

2017

A cross sectional analysis of the association between FGF19 tumor expression and serum AFP levels in advanced HCC patients

<https://hdl.handle.net/2144/23745>

"Downloaded from OpenBU. Boston University's institutional repository."

BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

**A CROSS SECTIONAL ANALYSIS OF THE ASSOCIATION BETWEEN FGF19
TUMOR EXPRESSION AND SERUM AFP LEVELS IN ADVANCED HCC
PATIENTS**

by

CORINNE CLIFFORD

B.S., Fairfield University, 2004

Submitted in partial fulfillment of the
requirements for the degree of
Master of Science

2017

© 2017 by
CORINNE CLIFFORD
All rights reserved

Approved by

First Reader

Stacey Hess Pino, M.S., M.S.
Instructor, Department of Medical Sciences & Education

Second Reader

Ben Wolf, M.D., Ph.D.
Vice President, Clinical Development, Blueprint Medicines

Third Reader

Hongliang Shi, M.S.
Director, Biostatistics, Blueprint Medicines

ACKNOWLEDGMENTS

I would like to thank Stacey Hess Pino, Hongliang Shi, and Ben Wolf for dedicating their time and efforts, as well as providing extremely helpful guidance and expertise, during this process. I would not have been able to complete my thesis without each of you!

**A CROSS SECTIONAL ANALYSIS OF THE ASSOCIATION BETWEEN FGF19
TUMOR EXPRESSION AND SERUM AFP LEVELS IN ADVANCED HCC
PATIENTS**

CORINNE CLIFFORD

ABSTRACT

Purpose: HCC is a complicated disease with high mortality rates and limited treatment options. No universal clinical or molecular classification established to inform better treatment options. There has been very limited success in determining a molecular profile that represent valid drivers in HCC patients and thus no targeted agents have obtained marketing approval. However, emerging data suggest the FGF19-pathway as a HCC driver and a potential therapeutic target. This research study aims to investigate whether the HCC prognostic risk factor, serum AFP, is predictive of FGF19 protein expression as assessed by immunohistochemistry in advanced HCC patients.

Methods: A cross-sectional analysis was performed from baseline data collected in a Phase 1 study conducted at various centers across the US, EU, and Asia. Only advanced HCC patients with adequate liver function were eligible for enrollment. Demographic data, detailed history of HCC, and any prior treatments or surgeries were recorded. Baseline laboratory values and prognostic factors including performance status (ECOG), lab values (i.e. bilirubin, albumin), and the number, size and biomarker status of the tumor(s) were collected. Differences between groups were assessed by t test, or Chi-square test, as appropriate. Multivariate logistic stepwise regression analyses were

performed including all parameters with highly significant correlations in the multivariate analysis.

Results: Only AFP, metastatic disease, and prior surgery met the criteria to be incorporated into the final model. Results indicated that high AFP had a statistically significant (p-value = .01) positive association (Wald chi-square statistic = 6.601) with positive FGF19 IHC status. The odds ratio for being FGF19 IHC+ was 12.216 among the high AFP subjects as compared to low AFP subjects, and also statistically significant but had a very wide 95% confidence interval (1.811, 82.79).

Conclusions: The results indicated that HCC patients with high serum AFP levels have a twelve fold higher chance of having a positive FGF19 IHC status than those with low AFP levels. Further studies are warranted in order to replicate the data in a larger sample size to understand future clinical implications once treatment options become available for FGF19 IHC positive patients.

TABLE OF CONTENTS

TITLE.....	i
COPYRIGHT PAGE.....	ii
READER APPROVAL PAGE.....	iii
ACKNOWLEDGMENTS	iv
ABSTRACT.....	v
TABLE OF CONTENTS.....	vii
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF ABBREVIATIONS.....	xi
INTRODUCTION	1
Background	1
Study Rationale and Purpose	8
METHODS	10
Study Design	10
Study Population	10
Data Collection	11
Statistical Analysis	11
RESULTS	13

Study Population.....	13
Linear Regression Analysis	15
Logistic Regression Analysis.....	16
Univariate Logistic Regression Analysis.....	16
Multivariate Stepwise Logistic Regression Analysis	18
Multivariate Stepwise Logistic Regression Analysis – Final Model.....	18
DISCUSSION	20
Limitations	22
Future Directions	24
CONCLUSION.....	25
REFERENCES	26
CURRICULUM VITAE.....	30

LIST OF TABLES

Table	Title	Page
1	2012 Liver Cancer Estimated Incidence, Prevalence, and Mortality by Region	2
2	FGFR4/FGF19 Inhibitors in Development	8
3	Key Exclusionary Values	11
4	Patient Demographics (N=45)	14
5	FGF19 IHC Results	15
6	Serum AFP levels by FGF19 IHC Status	16
7	Univariate Analysis of FGF19 IHC Status vs. HCC Risk Factors	17
8	Multivariate Analysis of FGF19 IHC Status vs. HCC Risk Factors	18

LIST OF FIGURES

Figure	Title	Page
1	FGF19 Role in Normal Liver and in HCC Liver	7
2	Phase 1 Study Design	10
3	Eligibility Profile	13
4	Fit Plot of FGF19 IHC vs. AFP Value	16

LIST OF ABBREVIATIONS

Abbreviation	Definition
ALP	Alkaline phosphatase
AFP	Alpha-fetoprotein
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ECOG	Eastern Cooperative Oncology Group
FGF19	Fibroblast growth factor 19
FGFR4	Fibroblast growth factor receptor 4
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
IHC	Immunohistochemistry
NASH	Nonalcoholic steatohepatitis
OS	Overall survival
PFS	Progression-free survival
PR	Partial response
PS	Performance status
SD	Standard deviation
TACE	Trans-arterial chemoembolization
ULN	Upper limit of normal

INTRODUCTION

Background

Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer. Most HCC patients (70%–90%) have underlying chronic liver disease and cirrhosis related to co-morbid conditions such as hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, alcoholic liver disease, or nonalcoholic steatohepatitis (NASH).¹ However, the prevalence of co-morbid conditions varies greatly between western and eastern populations.¹ For example, approximately 50% of HCC cases worldwide are associated with HBV compared to 10-15% in the US, whereas about half HCC patients in the US are infected with HCV compared to 25% worldwide.^{2,3}

