

Clinical Relevance of Serum Full-Length Glypican-3 in Unresectable Hepatocellular Carcinoma Treated with Atezolizumab and Bevacizumab

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Abstract

Biomarkers that can predict patient survival and the therapeutic response to atezolizumab and bevacizumab (Atezo/Bev) as first-line chemotherapy for unresectable hepatocellular carcinoma (HCC) have yet to be identified. We aimed to determine whether the serum full-length glypican-3 (FL-GPC3) concentration can be used as such a biomarker. We included 102 patients treated with Atezo/Bev therapy for unresectable HCC at our institute during 2020–2025. We investigated the association between baseline serum FL-GPC3 concentrations and the therapeutic effectiveness and survival outcomes. The baseline serum FL-GPC3 concentration was lower in patients in whom disease control was achieved (10.8 vs. 26.7 pg/mL, $p = 0.002$). An FL-GPC3 concentration ≥ 21.7 pg/mL was an independent negative predictor of disease control (odds ratio, 0.382; 95% confidence interval [CI], 0.151–0.970; $p = 0.043$). The FL-GPC3-high group had a shorter median progression-free survival (PFS; 3.0 vs. 5.6 months, $p = 0.031$) and overall survival (OS; 13.7 vs. 25.8 months, $p = 0.006$) than the FL-GPC3-low group. A high FL-GPC3 concentration was an independent negative prognostic factor for PFS (hazard ratio [HR], 1.592; 95% CI, 1.041–2.435; $p = 0.032$) and OS (HR, 1.807; 95% CI, 1.084–3.012; $p = 0.023$). In conclusion, the serum FL-GPC3 concentration may serve as a noninvasive prognostic biomarker in patients with HCC treated with immunotherapy.

Introduction

Liver cancer was the sixth most common cancer and the third leading cause of cancer-related deaths worldwide in 2022^[1, 2]. Hepatocellular carcinoma (HCC) accounts for approximately 90% of liver cancers and is the most common histological subtype^[3]. Recently, considerable advancements have been made in systemic therapy for HCC, particularly with the introduction of immunotherapy^[4–7]. Patients with intermediate- or advanced-stage HCC are likely to benefit from systemic therapies^[8]. Given the numerous clinical trials in which drug therapies are combined with other locoregional treatments, the role of systemic therapy in HCC management is expected to grow.

Atezolizumab and bevacizumab (Atezo/Bev) therapy is considered one of the most promising systemic therapies for unresectable HCC. This therapy, the first immune-checkpoint inhibitor (ICI)-based regimen for HCC, comprises a combination of a programmed cell death-ligand 1 inhibitor and an anti-vascular endothelial growth factor antibody. The IMbrave150 trial showed that Atezo/Bev significantly improves overall survival (OS) and progression-free survival (PFS) compared with sorafenib^[4, 5]. Atezo/Bev is one of the first-line systemic therapy regimens of choice for HCC, alongside other ICI-based regimens: tremelimumab plus durvalumab [6] and nivolumab plus ipilimumab^[9]. However, the confirmed objective response rate (ORR) was only 27.3% and the disease control rate (DCR) was 73.6% with Atezo/Bev therapy, according to an independent assessment following the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1^[4]. Thus, the identification of biomarkers predictive of progressive disease (PD) would be of great benefit.

Serum tumor markers, such as alpha-fetoprotein (AFP), des-gamma-carboxy prothrombin (DCP), and AFP bound to *Lens culinaris* agglutinin (AFP-L3), are commonly used in some countries for HCC diagnosis and treatment^[10]. These markers have also been investigated as predictive biomarkers for patients treated with Atezo/Bev. Serum concentrations of AFP, AFP-L3, and DCP, not only individually but also in combination, are reportedly related to the therapeutic response of HCC to or survival of patients upon Atezo/Bev therapy^[11–14]. Dynamic changes in these markers may therefore be associated with the clinical outcome of Atezo/Bev therapy. However, their predictive performance and threshold values vary considerably across reports^[11]. Therefore, a more universal predictive biomarker of the response to Atezo/Bev therapy needs to be identified.

