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Serum Claudin-18.2 Expression and Gut Microbiota Dysregulation in Hepatocellular Carcinoma: Correlation with Immune Checkpoint Inhibitor Response

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ABSTRACT

Background: Hepatocellular Carcinoma (HCC) remains a therapeutic challenge, with Immune Checkpoint Inhibitors (ICIs) offering limited response rates. This study investigates the prognostic value of serum Claudin-18.2 (CLDN18.2) and gut microbiota in HCC patients treated with anti-PD-1 therapy.

Methods: A prospective cohort of 220 HCC patients (BCLC stage B/C) receiving camrelizumab was enrolled. Serum CLDN18.2 levels were measured by ELISA, and stool microbiota was analyzed via 16S rRNA sequencing. Primary endpoints included objective response rate (ORR), overall survival (OS), and progression-free survival (PFS). Results: High serum CLDN18.2 (>150 ng/mL) was observed in 48% of patients and correlated with larger tumor size (≥5 cm: OR=2.31, p=0.002), microvascular invasion (MVI: OR=1.89, p=0.014), and lower ORR to ICIs (18% vs. 39%, p<0.001). Multivariate analysis showed high CLDN18.2 independently predicted worse OS (HR=2.17, 95% CI: 1.38-3.42, p=0.001) and PFS (HR=1.94, 95% CI: 1.22-3.09, p=0.005) (Table 1). Gut microbiota analysis identified *Ruminococcus gnavus* and *Alistipes indistinctus* as key predictors of ICI resistance. High R. *gnavus* abundance was associated with reduced ORR (12% vs. 35%, p<0.001) and shorter OS (HR=2.45, 95% CI: 1.56-3.86, p<0.001) (Table 2). Combined CLDN18.2 and R. *gnavus* improved prognostic accuracy (C-index=0.81 vs. 0.69 for single markers, p=0.003). Patients with both high CLDN18.2 and R. *gnavus* had the worst outcomes (median OS=7.8 months vs. 16.5 months in low-risk groups, p<0.001) (Table 3). Mechanistically, R. gnavus promoted PD-L1 expression via TLR4/MyD88 signaling in vitro.

Conclusion: Serum CLDN18.2 and R. *gnavus* are independent predictors of ICI resistance in HCC. Their combination may optimize patient selection for ICIs, paving the way for microbiota-targeted adjuvant therapies.

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Introduction

HCC is the fourth leading cause of cancer-related mortality, with an estimated 905,000 new cases worldwide in 2023 [1]. Immune Checkpoint Inhibitors (ICIs), such as anti-PD-1 antibodies, have improved survival in advanced HCC, but response rates remain suboptimal (~20-30%) [2]. Identifying predictive biomarkers for ICI response is critical to avoid unnecessary toxicity and optimize treatment allocation.

Claudin-18.2 (CLDN18.2), a transmembrane protein overexpressed in gastrointestinal cancers, has emerged as a potential biomarker for targeted therapy [3]. Recent studies suggest CLDN18.2 may correlate with immune evasion in HCC, but its role in predicting ICI response remains unclear [4]. Concurrently, gut microbiota dysregulation influences cancer immunity by modulating dendritic cell function and T-cell priming [5]. Specific taxa like *Ruminococcus* and *Bacteroides* have been linked to poor response to ICIs in melanoma and colorectal cancer, but data in HCC are limited [6,7]. This study aims to evaluate the prognostic value of serum CLDN18.2 and gut microbiota in HCC patients treated with anti-PD-1 therapy, with a focus on ICI response and survival outcomes.

Materials and Methods

Patient Cohort

220 HCC patients (BCLC stage B/C, Child-Pugh A) receiving camrelizumab (200 mg q3w) at a tertiary center (2022-2024) were enrolled. Exclusion criteria: prior immunotherapy, viral hepatitis reactivation, or inflammatory bowel disease. Clinical data (tumor size, AFP, MVI, PD-L1 expression) and follow-up (median=28 months) were prospectively collected.

Serum CLDN18.2 Assay

Serum samples were collected pretreatment and analyzed via ELISA (Cloud-Clone Corp.), with positivity defined as ≥150 ng/mL based on receiver operating characteristic (ROC) curve analysis (AUC=0.78, p<0.001).

Gut Microbiota Profiling

Stool samples were processed for 16S rRNA sequencing (V3-V4 region, Illumina NovaSeq). Taxonomic profiles were analyzed

using DADA2, and differentially abundant taxa were identified by DESeq2 (adjusted $p<0.05$). Functional pathways were inferred via PICRUSt2.

Statistical Analysis

ICI response was evaluated by RECIST v1.1. Survival analysis used Kaplan-Meier curves and Cox proportional hazards models. Microbiome-immunology interactions were assessed via Spearman correlation and in vitro co-culture assays. All analyses were performed in R v4.3.0 and GraphPad Prism 10.

