Comprehensive molecular and immunological characterization of CLDN18.2 in pancreatic cancer

Midhun Malla[1], Sachin Kumar Deshmukh[2], Timothy Samec[3], Sharon Wu[4], Joanne Xiu[4], Mehmet Akce[1], Garima Gupta[1], Qasim Hussaini[1], Darryl Outlaw[1], Rebecca Arrend[1], Aakash Desai[1], Arnab Basu[1], Purnachandra N. Ganji[1], Mark Evans[5], Heinz-Joseph Lenz[6], Emil Lou[7], Dani Castillo[5], Rachna Schroff[8], David Spetzler[5], Bassel El-Rayes[1]

[1]University of Alabama at Birmingham, Birmingham, AL,[2]Caris Life sciences, phoenix, AZ,[3]Caris Life sciences, Phoenix, AZ,[4]Caris Life Sciences, Phoenix, AZ,[5]Caris Life Sciences, phoenix, AZ,[6]University of South California, California, CA,

[7]University of Minnesotta, Minnesotta, MN,[8]University of Arizona, Tucson, AZ

BACKGROUND

- Claudin 18 (CLDN18) is an emerging biomarker and transmembrane protein that maintains a tight junction between cells in pancreatic cancer (PC).
- Due to the high expression of CLDN18.2, an isoform of CLDN18, genomic profiling has demonstrated CLDN18.2 as a potential target with prognostic impact.
- We aimed to characterize the molecular and immunological features associated with CLDN18.2 expression in PC.

METHODS

- 9,837 PC samples were tested by next-generation sequencing (592, NextSeq; WES, NovaSeq), Whole Transcriptome Sequencing (WTS; NovaSeq) (Caris Life Sciences, Phoenix, AZ).
- Tumor mutational burden (TMB) totaled somatic mutations per tumor (high>10 mt/MB).
- PD-L1 expression was assessed using immunohistochemistry
- Immune cell fractions were calculated by deconvolution of WTS: Quantiseq.
- Tumors with CLDN18.2-high(H) and CLDN18.2-low(L) RNA expression were classified by top and bottom quartiles, respectively.
- Real world overall survival (OS) was obtained from insurance claims and calculated from tissue collection to last contact using Kaplan-Meier estimates. Statistical significance was determined by chi-square and Mann-Whitney U test with p-values adjusted for multiple comparisons (q<0.05).

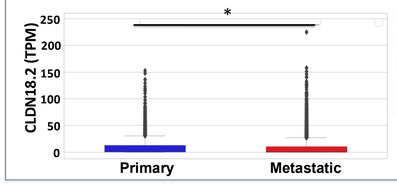
Table 1: Sample demographic information

		Primary		Metastatic	
		CLDN18.2 Low	CLDN18.2 High	CLDN18.2 Low	CLDN18.2 High
Counts (N)		540	1026	1704	1218
Age [Range]		66.5 [21 - >89]	68.5 [34 - >89]	66.5 [13 - >89]	67.5 [29 - >89]
Sex	Male	52.04% (281/540)	46.69% (479/1026)	58.33% (994/1704)	51.81% (631/1218)
	Female	47.96% (259/540)	53.31% (547/1026)	41.67% (710/1704)	48.19% (587/1218)
Race	White	75.11% (327/441)	75.26% (642/853)	75.43% (1013/1343)	74.7% (756/1012)
	Black/AA	14.85% (62/441)	11.02% (94/853)	12.12% (62/1343)	14.03% (142/1012)
	Asian/PI	5.1% (31/441)	6.21% (53/853)	4.47% (31/1343)	4.74% (48/1012)
	Other	4.93% (21/441)	7.5% (64/853)	4.99% (21/1343)	6.52% (66/1012)
Ethnicity	Not Hispanic or Latino	87.27% (384/440)	87.03% (718/825)	89.24% (1170/1311)	88.58% (869/981)
	Hispanic or Latino	12.73% (56/440)	12.97% (107/825)	10.76% (141/1311)	11.42% (112/981)