Liver cancer is one of the most common cancers worldwide (5th) and is only second to lung cancer in the number of cancer related deaths every year.⁴ In 2012, the annual incidence globally was estimated to be approximately 782,000 cases, with a mortality rate of approximately 746,000 per year.⁴ As shown in Table 1, there is a distinct distribution of the malignancy across geographic locations, with the highest incidence, prevalence, and mortality rates (by far) in China due to the high prevalence of HBV and HCV.⁴

Table 1: 2012 Liver Cancer Estimated Incidence, Prevalence, and Mortality by Region⁴

Estimated Numbers (thousands) ²	Men		Women		Both Sexes
	Cases	Deaths	Cases	Deaths	
Geographic Locations					5-year Prevalence
Africa	25	24	14	12	26
US	23	17	8	7	27
China	293	282	101	101	291
India	17	17	10	10	13
EU	36	32	16	17	47

Prognosis for these patients is extremely poor with an overall mortality to incidence ratio of 0.952.⁴ Despite all the advances in cancer research and development over recent years, HCC remains a high unmet medical need with its increasing number of cases each year and high mortality rates. The additional complexity of the decompensated liver combined with the malignant tumor(s), make HCC a difficult malignancy to diagnose and treat at an early cancer stage.

Treatment Options for HCC

The only curative treatments for HCC are by liver transplantation or surgical resection of the liver tumor(s).⁵ Most patients are not considered good candidates for these surgeries due to the advanced stage of HCC at diagnosis or degree of liver decompensation due to the underlying cirrhosis.⁵ Even for the few patients who are good candidates, recurrence rates are unfavorable (20% for transplants and 50% for resections).^{6,7} Other treatment options include localized therapies, which have shown

some long term success. These include radiofrequency ablation, trans-arterial chemoembolization (TACE), and radioembolization, but have only been effective for treating a select group of HCC patients.⁸

Non-surgical options for treating other malignancies usually include chemotherapy or systemic therapy. Unfortunately, HCC is known to be a cancer type that is one of the most resistant to chemotherapy treatment options. Currently, sorafenib, a multi-kinase inhibitor, is the only drug approved for systemic treatment of HCC.^{5,9} Additionally, sorafenib has a very low response rate (~2%) and was only shown to improve overall survival (OS) in pivotal trials by a meager 2.8 months.^{8,9} Even with sorafenib treatment, survival duration is still less than a year (10.7 months).⁸ Since its approval in 2007, no other therapies, including targeting agents, have been able to show clinically significant improvement in OS; consequently HCC remains a cancer that has a significant unmet medical need for better and more treatment options.

HCC Staging and Prognosis

Accurately staging cancer patients is essential regardless of the oncological indication. It contributes to prognosis, can help guide treatment decisions, and also informs clinical, epidemiologic, and health services research.¹⁰ Staging HCC patients is more complex than other solid tumor cancers due to cirrhosis and diversity of the underlying disease etiology.¹¹ Additionally, the lack of universal acceptance as how to best classify HCC patients poses another challenge. However most systems developed over the past 50 years do possess one commonality; they all account for both

characteristics of the tumor(s) as well as the overall health of the liver as the result of the underlying liver disease.¹¹

The goal of staging is to classify patients according to certain characteristics in order to define prognosis and guide treatment.¹² Important variables considered include the patient's overall health (i.e. performance status), tumor burden (i.e. size, number, presence of metastases), clinical symptoms (i.e. abdominal pain), liver function (i.e. AST/ALT levels), and presence of any other comorbidities.¹³ As mentioned previously, the degree of liver damage is an important part of classifying HCC patients, as measured by Child's Pugh status. Child's Pugh scores were not initially designed for cirrhosis assessments, but instead used to predict the risk of mortality during surgery.¹⁴ Since the original development, it has been modified and is broadly used to assess the prognosis of chronic liver disease and cirrhosis.¹⁴ It is calculated by measuring total bilirubin, serum albumin, and prothrombin time (prolongation) as well as the presences and severity ascites and hepatic encephalopathy.¹⁴

The current staging classification systems have many limitations. They have been based upon a population balanced between early and advanced staged HCC patients.¹⁵ Characteristics may vary if examining only advanced staged patients, consequently it may be more informative to separate the two.¹⁵ Furthermore, it's not clear whether additional variables that differ between these systems provide significant prognostic value.¹⁵ Since patients diagnosed with HCC with earlier-stage disease are much more likely to receive potentially curative treatment, it is important to be able to identify these patients early on and classify these patients consistently and correctly.¹⁴

Additionally, no clear molecular or genetic profiles have been identified to show prognostic value in HCC.¹⁶ There has been success in identifying these types of markers or mutations in other oncologic indications such as breast and lung cancer, but it has not yet translated to HCC.¹⁶ Treatment options in these patients are more defined and effective if they are positive for a particular marker. Additional research in HCC could yield molecular or genetic profiles that become the basis for a new HCC staging system; one that effectively separates patients into subgroups with homogeneous prognosis and response to treatment, which could aid in the selection of treatment.¹⁶

Role of Alpha-fetoprotein in HCC

Alpha-fetoprotein (AFP) was first recognized as a marker for HCC over 40 years ago, but its clinical implications are not entirely clear.¹⁷ It is one of the most abundant proteins present in fetal development that sharply declines after birth; therefore any elevations in adults could be indicative of underlying malignancy.⁸ Specifically, AFP is made by the yolk sac and the liver during gestation which could provide insight as to why it later emerged as a means to detect HCC preclinically.⁸ However, clinical studies have provided mixed results regarding the link to serum AFP elevations in diagnosing adults with HCC.⁸

Measuring AFP is relatively simple and is routinely used for HCC surveillance, and has been used in evaluating prognosis and monitoring recurrence following treatment.¹⁸ Studies have shown that elevated AFP levels in HCC patients is associated with decreased survival or a poor outcome; however, it is not as clear which is the driver after adjusting the analysis for tumor size.¹⁹ Findings suggest there is a complex

