UNIVERSITY of HAWAI'I SYSTEM

David Lassner
President

DEPT. COMM. NO. 123

December 7, 2020

The Honorable Ronald D. Kouchi,
President and Members of the Senate
Thirtieth State Legislature
Honolulu, Hawai'i 96813

The Honorable Scott Saiki, Speaker
and Members of the House of Representatives
Thirtieth State Legislature
Honolulu, Hawai'i 96813

Dear President Kouchi, Speaker Saiki, and Members of the Legislature:

For your information and consideration, the University of Hawai'i is transmitting one copy of the Report on the University of Hawai'i Cancer Center on the Etiologies of the High Incidence of Liver and Bile Duct Cancer in Hawai'i (Act 265, Session Laws of Hawai'i 2019) as requested by the Legislature.

In accordance with Section 93-16, Hawai'i Revised Statutes, this report may be viewed electronically at: https://www.hawaii.edu/offices/government-relations/2021-legislative-reports/.

Should you have any questions about this report, please do not hesitate to contact Stephanie Kim at 956-4250, or via e-mail at scskim@hawaii.edu.

Sincerely,

David Lassner
President

Enclosure

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UNIVERSITY OF HAWAIʻI SYSTEM REPORT

REPORT TO THE 2021 LEGISLATURE

Report on the University of Hawaiʻi Cancer Center
on the Etiologies of the High Incidence
of Liver and Bile Duct Cancer

Act 265, SLH 2019

November 2020

University of Hawai‘i Cancer Center
Report on High Incidence of Liver and Bile Duct Cancer in Hawai‘i

Pursuant to Act 265, Session Laws of Hawai‘i 2019, the Hawai‘i State Legislature appropriated funds to the University of Hawai‘i Cancer Center (UHCC) to determine the etiologies of the high incidence of liver and bile duct cancer in Hawai‘i. This report will contain UHCC's findings, including how the appropriated funds are being spent.

The appropriated funds were released in 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	**Project Titles**	**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	Smaller scale projects 1. Oral microbiome and HCC risk 2. Non-alcoholic steatohepatitis (NASH) and hepatocellular cancer 3. Identify signature of exosomal miRs transferred from TAMs to HCC and mechanism of PDL-1 upregulation in HCC by TAM-derived exosomal miRs 4. Pet imaging and liquid biopsy detection of CTNNB1 exon 3 mutations in HCC	 1. Hernandez 2. Kuwada 3. Fabbri 4. Kwee

Results on the various projects are attached to this memorandum. Multiple projects had very significant findings that are being prepared for peer-reviewed publications. In addition, several of the investigators are utilizing the preliminary data generated under this limited one-year funding mechanism to support applications for additional research grants through the National Institutes of Health.

Major findings include:

- No evidence of liver fluke infection in liver cancer patients in Hawai‘i, though testing for other forms of liver fluke is being considered.
- There is aflatoxin B food contamination, especially in raw peanuts in Hawai‘i that may contribute to liver cancer in the state.
- The composition of oral bacterial (microbiota) may contribute to liver and bile duct cancer through the disruption of the healthy flora of the mouth inducing insulin resistance, aberrant fatty acid metabolism and other metabolic disruption.

- Oral bacterial metabolites may induce liver toxins and act independently from other known risk factors.
- An association between tumorigenic Wnt signaling and PET/CT imaging phenotype has been confirmed in liver cancer.
- Non-alcoholic fatty liver disease (NAFLD) patients have an over-representation of Asians.
- Aspirin may be an effective anti-fibrotic agent in NAFLD patients and could be tested as a cancer prevention strategy for liver cancer.
- An investigator-initiated immunotherapy trial of combination immunotherapy for liver cancer patients has been initiated and remains ongoing. Two patients have experienced stable disease for >10 months and one patient had an 85% reduction in the extent of tumor.
- Micro-RNAs impact PDL1 expression in liver cancer cells in vitro, and the latter is a marker of clinical responsiveness to immune therapies.
- Fibroblasts in the tumor microenvironment play a role in transmitting micro-RNAs and affecting the immune-responsiveness of the cancer milieu.

Results from four of the projects were presented at the University of Hawai‘i Cancer Center Scientific Retreat, held in virtual format on October 9, 2020. These presentations, plus summaries from the other two projects, are attached.

A summary composite budget delineating expenditure of funds is also appended to this report.

Randall F. Holcombe, MD, MBA
Director, University of Hawai‘i Cancer Center
October 23, 2020

Investigation of Risk Factors of Liver Cancer in Hawaii

Herbert Yu

October 9, 2020

Background

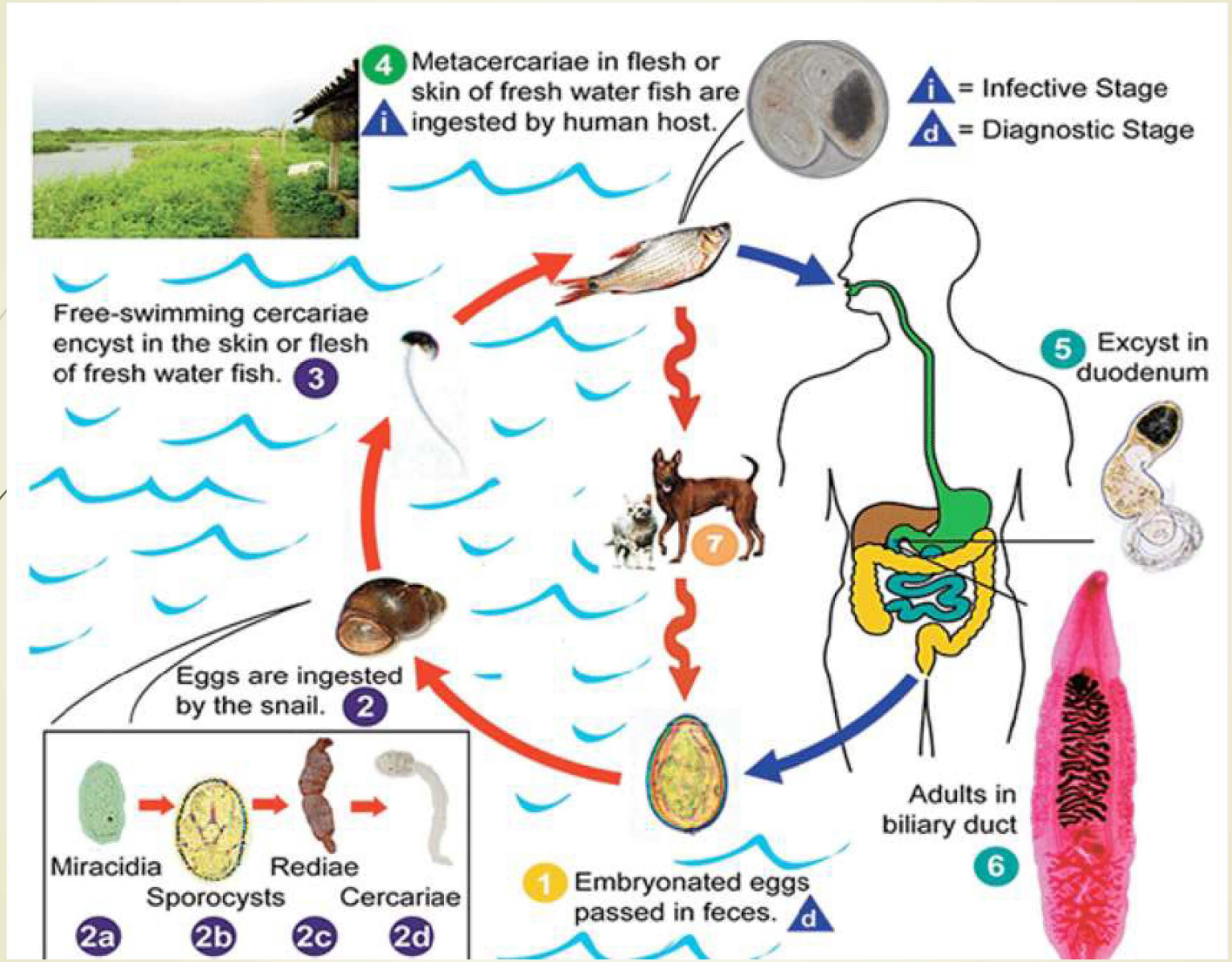
- Community request
- Legislature bill (SB2049 in 2018)
- Legislature bill (HB654 in 2019)
- State government funding (July, 2019)
- Research focus (local risk factors)
- Liver fluke infection
- Food contamination by Aflatoxin B1

Are There Liver Flukes in Hawaii?

A UH research study entitled *Parasite Infections of Man and Animals in Hawaii* by **Joseph E. Alicata** a professor at the Hawaii Agricultural Experiment Station, College of Tropical Agriculture, University of Hawaii, **November 1964**, clearly shows that **liver flukes are relatively common in Hawaii found in various animals and snails in and near fresh water habitats such as streams, ponds and wet soil**. https://www.ctahr.hawaii.edu/oc/freepubs/pdf/tb-61.pdf

According to one report: "***There are twelve watercress farms in Oahu*, each growing a different variety of watercress, but all twelve are in the vicinity of the Pearl Harbor Spring** (the rest around Pearl City and Waipahu).' http://hawaiiindependent.net/story/sumida-farms-embraces-the-past-and-future-of-agriculture-in-hawaii

Research should be done at the UH Cancer Center immediately to determine whether residents of Hawaii are at risk of liver/bile duct cancer by eating uncooked watercress which may be infected with liver flukes. There are other possible causes such as eating foods with aflatoxins, produced by a fungus abundant in warm and humid regions, that can grow on foods such as grains e.g. rice and nuts that have been stored improperly. This too should be researched by UHCC.


4 Metacercariae in flesh or skin of fresh water fish are i ingested by human host.
i = Infective Stage
d = Diagnostic Stage
Free-swimming cercariae encyst in the skin or flesh of fresh water fish. 3
5 Excyst in duodenum
7
Eggs are ingested by the snail. 2
Miracidia
Sporocysts
Rediae
Cercariae
2a
2b
2c
2d
1 Embryonated eggs passed in feces. d
Adults in biliary duct
6

How to examine liver fluke infection?

- Microscopic exam for parasite eggs in stool samples.
- Tissue analysis of parasite eggs in liver specimens.
- DNA/RNA analysis?
- Immunoassay for antigens?
- Immunoassay for human antibodies
- Tested 67 plasma samples from Dr. Wong's biorepository
- All results were negative.

DGR				Origene			DiagAuto	
Sample ID	Average	Final		Value	Final		Value	Final
L0009	0.074	Negative		0.102	Negative		0.187	Negative
L0010	0.068	Negative		0.071	Negative		0.054	Negative
L0011	0.130	Negative		0.184	Negative		0.084	Negative
L0012	0.246	Negative		0.302	Negative		2.129	Negative
L0013	-0.009	Negative		0.043	Negative		-0.005	Negative
L0014	0.217	Negative		0.279	Negative		0.130	Negative
L0015	0.392	Negative		0.405	Negative		0.139	Negative
L0016	0.120	Negative		0.158	Negative		0.200	Negative
L0017	0.182	Negative		0.182	Negative		0.159	Negative
L0018	0.375	Negative		0.248	Negative		1.771	Negative
L0019	0.166	Negative		0.170	Negative		0.093	Negative
L0020	-0.013	Negative		0.032	Negative		-0.003	Negative
L0021	0.441	Negative		0.427	Negative		0.218	Negative
L0022	0.183	Negative		0.219	Negative		0.183	Negative
L0023	0.296	Negative		0.286	Negative		0.276	Negative
L0024	0.263	Negative		0.332	Negative		0.144	Negative
L0025	0.154	Negative		0.159	Negative		0.168	Negative
L0026	0.287	Negative		0.231	Negative		0.204	Negative
L0027	0.328	Negative		0.229	Negative		0.560	Negative
L0028	0.033	Negative		0.089	Negative		0.120	Negative
L0029	0.182	Negative		0.132	Negative		0.384	Negative
L0030	0.144	Negative		0.146	Negative		0.419	Negative
L0031	0.217	Negative		0.199	Negative		0.046	Negative
L0032	0.174	Negative		0.188	Negative		0.136	Negative
L0033	0.173	Negative		0.156	Negative		0.201	Negative
L0034	0.492	Negative		0.486	Negative		0.560	Negative
L0035	0.129	Negative		0.011	Negative		0.062	Negative
L0036	0.094	Negative		0.129	Negative		0.073	Negative
L0037	0.152	Negative		0.074	Negative		0.052	Negative
LW101	0.256	Negative		0.229	Negative		0.093	Negative
LW102	0.200	Negative		0.136	Negative		0.047	Negative
LW103	0.237	Negative		0.236	Negative		0.220	Negative
LW104	0.243	Negative		0.201	Negative		0.271	Negative
LW105	0.303	Negative		0.255	Negative		0.298	Negative
LW106	0.203	Negative		0.012	Negative		0.551	Negative
LW107	0.233	Negative		0.166	Negative		0.158	Negative
LW108	0.288	Negative		0.088	Negative		0.454	Negative
LW109	0.280	Negative		0.212	Negative		0.160	Negative
LW110	0.439	Negative		0.171	Negative		0.285	Negative
LW111	0.213	Negative		0.165	Negative		0.807	Negative
LW112	0.246	Negative		0.195	Negative		0.390	Negative
LW113	0.217	Negative		0.199	Negative		1.404	Negative
LW114	0.290	Negative		0.061	Negative		0.164	Negative
LW115	0.266	Negative		0.222	Negative		0.079	Negative
LW116	0.130	Negative		0.029	Negative		0.098	Negative
LW118	0.420	Negative		0.270	Negative		1.342	Negative
LW119	0.267	Negative		0.159	Negative		0.472	Negative
LW120	0.244	Negative		0.163	Negative		0.392	Negative
LW121	0.202	Negative		0.153	Negative		0.095	Negative
LW122	0.138	Negative		0.148	Negative		0.245	Negative
LW123	0.257	Negative		0.114	Negative		0.406	Negative
LW124	0.340	Negative		0.243	Negative		0.078	Negative
LW125	0.189	Negative		0.096	Negative		0.259	Negative
LW126	0.324	Negative		0.269	Negative		2.222	Negative
LW127	0.443	Negative		0.219	Negative		1.930	Negative
LH0001	0.208	Negative		0.156	Negative		1.425	Negative
LH0002	0.415	Negative		0.193	Negative		0.850	Negative
LH0003	0.467	Negative		0.133	Negative		0.076	Negative
LH0004	0.370	Negative		0.149	Negative		0.784	Negative
LH0005	0.377	Negative		0.250	Negative		0.304	Negative
LH0006	0.205	Negative		0.069	Negative		0.259	Negative
LH0007	0.296	Negative		0.200	Negative		0.182	Negative
LH0008	0.251	Negative		0.075	Negative		0.113	Negative
LH0009	0.383	Negative		0.279	Negative		0.515	Negative
LH0010	0.146	Negative		0.168	Negative		0.046	Negative
LH0011	0.225	Negative		0.204	Negative		0.057	Negative
LH0012	0.184	Negative		0.201	Negative		0.048	Negative

Fasciola IgG ELISA RUO

RUO

REF EIA-4503R

Σ 96

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IMMUNO DIAGNOSTICS

AccuDiag™
Fasciola IgG
ELISA Kit

Cat # 8119-35

uncooked water vegetation in endemic areas, the spread of
reduced.[1,4] Recent estimates report that as many as 2.4 million
worldwide.[3]

TEST PRINCIPLE

The micro test wells are coated with *Fasciola* antigen. During the
the diluted patients' sera, any antibodies that are reactive with the
the coated wells. After washing to remove the rest of the sa
Conjugate is added. If antibodies have been bound to the wells, the
will then bind to these antibodies. After another series of wa
(tetramethylbenzidine or TMB) and a substrate (hydrogen peroxid

ORIGENE

OriGene Technologies, Inc.
9620 Medical Center Dr., Suite 200, Rockville, MD 20850
Phone: 1.888.267.4436 Fax: 301-340-9254
Email: techsupport@origene.com Web: www.origene.com

Product Information

***Fasciola hepatica* IgG ELISA Kit**
Catalog Number: EA101104
Storage Temperature: 2 – 8°C

Instruction for Use

Liver Fluke Parasites

- Opisthorchis viverrini (Opisthorchis felineus)
- Clonorchis sinensis
- Fasciola hepatica

How to evaluate aflatoxin exposure?

