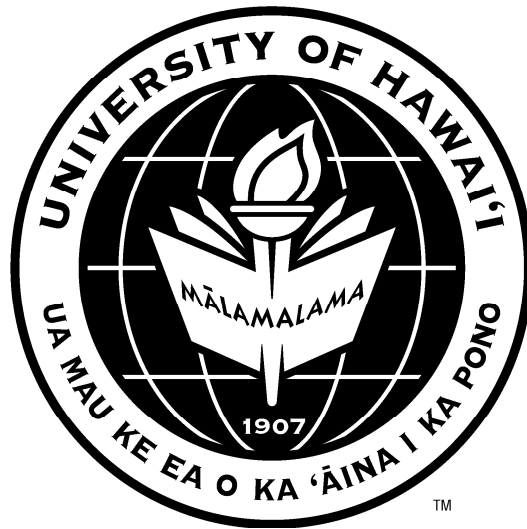


# UNIVERSITY OF HAWAI‘I SYSTEM REPORT



REPORT TO THE 2020 LEGISLATURE

Interim Report on High Incidence of Liver and  
Bile Duct Cancer in Hawai'i

Act 265, SLH 2019

February 2020

**University of Hawai'i Cancer Center's  
Report on High Incidence of Liver and Bile Duct Cancer in Hawai'i  
Pursuant to Act 265, Session Laws of Hawai'i 2019**

The 2019 Hawai'i State Legislature appropriated funds to the University of Hawai'i Cancer Center to determine the etiologies of the high incidence of liver and bile duct cancer in Hawai'i. The report will contain UHCC's findings, including how the appropriated funds will be spent.

The appropriated funds were not released until late October 2019. Funds were allocated to projects to conform to the guidelines and intent of the legislation, in order to conduct research to determine the etiologies of the high incidence of liver and bile duct cancer in Hawai'i. The projects are as outlined in the Table below.

**Liver Cancer Legislative Allocation**

| Year | Title   | UHCC Principal Investigators  |
|------|---|---|
| 2019 | Liver Fluke and Aflatoxin as an etiology for liver and bile duct cancer   | Yu, Jia   |
| 2019 | Immunotherapy of liver cancer (IIT)   | Acoba   |
| 2019 | Solicited proposals <ol style="list-style-type: none"> <li>1. Oral microbiome and HCC risk</li> <li>2. Non-alcoholic steatohepatitis (NASH) and hepatocellular cancer</li> <li>3. identify signature of exosomal miRs transferred from TAMs to HCC and mechanism of PDL-1 upregulation in HCC by TAM-derived exosomal miRs</li> <li>4. Pet imaging and liquid biopsy detection of CTNNB1 exon 3 mutations in HCC</li> </ol> | <ol style="list-style-type: none"> <li>1. Hernandez</li> <li>2. Kuwada</li> <li>3. Fabbri</li> <li>4. Kwee</li> </ol> |

Interim results on the various projects are attached to this memorandum. A final report for the legislature for this year of funding will be prepared for submission by the end of June 2020.

Randall F. Holcombe, MD, MBA  
Director, University of Hawai'i Cancer Center  
February 2020

## **Epidemiological Study of Liver Cancer in Hawaii**

**Wei Jia, Herbert Yu, Brenda Hernandez, and Linda Wong**

The preparation of our epidemiological study is under way, and we have initiated and completed the following tasks.

- Hired a research scientist and a graduate student to assist the study preparation and development
- Revised our study protocol (attached)
- Prepared an IRB application for the human study
- Submitted the IRB application to WIRB
- Revised the IRB application following an initial review by WIRB
- Resubmitted the IRB application for approval
- Searched for ELISA kits for testing aflatoxin antigen (total, B1)
- Set up lab instruments for ELISA testing
- Ordered lab suppliers and reagents for ELISA testing
- Identified ELISA kits for testing aflatoxin B1 and aflatoxin total from several companies
- Ordered ELISA kits for aflatoxin B1 from 4 different companies
- Received 2 kits for aflatoxin B1 and tested, one worked and one did not
- Ordered ELISA kits for aflatoxin total from 5 different companies
- Received 1 kit for aflatoxin total and tested one which did not work
- Identified ELISA kits for testing human antibodies against liver fluke from several companies
- Ordered ELISA kits for human liver fluke antibodies from 6 different companies
- Received one kit yesterday (Jan. 28) and one is expected to arrive today (Jan. 29)