Race/ethnicity data is self-reported

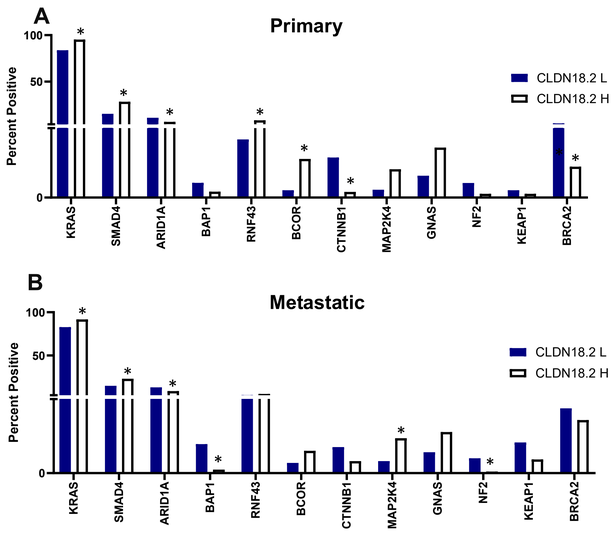
RESULTS

Figure 1. CLDN18.2 expression in primary and metastatic PC



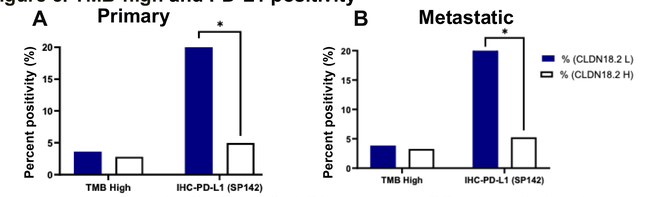
Primary PC had a higher expression of CLDN18.2 (3.45 TPM vs 1.15 TPM) compared to metastatic PC. * q<0.05.

Figure 2. Mutation analysis CLDN18.2 high vs low PC



Primary and metastatic CLDN18.2 H PC had higher frequency of *KRAS* (95.3% vs 83.8%, 91.7% vs 82.8%), *SMAD4* (28.4% vs 15.3%, 23.1% vs 14.9%), but lower frequency of *ARID1A* (6.6% vs 11.1%, 8.9% vs 13.2%) compared to *CLDN18.2* L PC. Primary CLDN18.2 H PC had higher frequency of *RNF43* (8.3% vs 3.4%), *BCOR* (2.2% vs 0.4%), but lower frequency of *CTNNB1* (0.3% vs 2.3%), and *BRCA2* (5.1% vs 1.8%). Metastatic CLDN18.2 H PC had higher frequency of *MAP2K4* (1.8% vs 0.6%), but lower frequency of *BAP1* (0.2 vs 1.5%) and *NF2* (0.1 vs 0.8%) compared to CLDN18.2 L patients. *q<0.05

Figure 3. TMB-high and PD-L1 positivity



Primary and metastatic CLDN18.2 H PC had lower PD-L1 positivity (4.1% vs 27.9%, 5.2% vs 29.8%). There was no difference in TMB high. * q<0.05.

Figure 4. Immune cell infiltration

A. Primary

	CLDN18.2 L	CLDN18.2 H
B cell	3.81	5.1 *
Mφ M1	5.09	5.37
Mφ M2	3.25	3.6 *
Neutrophil	6.19	6.27
NK cell	2.46	2.76 *
CD8+ T cell	0.17	0
Tregs	2.26	2.25

B. Metastatic

	CLDN18.2 L	CLDN18.2 H
B cell	3.31	4.44 *
Mφ M1	5.21	5.07
Mφ M2	3.16	3.72 *
Neutrophil	5.61	5.6 *
NK cell	2.31	2.55
Tregs	1.51	1.44
DC	0.04	0.46

Low Median% High Median%

A. CLDN18.2-H primary and metastatic tumors had higher infiltration of B cells (5.1% vs 3.8%, 4.4% vs 3.3%), M2 Mφ (3.6% vs 3.3%, 3.7% vs 3.1%), and NK cells (2.8% vs 2.5%, 2.5% vs 2.3%). For monocyte and CD4+ T cells median was 0 in both groups, DC median was 0 in primary and CD8+ T cell median was 0 in metastatic PC. * q<0.05.