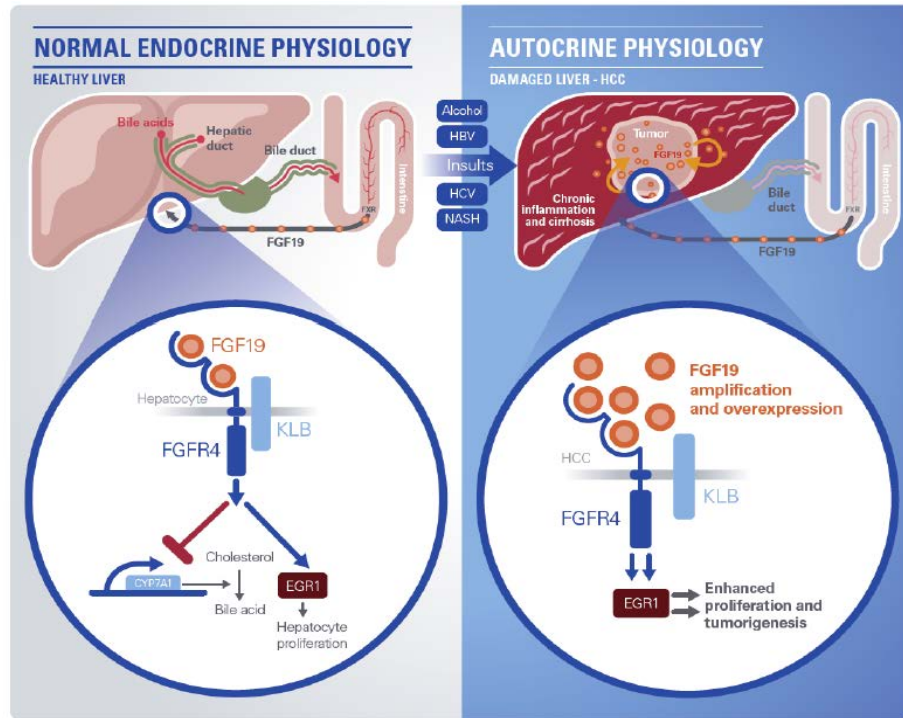
interaction with other risk factors for HCC since high AFP values are also correlated with other major clinical factors including age, HBV related HCC, tumor size, and vascular invasion.¹⁹ Additionally, AFP serum levels can also be increased in patients with other non-malignant liver disorders.¹⁹

One large study investigated the presence or absence of any malignancy among adults with elevated AFP levels (>20 ng/ml) and found that 68% had a malignancy (most, but not all HCC) while 32% did not have nor develop any malignancy.¹⁸ For reference, both AFP levels of 20 and 400 ng/mL are considered feasible cutoffs for predicting outcome in HCC patients.²⁰ The mean serum AFP level of HCC patients identified in this study was 1,030 ng/ml (SD +/- 1,890) with a range of 20 to 3,268 ng/ml.¹⁸ Conversely among patients diagnosed with HCC, some possessed high serum levels, however, approximately 30% had normal levels that were maintained over the course of their disease.¹⁷

Fibroblast Growth Factor 19 Tumor Expression in HCC patients

Fibroblast growth factor 19 (FGF19) is a protein produced in the small intestine that regulates bile acid synthesis in the liver. Normal liver does not express the FGF19 protein; therefore, any expression is a tumor marker.²¹ Preclinical studies investigating FGF19 protein levels in mice have shown that genomic amplification and/or over-expression was associated with the development of HCC and has been implicated to play a similar role in humans.²¹

Figure 1: FGF19 Role in Normal Liver and in HCC Liver²²



The function of FGF19 in normal liver vs. a liver with FGF19 driven HCC is depicted in Figure 1. It is estimated that about 20-30% of HCC tumors have aberrant FGF19 expression.²¹ A list of drugs currently in development to treat HCC by targeting the FGFR4/FGF19 pathway are listed in Table 2.

Table 2: FGFR4/FGF19 Inhibitors in Development²³

NCT Number	Protocol Title	Intervention	Sponsor	Phase
NCT02325739	FGF401 in HCC and Solid Tumors Characterized by Positive FGFR4 and KLB Expression	Drug: FGF401	Novartis	1/2
NCT02690350	An Open Study Assessing the Safety and Tolerability of U3-1784	Drug: U3-1784	Daiichi Sankyo Inc.	1
NCT02834780	Phase 1 Study to Evaluate the Safety, Pharmacokinetics and Pharmacodynamics of H3B-6527 in Subjects With Advanced HCC	Drug: H3B-6527	H3 Biomedicine/ Eisai	1
NCT02508467	A Phase 1 Study of BLU-554 in Patients With HCC	Drug: BLU-554	Blueprint Medicines	1

Study Rationale and Purpose

Since HCC patients have such a poor survival rate, striving to better characterize and classify them is imperative to identify potential subsets that may gain substantial benefit from any new interventions in development.¹¹ Therefore this study aims to investigate whether AFP levels are different in FGF19 over-expressed advanced stage HCC patients. This could provide additional information to further investigate its prognostic value in this subset of HCC patients and whether this difference can help guide better treatment decisions. Specifically, the association between high serum AFP levels and FGF19 tumor expression in patients with advanced HCC will be investigated.

Study Question

Are high serum AFP levels associated with tumor FGF19 protein expression as assessed by IHC in patients with advanced HCC?

Study Objectives

To determine whether serum AFP levels were predictive of FGF19 IHC tumor status in HCC patients.

Study Endpoints/Outcomes of Interest

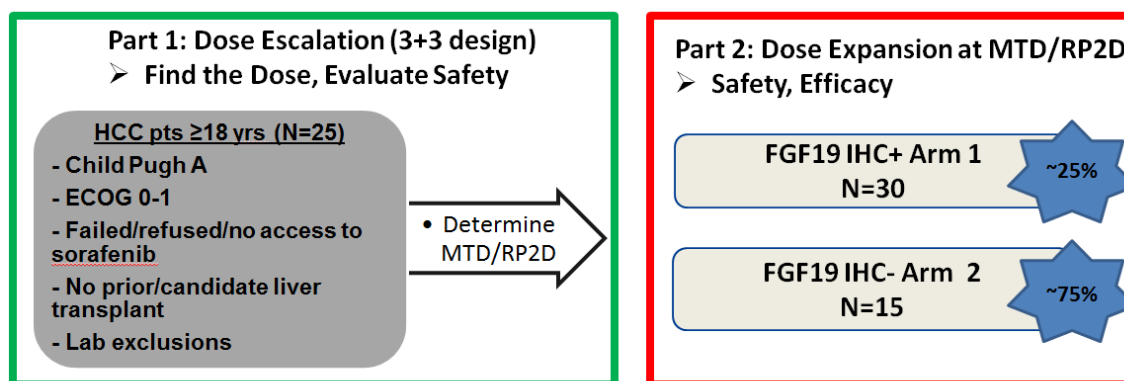
The “exposure” or outcome variable was tumor FGF19 IHC tumor expression status and the primary risk factor or predictor variable for this study was AFP levels.