- Human who have consumed the food contaminated by aflatoxin b1.
  - Antibody?
  - Antigen?
  - DNA-adducts
- Food contaminated by aflatoxin

8th Edition, revised in February, 2018

AF (Total Aflatoxin) ELISA Kit
Catalog No: MBS2556985
96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Aflatoxin B1 (AFB1) ELISA Kit

【*INTENDED USE*】

For the quantitative detection of **Aflatoxin B1 (AFB1)** concentration in **cereal, compound feed, cooking oil, peanut, sauce, wheat and other feed, beer, wine, soy sauce, vinegar.**

This package insert must be read in its entirety before using this product.

Aflatoxin B1 ELISA Assay Kit

(For Research Use Only)

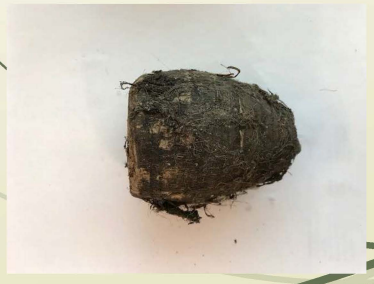
Instructions

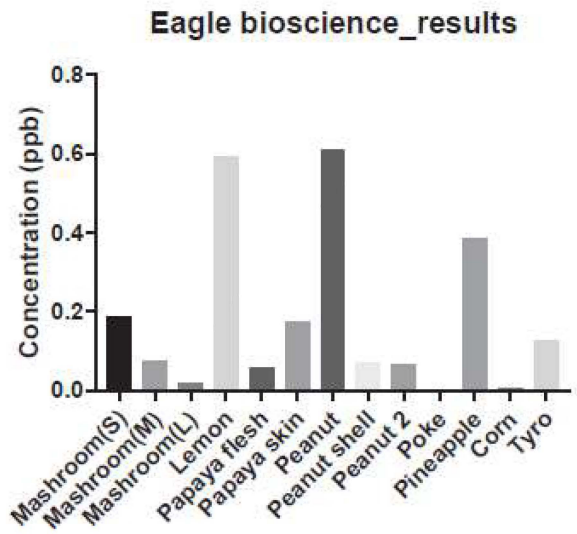
Competitive enzyme immunoassay for the quantitative detection of Aflatoxin B1

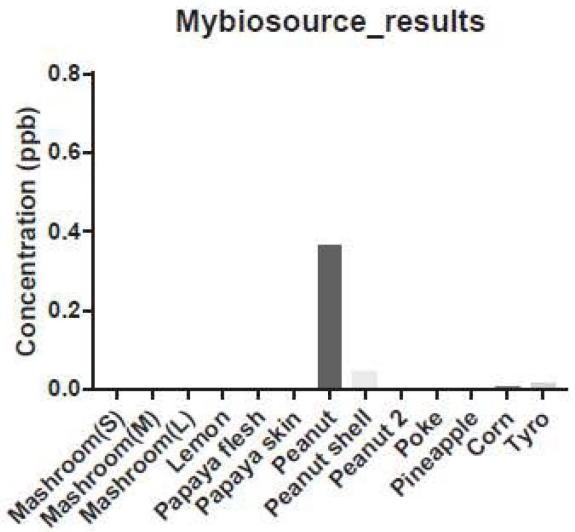
Catalog number: BTAFEK-001


Go Organic
Short Grain
Brown Rice
PLU
21050
Nutrition
Facts
Calories
160


Go Organic
Arborio Rice
PLU
26562
Nutrition
Facts
Calories
160







Collection 1			Collection 2	
Samples	Con.		Samples	Con.
Mashroom(S)	0.0071		Cake (white)	0.0136
Mashroom(M)	0.0150		Cake (brown)	0.0082
Mashroom(L)	0.0083		Honey	0.0804
Lemon	0.1059		Papaya	0.0206
Papaya	0.0125		Papaya skin	0.0070
Papaya skin	0.0119		Corn	0.0077
Peanut (fresh)	**0.3810**		Peanut (dry)	0.0300
Peanut shell	0.1473		Rice 26562 (Safeway	0.0115
Peanut 2	0.0065		Rice 20105 (Safeway)	0.0178
Poke (From Safeway)	0.0113		Rice 28560 (Safeway)	0.0099
Pineapple	0.0106			
Corn (From Safeway)	0.0076			
Tyro (From Safeway)	0.0440			

the Hawai'i Cancer Research Survey

University of Hawai'i
Cancer Research Center
of Hawai'i

DESSERTS AND SNACKS	AVERAGE USE DURING LAST YEAR								YOUR USUAL SERVING SIZE
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day	
Ice Cream	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 scoop (1/2 cup) or less **OR** ○ 2 scoops (1 cup) or 1 bar **OR** ○ 3 to 4 scoops (1 pint) or more
Ice Milk, Frozen Yogurt, or Sherbet	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 scoop (1/2 cup) or less **OR** ○ 2 scoops (1 cup) or 1 bar **OR** ○ 3 to 4 scoops (1 pint) or more
Cookies, Brownies, or Fruit Bars	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 to 2 average size cookies **OR** ○ 3 to 4 average or 1 extra large cookie or 1 brownie or fruit bar **OR** ○ 2 large cookies or brownies or more
Crackers and Pretzels (such as soda, graham, Japanese rice crackers, wheat thins)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 4 to 5 snack or 1 large cracker **OR** ○ 6 to 10 snack or 2 large crackers **OR** ○ 3 large crackers or more
Peanuts or Other Nuts	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 12 nuts or less **OR** ○ 1/4 cup **OR** ○ 1/2 cup or more

Liver cancer and peanut/corn consumption in MEC HI

Adjusted for sex, ethnicity, energy and alcohol consumption, Healthy Eating Index, smoking status, pack-years, log BMI							
Hazard ratio (95% CI)							
n=412 cases							
ANALYSIS I	**Not adjusted for Healthy Eating Index**						
	T1	T2		T3		p for tertile trend	p for continuous trend
Peanuts and other nuts (g/day)	1.00	0.724	(0.574-0.913)	0.882	(0.672-1.158)	0.7516	0.1857
Corn (g/day)	1.00	0.922	(0.731-1.161)	1.009	(0.781-1.304)	0.9979	0.7602
Peanut butter (g/day)	1.00	1.024	(0.779-1.345)	1.019	(0.819-1.269)	0.9037	0.7825
ANALYSIS II	**Further adjusted for Healthy Eating Index**						

Summary

- We found no evidence of liver fluke infection in liver cancer patients in Hawaii, but our evaluation was preliminary, testing only one form of parasite. We need to test other forms of liver fluke if possible, and search for other evidence of live fluke infection.

- We found evidence of food contamination by aflatoxin b1, but did not know if the contaminated food is being consumed regularly. We need to collect more evidence on food contamination and human intake.

Acknowledgement

- Wei Jia
- Linda Wong
- Brenda Hernandez
- Lynne Wilkens
- Zhanwei Wang
- Jason Wang

UHCC Liver Cancer Initiative Report:

Oral microbiome and hepatocellular carcinoma risk (PI B. Hernandez, Co-I: H. Yu, L. Wong)

The overall objective of our study was to investigate the role of the oral bacterial microbiome in the development of liver cancer and to identify novel biomarkers of liver cancer that may inform prevention and early detection strategies.

Bacterial 16S rRNA sequences were evaluated in oral samples from 90 hepatocellular carcinoma (HCC) cases and 90 matched controls from the U.S. mainland (R01CA138698, PI Yu). Compared to controls, HCC cases showed significantly reduced oral bacterial diversity (Shannon diversity index p-value =0.002) including reductions in common commensal species. HCC cases had a significantly higher relative abundance of certain taxa including the phyla, Cyanobacteria (p=0.02), and the genus, Aggregatibacter (p-0.002), relative to controls. Cyanobacteria are found in all terrestrial and aquatic ecosystems and include a wide range of species producing toxins with tumor-promoting properties, including the potent liver toxins—microcystins (MC), nodularins (NOD), and cylindrospermopsin (CYN). Aggregatibacter include the periodontal pathogen, *A. actinomycetemcomitans*, which has been positively correlated with visceral fat, fasting plasma insulin, and insulin resistance in patients with non-alcoholic fatty liver disease (NAFLD), a common condition that can lead to liver damage and progress to cancer.

We then evaluated the presence of MC/NOD and CYN in serum from 57 HCC patients from Hawaii. Mean levels of MC/NOD in HCC patients exceeded international standards for water designated for human use. MC/NOD levels significantly varied by etiology with the highest levels in HCC cases of unknown etiology and alcohol-associated disease; levels were intermediate in metabolic/NAFLD-associated HCC cases and lowest in hepatitis C associated HCC cases (p=0.0082). Uptake of MC-LR into hepatocytes has been previously shown to be reduced in HCV and increased in NAFLD through shared substrates including OATP transporters. Moreover, positive interactions of MC-LR and alcohol exposure have been shown.

We next compared tumor gene expression and cyanotoxin levels in a subset of 16 HCC cases. Expression of 770 genes were evaluated using the Nanostring nCounter platform. Serum levels of MC/NOD and

CYN were significantly correlated with tumor expression of three host genes functioning in fatty acid metabolism, CD36 (cluster of differentiation 36), FABP4 (fatty acid binding protein 4), and LPL (lipoprotein lipase). Fatty acid metabolism is a key source of energy and anabolism in cancer development as transformed cells switch to aerobic glycolysis.

Our study results provide evidence that oral bacteria may contribute to liver cancer through the disruption of the healthy flora of the mouth characterized by reduced levels of commensal bacteria and growth of both exogenous and resident pathogenic bacteria including toxin-producing species and species inducing insulin resistance, aberrant fatty acid metabolism, and other metabolic disruption. Bacteria and bacterial metabolites, including liver toxins, may act independently or with known risk factors to influence liver cancer risk.

PET imaging and liquid biopsy detection of CTNNB1 exon 3 mutations in HCC -A Pilot Study-

Sandi Kwee, Maarit Tiirikainen, Karolina Peplowska, Chris Farrar, Linda Wong

October 9, 2020 UHCC Scientific Retreat

Background

- Immune checkpoint inhibitors FDA approved for hepatocellular carcinoma (HCC)
  - Objective response occurs in minority of patients
    - 20% experience objective response to anti-PD1 used as second-line (2017, 2018)
    - 36% experience objective response to anti-PD-L1+bevacizumab used as first-line (2020)

- Biomarkers that can predict response to ICI therapy in other cancers
    - Tumor PD-L1 Expression
    - Tumor Mutation Burden
    - Microsatellite Instability-High

Background

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- Biomarkers that can predict response to ICI therapy in other cancers
    - Tumor PD-L1 Expression
    - Tumor Mutation Burden
    - Microsatellite Instability- High

Background

- Biomarkers that can predict clinical response to immune-checkpoint inhibitors in other cancers don't seem to work for HCC.

- GEMM models of in-situ melanoma and HCC reveal a novel mechanism of immune avoidance mediated by Wnt/beta-catenin signaling.

    - Beta-catenin is a transcription co-activator that binds nuclear T-cell factor/lymphoid enhancing factor (TCF/LEF) to initiate multiple cellular programs including immune programming.

    - This results in altered cytokine signaling with diminished recruitment of dendritic antigen presenting cells and T-cells to the tumor microenvironment.

    - This in turn limits the effectiveness of immune-checkpoint inhibition.

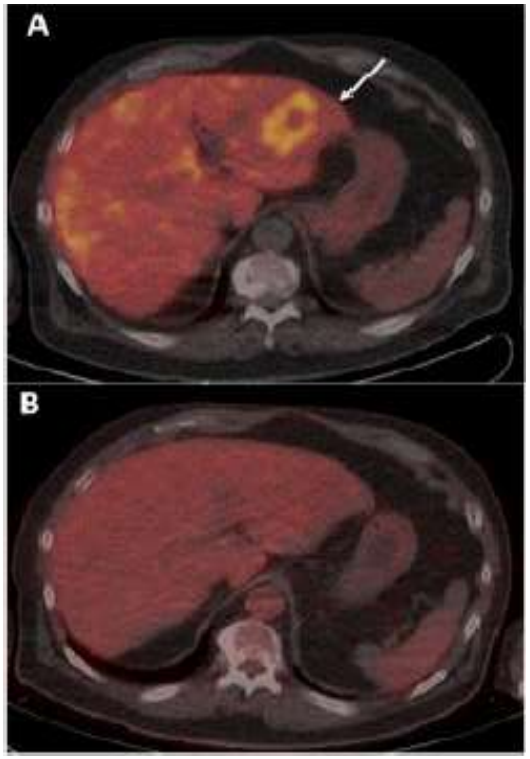
- Aberrant beta-catenin activation present in up to 2/3 of HCCs.
    - CTNNB1 exon 3 mutations (present in 30-40% of HCCs) are the most common cause.

Specific Aims

1. Perform targeted next-generation DNA sequencing of previously collected surgically-resected hepatocellular carcinoma (HCC) samples to profile mutations associated with Wnt/beta-catenin activation.
2. Conduct targeted sequencing on cell-free DNA (cfDNA) from patient blood samples (ie. liquid bx) corresponding to the tumor samples
3. Explore the associations with biomarkers (including PET imaging) that have already been applied to our samples.

Goal: Assess the feasibility of non-invasively measuring beta-catenin activation in HCC as a potential predictive biomarker for HCC immunotherapy.

