enrollment to nearly every patient presenting to the Liver Care Center at Children's Mercy Kansas City for post-transplant care. This study will include consent forms written in both English and Spanish. We will attempt to use the short form if there is a patient whose primary language is not Spanish or English and have a certified translator present to translate the consent form.

7.3 Vulnerable Populations: *Check any vulnerable populations that are being targeted for enrollment into the study: (Members of the following populations may not be included as participants in the research unless selected here.)*

☒ Children/Minors (under 7 years of age)
☒ Children/Minors (7-17 years of age)
☐ Neonates (infants less than 30 days old)
☐ Neonates of Uncertain Viability (infants less than 30 days old)
☐ Non-Viable Neonates (infants less than 30 days old)
☐ Wards of the State
☐ Fetuses
☐ Pregnant Women
☐ Adults with impaired decision-making capacity

☐ CM Employees
☐ CM Students/Residents/ Fellows
☐ Economically or Educationally Disadvantaged Persons
☐ Prisoners

- We will obtain parental permission and (where applicable) the assent of the child/subject. The study presents no more than minimal risk as blood draw for GEP will be done at the same time as standard of care laboratory studies and will adhere to daily/weekly blood draw limits. Potential participants will be given ample time to ask questions and it will be iterated that participation is optional and will not affect clinical care.

8.0 Local Number of Participants

8.1 Our enrollment goal will be 25 patients. We will enroll at least 8 patients obtaining biopsy "for cause" (higher risk of rejection) and at least 8 surveillance biopsy patients.

	Total	**Sub-group "For Cause"**	**Sub-group "Surveillance"**
Enrollment Goal: *Number of participants to be enrolled = the number of participants to be consented.*	25	At least 8	At least 8

9.0 Identification and Recruitment of Potential Participants*

9.1 Identification of Potential Participants:

How will participants be identified? (Check all that apply)

☒ Chart reviews

☒ By their treating physician who will then provide the study team's contact information to the potential participant/family

☒ By their treating physician who will obtain patient/family permission to share contact information with the study team

☐ Self-refer in response to IRB approved advertisements or websites

☐ Through Cerner or other CM sources (e.g. databases, billing records, pathology reports, admission logs, etc.) May involve access of records by individuals not involved in the patient's care.

☐ List of candidates provided through the Data Report Request Form

☐ Registry of individuals interested in research opportunities

☐ Past participant list

☐ Participants will roll-over from another research study: Study #

☐ Other:

9.2 Pre-Screening prior to HIPAA Authorization

Will any of the identification methods checked above involve access to Protected Health Information (PHI) prior to obtaining HIPAA Authorization?

☒ Yes

☐ No

- *If yes, a "Partial Waiver of HIPAA Authorization" is required. Be sure to make this selection in the "HIPAA & Confidentiality" section below and complete* ***Addendum E: Waiver/Alteration of HIPAA Authorization***

9.3 Recruitment of Potential Participants:

- Patients will be recruited from the Pediatric Liver Transplant Program clinic. The clinic cares for more than 100 pediatric recipients of liver transplant. Patients are seen in person in clinic on at least an annual basis. Patients undergo biopsy on a bi-annual schedule. An estimated 50 liver biopsies are done for surveillance or for cause annually. Patients undergoing liver biopsy are scheduled at least 2-3 days in advance. This will allow adequate time for the transplant team to notify the physician and/or research team of the upcoming biopsy.

- A member of the study team will introduce the study to eligible subjects/families during a clinic visit or clinical phone call that is prior to liver biopsy. If this is not feasible, a member of the study team will call eligible families prior to liver biopsy and introduce to the study.

10.0 Surveys and Psychometric Testing:

11.0 Additional Study Activities

15.0 Risks to Participants

16.0 Potential Benefits