## **Immunotherapy of Liver Cancer**

**Jared Acoba, MD**

**January, 2020 Interim Report**

### Research Activities

- Obtained IRB approval, developed case report forms.
- Listed protocol on [www.clinicaltrials.gov](http://www.clinicaltrials.gov)
- Consented three patients within a month of opening trial.
- Two patients have received study treatment (one patient has received two doses) and third one starts treatment this January 31.
- No concerning side effects noted as yet.
- First patient will undergo scans for disease assessment in about a month (end of February).
- Collecting tissue and blood samples to help predict which patients are most likely to benefit from treatment. Samples are being stored for subsequent analysis.

**UHCC Liver Cancer Initiative project progress report: Oral microbiome and hepatocellular carcinoma risk (PI B. Hernandez, Co-I: H. Yu, L. Wong)**

**January 29, 2020**

The overall objective of our study is to investigate the role of the oral bacterial microbiome and metagenome in the development of liver cancer. The long-term goal is to understand the pathogenesis of liver disease and to identify novel biomarkers of liver cancer that may inform prevention and early detection strategies. Our study aims are: 1) To evaluate the relationship of oral bacteria composition with HCC risk overall and by etiology; and 2) To evaluate the relationship of oral bacteria gene functional pathways with HCC risk overall and by etiology.

Progress to date:

- Protocol submitted to and approval received from the IRB (UH Committee on Human Studies).
- Reagents for PCR and Illumina MiSeq 16S rRNA sequencing purchased.
- Preliminary work done (funded through another grant) to inform the bacterial gene functional analyses. Expression of 770 genes in 23 HCC tumors were evaluated using the Nanostring nCounter platform. Twenty-one genes were most prominent including apolipoprotein genes (APOA1, APOA2, APOC3, APOB, APOH), alcohol dehydrogenase 1B, class I, beta polypeptide (ADH1B), aldolase, fructose-bisphosphate B (ALDOB), and stearyl-CoA desaturase (SCD). The corresponding top gene pathways were related to cholesterol metabolism, fatty acid metabolism, gluconeogenesis, alcohol metabolism, and peroxisome proliferator-activated receptor (PPAR) signaling. These results are consistent with our prior work in a healthy adult population where we found that alteration of the oral microbiome is linked to alcohol consumption and type 2 diabetes, two major liver cancer risk factors.
- 80 HCC cases and 80 controls selected from case-control study (R01CA138698, PI Yu) for comparison of the oral microbiome.

## **Non-alcoholic Steatohepatitis (NASH) and Hepatocellular Cancer**

**Scott Kuwada, MD**

**January, 2020 Interim Report**

### Research Activities

This project requires identification of individual with NASH that has developed in the exclusion of other chronic liver disease. Following identification and verification of potential participants, an dietary intervention designed to reduce the fat content in the liver will be offered.