METHODS

Study Design

A cross-sectional analysis was performed from baseline data collected in a Phase 1 study conducted at various centers (21) across the US, EU, and Asia as shown in Figure 2. The trial was approved at the local Institutional Review Board or Ethics Committee prior to study initiation and all patients provided written informed consent prior to any study specific procedures.

Figure 2: Phase 1 Study Design



Study Population

A total of 52 patients were enrolled at the time of analysis. Only patients 18 years or older with advanced HCC, adequate liver function (Child Pugh score 5-6 points, class A), and good performance status (ECOG 0-1) were enrolled in the study. Patients must have failed, refused, or did not have access to sorafenib. Patients were excluded if they underwent liver transplant or if they were candidates for liver transplant or resection. Details of laboratory exclusion criteria are shown in Table 3.

Table 3: Key Exclusionary Values

Test	Value
Platelet count	$<75 \times 10^9/L$
Absolute neutrophil count	$<1.0 \times 10^9/L$
Hemoglobin	$<8.0 \text{ g/dL}$
Aspartate aminotransferase (AST)	$>5 \times$ the upper limit of normal (ULN)
Alanine aminotransferase (ALT)	$>5 \times$ the ULN
Total bilirubin	$>2.5 \text{ mg/dL}$
Prothrombin time	>6 seconds above control
Measured creatinine clearance	$<40 \text{ mL/min}$
QT interval corrected using Frederica's formula	$>450 \text{ msec}$

Data Collection

Demographic data such as age, race, and gender were recorded electronically using medidata RAVE EDC database. Detailed history of HCC and any prior treatments or surgeries were collected for all subjects. Baseline blood count, AFP levels, performance status (ECOG), lab values (i.e. AST, ALT, alkaline phosphatase (ALP), bilirubin, albumin, etc.), tumor characteristics (i.e. the number, size, etc.), and FGF19 tumor expression levels were also recorded. All tests were performed locally per the site's local standards, except FGF19 tumor status was determined by central laboratory immunohistochemistry (IHC) testing. FGF19 IHC tumor status was reported by percent positivity and positive/negative based on a 1% cutoff.

Statistical Analysis

Data were summarized using descriptive statistics. Continuous data variables were described by N, mean, standard deviation, minimum, and maximum. Categorical data variables were shown using the number and percentage (N, %) of patients within each classification. All statistical analyses were performed using SAS version 9.4.

Initially, to understand the correlation between AFP and FGF19, a linear regression model was built to explore whether the FGF19 IHC value was predictable by AFP value. Both FGF19 IHC results and AFP levels were treated as continuous variables in the initial linear regression model. The outcome/ dependent variable was FGF19, while AFP served as the predictor/independent variable. A t-test was used to show whether the relationship was significant. A p-value < 0.05 was considered statistically significant.

A logistic regression model was built using FGF19 IHC as a binary outcome variable (positive or negative) and AFP as a continuous predictor variable. Odds ratios estimates were calculated with 95% Wald confidence intervals. By dichotomizing FGF19 IHC levels, univariate logistic regression analysis was then conducted to examine its association with various HCC risk factors. For those variables exhibiting a significant association in univariate logistic regression analysis, multivariate logistic stepwise regression analysis was then built.

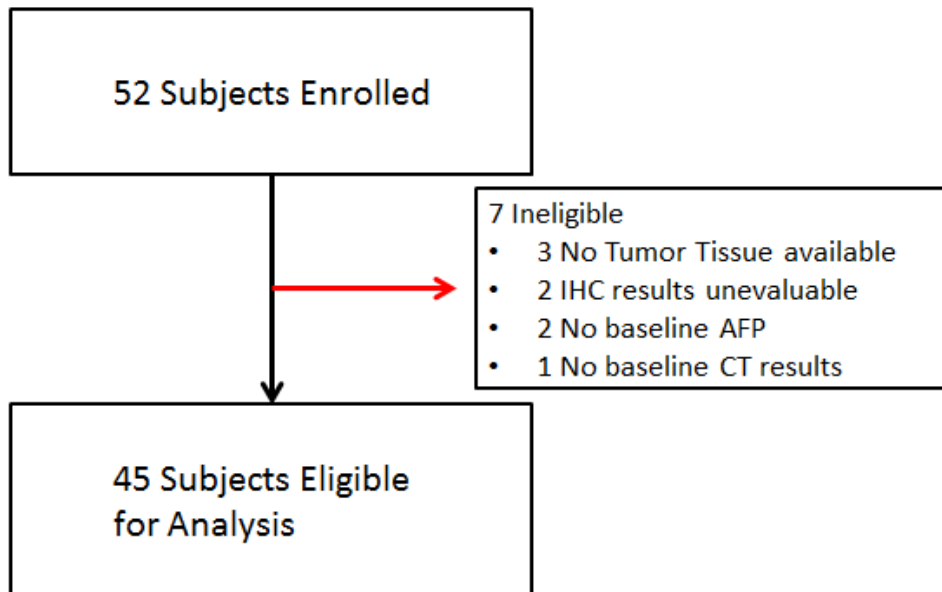
Variables selected by the multivariate stepwise logistic regression model were further examined in the final model. Group comparisons were conducted using the chi-square test. Odds ratios estimates were calculated with 95% Wald confidence intervals. A p-value < 0.05 was considered statistically significant.

RESULTS

Study Population

From September 2015 to February 2017, a total of 52 patients with advanced HCC were enrolled at 11 centers in Hong Kong, Singapore, South Korea, Spain, the United Kingdom, and the US. Of these patients, only 45 were eligible for analysis in this study as detailed in Figure 3.