Glypican-3 (GPC3), a cell surface proteoglycan, enhances the proliferation of HCC cells, epithelial-to-mesenchymal transition, and angiogenesis^[15, 16]. Its re-expression due to malignant hepatocyte transformation is known to be correlated with a poor prognosis in patients with HCC^[16–18]. Previous reports have confirmed the validity of GPC3 immunohistochemistry (IHC) for the diagnosis of HCC^[16]. Circulating GPC3 has also been investigated as a tumor marker of HCC^[16]. A system to quantify full-length (FL) GPC3 was recently established^[19]. Its developers reported that the plasma FL-GPC3 concentration may be a predictive marker of tumoral GPC3 expression in patients with hepatitis C virus-related HCC^[20], and that the preoperative plasma FL-GPC3 level is a potential predictive marker of HCC recurrence in patients scheduled to undergo radical surgery^[19]. However, whether circulating FL-GPC3 levels are predictive of the effectiveness or prognosis associated with systemic chemotherapy for HCC is unclear.

Therefore, we conducted a study on circulating FL-GPC3 as a potential prognostic biomarker for the response to Atezo/Bev therapy. Its aim was to evaluate the ability of the serum FL-GPC3 concentration to predict the therapeutic effectiveness of this therapy and the prognosis of patients treated for unresectable HCC.

Results

Patient characteristics

Table 1 describes the baseline characteristics of the study sample (N = 102). Briefly, their median age was 72 years, and males (n = 83, 81.4%) comprised the majority of the sample. The etiologies of liver disease were hepatitis B virus (n = 18, 17.6%), hepatitis C virus (n = 34, 33.3%), alcohol (n = 24, 23.5%) and other causes (n = 26, 25.5%). Regarding liver functional reserve, 95 patients (93.1%) were classified as Child–Pugh grade A, and the median ALBI score was – 2.48. The median AFP, AFP-L3, and DCP levels were 55.1 ng/mL, 11.4%, and 332.0 mAU/mL, respectively. The majority of patients had Barcelona Clinic Liver Cancer^[21] stage C HCC (n = 53, 52.0%). While most patients had undergone some form of therapy for HCC, 21 (20.6%) did not.

Table 1
Characteristics of patients at baseline.

Characteristics	Particulars/Units	Overall (N = 102)
Age	Years	72 (66–80)
Sex	Male	83 (81.4)
Etiology	HBV/HCV/Alcohol/Others	18 (17.6)/34 (33.3)/24 (23.5)/26 (25.5)
ECOG PS	0–1	96 (94.1)
1st-line therapy	Yes	80 (78.4)
Child–Pugh grade	A/B	95 (93.1)/7 (6.9)
ALBI score		-2.48 (-2.86 to -2.26)
mALBI grade	1/2a/2b/3	45 (44.1)/30 (29.4)/25 (24.5)/2 (2.0)
NLR		3.2 (2.3–4.7)
Bilirubin	mg/dL	0.9 (0.6–1.2)
Albumin	g/dL	3.9 (3.5–4.2)
PT	%	87 (79–93)
AST	U/L	40 (29–54)
ALT	U/L	26 (18–39)
AFP	ng/mL	55.1 (7.0–475.7)
AFP-L3	%	11.4 (2.4–46.3)
DCP	mAU/mL	332.0 (78.3–5100.0)
FL-GPC3	pg/mL	15.7 (6.6–48.3)
Maximum tumor diameter	mm	35 (20–65)
Number of tumors	At least four	61 (59.8)
BCLC stage	A/B/C	8/41/53
Data are shown as medians (quartiles 1–3) or frequencies (%). Abbreviations: AFP, alpha-fetoprotein; AFP-L3, alpha-fetoprotein bound to <i>Lens culinaris</i> agglutinin; ALBI, albumin–bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; DCP, des-gamma-carboxy prothrombin; ECOG PS, Eastern Cooperative Oncology Group performance status; FL-GPC3, full-length glypican-3; HAIC, hepatic arterial infusion chemotherapy; HBV, hepatitis B virus; HCV, hepatitis C virus; mALBI, modified albumin–bilirubin; NLR, neutrophil–lymphocyte ratio; PEI, percutaneous ethanol injection; PT, prothrombin time; RFA, radiofrequency ablation; TACE, transarterial chemoembolization.		

