VANDERBILT-INGRAM CANCER CENTER

GDF15 MRNA EXPRESSION IN BILIARY TRACT CANCER (BTC): A MULTIOMIC ANALYSIS OF ITS PROGNOSTIC RELEVANCE AND ASSOCIATION WITH TUMOR-IMMUNE STATES IN A LARGE REAL-WORLD COHORT

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INTRODUCTION

- Growth/Differentiation Factor 15 (*GDF15*) has been implicated in cancer carcinogenesis, neoangiogenesis, and cancer cachexia
- High circulating or tissue protein expression levels of *GDF15* have been associated with worse clinical outcomes in a wide range of tumor types, correlating with platinum resistance and immune exclusion in preclinical models
- GDF15 antagonist antibodies are currently in clinical development for both cachexia and augmentation of immunotherapies
- Limited studies on *GDF15* overexpression in biliary tract cancers (BTCs)

METHODS

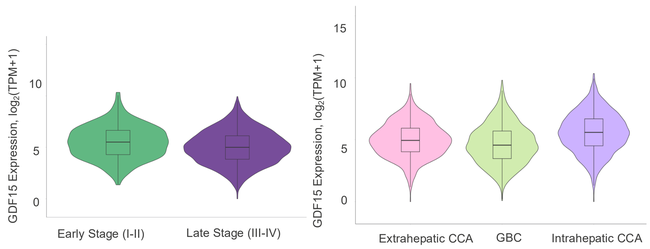
- Tempus Lens used to identify pts with primary diagnosis of BTC who had Tempus xT DNA and xR RNA testing (n=4479)
- RNA-Seq data normalized to correct for assay/batch effects, quantified as transcripts per million (TPM) and reported as $\log_2$(TPM+1)
- *GDF15* high (n=1120) vs. low (n=1120) based on top and bottom quartiles of GDF15 mRNA expression
- Immune cell proportions and cytolytic, cytotoxic, and interferon-γ immune scores estimated from RNA expression
- Samples were assessed for actionable for which an additional (FDA-approved) targeted therapy is available beyond standard all-comer BTC therapies: IDH1, BRCA 1&2, HER2/ERBB2 (Amplification [NGS] or 3+ [IHC]), BRAF (V600E only), KRAS (G12C only), MSI-H, dMMR (by IHC), and fusions for FGFR2, NTRK, ROS1, or RET
- Real-world overall survival (rwOS) defined as time from sample collection to death or loss to follow up

RESULTS

- BTC *GDF15*-Hpts had a higher median expression compared to *GDF15*-L expressors (7.09 vs 3.32)
- *GDF15*-H cases comprised a higher proportion of intrahepatic CCA primaries (61% vs. 32%) and lower proportion of gallbladder cancer (GBC) compared to *GDF15*-L BTC samples (15% vs. 35%)
- *GDF15*-H pts had a higher frequency stage I/II disease (17.7% vs. 8.4%) and lower frequency of stage IV disease compared to *GDF15*-L BTC (69% vs. 78%)
- *GDF15*-H tumors also had a higher frequency of concomitant actionable mutations eligible for FDA-approved therapies (39% vs. 17%, p=<0.001)
- The TME *GDF15*-H tumors of had significantly lower cytolytic, cytotoxic, and interferon-γ immune scores (all p<0.001) compared to *GDF15*-L tumors
- After controlling for overall stage, TP53 status, and presence of actionable mutation, there was no significant difference in OS outcomes between *GDF15*-H and *GDF15*-L BTC cohorts

GDF15 EXPRESSION

	Overall N = 4,479	GDF15		p-value
		GDF15-High N = 1120	GDF15-Low N = 1120	
RNA expression levels of GDF15				<0.001
Median (Q1, Q3)	5.20 (3.32, 7.09)	7.09 (6.67, 7.66)	3.32 (2.76, 3.71)	
Min, Max	0.00, 10.35	6.33, 10.35	0.00, 4.06	