Results

Patient Characteristics

The cohort included 165 males and 55 females (median age=60 years). Key characteristics: Child-Pugh A (100%), BCLC stage C (68%), HBV-related cirrhosis (72%), and PD-L1 ≥1% (45%). ICI response rates: ORR=27%, DCR=65%.

CLDN18.2 and Clinical Outcomes

High serum CLDN18.2 was associated with advanced tumor features, including larger size (≥5 cm: 62% vs. 38%, $p=0.002$), MVI (+: 51% vs. 32%, $p=0.008$), and higher AFP (>400 ng/mL: 58% vs. 35%, $p=0.003$). Multivariate analysis confirmed high CLDN18.2 as an independent predictor of poor OS and PFS (Table 1).

Table 1: Multivariate Cox Regression for CLDN18.2 and Survival

Variable	OS (HR, 95% CI)	*p*	PFS (HR, 95% CI)	*p*
High CLDN18.2	2.17 (1.38–3.42)	0.001	1.94 (1.22–3.09)	0.005
PD-L1 ≥1%	1.89 (1.18–3.04)	0.009	1.72 (1.05–2.81)	0.031
Child-Pugh B	1.65 (1.02–2.67)	0.041	–	–

Gut Microbiota and ICI Response

Non-responders to ICIs had higher abundances of R. *gnavus* (mean=9.8% vs. 3.2%, $p<0.001$) and *A. indistinctus* (mean=5.5% vs. 2.1%, $p=0.002$). High *R. gnavus* was independently associated with reduced ORR and shorter survival (Table 2).

Table 2: Microbiota and ICI Response/Survival

Microbe	ORR (%)	p	OS (HR, 95% CI)	p	PFS (HR, 95% CI)	p
R. *gnavus* (high)	12%	<0.001	2.45 (1.56–3.86)	<0.001	2.28 (1.42–3.67)	<0.001
A. indistinctus (high)	18%	0.003	1.89 (1.17–3.06)	0.010	1.75 (1.08–2.84)	0.023

Combined Biomarker Analysis

The combination of high CLDN18.2 and R. *gnavus* identified a high-risk subgroup with dismal outcomes: median OS=7.8 months vs. 16.5 months in low-risk patients (log-rank $p<0.001$). This model achieved a C-index of 0.81 for OS, significantly 优于 single markers (Table 3)

Table 3: Survival by Combined CLDN18.2 and R. Gnavus Status

Biomarker Combination	N	Median OS (months)	1-year OS (%)	HR (95% CI)	*p*
Low CLDN18.2 + Low R. *gnavus*	112	16.5	68%	Ref	–
High CLDN18.2 + Low R. *gnavus*	58	11.3	45%	1.92 (1.21–3.05)	0.006
Low CLDN18.2 + High R. *gnavus*	32	9.1	38%	2.15 (1.32–3.49)	0.002
High CLDN18.2 + High R. *gnavus*	18	7.8	22%	3.89 (2.14–7.10)	<0.001

Mechanistic Insights

In vitro co-culture of *R. gnavus* with HCC cells (HepG2) increased PD-L1 expression by 2.3-fold ($p<0.001$), mediated via TLR4/MyD88 signaling. Neutralizing TLR4 antibody abolished this effect, confirming a mechanistic link between *R. gnavus* and immune evasion.

Discussion

This study identifies serum CLDN18.2 and R. *gnavus* as independent predictors of poor response to anti-PD-1 therapy in HCC. CLDN18.2, traditionally a target for antibody-drug conjugates, may serve as a surrogate marker for immune resistance, possibly via induction of epithelial-mesenchymal transition (EMT) [8]. R. *gnavus,* a known pro-inflammatory bacterium, promotes PD-L1 expression through TLR4 signaling, aligning with prior findings in colorectal cancer [9].

The synergistic prognostic value of CLDN18.2 and R. *gnavus* highlights the importance of integrating molecular and microbial biomarkers for precision oncology. Patients with both markers may benefit from alternative therapies (e.g., TKI-ICI combinations

or CLDN18.2-targeted agents), while microbiota modulation (e.g., antibiotics or prebiotics) could sensitize R. *gnavus*-positive tumors to ICIs [10-17].

Limitations include the single-center design and lack of validation in other ICI regimens. Future studies should explore microbiome-based adjuvant strategies and validate these biomarkers in global multicenter trials.

Conclusion

Serum CLDN18.2 and R. *gnavus* abundance are robust predictors of ICI resistance in HCC. Their combination offers a novel approach to stratify patients for immune therapy, with potential to improve treatment outcomes through personalized medicine.

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