Characteristics	Particulars/Units	Overall (N = 102)
Up-to-seven criteria	Out	69 (67.6)
Previous therapy	RFA/PEI	32 (31.4)
	Resection	33 (32.4)
	Radiation	9 (8.8)
	TACE/HAIC	76 (74.5)
	Chemotherapy	21 (20.6)
	None	21 (20.6)

Data are shown as medians (quartiles 1–3) or frequencies (%). Abbreviations: AFP, alpha-fetoprotein; AFP-L3, alpha-fetoprotein bound to *Lens culinaris* agglutinin; ALBI, albumin–bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; DCP, des-gamma-carboxy prothrombin; ECOG PS, Eastern Cooperative Oncology Group performance status; FL-GPC3, full-length glypican-3; HAIC, hepatic arterial infusion chemotherapy; HBV, hepatitis B virus; HCV, hepatitis C virus; mALBI, modified albumin–bilirubin; NLR, neutrophil–lymphocyte ratio; PEI, percutaneous ethanol injection; PT, prothrombin time; RFA, radiofrequency ablation; TACE, transarterial chemoembolization.

The median serum concentration of FL-GPC3 was 15.7 pg/mL. We evaluated the correlation between serum FL-GPC3 levels and levels of commonly used tumor markers for HCC (Fig. 1). Serum FL-GPC3 levels did not significantly correlate with AFP-L3 or DCP levels but did correlate with AFP levels ($r = 0.249$, $p = 0.012$). To confirm GPC3 expression in tissues, we analyzed formalin-fixed HCC tissues of 10 patients who had undergone surgical resection in the 4 years prior to Atezo/Bev therapy. We confirmed GPC3 expression in tumor cells of nine of them (Supplementary Fig. S1 and Supplementary Table S1).

Efficacy and prognosis of patients treated with Atezo/Bev for HCC

The best radiological responses were as follows: one patient (1.0%) had a CR, 26 (25.5%) had a PR, 40 had SD (39.2%), and 35 (34.3%) had PD. The ORR and DCR were 26.5% and 65.7%, respectively (Supplementary Table S2). The median PFS was 4.1 months (95% confidence interval [CI], 3.4–5.5), and the median OS was 21.2 months (95% CI, 15.8–26.7; Supplementary Fig. S2).

Relationship between baseline concentration of FL-GPC3 and effectiveness of Atezo/Bev

To evaluate the usefulness of the baseline FL-GPC3 concentration in predicting the therapeutic response to Atezo/Bev, we examined the baseline FL-GPC3 concentrations in patients for whom an objective response was achieved or disease control was the best response. The baseline median serum concentration of FL-GPC3 was lower, albeit not significantly, in patients in whom an objective response was achieved (yes vs. no = 11.9 vs. 18.7 pg/mL, $p = 0.059$; Supplementary Table S3 and Fig. S3). Additionally, the baseline median serum concentration of FL-GPC3 was lower in patients in whom

disease control was achieved (yes vs. no = 10.8 vs. 26.7 pg/mL, $p = 0.002$; Supplementary Table S4 and Fig. S3).

We also examined the ability of serum FL-GPC3 concentrations to predict disease control by using an ROC curve. The value that maximized the sum of sensitivity and specificity was determined using the Youden index. Disease control could be predicted with 70.1% sensitivity and 60.0% specificity (area under the ROC curve: 0.688; 95% CI: 0.580–0.795) when the cutoff value was set to 21.7 pg/mL (Fig. 2). Bootstrap internal validation showed minimal optimism; the optimism-corrected area under the curve was also 0.688, indicating robust discriminative performance without substantial overfitting (Supplementary Table S5). Multivariable analyses in which we used this cutoff value revealed that the serum FL-GPC3 concentration (odds ratio, 0.382; $p = 0.043$), rather than the AFP concentration, is independently associated with disease control via Atezo/Bev treatment (Table 2).

Table 2

Univariate and multivariable logistic regression analysis for prediction of disease control via Atezo/Bev therapy.