Prior Data



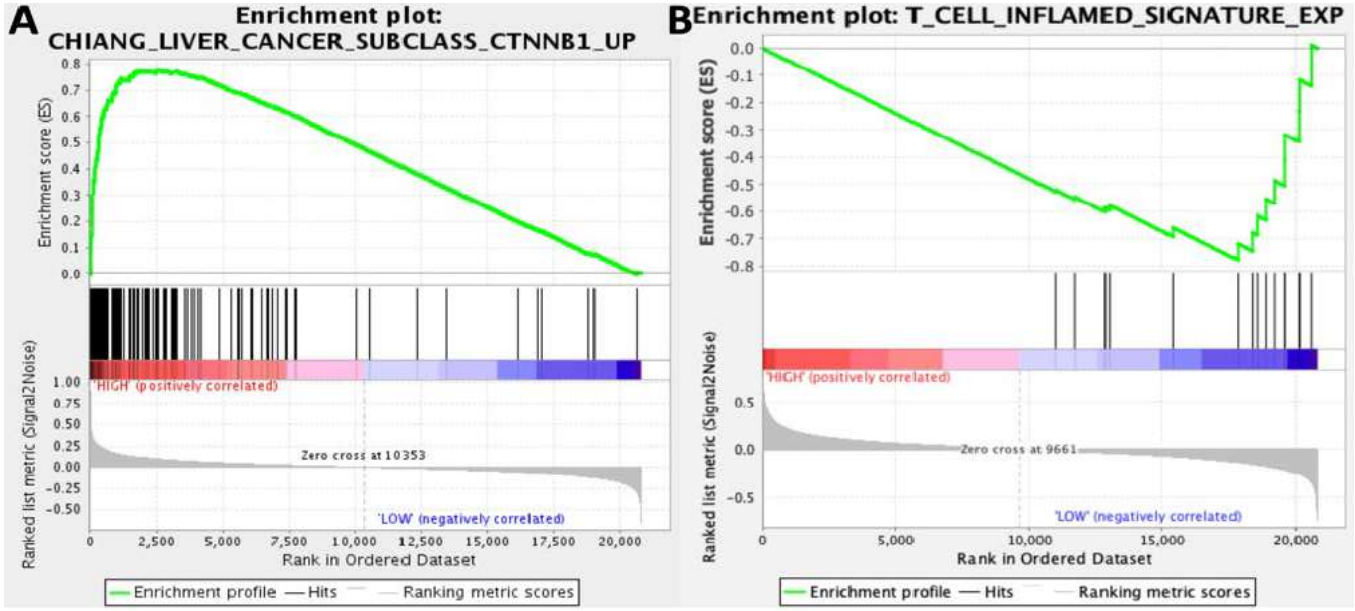


Figure 1: Tumor FCH uptake corresponds with immunotherapy-relevant expression profiles. Gene set enrichment plots are based on 41 tumor samples (31 FCH-avid, 10 FCH non-avid): **A)** Tumors showing high FCH metabolism were significantly enriched for genes from a CTNNB1 activation signature (FDR 0.062). **B)** A signature of T-cell inflammation that can predict immunotherapy response in several different tumor types was enriched by tumors that showed low FCH metabolism (FDR 0.116).

Kwee et al. Cancer Research, 2019.

Activities Performed

- Deparaffinization, laser capture microdissection of FFPE sections
  - Christine Farrar
  - Thanks also to Owen Chang and the PSR Team

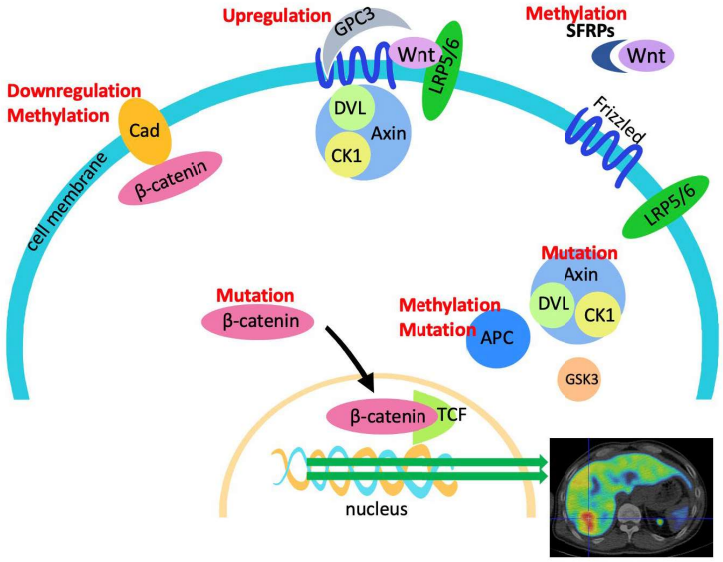
- DNA extraction, dual replicant targeted sequencing using Accel-Amplicon 56G Cancer Panel v2 (Swift) (44 cfDNA, 8 FFPE, 4 fresh frozen)
  - Maarit Tiirikainen and Karolina Peplowska

- Variant Calling using ERASE-Seq algorithm on replicated samples
  - Peplowska via Cloud-based Data Analysis Pipeline (Fluxion)

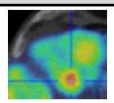
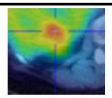
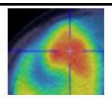
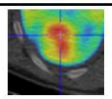
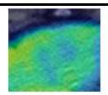
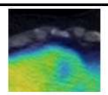
Wnt/beta-catenin pathway mutations of interest

Gene ID	Mutation Freq.	Panel Coverage	Mecha-nism	Published References
CTNNB1	25-40%	Exon 3	GOF	[14, 17, 27, 28]
AXIN1 *	6-19%	Hotspot	LOF	[14, 27, 32-35]
APC	5-7%	Complete	LOF	[17, 107]
GNAS	< 2%	Exons 1,8	LOF	[99]

Table 2. Mutations that impact β-catenin signaling in HCC. Coverages are validated by Foundation Medicine. Only CTNNB1 will be evaluate individually in Aim 2. GOF= gain of function, LOF = loss of function.



Highlighted Results

ID	PET/CT	Detected in Tumor by GTC 434-gene Cancer Panel (VAF %)	cfDNA Detected (VAF %)
P1 (FCH+)		CTNNB1 missense (5.7%), TP53 missense (9.1%), TET2 stop-gain (6.4%), and missenses of CSF3R (5.1%), BRAF (4.8%), PTEN (5.8%)	CTNNB1 missense (3.8%), TP53 missense (7.0%)
P2 (FCH+)		CTNNB1 missense (36.3%), INHBA/INHBA-AS1 insertion (12%)	CTNNB1 missense (17%)
P3 (FCH+)		CTNNB1 missense (34.4%), PBRM1 missense (12.2%), NUP93 stop-gain (13.1%), DNM2 (11.3%), SMARCA4 missense (29.6%)	CTNNB1 missense (0.8%)
P4 (FCH+)		GNAS missense (42.1%), EPHA3 stop-gain (38.7%), ATM stop-gain (4.6%), STAG2 stop-gain (4.8%)	GNAS missense (10.3%)
N1 (FCH-)		SPTA1 stop-gain (12.6%), STAT4 stop-gain (10.8%), FANCM stop-gain (11.5%), SMAD4 missense (3.8%)	No mutation loci detected on the 56 gene panel.
N2 (FCH-)		ARID1A frameshift (6.6%), LRP1B stop-gain(13%), PDGFRA stop-gain(8.4%), PTEN frameshift (7.1%), BRIP1 stop-gain/splice (11.2%)	DNMT3A missense (1.5%)

Summary

- Demonstrated feasibility of an in-house liquid biopsy approach employing targeted cfDNA sequencing to profile mutations of 56 oncology related genes.
- Mutations detected in cfDNA corroborated with tumor DNA.
- Preliminary affirms an association between Wnt/beta-catenin activating mutations and a PET/CT imaging phenotype that we previously found associated with beta-catenin activation in HCC.

Outcomes

- NIH R01 Grant Application
  - PAR 19-363:Integration of Imaging and Fluid-Based Tumor Monitoring in Cancer Therapy (R01 Clinical Trial)
- Working on concept/protocol for review by Foundation Medicine Study Committee
  - Foundation One CDx Liquid Biopsy (309 genes, FDA Approved August 2020)

Acknowledgement

- Hawaii Legislative Act 265, SLH 2019 (HB654 HD1 SD1 CD1)

Scott Kuwada, MD

Professor of Medicine & Chief of Gastroenterology, JABSOM

Clinical Member, UHCC

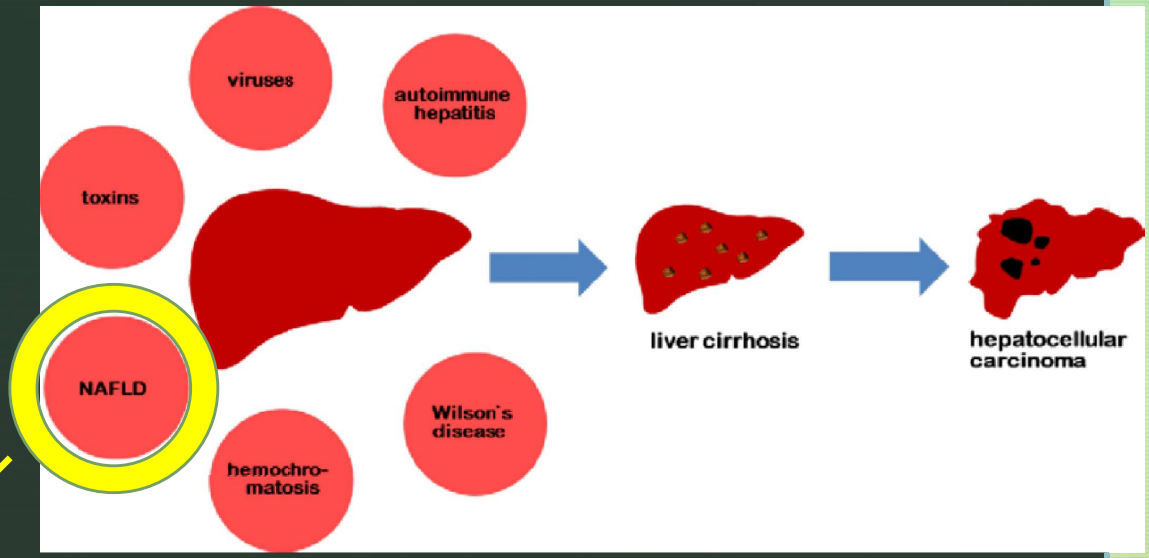
Non-Alcoholic Steatohepatitis & Hepatocellular Carcinoma

Hepatocellular Carcinoma (Hepatoma)

- 4th leading cause of cancer deaths in US
- 5-year survival in US = 10%
- Incidence of liver cancer in the U.S. is second highest in Hawaii and is highest in Native Hawaiians
  - Hawaii Tumor Registry (University of Hawaii Cancer Center), *Hawai'i Cancer at a Glance (2009-2013)*. 2017.
- >90% of cases occur in patients with cirrhosis (severe liver scarring)

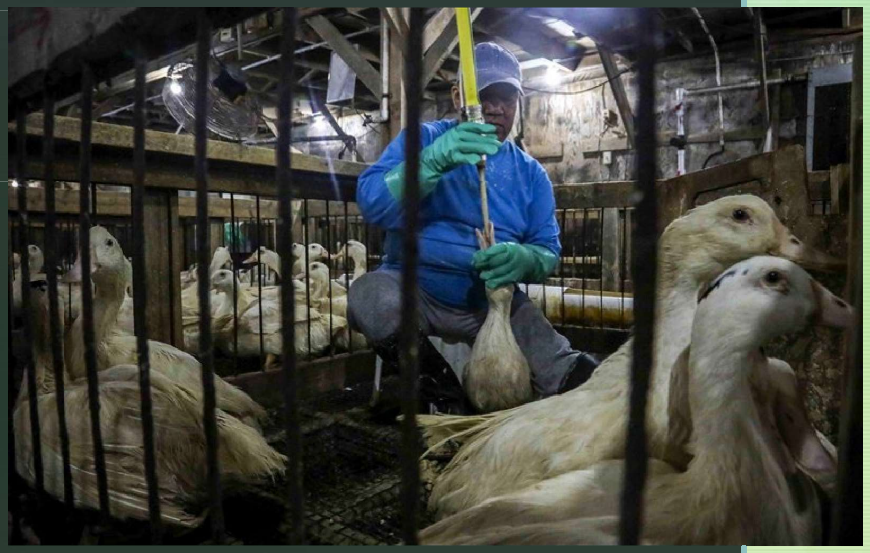
Liver Cancer

Chronic liver inflammation (hepatitis) → fibrosis → cirrhosis → hepatocellular carcinoma



Non-alcoholic fatty liver disease

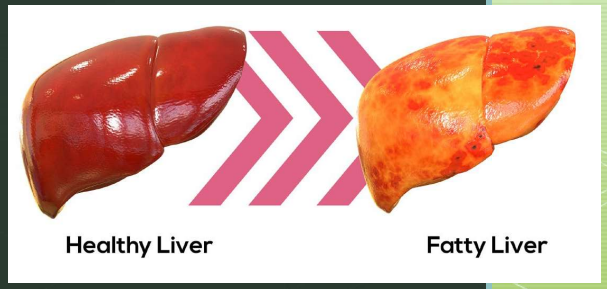
NAFLD (non-alcoholic fatty liver disease) in Hawaii



Prevalence of non-alcoholic fatty liver disease (NAFLD- >5% liver fat in the absence of high alcohol intake):

- Latinos (56% in men, 47% in women)
- Japanese Americans (38%, 46%)
- Native Hawaiians (35%, 42%)
- whites (23%, 21%)
- African Americans (21%, 18%)

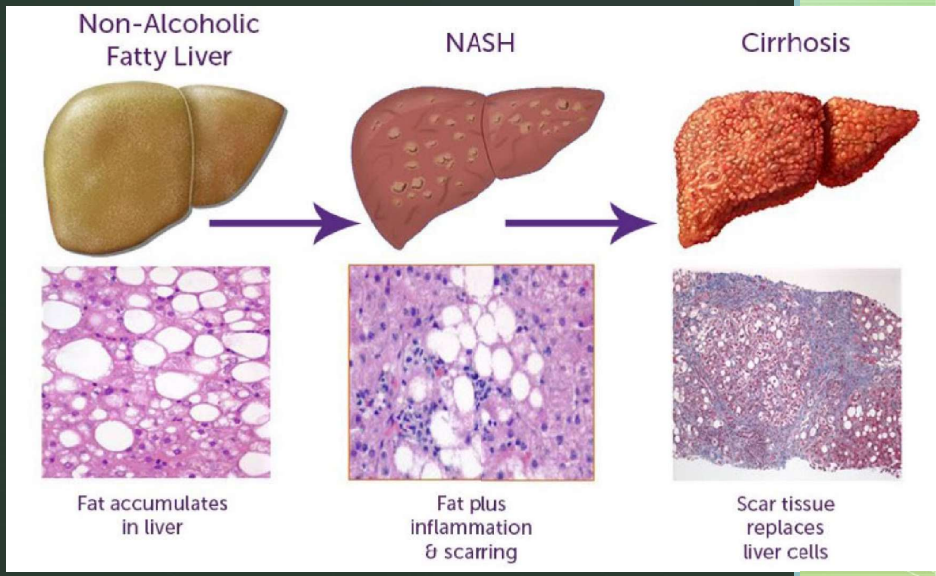
NAFLD is a more common cause of cirrhosis in Japanese Americans (32.3%), Native Hawaiians (31.5%), & Latinos (31.9%) than whites (21.7%)



Lim, U., et al. Gastroenterology, 2019. 156(4): p. 966-975 e10.
Setiawan VW et al. Hepatology. 2016;64((6)):1969–1977