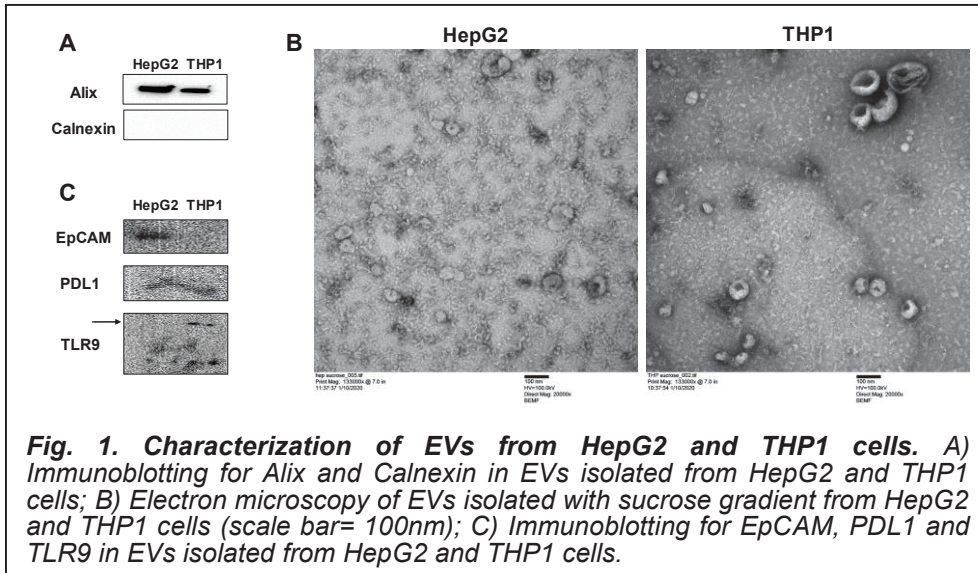
Activities to date include:

- Identification of over 150 patients in the fibroscan database
- Review of medical records of identified individuals to:
  - Verify diagnosis
  - Exclude other chronic liver disease
  - Record ethnicity and other demographics

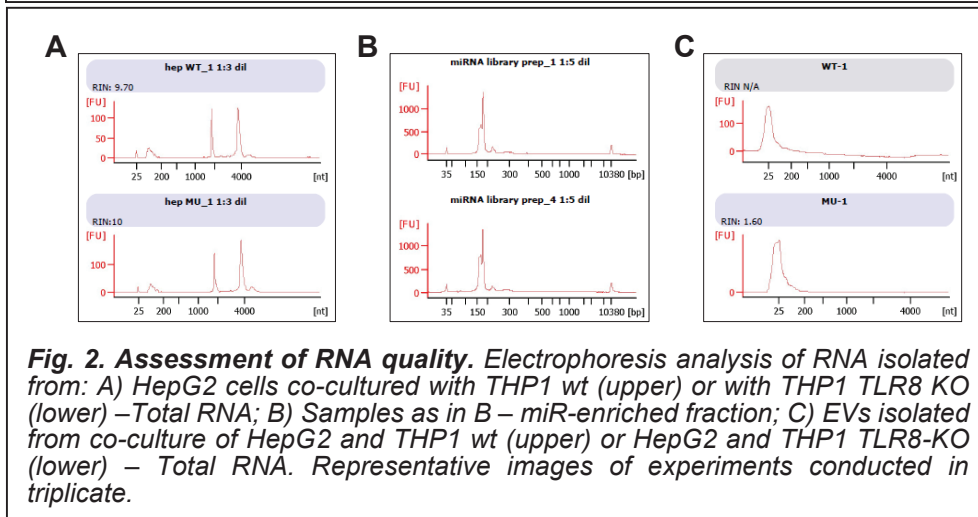
These activities will continue until sufficient candidates for an interventional dietary program are identified and enrolled.

Title: Identify signature of exosomal miRs transferred from TAMs to HCC and mechanism of PDL1 upregulation in HCC by TAM-derived exosomal miRs.

PI: Muller Fabbri, MD, PhD



During these first 2.5 months of work we have focused on the identification of a signature of dys-regulated miRs in Hepatocellular Carcinoma (HCC) cells co-cultured with human monocytes (THP1 cells) wild-type or with CRSPR-deletion of Toll-like receptor 8 (TLR8). HepG2 and THP1 (wt or TLR8-KO) have been co-cultured for 7 days in a trans-well system with pores 0.4  $\mu\text{m}$  in diameter, so to allow only the exchange of soluble factors and not cell-cell contact. We reasoned that 5 days of co-culture are necessary to polarize THP1 cells into M2-polarized macrophages (or Tumor-Associated Macrophages, TAMs). The additional 2 days were required to assess the miR exchange between TAMs and HepG2 after 48h. Extracellular vesicles (EVs) have been collected from the following groups: HepG2 alone, THP1 wt alone, THP1 TLR8-KO alone, HepG2 co-cultured with THP1 wt, HepG2 co-cultured with THP1 TLR8-KO. EVs have