Figure 2: Eligibility Profile



Baseline characteristics are described in Table 4. The average age in this subject population was 60 years old, and comprised of mostly white or Asian men. Over half of the subjects had a history of underlying hepatitis B or C, while all had advanced disease as indicated by the average number of lesions (about 3), largest diameter of target lesion (~6 cm), and metastatic disease (78%). The majority of subjects (78%) also had metastatic disease, but with preserved liver function (i.e. all subjects are Child's Pugh A) as shown by the mean averages of liver function lab values. Of the subjects who were

evaluable for analysis, 47% were found to be FGF19 IHC positive as defined by a cutoff of 1% or higher. Also, the proportion of subjects with high serum AFP levels was similar to what has been reported previously (67% vs. 68%) indicating that this study cohort was representative of the HCC population.

Table 4: Patient Demographics (N=45)

Category		Frequency	
		N	% Total
Sex	Male	38	84.4
	Female	7	15.6
Race	Asian	22	48.9
	Black/African	1	2.2
	White	21	46.7
	Unknown	1	2.2
Etiology	HBV	22	48.9
	HCV	4	8.9
	Non-Viral	11	24.4
	Unknown	8	17.8
ECOG	0	7	15.6
	1	38	84.4
FGF19 IHC Results	Positive	21	46.7
	Negative	24	53.3
Metastatic Disease	No	10	22.2
	Yes	35	77.8
Vascular Invasion	No	21	46.7
	Yes	11	24.4
	Unknown	13	23.9
		Mean (SD)	Min, Max
Age		59.8(15.6)	18, 85
Target Lesions	Number	2.8 (1.1)	1, 5
	Largest (cm)	5.7 (2.9)	2.1,14.2
AFP (ng/mL)		11,907.9 (34,282.3)	2.2, 192084
Laboratory Values	ALT (IU/L)	46.9 (32.2)	7, 167
	AST (IU/L)	61.3 (36.4)	10, 162
	ALP (IU/L)	233.4 (245.7)	35, 1178
	Bilirubin (μ mol/L)	14 (8.2)	5, 39.3
	Albumin (g/L)	39 (3.9)	32, 49

Linear Regression Analysis

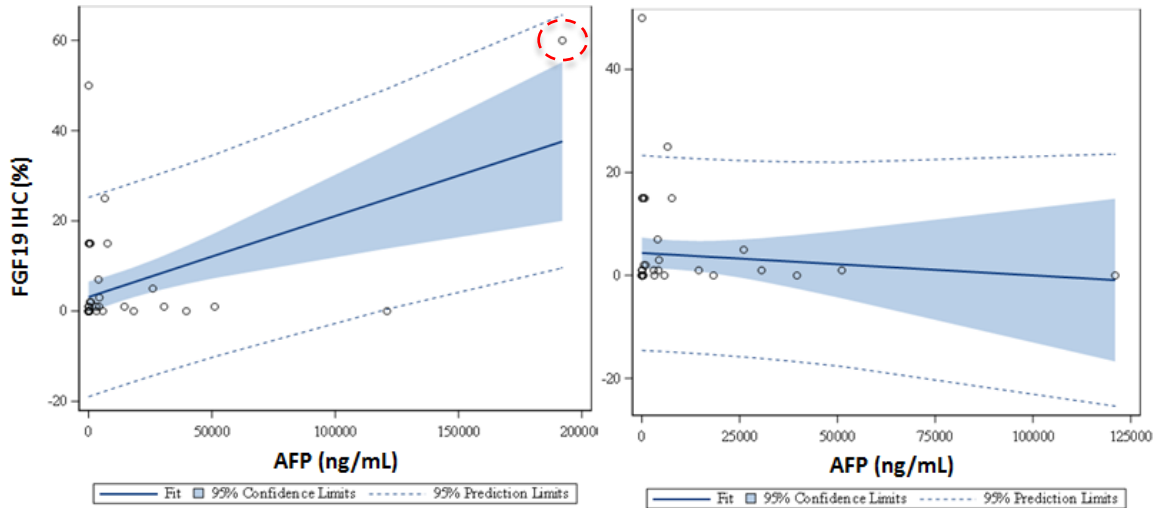
FGF19 IHC and AFP values were treated as continuous variables in the linear regression analysis. The range in FGF19 IHC results for the 45 subjects included in the analysis are detailed in Table 5. Of the patients who were FGF19 IHC+, most were either 1% or 15% positive.

Table 5: FGF19 IHC Results

FGF19 IHC Results (%)	N (45)	% Total
0	24	53.3
1	8	17.8
2	2	4.4
3	1	2.2
5	1	2.2
7	1	2.2
15	5	11.1
25	1	2.2
50	1	2.2
60	1	2.2

Linear regression results show that there was a statistically significant positive relationship between AFP levels and FGF19 IHC values (p value = 0.0005). Upon evaluation of the fit plot of the linear regression model as shown on the left in Figure 3, there was one data point that was an outlier that could potentially be driving the line in an upward linear direction. Consequently, the model was rerun excluding the outlier value as shown on the right in Figure 4, and the relationship no longer held. The fit plot line no longer supported a positive relationship; it was slightly negative and not statistically significant (p value = 0.53).

Figure 3: Fit Plot of FGF19 IHC vs. AFP Value



Logistic Regression Analysis

As previously mentioned, FGF19 IHC results were reported as positive or negative as defined by a 1% cutoff. Using FGF19 IHC status as a binary outcome variable and AFP as a continuous predictor variable, a logistic regression model was run to analyze the relationship between the two. Odds ratio estimates showed no association and the results were not statically significant (p value = 0.42).

Univariate Logistic Regression Analysis

Based on a cutoff of 20 ng/mL, AFP levels were grouped and treated as a binary predictive variable, then categorized by FGF19 IHC status as shown in Table 6.

Table 6: Serum AFP levels by FGF19 IHC Status

FGF19 IHC Status	Serum AFP Levels (N, % total)		
	≤20 ng/mL	>20 ng/mL	Total
Positive	3 (6.7)	18 (40.0)	21 (46.7)
Negative	12 (26.7)	12 (26.7)	24 (53.3)
Total	15 (33.3)	30 (66.7)	45

A univariate logistic regression of FGF19 on categorical AFP was performed and analysis results are shown in Table 7. In addition to AFP, the model was run using various HCC risk factors against FGF19 IHC status.