Non-alcoholic Steatohepatitis (NASH) = subset of NAFLD w/ liver inflammation

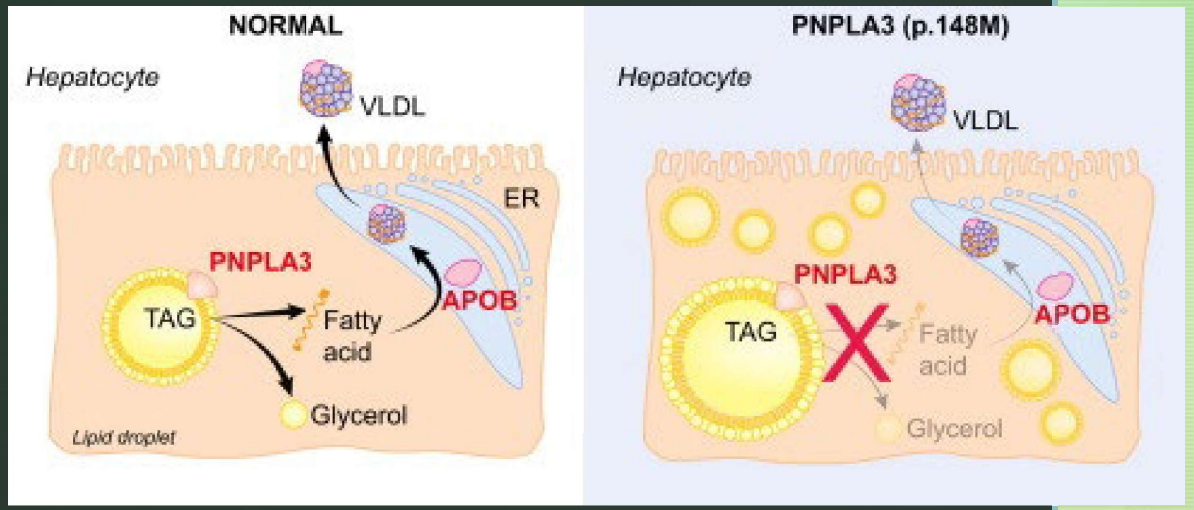
- Rapidly becoming the leading cause of liver transplantation and hepatocellular carcinoma in the U.S.
- Occurs in 30% of NAFLD (non-alcoholic fatty liver disease)
  - NAFLD affects up to 90 million Americans
  - Approximately 5% (1,500,000) of NASH patients progress to cirrhosis



Genetics and NAFLD & NASH - *PNPLA3*

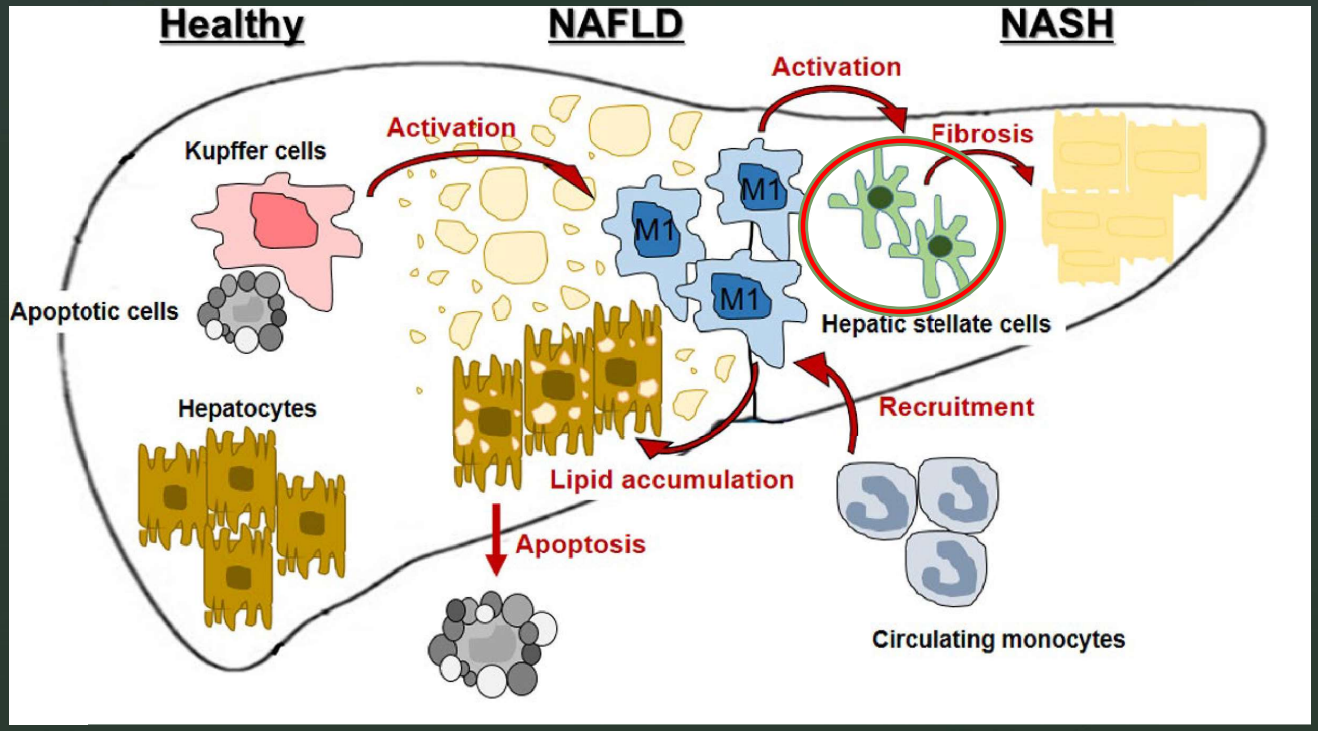
I148M variant of the palatin-like phospholipase domain-containing 3 (*PNPLA3*) gene: 13%-19% of Asians, compared to only 4% of whites and 2% of African-Americans

- Confers 73% higher hepatic fat content and 3-fold increased risk of severe inflammation and fibrosis



Pattison et al. Hawaii J Health Soc Welf. 2020 Jun 1; 79(6): 180–186

Liver Injury → Activation of Hepatic Stellate Cells → Fibrosis



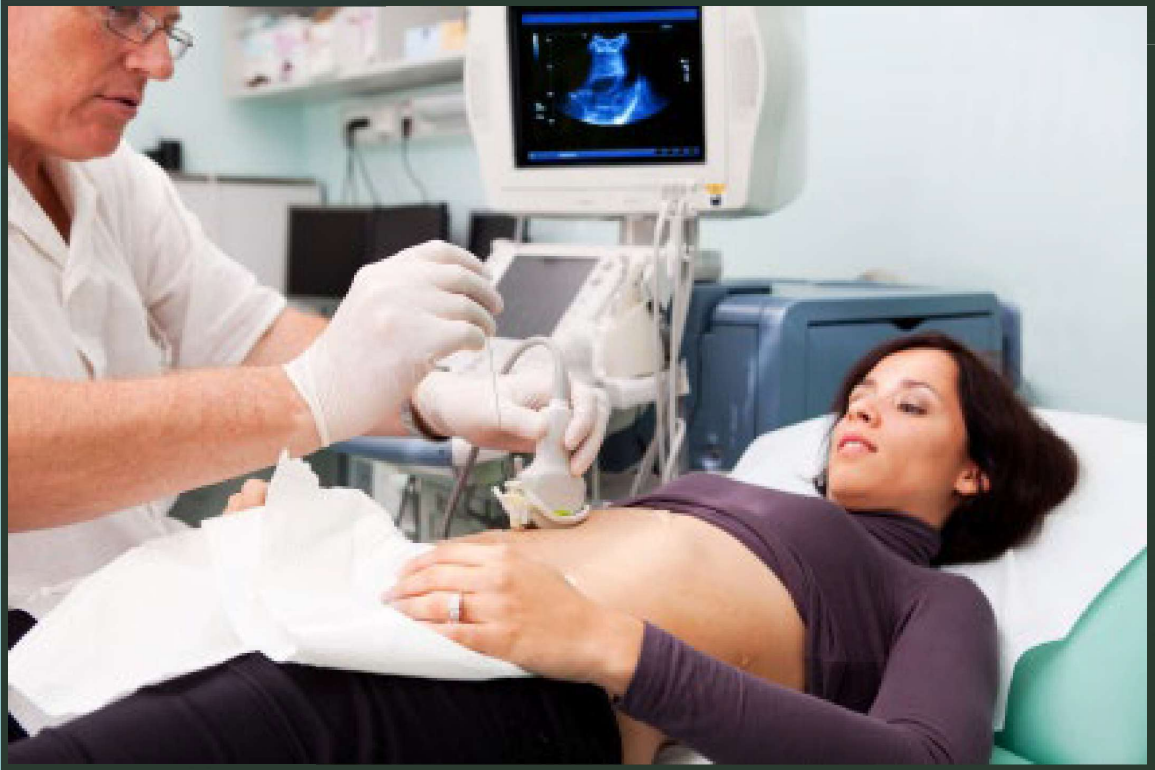
Our Study

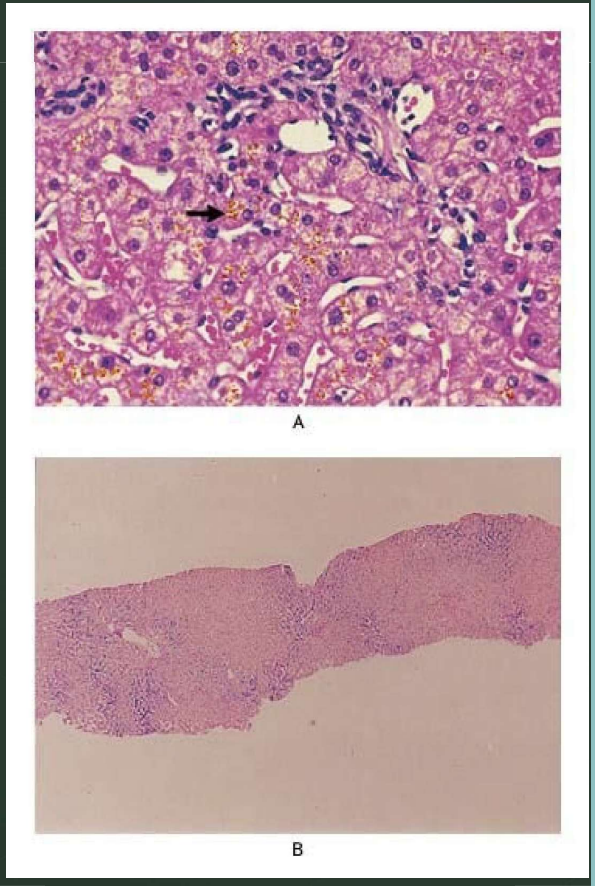
- Goals:
  - Identify NASH patients in Hawaii for dietary intervention trial (P20 Specific Aim #1)
  - Characterize NASH in our multi-ethnic population

Diagnosis & Treatment of NASH Patients

- Suspected cases of NASH – chronic elevation of transaminases (ALT, AST) in the absence of chronic heavy ethanol use, HBV, HCV, autoimmune hepatitis, hereditary liver diseases
- Risk stratification → high risk for progression →
  1. Evaluation for hepatic fibrosis: liver biopsy, elastography
  2. Weight loss
  3. Pharmacological Rx: Vitamin E, aspirin, investigational drugs

Ultrasound guided liver biopsy- gold standard for liver fibrosis



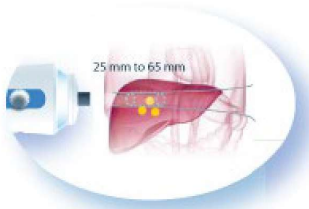


Elastography – Fibroscan®

How FibroScan® measure stiffness?

Hard liver	Soft liver
Pathologics status	Normal status
High kPa value	Low kPa value

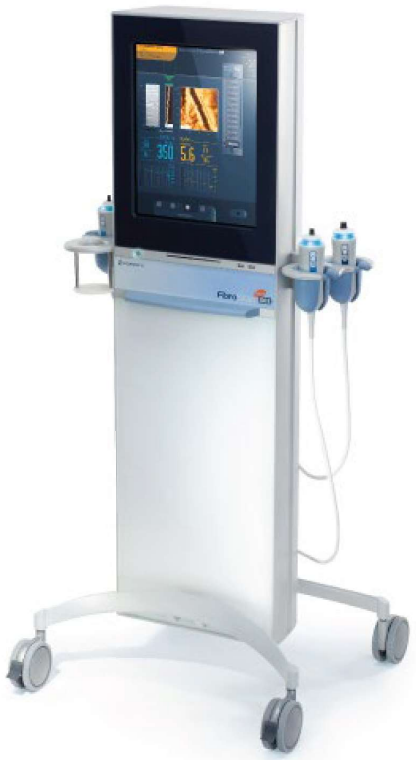
How FibroScan® measure steatosis?