been characterized for the expression of Alix and absence of Calnexin expression (Fig. 1A) and analyzed at the electron microscopy for their size, lipidic membrane bilayer and cup-shaped morphology (Fig. 1B). These data suggest that the EVs that we isolated belong to the category of “exosomes”, according to the ISEV guidelines (characterization by qNANO is ongoing). We also investigated whether EpCAM1, PDL1 and TLR9 expression in EVs could distinguish between exosomes derived from HepG2 cells and exosomes derived from THP1 cells. While these data need further confirmation, preliminary evidence suggests that EpCAM1 could be selectively present in exosomes derived from HepG2 cells (Fig. 1C). Next, we isolated total RNA and the miR-enriched RNA fraction from HepG2 cells in co-culture with THP1 wt and from HepG2 cells in co-culture with THP1 TLR8-KO. The RNA quality by electrophoresis resulted excellent with a pure population of small RNAs in the fraction enriched for miRs (Fig. 2A-B). Moreover, we isolated total RNA from exosomes from co-cultures of HepG2 and THP1 wt (or HepG2 and THP1 TLR8-KO) and the quality assay revealed the presence, in EVs of small RNAs with the absence of large ribosomal RNAs (Fig. 2C). Given the good quality of the isolated RNAs, we decided to proceed with the RNA sequencing and the samples are currently running. We are expecting the results in the next few days, followed by the bioinformatics analysis that will allow to focus on the top dys-regulated candidates and proceed with the functional experiments in Aim 2 of the proposal. We currently do not foresee any delays in the successful completion of this project by June 2020.

**PET imaging and liquid biopsy detection of CTNNB1 exon 3 mutations in HCC**  
(PI-Sandi Kwee, Team: Maarit Tiirikainen, Karolina Peplowska, Chris Farrar, Linda Wong)  
**January 2020 Project Progress Report**

The specific aims are unchanged from the submitted proposal. For a summary of the background and rationale of the project, please refer to the proposal. Our progress towards completing our aims are as follows: Aim 1. We have completed macro-dissection and laser-capture microdissection of >24 tumor tissue samples from HCC patients and have extracted the tumor DNA. Tumor DNA sequencing is planned for early next month. Aim 2. We have completed the targeted DNA sequencing and mutation profiling of cell-free DNA from the pre- and post- surgery blood samples. Bioinformatic analysis of the sequencing data is not yet finished, but some preliminary results are shown below. Completion of Aim 3 must await completion of aims 1 and 2.

Commercial tumor mutational profiles were clinically available from 6 of the patients. We preliminarily examined their correspondence with our liquid biopsy results and PET imaging results. Below is a summary of the results:

| ID                | Tumor mutations detected in tissue using the Genomic Testing Co-op 434 gene panel (VAF %)   | cfDNA mutations (VAF %)                         |
|-------------------|---|---|
| P1 (PET-positive) | CTNNB1 missense (5.7%), TP53 missense (9.1%), CSF3R missense (5.1%), TET2 stopgain (6.4%), BRAF missense (4.8%), PTEN missense (5.8%)         | CTNNB1 missense (3.8%), TP53 missense (7.0%)    |
| P2 (PET-positive) | CTNNB1 missense (36.3%), TP53 missense (76.2%, a putative germline variant), INHBA/INHBA-AS1 insertion (12%)                                  | CTNNB1 missense (17.1%), TP53 missense (28-35%) |
| P3 (PET-positive) | CTNNB1 missense (34.4%), PBRM1 missense (12.2%), NUP93 stopgain (13.1%), DNMT2 (11.3%), SMARCA4 missense (29.6%)                              | CTNNB1 missense (0.8%)                          |
| P4 (PET-positive) | GNAS missense (42.1%), EPHA3 stopgain (38.7%), ATM stopgain (4.6%), STAG2 stopgain (4.8%)   | GNAS missense (10.3%)                           |
| N1 (PET-negative) | SPTA1 stopgain (12.6%), STAT4 stopgain (10.8%), FANCM stopgain (11.5%), SMAD4 missense (3.8%)   | No 56G panel gene mutation loci detected.       |
| N2 (PET-negative) | ARD1A frameshift (6.6%), LRP1B stopgain (12.7%), PDGFRA stopgain (8.4%), PTEN frameshift (7.1%), BRIP1 stopgain/splice region variant (11.2%) | DNMT3A missense (1.5%)                          |