Table 7: Univariate Analysis of FGF19 IHC Status vs. HCC Risk Factors

Variable	Point Estimate	Standard Error	Wald Chi-Square	P-value	Odds Ratio	95% Confidence Interval (min, max)
AFP	0.896	0.373	5.789	0.016	6.000	1.392, 25.857
Age	-0.008	0.020	0.170	0.681	0.992	0.955, 1.031
Albumin	0.099	0.081	1.480	0.224	1.104	0.942, 1.294
ALP	0.001	0.001	0.793	0.373	1.001	0.999, 1.004
ALT	-0.002	0.009	0.044	0.833	0.998	0.980, 1.107
AST	-0.004	0.008	0.272	0.602	0.996	0.980, 1.012
Bilirubin	-0.010	0.037	0.072	0.788	0.990	0.921, 1.065
Etiology- HCV	9.030	168.000	0.003	0.957	>999.999	<0.001, >999.999
Etiology -Non -viral	-2.621	56.008	0.002	0.963	1.050	0.159, 6.924
Etiology- HBV	-3.740	56.007	0.005	0.947	0.343	0.064, 1.829
ECOG – 0	0.091	0.415	0.048	0.826	1.200	0.236, 6.105
Largest Target Lesion Size	0.002	0.010	0.043	0.836	1.002	0.982, 1.023
Number target lesions	0.405	0.300	1.822	0.177	1.499	0.833, 2.699
Metastatic- N	-0.626	0.386	2.635	0.104	0.286	0.063, 1.296
Prior Surgery- N	0.549	0.380	2.094	0.148	3.000	0.677, 13.285
Race – Black	10.121	130.300	0.006	0.938	>999.99	<0.001, >999.99
Race - UNK	-10.299	130.300	0.006	0.937	<0.001	<0.001, >999.99
Race –Asian	-0.649	58.286	0.000	0.991	0.229	0.063, 0.826
Sex	0.091	0.415	0.048	0.826	1.200	0.236, 6.105
Vascular Invasion –No	0.738	0.425	3.014	0.083	5.333	1.069, 26.613
Vascular Invasion – UNK	0.199	0.461	0.186	0.666	3.111	0.559, 17.330

Only AFP levels showed a statistically significant association for FGF19 IHC status (p-value = 0.016) by Wald Chi-square test, and the odds ratio was also significant

(lower limit 1.392). There was a trend for a positive association of vascular invasion to FGF19 IHC status with a p-value of 0.083 with the odds ratio being significant (lower limit 1.069).

Multivariate Stepwise Logistic Regression Analysis

A stepwise logistic regression model was built including all HCC risk factors investigated from Table 7. In order for a variable to be selected into the model it must be significant at 0.1 level, and the significant level of 0.2 to stay in the model. Age, sex, race, ECOG, vascular invasion, etiology largest tumor diameter, total lesion size, albumin, ALT, AST, ALP, and bilirubin were excluded from the stepwise selection. AFP, metastatic disease, and prior surgery were selected into the model.

Multivariate Stepwise Logistic Regression Analysis – Final Model

The Final Model was run after the stepwise regression with results shown in Table 8. The final model included the 3 variables selected in the stepwise regression: AFP, metastatic disease, and prior surgery. All were binary high/low (AFP), yes/no (metastatic disease and prior surgery), or positive/negative (FGF19 IHC status).

Table 8: Multivariate Analysis of FGF19 IHC Status vs. HCC Risk Factors

Parameter	Analysis of Maximum Likelihood Estimates				Odd's Ratio Estimate		
	Estimate	Standard Error	Wald Chi-Square	P-value	Point Estimate	95% Wald Confidence Limits	
Intercept	0.569	0.546	1.087	0.30	NA	NA	NA
AFP	1.253	0.488	6.601	0.01	12.246	1.811	82.790
Metastatic Disease	-1.253	0.543	5.323	0.02	0.082	0.010	0.686
Prior Surgery	1.049	0.539	3.798	0.05	8.155	0.988	67.320

Based on the final model comparing the Wald chi-square test statistics, both high AFP levels and having a prior surgery were found to have a positive association on FGF19 IHC status and were found to be statistically significant (p-value = .01, .05 respectively). Metastatic disease was found to have a statistically significant negative association on FGF19 IHC (p-value = .02).

When comparing the odds ratio for each risk factor, only AFP levels remained significant since the lower limit of 95% confidence interval was not less than 1. The point estimate suggests that with a high AFP value (>20 ng/mL), there is a 12-fold chance of having a positive FGF19 IHC value. The confidence limit was quite large (1.18-82.79) due to the fact that the sample size was small (45). The point estimates for metastatic disease and prior surgery on FGF19 IHC status were not significant since the lower limit of the confidence intervals were less than 1 (0.01 and 0.99, respectively); however the trend for prior surgery was stronger with the lower limit just slightly less than 1. This suggests that there was an 8-fold chance, if you had a prior surgery, of having a positive FGF19 IHC value. Although metastatic disease was not significant, the point estimate suggests that if a subject has metastatic disease, the odds of having a positive FGF19 IHC value were 0.08 as compared to subjects who do not have metastatic disease.

DISCUSSION

The aim of this study was to investigate the association of serum AFP levels and FGF19 IHC tumor status in advanced HCC patients. HCC is a complex disease and little consensus has been obtained universally on how to classify these patients.¹¹ While treatment options are limited, new targeted agents, such as FGFR4/FGF19 inhibitors, are currently in clinical development so it is important to more thoroughly understand these patients to better inform treatment options once additional options become available. Findings from this study suggest that high serum AFP levels could be a relevant pre-screen or surrogate for the FGF19 tumor marker and guide treatment for targeted therapies in development such as FGFR4 inhibitors.

The study population as described in Table 4 was representative of HCC patients worldwide in terms of sex, etiology, and age. The disease primarily affects men, and 84% of the subjects analyzed in this study were men.⁴ The HCC etiology predominantly was viral (48.9% HBV and 8.9% HCV) which is generalizable to the worldwide breakdown.⁴ Lastly, the average age was 59.8 years old in this study, which is slightly younger than the average age at HCC diagnosis (65 years old), but there has been a recent shift toward diagnosis at an earlier age that could account for this difference.²⁵ Additionally, the youngest patient enrolled at 18 years, could have pulled the average down. Therefore, this study represents a generalizable population of HCC patients with respect to age, HCC etiology, and gender.