- Quantify the decrease in amplitude of ultrasound waves

More Steatosis

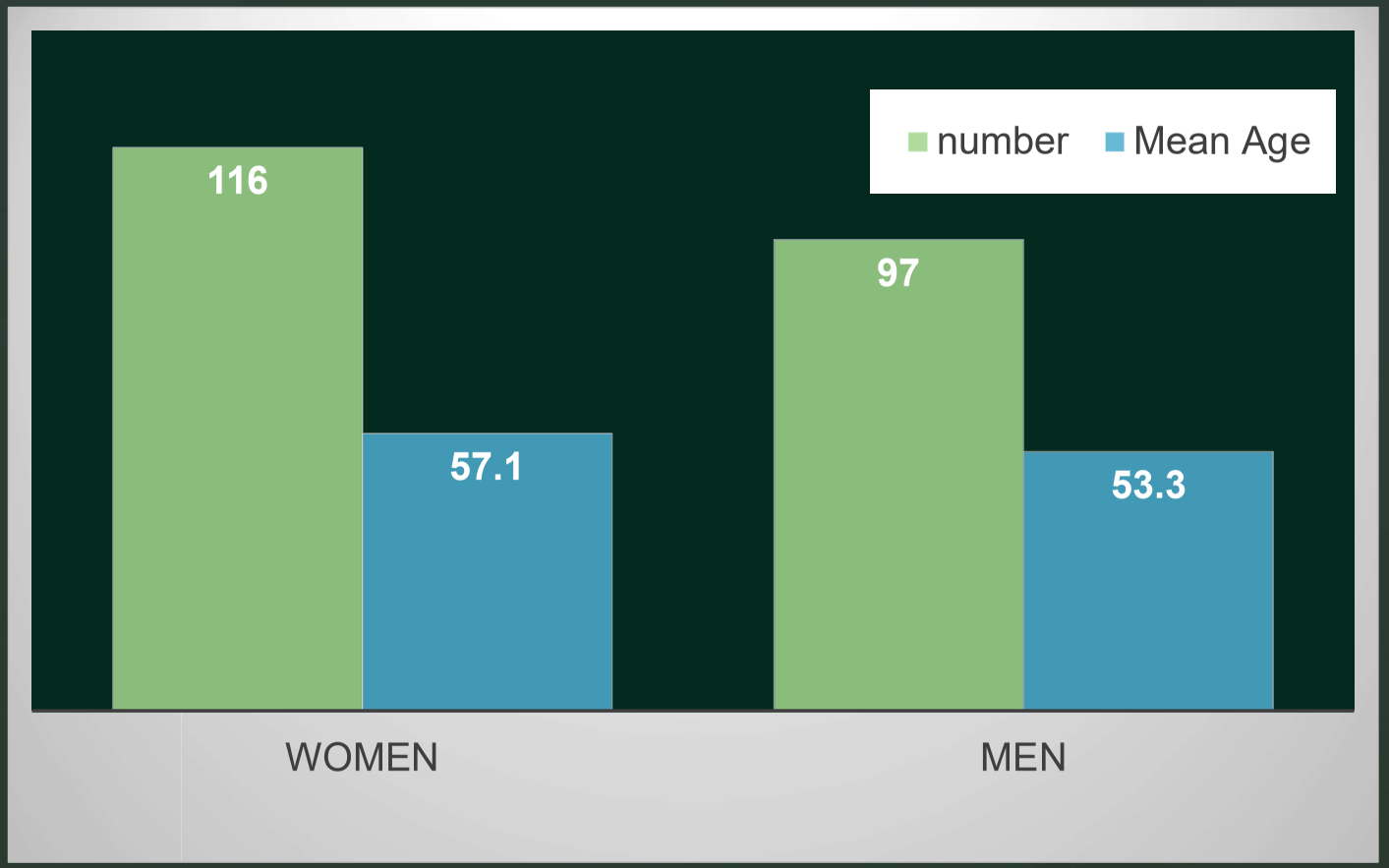
Higher CAP value



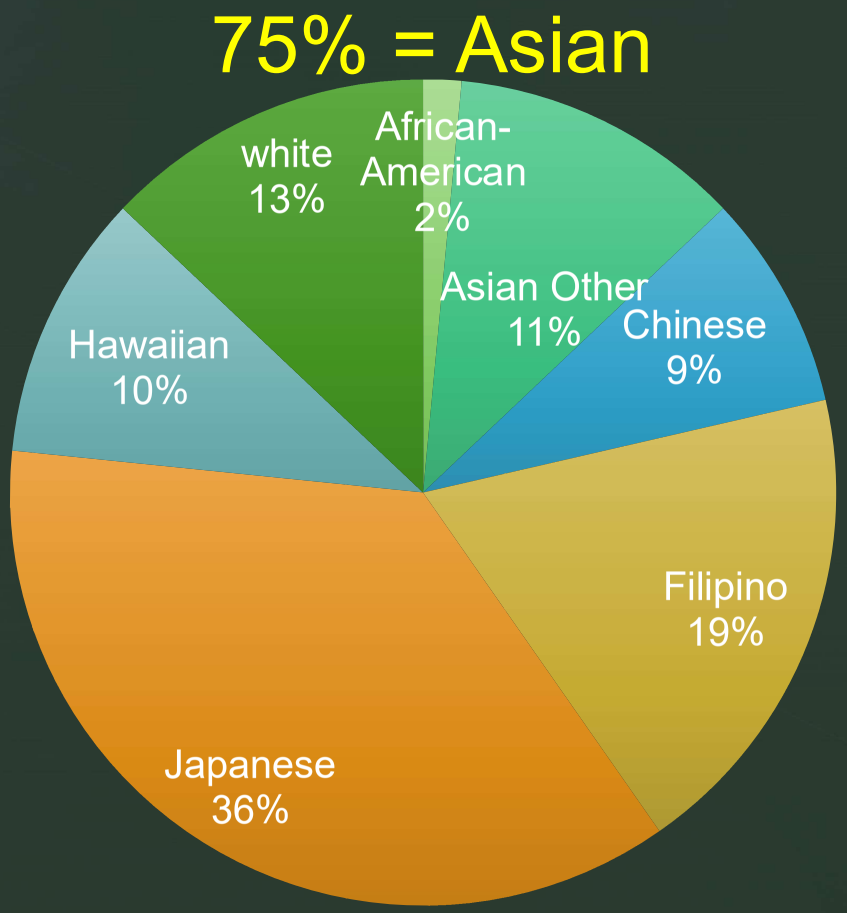
NASH Patient Selection

- Searched Queen's Medical Center Fibroscan and Liver Biopsy databases over the past 5 yrs for NASH cases
- Identified 175 Fibroscans and 76 Liver Biopsies (54 had both)

NASH – Gender & Age



- NASH & Ethnicity – Asians are overrepresented

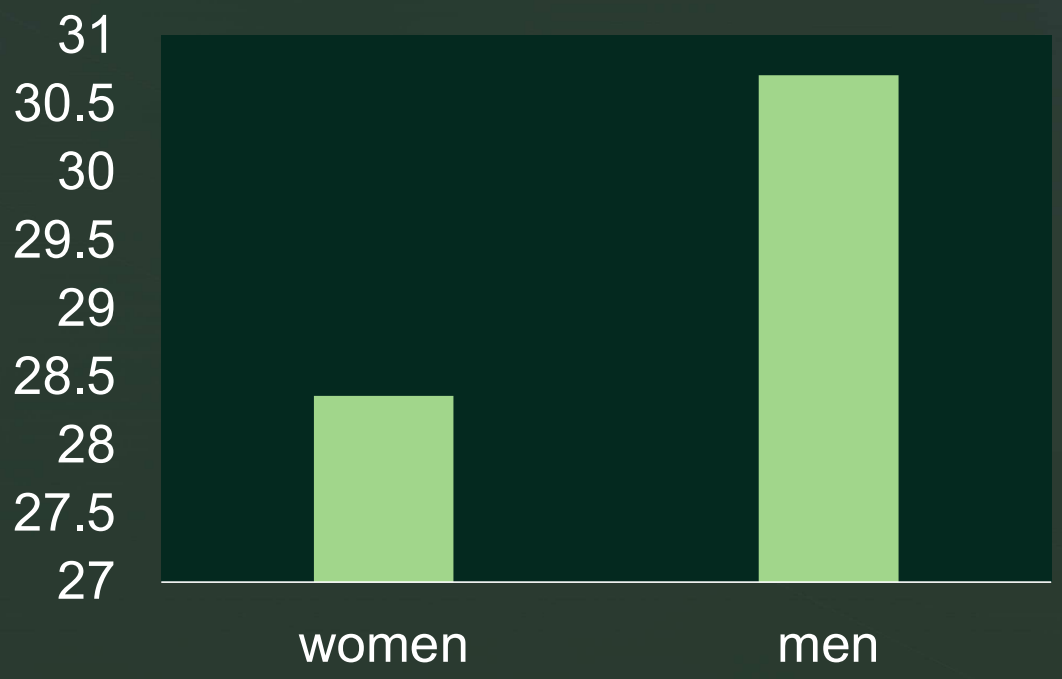


US Census (2019)
Hawaii-Asians = 57.7%

Body Mass Index

= weight (kg) / [height (m)]^2

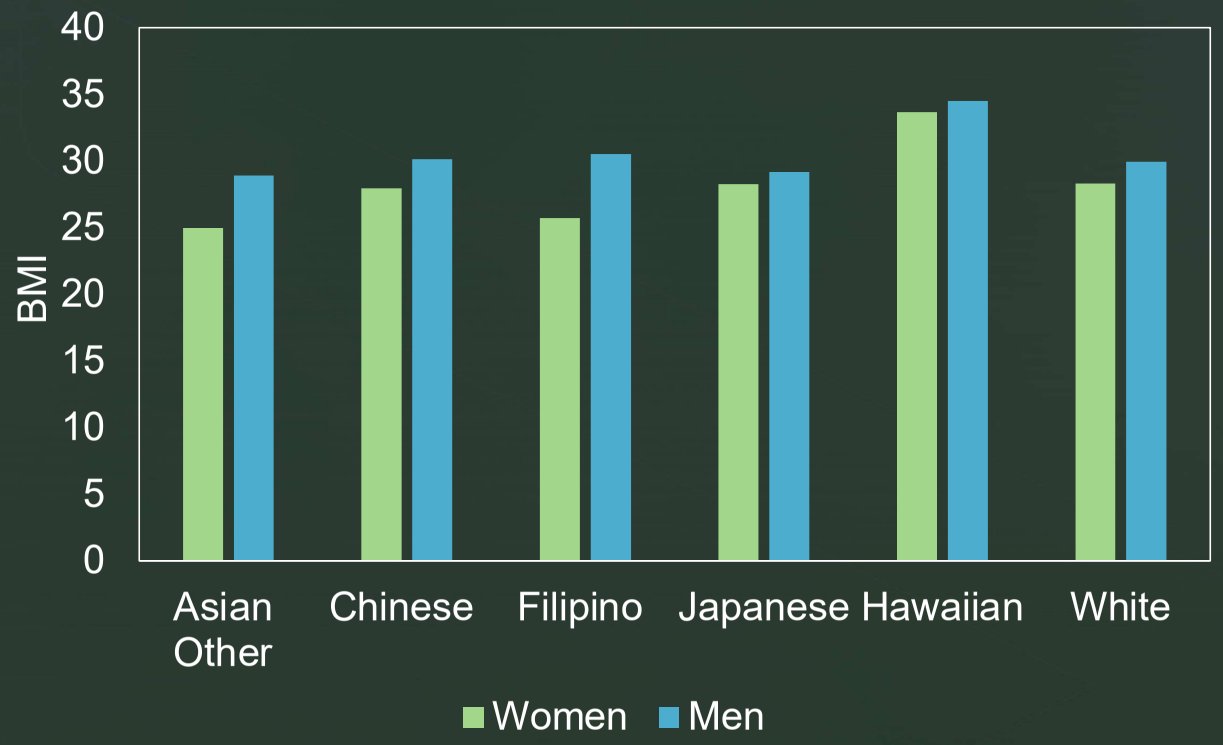
BMI
<25 = nml
25-29 = overweight
30+ = obese



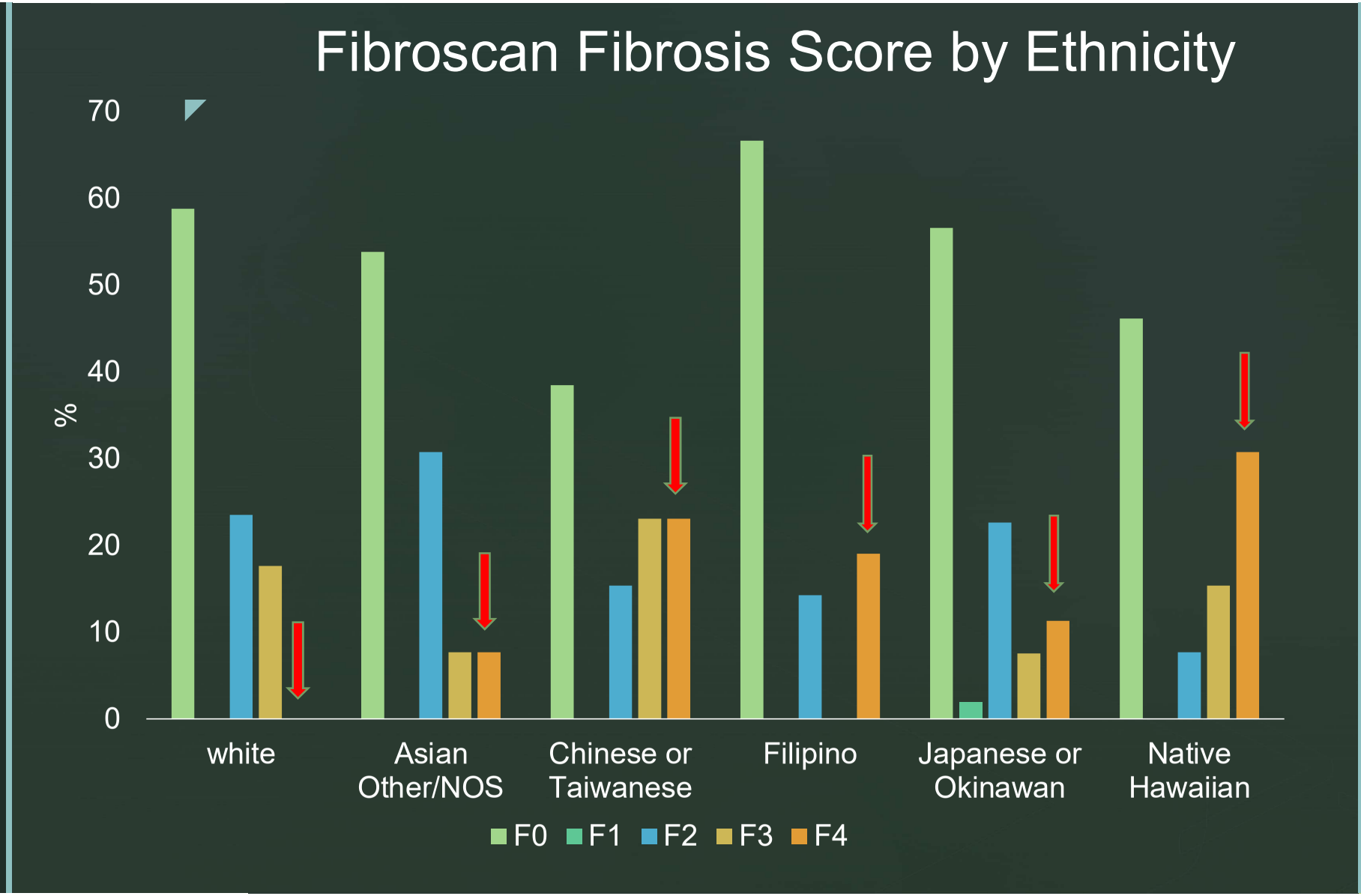
p = 0.07

Body Mass Index & Ethnicity

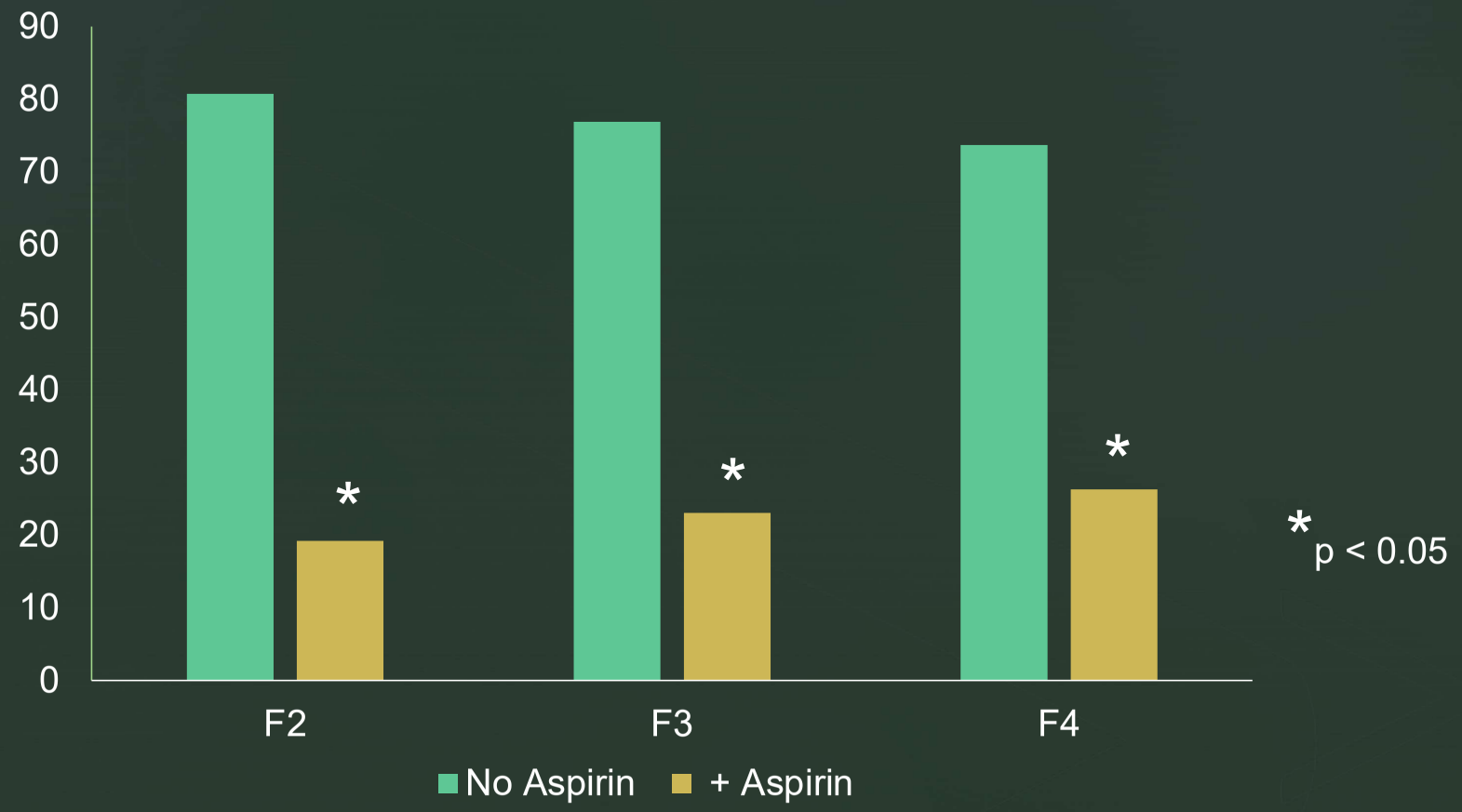
BMI
<25 = nml
25-29 = overweight
30+ = obese