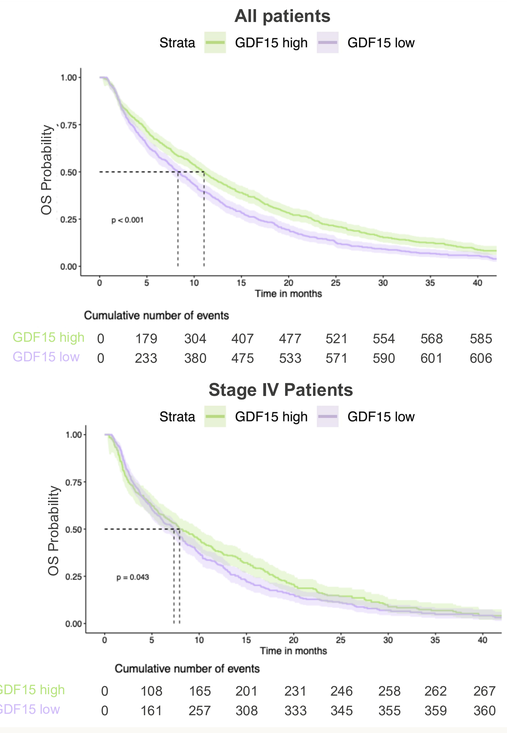


PATIENT AND TUMOR CHARACTERISTICS

	Overall N = 2240[1]	GDF15 GDF15-High N = 1120[1]	GDF15 GDF15-Low N = 1120[1]	p-value[2]
Age[3]				0.20
Median (Q1, Q3)	67 (58, 74)	67 (58, 74)	67 (59, 74)	
Sex				0.20
Female	1,272 (57%)	652 (58%)	620 (55%)	
Male	968 (43%)	468 (42%)	500 (45%)	
Race				<0.001
White	999 (77%)	549 (82%)	450 (72%)	
Black or African American	145 (11%)	56 (8.3%)	89 (14%)	
Asian	95 (7.3%)	37 (5.5%)	58 (9.3%)	
Other Race	55 (4.3%)	29 (4.3%)	26 (4.2%)	
BTC Stage3				<0.001
I	63 (4.5%)	42 (6.7%)	21 (2.7%)	
II	111 (7.9%)	67 (11%)	44 (5.7%)	
III	190 (14%)	85 (14%)	105 (14%)	
IV	1,035 (74%)	435 (69%)	600 (78%)	
Unknown	841	491	350	
Tumor Site				<0.001
Intrahepatic biliary tract	1,043 (47%)	681 (61%)	362 (32%)	
Extrahepatic duct	301 (13%)	122 (11%)	179 (16%)	
Gallbladder	552 (25%)	164 (15%)	388 (35%)	
Biliary tract (unspecified)	344 (15%)	153 (14%)	191 (17%)	
Resection Status				0.13
Non-Resected	1,732 (77%)	851 (76%)	881 (79%)	
Resected	508 (23%)	269 (24%)	239 (21%)	
Actional Mutation[4]				<0.001
Present	628 (28%)	435 (39%)	193 (17%)	
Absent	1,612 (72%)	685 (61%)	927 (83%)	

[1] n (%); [2] Pearson's Chi-squared test; [3] at time of sample collection (within 90 days); [4] Pathologic variance in IDH1, BRCA 1&2, HER2/ERBB2 (Amplification [NGS] or 3+ [IHC]), BRAF (V600E only), KRAS (G12C only), MSI-H, dMMR (by IHC), and/or fusions for FGFR2, NTRK, ROS1, or RET

OVERALL SURVIVAL



Multivariable regression of GDF15 expression and Overall Survival

Characteristic	N	Event N	HR	95% CI	p-value
GDF15 Status1					
High (Q4)	526	351	—	—	
Low (Q1)	635	449	1.09	0.93, 1.29	0.3
BTC Stage					
Early stage (I/II)	125	70	—	—	
Late stage (III/IV)	1,036	730	1.7	1.32, 2.19	**<0.001**
TP53 Status					
TP53 altered	560	400	—	—	
TP53 WT	601	400	0.84	0.72, 0.99	**0.034**
Actionable Mutation					
Absent	862	606	—	—	
Present	299	194	0.79	0.67, 0.93	**0.005**

[1]Multivariate regression controlling for stage, presence of actionable molecular feature, and TP53 status

SOMATIC ALTERATIONS

	Overall N = 2240	GDF15-High N = 1120	GDF15-Low N = 1120	p-value
Actionable1				
FGFR2 (Fusion)	209 (9.3%)	177 (15.8%)	32 (2.9%)	<0.001
IDH1	219 (9.8%)	171 (15%)	48 (4.3%)	<0.001
ERBB2 (Amp)	84 (3.8%)	30 (2.7%)	54 (4.8%)	0.008
BRAF V600E	29 (1.3%)	15 (1.3%)	14 (1.3%)	0.9
KRAS G12C	23 (1.0%)	12 (1.1%)	11 (1.0%)	0.8
BRCA 1	6 (0.7%)	7 (0.6%)	9 (0.8%)	0.6
BRCA 2	42 (1.9%)	23 (2.1%)	19 (1.7%)	0.5
MSI-High	33 (1.5%)	21 (1.9%)	12 (1.1%	0.10
Deficient MMR	17 (2.1%)	13 (3.1%)	4 (1.0%)	0.046
NTRK (Fusion)	3	2	1	?
ROS1 (Fusion)	2	0	2	?
Other				
TP53	1,024 (46%)	256 (23%)	768 (69%)	<0.001
KRAS	414 (18%)	151 (13%)	263 (23%)	<0.001
BAP1	206 (9.2%)	157 (14%)	49 (4.4%)	<0.001
CDKN2A	517 (23%)	270 (24%)	247 (22%)	0.2
CDKN2B	377 (17%)	234 (21%)	143 (13%)	<0.001
ARID1A	342 (15%)	214 (19%)	128 (11%)	<0.001
FGFR2 (mut)	56 (2.5%)	45 (4.0%)	11 (1.0%)	<0.001
IDH2	57 (2.5%)	44 (3.9%)	13 (1.2%)	<0.001