**Table 1. Preliminary data comparing targeted NGS-based mutation profiles of cfDNA and laser-capture microdissected tumor DNA from HCC patients imaged by 18F-FCH PET/CT (4 PET positives and 2 PET negatives for tumor beta-catenin activation).** VAF = variant allele frequency. Mutations that have correspondence between tumor and cfDNA are shown in yellow: Mutations associated with aberrant Wnt/beta-catenin pathway activation were detected in the cfDNA and corresponding tumor DNA of all 4 patients with PET-positive tumors (P1-P4). In P1-P3, these were missense mutations of CTNNB1. In P4, a GNAS mutation was identified. GNAS mutations have been reported to activate W/β-c pathways and upregulate lipid metabolism in other digestive tract cancers, but not yet in HCC. No Wnt/beta-catenin activating mutations were detected in the cfDNA or tumor DNA of the 2 patients with PET-negative tumors. In N1, an absence of mutations detected by liquid biopsy is consistent with a true-negative result based on the corresponding profile from tumor tissue. Interestingly, tumor profiling detected a mutation causing inactivation of SMAD4. SMAD4 mutations are associated with Wnt/beta-catenin pathway downregulation, and could in this case be contributing to the low tumor FCH uptake. A “false positive” mutation (detected in cfDNA but not tumor DNA) was noted in N2. This mutation, detected in cfDNA at VAF 1.5%, may be due to clonal hematopoiesis, an age related hematologic phenomenon that frequently manifests as DNMT3A mutations. No β-catenin activating mutations were detected, thus PET was correct.

To summarize, mutations known to cause Wnt/beta-catenin activation (yellow) were detected in the cfDNA and tumor DNA of all 4 patients with PET-positive tumors (green). No Wnt/beta-catenin activating mutations were detected in the samples of 2 patients with FCH non-avid tumors (red). A DNMT3A mutation detected in cfDNA but not tumor DNA in one patient (blue) is likely the result of clonal hematopoiesis, a recently discovered phenomenon that may have implications for clinical development of a FDA-approved liquid biopsy assay. Although this is only preliminary data from 6 patients, it establishes our capabilities for performing liquid biopsy, and for detecting Wnt/beta-catenin activating mutations by cfDNA-sequencing, as well as an association between Wnt/beta-catenin activating mutations and tumor avidity for FCH. Thus, these preliminary results are of value to support our R01 proposal for a clinical diagnostic trial to evaluate NGS-based liquid biopsy and FCH PET/CT as sources of Wnt/beta-catenin pathway biomarkers for predicting the efficacy of ICI therapy in HCC. The sequencing coverage in these pilot experiments exceeded 24,000x. These results also showed that our platform could accurately detect cancer mutations in cfDNA at below 1% allele frequency. This platform therefore meets contemporary benchmarks for current-generation NGS-based liquid biopsy assays.

**Next Steps:** Based on these results, we began writing a Clinical Trials R01 grant proposal (responding to either NIH/NCI PAR-18-560 or PAR 19-363) to conduct a phase II clinical trial evaluating the combined use of FCH PET/CT and NGS-based liquid biopsy as diagnostic tools for predicting objective therapeutic response to anti-PD1 immunotherapy in unresectable HCC. One of our personal goals is to establish a clinically-translatable liquid biopsy platform that can exceed the current benchmarks for NGS-based liquid biopsy by a factor of two with regards to not only sensitivity and depth of coverage, but also false positive rate control. This R01 grant proposal will be submitted February 4, 2020. Despite the added effort required in writing this grant proposal, we expect to complete our stated aims by the end of the project period.