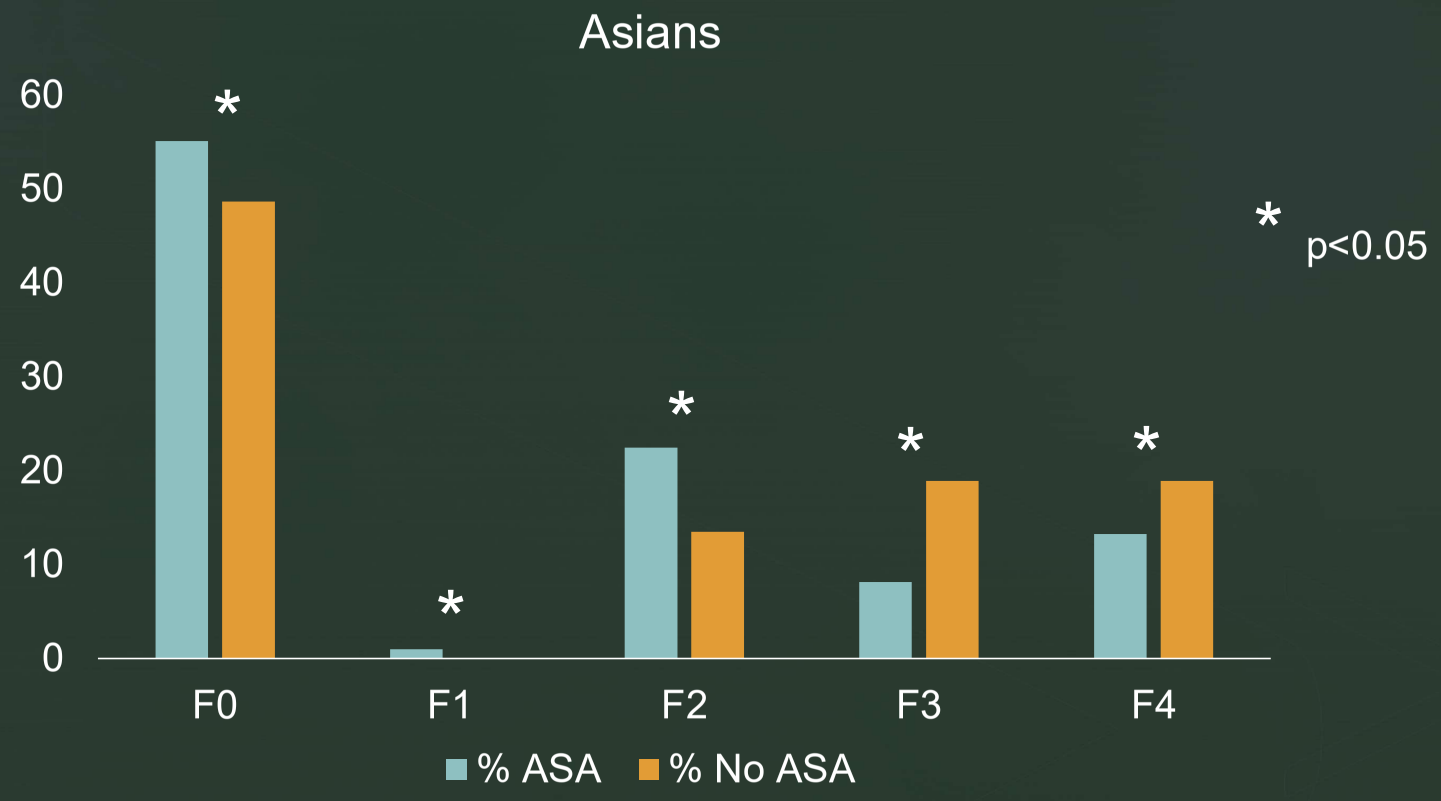
c/w Whites
n.s. differences in Ave BMI


Fibroscan Fibrosis Score by Ethnicity
70
60
50
40
30
20
10
0
%
white
Asian Other/NOS
Chinese or Taiwanese
Filipino
Japanese or Okinawan
Native Hawaiian
F0
F1
F2
F3
F4

Aspirin use and fibrosis score – all ethnicities



Aspirin & Fibrosis Score



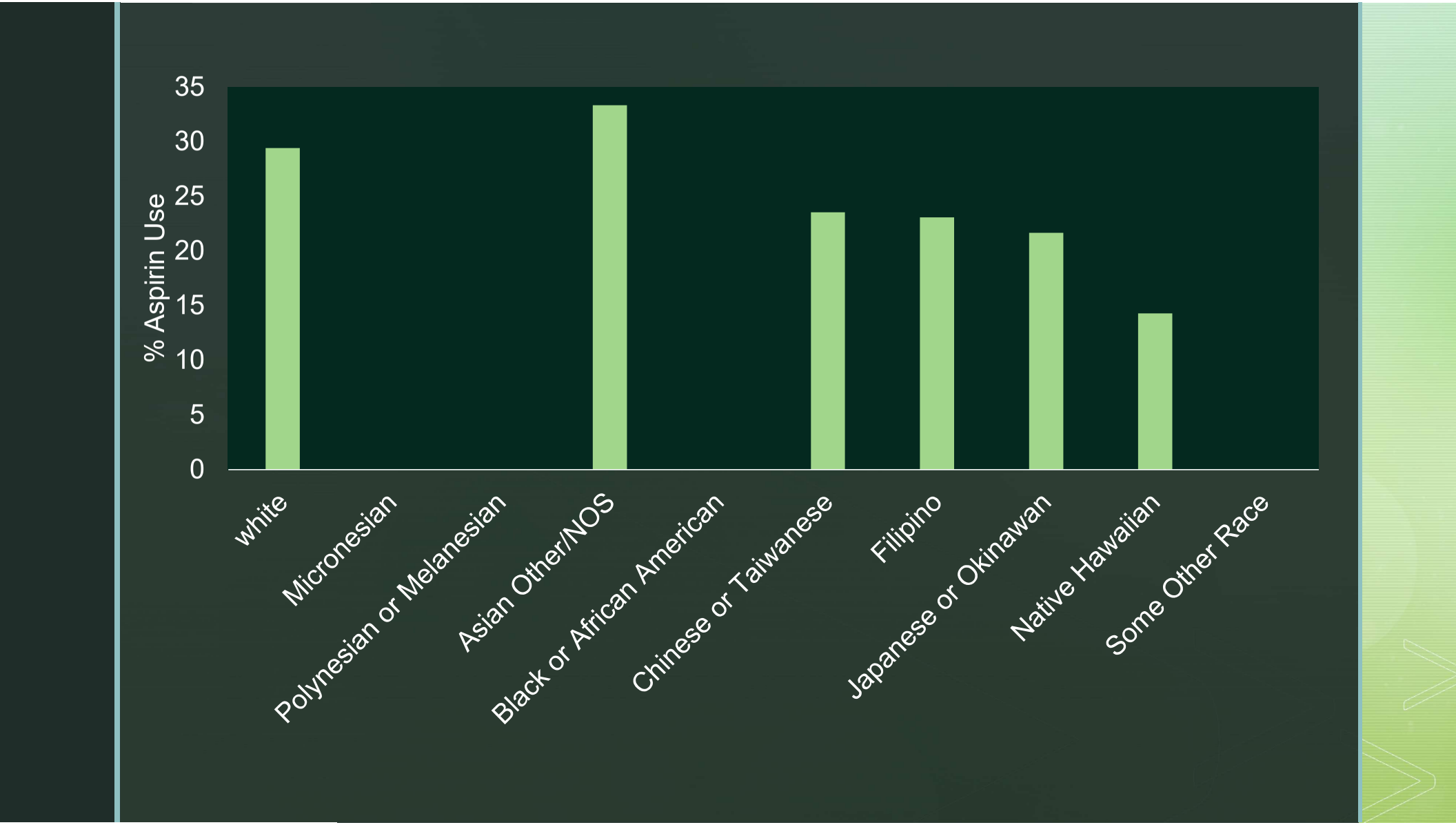
Conclusions

- NAFLD pts referred for Fibroscan & liver biopsies show overrepresentation of Asians
- Aspirin may be an effective anti-fibrotic agent in NASH patients.
  - And if so, can it be an effective chemopreventive agent for hepatocellular carcinoma?
  - Is aspirin effective in patients with I148M *PNPLA3*?

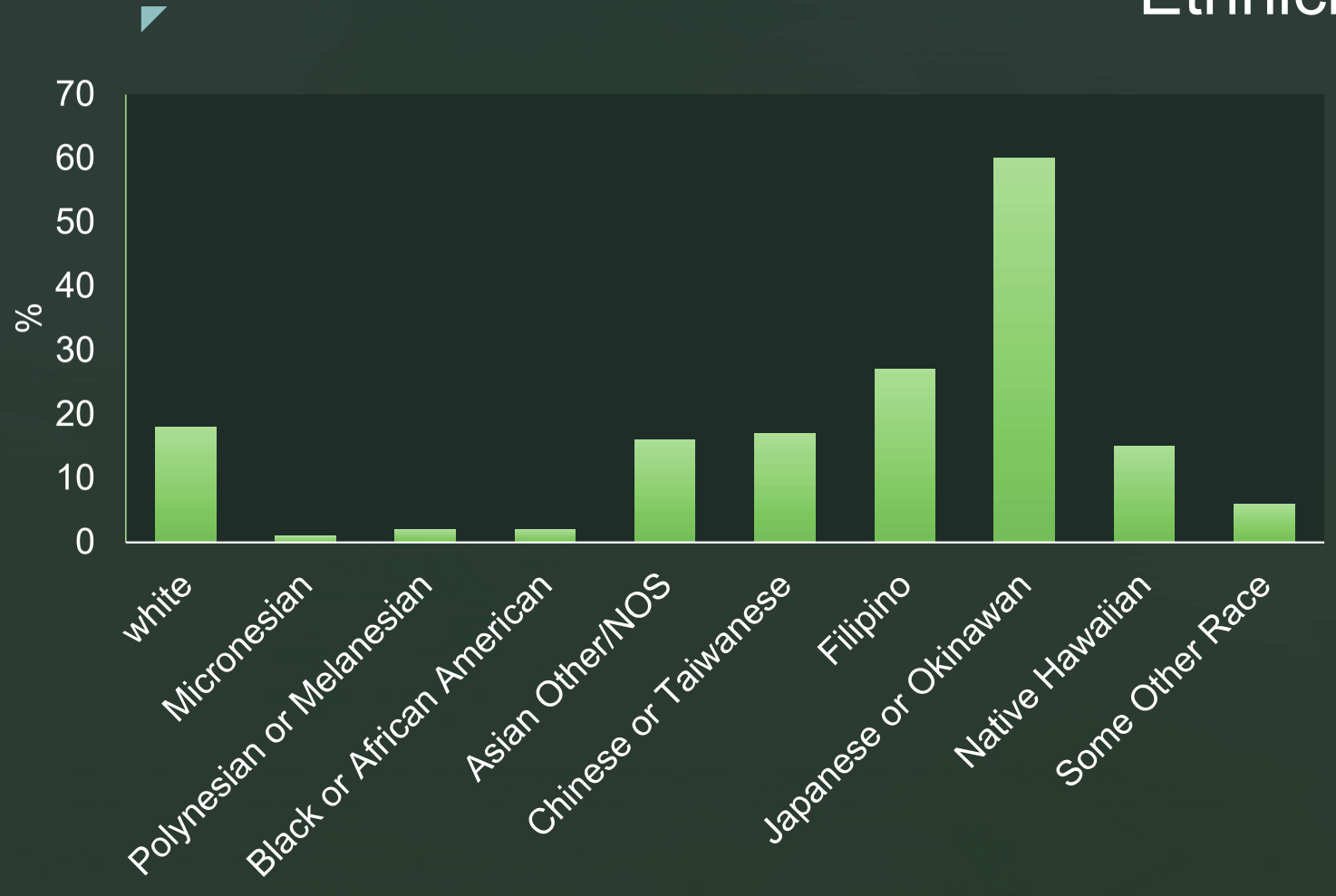
Acknowledgements

- CTO:
  - Kate Bryant-Greenwood
  - Nate Ramos
  - Anita Cheung
- OnCore:
  - Robert Schuetz
- Hawaii Pathology Lab
  - Casey Phan

- Loic Le Marchand
- Unhee Lim
- Carol Boushey
- Brenda Hernandez
- Kevin Cassel
- Linda Wong


% Aspirin Use
35
30
25
20
15
10
5
0
white
Micronesian
Polynesian or Melanesian
Asian Other/NOS
Black or African American
Chinese or Taiwanese
Filipino
Japanese or Okinawan
Native Hawaiian
Some Other Race

Ethnicity


70
60
50
40
30
20
10
0
%
white
Micronesian
Polynesian or Melanesian
Black or African American
Asian Other/NOS
Chinese or Taiwanese
Filipino
Japanese or Okinawan
Native Hawaiian
Some Other Race

Phase II study of TSR-022 in combination with TSR-042 for the treatment of advanced hepatocellular carcinoma

PI: Jared Acoba

This is a phase II, single arm study for patients with advanced and incurable hepatoma (cancer of the liver). Hawaii has a very high incidence of hepatoma, so novel therapies are incredibly valuable to our patients. Immunotherapy in the form of immune checkpoint inhibitors is a recently established strategy for treating hepatoma. I developed a protocol that combines two immune checkpoint inhibitors, an anti-PD-1 antibody (TSR-042) and an anti-TIM-3 antibody (TSR-022), with a primary objective of improving the objective response rate. Highlights of the trial so far:

- Four patients have been enrolled on the trial; one from a neighbor island.
- No serious treatment-associated side effects have occurred.
- Two patients have not had growth of their cancer for >10 months. One patient has had over 85% of his cancer disappear.