Elevated AFP levels have been found to be associated with poor outcomes or a worse prognosis in HCC patients compared to those with normal or low levels.¹⁹ Results

from both the univariate and multivariate analyses showed that there was a statistically significant association between the two variables, AFP and FGF19 tumor status. Those subjects with high serum AFP levels (>20 ng/mL) had a significantly higher chance of also having a positive ($\geq 1\%$) FGF19 IHC status. Specifically, AFP levels were predictive of FGF19 IHC tumor status in this study population and the strength of this relationship is strong (twelve fold).

There have been many studies over the years that have studied the clinical role of AFP in HCC patients, but very few have actually investigated the prognostic impact of AFP levels.²⁰ The findings suggest that if high AFP levels are in fact predictive of poor prognoses, then patients with positive FGF19 IHC status are worse off than those that are negative. These results make sense given the FGFR4/FGF19 pathway is considered to be an oncogenic driver and contributes significantly to HCC progression.²⁴ Results could also suggest that the FGFR4/FGF19 pathway may regulate AFP levels.

AFP levels were the only risk factor that showed a statistically significant correlation with FGF19 IHC status in the multivariate stepwise logistic regression, but there were trends in the analysis for both metastatic disease and prior surgery. With respect to metastatic disease, the chi-square test static showed a statistically significant negative association with FGF19 IHC status. The negative relationship is counterintuitive given the presence of metastatic disease is an HCC risk factor, but the strength of the association was weak and not significant. Additionally, prior surgery showed a trend in association with FGF19 IHC status. This would suggest that patients who have had one or more liver resections or TACE procedures were more likely to also

be FGF19 IHC positive; but despite having a stronger relationship (8.155), it was not still significant. These two findings may be explained by the study population. Since the study only included advanced stage HCC patients, it may have been associated with the status of the disease at inclusion in the study more than FGF19 IHC status. It could also be due to the small sample size.

Studies have shown that tumor size can be a confounding factor to AFP levels indicating that tumor size has a significant impact on outcome.¹⁹ The results in this study showed that there was not a significant difference in tumor size between the FGF19 positive and FGF19 IHC negative groups, whereas there was a significant difference in AFP levels between the groups. While this doesn't provide any information regarding an association with FGF19 IHC status, it does help provide evidence that the findings were not confounded by tumor size. Similarly, other major risk factors as described in Table 4 did not have an association with FGF19 IHC status. This could be due to the small sample size analyzed in this study.

Limitations

A cross-sectional study design has inherent limitations that can be noted in this study. For example, a cross sectional analysis is only a snapshot of the data. Since the data for the exposure and outcome are obtained simultaneously, it is difficult to understand whether the outcome came before the exposure and vice versa. While the primary outcome variable was tumor FGF19 IHC tumor expression status and the primary predictor variable for this study was serum AFP levels, this relationship could reverse but causation was not investigated given the limitations of a cross-sectional study

design. Also, AFP levels could have been different if measured at a various points in time over the course of HCC progression, which could have impacted the strength of the association of the data.

Baseline FGF19 IHC status and AFP levels could also have been measured at different time points since the study allowed for archival tissue to be submitted for FGF19 IHC testing. Therefore the biopsy or resection may have occurred at a much different point in time that the AFP levels that were measured within locally at baseline. AFP levels may or may not have changed as a result of the advanced disease population. However one would expect to show less of an association between the two groups of FGF19 IHC status if the AFP levels were driven only by the advanced stage in disease.

The study population was also bound by the selection criteria from the Phase 1 Study; therefore, the analysis could not include other important HCC risk factors such as performance status or liver function (as measured by Child's Pugh score). Performance status is known to have a significant effect on a patient's outcome, regardless of disease.¹³ The study inclusion criteria limited to fit HCC patients since inclusion criteria specified an ECOG status of 0-1 for enrollment into the Phase 1 study. Because subjects with a 2-4 status were excluded from the study, this study could not measure whether the performance played a role in the results. Additionally, only HCC patients with adequate liver function as noted by the Child's Pugh A inclusion criteria were enrolled in the study and consequently included in the analysis. Because of the complex interaction of the liver cirrhosis with HCC, there was not a way to understand whether the degree of liver damage had an effect on the AFP levels.

Future Directions

The small sample size was noted as a limitation in the study design. Because the phase 1 study is still ongoing, the next step would be to re-analyze the study population after the study was fully enrolled. It would be informative to understand whether the strength of the association remains and whether or not the confidence interval become more narrow with the increased sample size. Also, the increased sample size could help better define the relationship of metastatic disease and prior surgery to FGF19 IHC status. Lastly, with a larger sample size, various AFP cutoffs could be explored in addition to 20 ng/mL to investigate if the cutoff affects the relationship to FGF19 tumor expression. Considering most of the modes of classification for HCC patients do not include a mixed population of both early and late stage disease, another possibility would include investigating the association of AFP and FGF19 IHC status in a mixed HCC population to see if results are similar.

CONCLUSION

In conclusion, this study provided useful information to better understand serum AFP levels in a molecular subset of HCC patients. The limitations described above have made it so that the results may not be entirely conclusive; however, they provide a good starting point for future studies. The results need to be replicated with a larger patient population after enrollment is complete for the Phase 1 study and could prove to be helpful in the clinical setting if similar conclusions are drawn.