TUMOR IMMUNE MICROENVIRONMENT

	Median % (Q1, Q3) Overall N = 2240	GDF15-High N = 1120	GDF15-Low N = 1120	p-value[1]
Cell types				
B cells	4.08 (3.17, 5.50)	3.87 (3.09, 4.92)	4.40 (3.26, 6.49)	<0.001
CD4 T cells[2]	0.00 (0.00, 41.49)	0.00 (0.00, 37.16)	0.00 (0.00, 41.49)	<0.001
CD8 T cells	0.21 (0.00, 1.03)	0.01 (0.00, 0.54)	0.54 (0.00, 1.54)	<0.001
Treg cells	3.39 (2.37, 4.93)	3.17 (2.24, 4.47)	3.70 (2.50, 5.31)	<0.001
NK cells	2.63 (2.05, 3.32)	2.76 (2.19, 3.40)	2.51 (1.93, 3.20)	<0.001
M1 macrophages	7.15 (5.22, 9.94)	7.66 (5.72, 10.35)	6.69 (4.70, 9.56)	<0.001
M2 macrophages	4.00 (2.67, 5.62)	3.57 (2.39, 4.87)	4.51 (3.00, 6.28)	<0.001
Monocytes[2]	0.00 (0.00, 20.43)	0.00 (0.00, 12.15)	0.00 (0.00, 20.43)	<0.001
Neutrophils	6.69 (5.09, 8.95)	6.53 (5.04, 8.64)	6.94 (5.12, 9.31)	0.009
Dendritic Cells[2]	0.00 (0.00, 17.19)	0.00 (0.00, 0.42)	0.00 (0.00, 17.19)	<0.001
Other Cells	68.74 (61.76, 73.79)	70.29 (64.61, 74.58)	66.67 (58.40, 72.65)	<0.001
Immune Scores				
Cytolytic Score	3.50 (2.85, 4.25)	3.30 (2.68, 3.93)	3.78 (3.07, 4.51)	<0.001
Cytotoxic Score	3.68 (3.22, 4.25)	3.49 (3.09, 3.95)	3.96 (3.43, 4.48)	<0.001
IFNgamma Score	3.52 (2.96, 4.19)	3.29 (2.77, 3.84)	3.83 (3.18, 4.50)	<0.001

[1] Wilcoxon rank sum test; [2] Median% (Min, Max)

Univariate regression of Tempus IO Scores and Overall Survival in Biliary Tract Cancers

Characteristic	N	Event N	HR	95% CI	p-value[2]
Cytolytic Score					
Low (Q1)	925	652	—	—	
High (Q4)	889	561	0.76	0.68, 0.85	**<0.001**
Cytotoxic Score					
Low (Q1)	913	652	—	—	
High (Q4)	891	544	0.73	0.65, 0.82	**<0.001**
INFgamma Score					
Low (Q1)	927	672	—	—	
High (Q4)	874	530	0.68	0.61, 0.77	**<0.001**

CONCLUSIONS

- In this large, real-world, analysis of biliary tract cancers, tumor *GDF15* mRNA levels did not significantly correlate with rwOS
- BTC pts with *GDF15*-H mRNA expression had less immunologically active tumors compared to *GDF15*-L tumors
- These findings diverge from previously published literature on *GDF15* as a prognostic biomarker/therapeutic target when it is assessed based on local or circulating protein level
- Given our findings and the fundamental complexities of *GDF15*, including extensive post-translational processing and source of production, future studies are needed to reconcile our understanding of the functional and prognostic role of *GDF15* in BTC

ACKNOWLEDGMENTS

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References: *Nishioka et al., 2025 Thoracic Cancer; Sugimoto et al 2022 Cancer Cell Int; Wang et al 2023 Cancer Letters; Yan et al 2021 JBUON; Zhang et al. 2023 Oncol Rep.*