University of Hawai'i Cancer Center

Scientific Retreat

RNA in Extracellular Vesicles affects the microenvironment of Liver Cancer

Muller Fabbri, MD, PhD

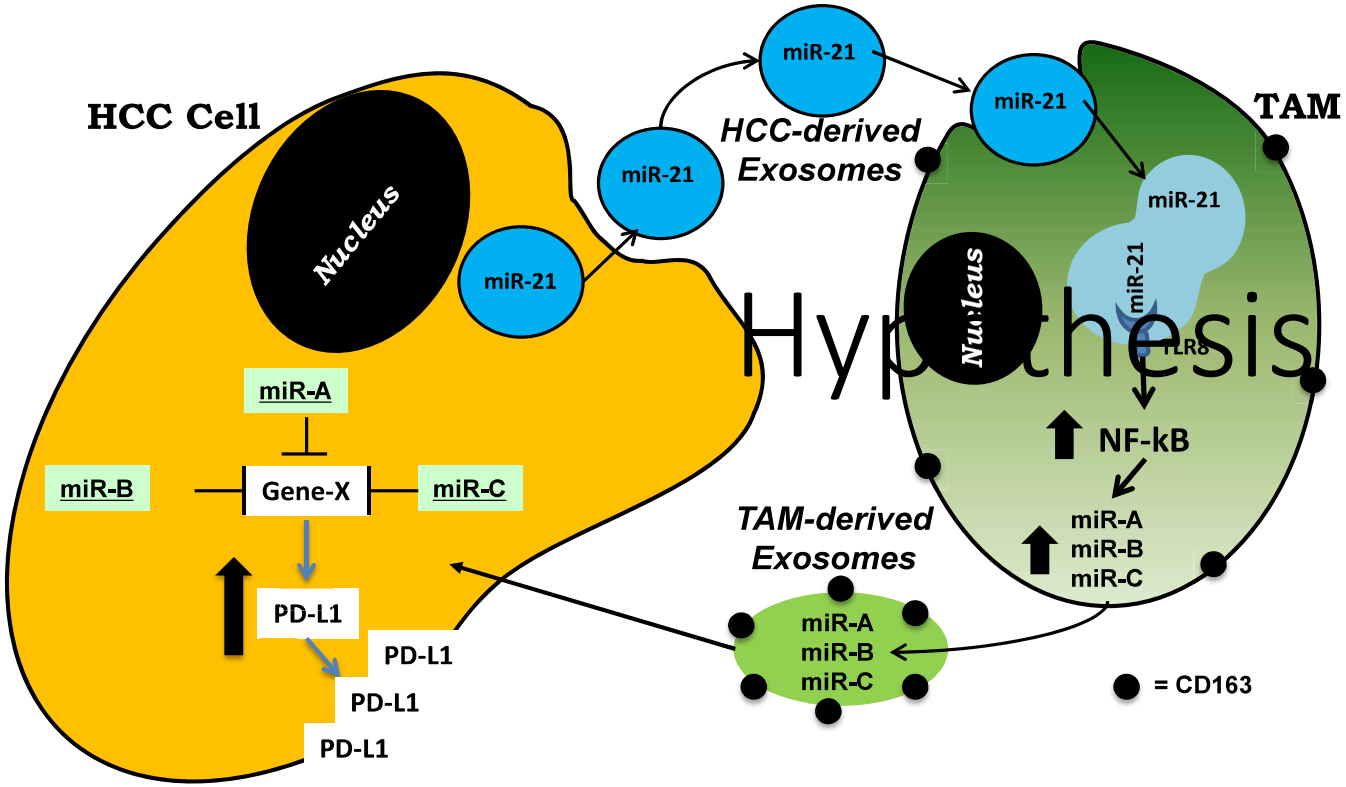
Associate Professor
Cancer Biology Program

University of Hawai'i
Cancer Center
Honolulu, HI

UNIVERSITY OF HAWAI'I
CANCER CENTER

Honolulu, HI – Friday October 9, 2020

Hypothesis



1. High TAMs poor prognosis in HCC patients
(Zhang J et al; J Invest Surg, 2019)

2. Significant direct correlation between TAM
Markers (CD163, CD206) and PDL1 expression in
HCC patients
(r2 database)

3. High TLR8 expression directly correlates with
High PDL1 expression in 371 HCC patients
(r2 database)

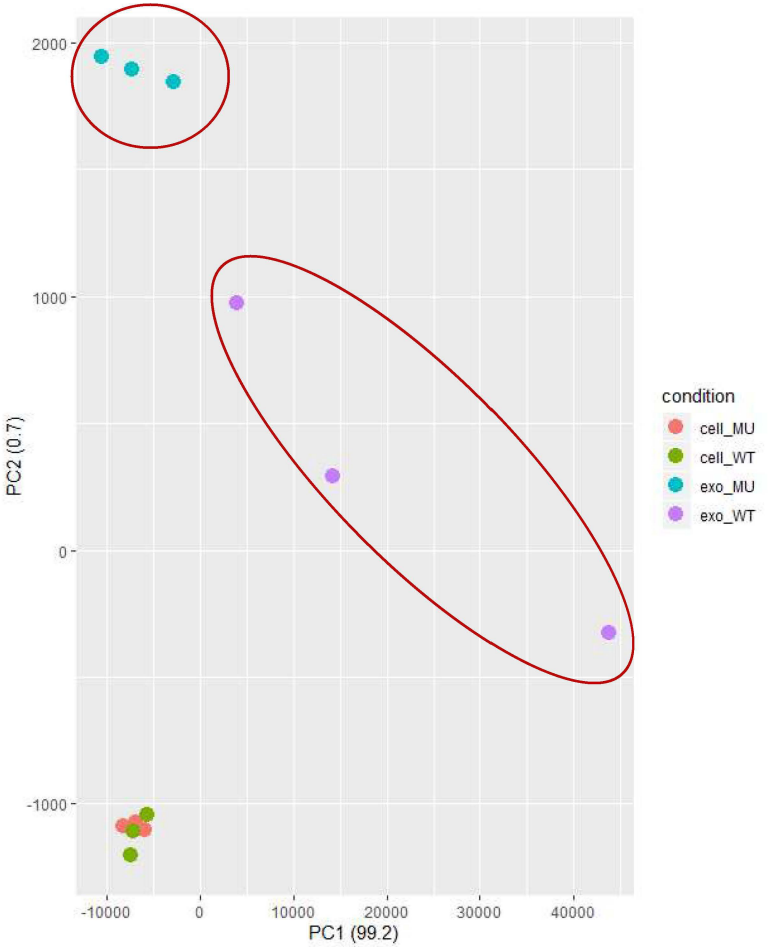
HepG2+THP1 co-culture – RNA sequencing

THP1 CRISPR TLR8
THP1 wt TLR8

Co-cultured with HepG2

RNAseq (**Genomics and Bioinformatics Shared Resource**)

Ongoing analysis (**collaboration with Youping Deng and Vedbar Khadka**)



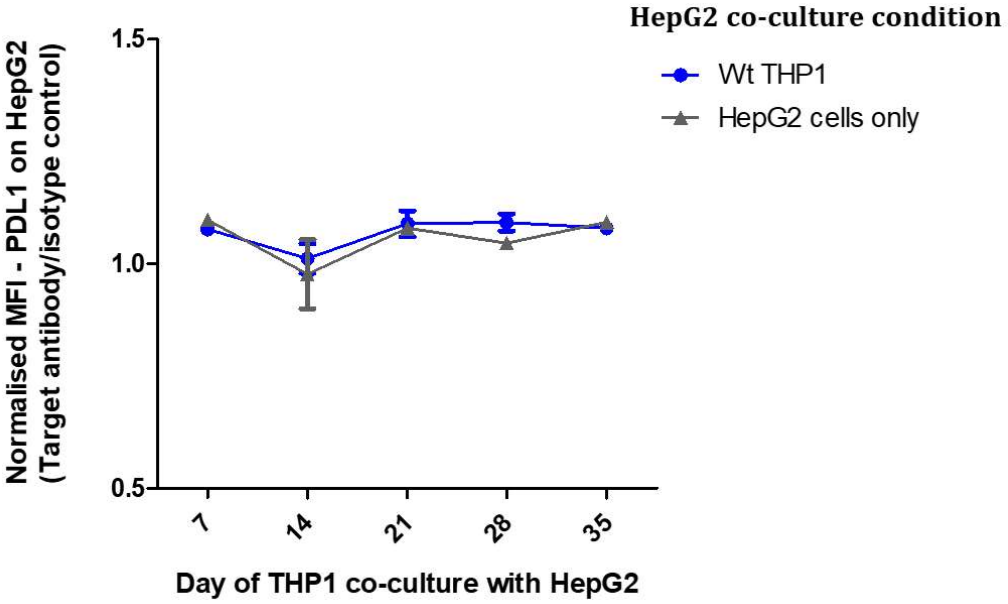
Monocyte co-culture

Does HepG2-THP1 co-culture generate a THP1 phenotype capable of inducing PDL1 expression in HepG2 cells?

THP1s co-cultured with HepG2s for 35 days
- THP1s seeded onto fresh HepG2s every 7 days
- Transwell setup

PDL1 measured on HepG2 cells every 7 days by flow cytometry

Co-culture of THP1 with HepG2 does not upregulate HepG2 PDL1



Role of THP1 polarization

M1 differentiation and characterisation

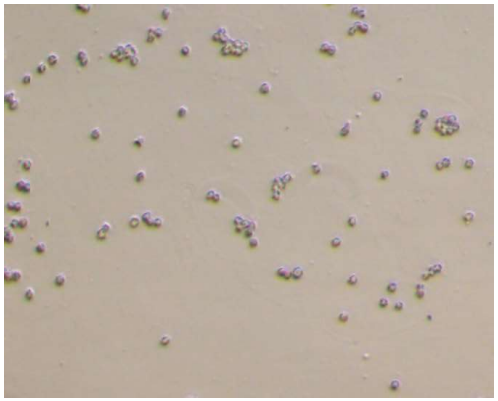
THP1s differentiated into M1-THP1s (pro-inflammatory cells):

- 5ng/mL PMA for 24 hours
- IFN-γ (20ng/mL) and LPS (100ng/mL) for 48 hours

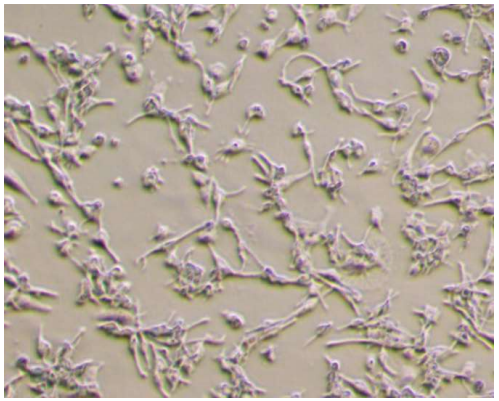
Differentiation confirmed by:

- Adherence and flattening of cells
- Increase in expression of CD80 (M1 marker), measured by flow cytometry

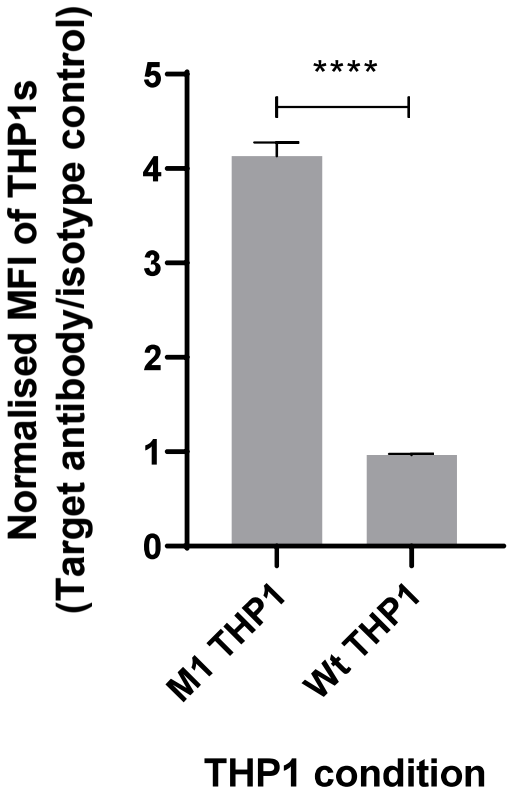
WT THP1s



M1 THP1s



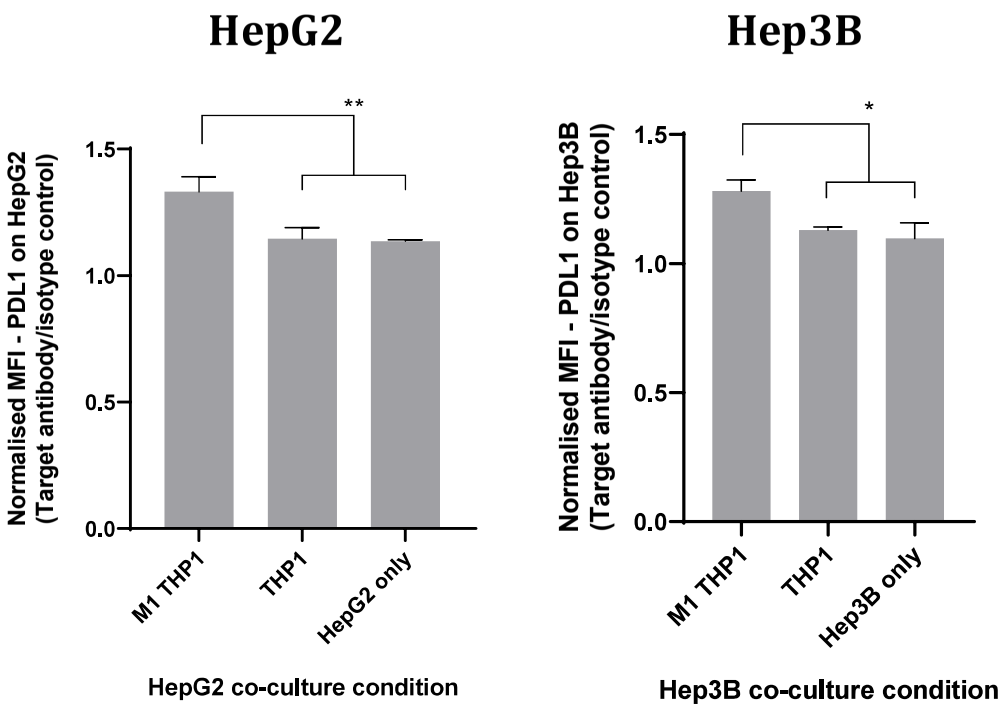
Expression of CD80 on THP1s



M1-THP1 – HepG2/Hep3B co-culture

HepG2/Hep3B co-cultured with M1 differentiated THP1s for 72 hours
- Transwell setup

PDL1 expression measured on HepG2/Hep3B by flow cytometry



Co-culture with M1-THP1 increases HepG2/Hep3B PDL1

Do EVs derived from the M1-THP1s contribute to this PDL1 upregulation?