Figure 5. Immune gene expression

A. Primary

	CLDN18.2 L	CLDN18.2 H
CD274	4.05	3.65 *
PDCD1	0.53	0.49
CTLA4	1.43	1.7 *
LAG3	1.94	1.7 *
HAVCR2	14.51	15.97 *
IDO1	1.98	1.58 *
FOXP3	1.66	1.97 *
CD47	44.31	64.53 *
BTLA	1.05	1.51 *
TIGIT	0.79	0.94 *
CD160	1.76	2.26 *
CD86	6	7.32
CD80	3.57	3.98
CD276	24.75	23.29 *

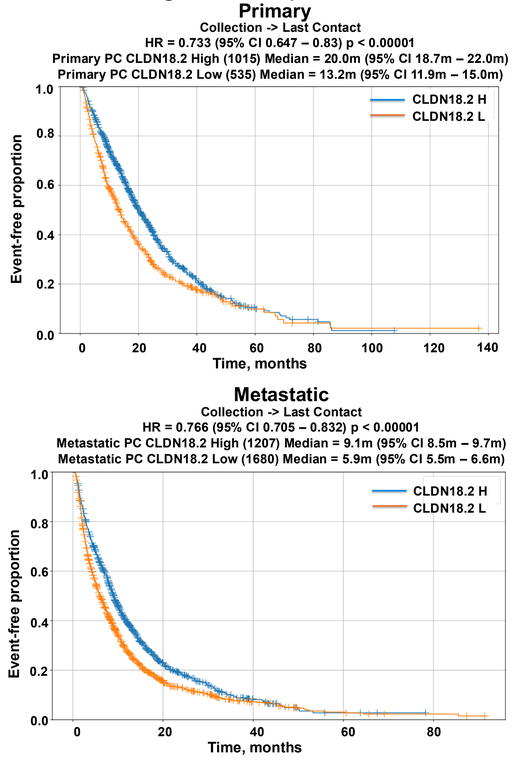
B. Metastatic

	CLDN18.2 L	CLDN18.2 H
CD274	4.69	3.6 *
PDCD1	0.44	0.31
CTLA4	1.08	1.17 *
LAG3	2.07	1.78 *
HAVCR2	18.38	19.84 *
IDO1	1.3	1.13 *
FOXP3	1.66	1.79 *
CD47	62.09	79.19 *
BTLA	0.75	0.97 *
TIGIT	0.66	0.72 *
CD160	1.84	2.05 *
CD86	7.8	8.09
CD80	4.87	4.68
CD276	26.2	23.25 *

Low TPM (Median) High TPM (Median)

CLDN18.2-H primary and metastatic PC had differential expression of immune checkpoint genes (upregulation: *CTLA4, HAVCR2, FOXP3, CD47, BTLA, TIGIT, CD160*; fold change (FC): 1.1 - 1.46; downregulation: *CD274, LAG3, IDO1, CD276*, FC: 1.1 - 1.3). * q<0.05.

Figure 6. CLDN18.2 high vs low PC patients' survival



CLDN18.2-H was associated with better OS compared to CLDN18.2-L in primary (mOS: 20 vs 13.2 months; HR: 0.73, 95% CI 0.64 - 0.83, p <0.00001) and metastatic (mOS: 9.1 vs 5.9 months; HR: 0.76, 95% CI 0.7 - 0.83, p <0.00001) PC.

CONCLUSIONS

CLDN18.2 expression is associated with distinct genomic alterations, a differentially modulated immune microenvironment and PC patients' survival. Prospective clinical trials using novel approaches to target CLDN18.2 in PC are underway. Further studies are required to study the impact of CLDN18.2 expression and targeted strategies on survival outcomes of patients with PC.