REFERENCES

1. Sanyal AJ, Yoon SK, Lencioni R. The Etiology of Hepatocellular Carcinoma and Consequences for Treatment. *The Oncologist*. November 2010; 15: Supplement 4, 14-22. http://theoncologist.alphamedpress.org/content/15/suppl_4/14.long#ref-12. Assessed February 16, 2017.
2. El-Serag HB, Kanwal F. Epidemiology of hepatocellular carcinoma in the United States: Where are we? Where do we go? *Hepatology*. 2014;60(5):1767-75
3. World Health Organization. Population Fact sheet [updated 2015 Feb]. http://globocan.iarc.fr/Pages/fact_sheets_population.aspx. Assessed January 27, 2017.
4. Bodzin AS, Busuttil RW. Hepatocellular carcinoma: Advances in diagnosis, management, and long term outcome. *World Journal of Hepatology*. 2015; 7(9):1157–1167. doi:10.4254/wjh.v7.i9.1157
5. Zimmerman MA, Ghobrial RM, Tong MJ, et al. Recurrence of Hepatocellular Carcinoma Following Liver Transplantation: A Review of Preoperative and Postoperative Prognostic Indicators. *Archives of Surgery*. 2008; 143(2):182-188. doi:10.1001/archsurg.2007.39
6. Shah SA, Cleary SP, Wei AC, et al. Recurrence after liver resection for hepatocellular carcinoma: Risk factors, treatment, and outcomes. 2007; 141(3):330-339. doi:10.1016/j.surg.2006.06.028
7. Raza A, Sood GK. Hepatocellular carcinoma review: Current treatment, and evidence-based medicine. *World Journal of Gastroenterology*. 2014; 20(15):4115-4127. doi:10.3748/wjg.v20.i15.4115.

8. National Cancer Institute (Updated November 26, 2013). FDA Approval for Sorafenib Tosylate. <https://www.cancer.gov/about-cancer/treatment/drugs/fda-sorafenib-tosylate> Accessed January 10, 2017.
9. Køstner AH, Sorensen M, Olesen RK, Grønbæk H, Lassen U, Ladekarl M. Sorafenib in Advanced Hepatocellular Carcinoma: A Nationwide Retrospective Study of Efficacy and Tolerability. *The Scientific World Journal*. 2013; 2013: Article ID 931972, 6 pages. doi:10.1155/2013/931972
10. Subramaniam S, Kelley RK, Venook AP. A review of hepatocellular carcinoma (HCC) staging systems. *Chinese Clinical Oncology*. 2013; 2(4):33. doi: 10.3978/j.issn.2304-3865.2013.07.05
11. Sala M, Forner A, Varela M, Bruix J. Prognostic Prediction in Patients with Hepatocellular Carcinoma. *Seminars in Liver Disease*. 2005; 25(2):171-180.
12. Marrero JA, Kudob M, Bronowickic JP. The Challenge of Prognosis and Staging for Hepatocellular Carcinoma. *The Oncologist*. November 2010; 15: Supplement 4, 23-33. doi: 10.1634/theoncologist.2010-S4-23
13. Huitzil-Melendez FD, Capanu M, O'Reilly EM, et al. Advanced Hepatocellular Carcinoma: Which Staging Systems Best Predict Prognosis? *Journal of Clinical Oncology*. 2010; 28(17):2889-2895. doi:10.1200/JCO.2009.25.9895.
14. Cheung A, Cheung A. The Child-Pugh score: Prognosis in chronic liver disease and cirrhosis [Classics Series]. Website <http://www.2minutemedicine.com/the-child-pugh-score-prognosis-in-chronic-liver-disease-and-cirrhosis-classics-series/>. July 16, 2013. Accessed on March 2, 2017.

15. Pons F, Varela M, Llovet JM. Staging systems in hepatocellular carcinoma. *HPB : The Official Journal of the International Hepato-Pancreato-Biliary Association*. 2005; 7(1), 35–41. doi:10.1080/13651820410024058.
16. Bialecki ES, DiBisceglie AM. Diagnosis of hepatocellular carcinoma. *HPB : The Official Journal of the International Hepato-Pancreato-Biliary Association*. 2005; 7(1), 26–34. doi:10.1080/13651820410024049 .
17. Park H, Park JY. Clinical Significance of AFP and PIVKA-II Responses for Monitoring Treatment Outcomes and Predicting Prognosis in Patients with Hepatocellular Carcinoma. *BioMedical Research International*. 2013; 2013:310427. doi:10.1155/2013/310427.
18. Kashyap R, Join A, Nalesnik M, Carr B, Barnes J, Vargas HE, et al. Clinical significance of elevated alpha-fetoprotein in adults and children. *Digestive Diseases and Sciences*. 2001; 46:1709–13.
19. Peng SY, Chen WJ, Lai PL, Jeng WM, Sheu JC, Hsu HC. High α -fetoprotein level correlates with high stage, early recurrence and poor prognosis of hepatocellular carcinoma: Significance of hepatitis virus infection, age, p53 and β -catenin mutations. *Cancer Cell Biology*. 2004; 112(1):44-50. doi: 10.1002/ijc.20279
20. Hsu C-Y, Liu P-H, Lee Y-H, et al. Using Serum α -Fetoprotein for Prognostic Prediction in Patients with Hepatocellular Carcinoma: What is the Most Optimal Cutoff? *PLoS ONE*. 2015; 10(3):e0118825. doi:10.1371/journal.pone.0118825.

21. Hagel M, Miduturu C, Sheets M, Rubin N, Weng W, Stransky N, et al. First selective small molecule inhibitor of FGRF4 signaling pathway. *Cancer Discovery* 2015;5(4):424-37.
22. Kim R, Sharma S, Meyer T, Sarker D, Macarulla T, Sung M, Choo, SP, Shi H, Schmidt-Kittler O, Clifford C, Wolf B, Kang YK, Llovet J. First-in-human study of BLU-554, a potent, highly-selective FGFR4 inhibitor designed for hepatocellular carcinoma (HCC) with FGFR4 pathway activation. Poster, 28th EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics, Munich, Germany (December, 2017).
23. ClinicalTrials.gov: A service of the U.S. National Institutes of Health. Website: <https://clinicaltrials.gov/ct2/home>. Accessed on March 15, 2017.
24. Ho HK, Pok S, Streit S, et al. Fibroblast growth factor receptor 4 regulates proliferation, anti-apoptosis and alpha-fetoprotein secretion during hepatocellular carcinoma progression and represents a potential target for therapeutic intervention. *Journal of Hepatology*. 2009; 50(1): 118-127. doi:10.1016/j.jhep.2008.08.015.
25. El-Serag HB, Kanwal F. Epidemiology of hepatocellular carcinoma in the United States: Where are we? Where do we go? *Hepatology* 2014; 60(5):1767-75. doi: 10.1002/hep.27222.

CURRICULUM VITAE