EV isolation

For EV studies, EV isolation from cell supernatant must be carried out

THP1 and M1-THP1 conditioned media collected

EVs isolated by ultracentrifugation on a sucrose cushion

- To concentrate and purify EVs

THP1/M1-THP1 conditioned media

↓

Differential centrifugation

↓ *To remove cells and large debris*

Sucrose cushion ultracentrifugation

↓ *100,000g, 90min*

Ultracentrifugation to pellet EVs

↓ *100,000g, 90min*

Resuspend EVs in PBS and freeze

↓

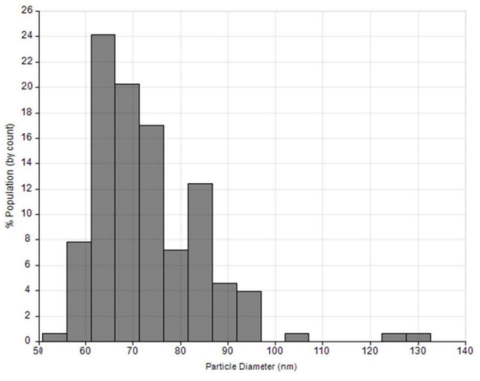
EV characterisation

EV isolation

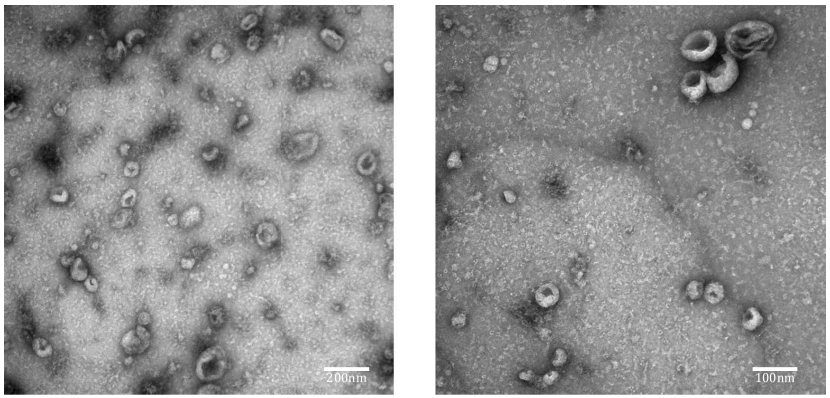
Following isolation of EVs, characterisation is carried out to demonstrate purity of vesicle preparation

1. Tunable resistive pulse sensing (TRPS) shows EVs are 50-100nm in size
2. Electron microscopy displays typical “cup-shaped” morphology
3. Preparations are enriched in EV markers (TSG101 and CD81)
   Negative for non-EV component (Calnexin)

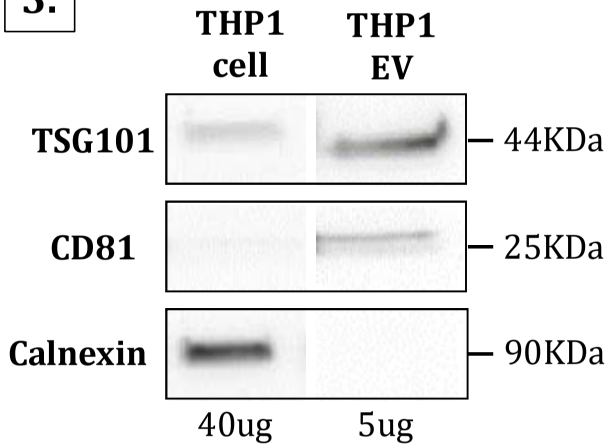
1.



2.



3.

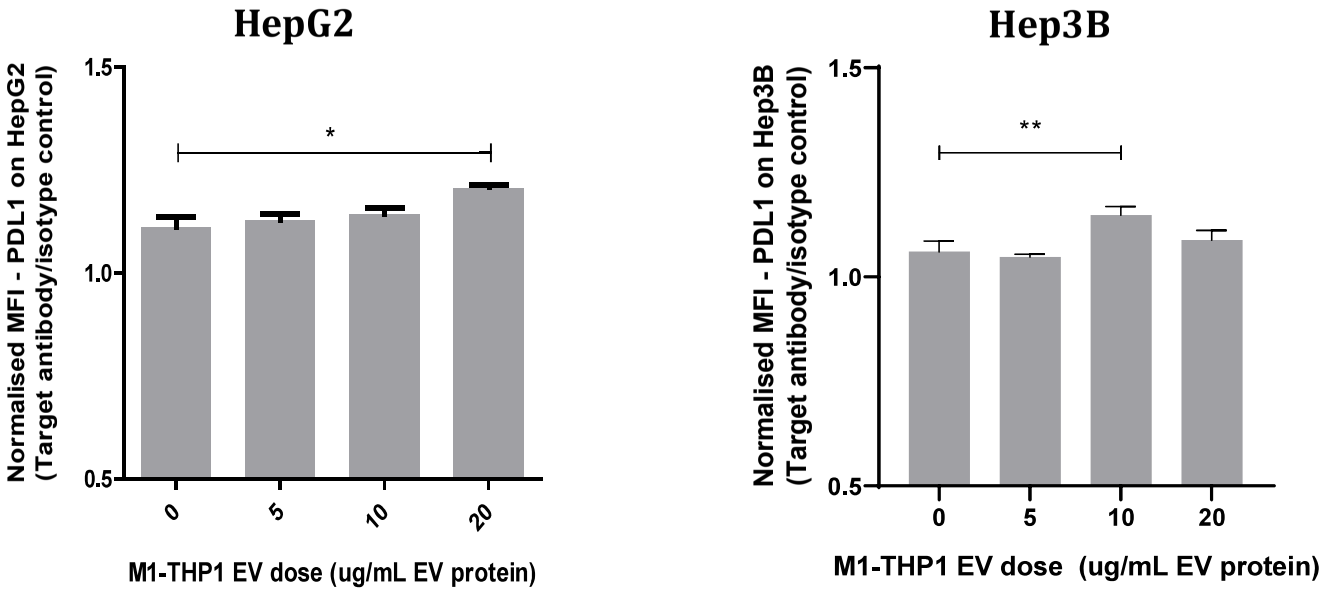


M1 EV effect on PDL1

HepG2/Hep3B cells were treated with increasing doses of isolated M1-THP1 derived EVs

PDL1 expression on the cancer cells measured by flow cytometry

These EVs could significantly increase PDL1 in both HepG2 and Hep3B cells



What EV cargo is responsible for the upregulation of PDL1?

Expression of PDL1 on M1 THP1s and EVs

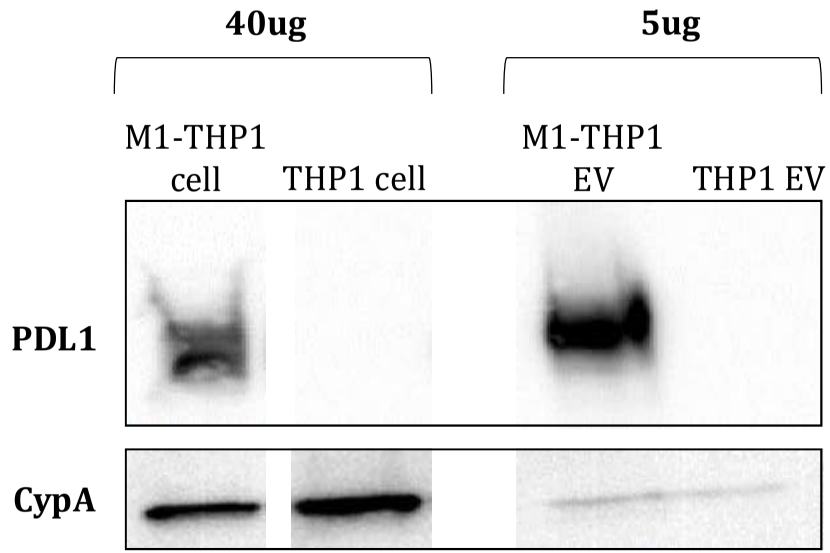
Pro-inflammatory macrophages are known to express PDL1
- EVs carrying proteins can directly transfer these to recipient cells
- Do M1-THP1s express PDL1? And do their EVs?

Western blot performed on M1-THP1s and their EVs to determine whether they express PDL1

- **M1-THP1s express PDL1**
- **EVs derived from M1-THP1s are enriched in PDL1**

Can PDL1 protein be transferred directly from M1-THP1 EVs to HepG2/Hep3B cells?

Do the EVs deliver miRNAs that drive PDL1 expression?



Cyclophilin A (CypA) used as loading control

Drosha KO M1-THP1 – HepG2 co-culture

The role of THP1 miRNAs in driving PDL1 expression in HCC cells was determined using Drosha KO THP1s

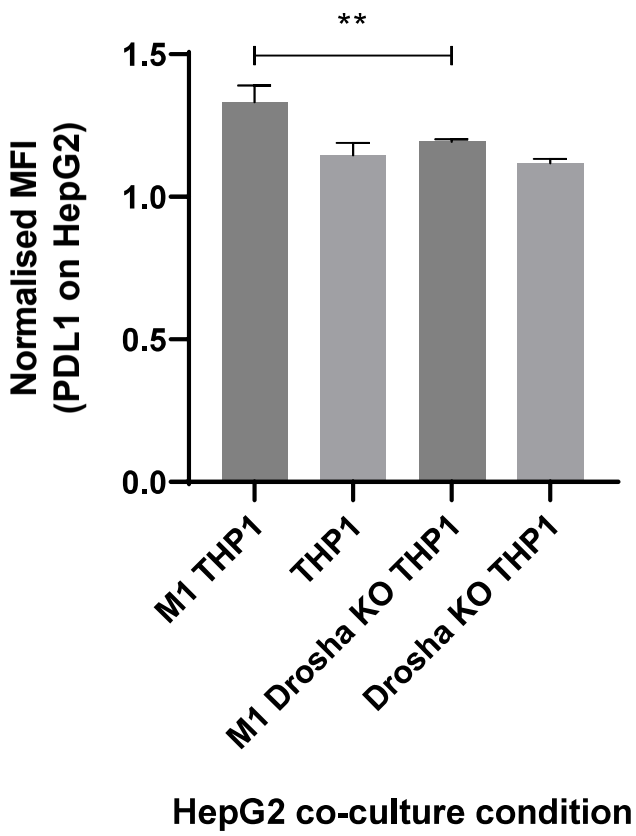
Drosha KO THP1s were differentiated into an M1 phenotype

- Then co-cultured with HepG2 cells for 72 hours

PDL1 expression on HepG2 was measured by flow cytometry

KO of Drosha in the THP1 impedes their ability to drive PDL1 expression in HepG2

This suggests a role for THP1 miRNAs in HepG2 PDL1 induction

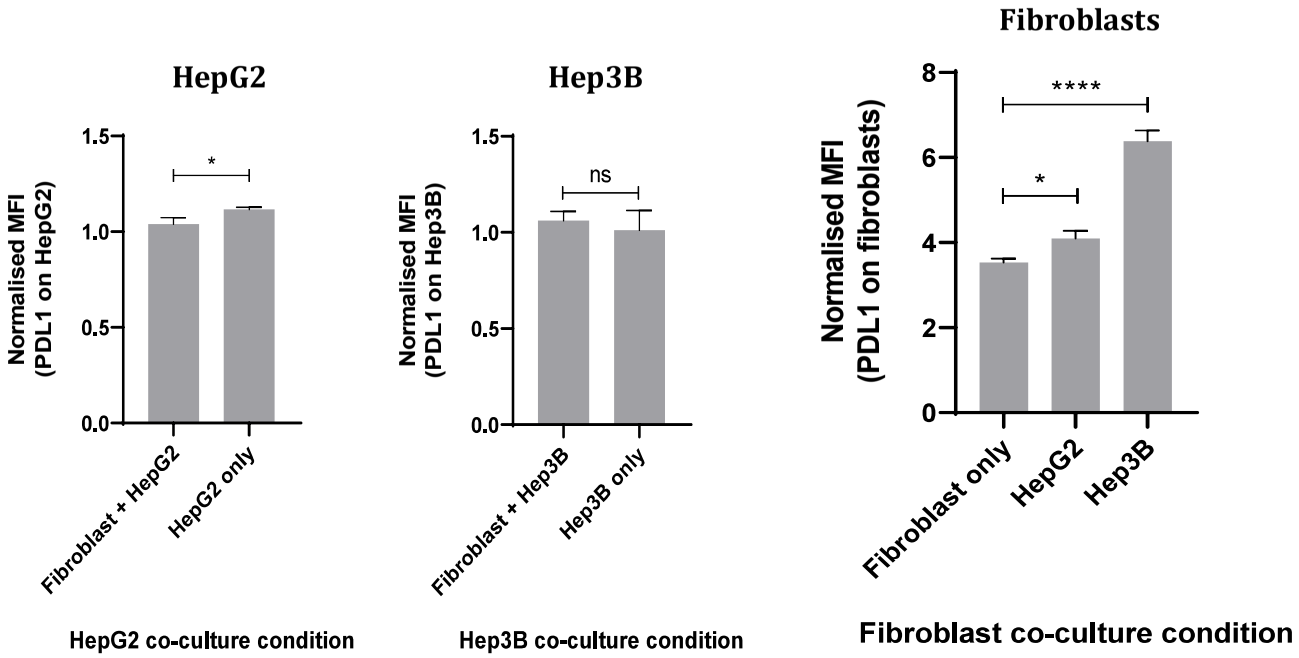


Role of fibroblasts in PDL1 expression

Fibroblasts form a large part of the tumour mass and are involved in inflammation

Do fibroblasts contribute to tumour PDL1?

Primary lung fibroblasts co-cultured with HepG2/Hep3B for 72 hours
- PDL1 measured on the fibroblasts and liver cancer cells by flow cytometry



Future directions

1. Identify a “signature of miRs/RNAs involved in PDL1 up-regulation in HCC”
2. Assess their mechanism of action
3. Better understand the role of PDL1 in cancer-associated fibroblasts
4. Determine the effects on checkpoint inhibitor therapy

SUMMARY BUDGET	Year 1	Year 2	Year 3	Year 4	Year 5	Total All Years
	7/1/19					7/1/19
	6/30/20					6/30/20
A. Senior/Key Person	158,391	0	0	0	0	158,391
B. Other Personnel	0	0	0	0	0	0
C. Equipment	0	0	0	0	0	0
D. Travel - Domestic Travel	0	0	0	0	0	0
E. Participant/Trainee Support Costs	0	0	0	0	0	0
F. Other Direct Costs						
1. Materials and Supplies	33,683	0	0	0	0	33,683
2. Publication Costs	0	0	0	0	0	0
3 Consultant Services	0	0	0	0	0	0
4. ADP/Computer Services (N/A)						
5a. Subawards/Consortium/Contract	0	0	0	0	0	0
5b. Subawards/Consortium/Contract	0	0	0	0	0	0
7. Alterations and Renovations	0	0	0	0	0	0
8. Other Expenses	122,794	0	0	0	0	122,794
9. Patient Care Costs	0	0	0	0	0	0
G. Direct Costs (A thru F)						
1. Total Direct Costs (A thru F)	**314,867**	**0**	**0**	**0**	**0**	**314,867**