Variables		Univariate analysis			Multivariable analysis		
		OR	95% CI	p-value	OR	95% CI	p-value
Age	≥ 75 years	1.370	0.594–3.170	0.459	-	-	-
Sex	Male	0.631	0.207–1.920	0.418	-	-	-
Etiology	Viral	2.360	1.020–5.460	0.046	2.130	0.854–5.330	0.105
BCLC stage	C	0.817	0.360–1.850	0.630	-	-	-
1st-line therapy	Yes	1.440	0.545–3.790	0.463	-	-	-
mALBI grade	2b or 3	0.291	0.116–0.727	0.008	0.359	0.135–0.954	0.040
NLR	≥ 3.03	0.686	0.301–1.560	0.369	-	-	-
AFP	≥ 100 ng/mL	0.671	0.294–1.530	0.344	-	-	-
AFP-L3	≥ 10%	0.728	0.320–1.660	0.450	-	-	-
DCP	≥ 186 mAU/mL	0.366	0.152–0.878	0.024	0.521	0.200–1.360	0.182
FL-GPC3	≥ 21.7 pg/mL	0.304	0.130–0.713	0.006	0.382	0.151–0.970	0.043

Abbreviations: AFP, alpha-fetoprotein; AFP-L3, alpha-fetoprotein bound to *Lens culinaris* agglutinin; Atezo/Bev, atezolizumab and bevacizumab; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; DCP, des-gamma-carboxy prothrombin; FL-GPC3, full-length glypican-3; mALBI, modified albumin–bilirubin; NLR, neutrophil–lymphocyte ratio; OR, odds ratio.

Relationship between baseline concentration of FL-GPC3 and PFS

Next, we analyzed the relationship between the baseline FL-GPC3 concentration and the prognosis of patients treated with Atezo/Bev. We divided all patients into two groups according to a cutoff serum FL-GPC3 concentration of 21.7 pg/mL. The patient characteristics of each group are shown in Supplementary Table S6. The Kaplan–Meier curve showed that PFS was shorter in the FL-GPC3-high group (3.0 vs. 5.6 months, $p = 0.031$; Fig. 3a). Multivariable Cox regression analysis revealed that the modified albumin–bilirubin (mALBI) grade (hazard ratio [HR], 2.140; $p = 0.002$), baseline AFP

concentration (HR, 1.753; $p = 0.014$), and baseline FL-GPC3 concentration (HR, 1.592; $p = 0.032$) were independent predictors of PFS (Table 3).

Table 3

Univariate and multivariable Cox regression analysis of factors associated with progression-free survival of patients with HCC.

Variables		Univariate analysis			Multivariable analysis		
		HR	95% CI	<i>p</i> -value	HR	95% CI	<i>p</i> -value
Age	≥ 75 years	0.848	0.558–1.286	0.437	-	-	-
Sex	Male	1.057	0.631–1.771	0.834	-	-	-
Etiology	Viral	0.816	0.543–1.227	0.328	-	-	-
BCLC stage	C	0.883	0.585–1.333	0.555	-	-	-
1st-line therapy	Yes	0.779	0.477–1.272	0.318	-	-	-
mALBI grade	2b or 3	2.050	1.289–3.261	0.002	2.140	1.331–3.438	0.002
NLR	≥ 3.03	1.262	0.838–1.900	0.266	-	-	-
AFP	≥ 100 ng/mL	1.888	1.217–2.926	0.005	1.753	1.122–2.738	0.014
AFP-L3	≥ 10%	1.001	0.665–1.508	0.995	-	-	-
DCP	≥ 186 mAU/mL	1.321	0.864–2.020	0.198	-	-	-
FL-GPC3	≥ 21.7 pg/mL	1.575	1.039–2.388	0.032	1.592	1.041–2.435	0.032
Abbreviations: AFP, alpha-fetoprotein; AFP-L3, alpha-fetoprotein bound to <i>Lens culinaris</i> agglutinin; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; DCP, des-gamma-carboxy prothrombin; FL-GPC3, full-length glypican-3; HR, hazard ratio; mALBI, modified albumin–bilirubin; NLR, neutrophil–lymphocyte ratio.							

Relationship between baseline concentration of FL-GPC3 and OS

Kaplan–Meier curves showed that the OS was shorter in the FL-GPC3-high group (13.7 vs. 25.8 months, $p = 0.006$; Fig. 3b). Multivariable Cox regression analysis revealed that the mALBI grade (HR, 1.972; $p =$

0.014), neutrophil–lymphocyte ratio (HR, 1.711; $p = 0.035$), baseline AFP concentration (HR, 2.211; $p = 0.003$), and baseline FL-GPC3 concentration (HR, 1.807; $p = 0.023$) were independent predictors of OS (Table 4).

Table 4
Univariate and multivariable Cox regression analysis of factors associated with overall survival of patients with HCC.

Variables		Univariate analysis			Multivariable analysis		
		HR	95% CI	<i>p</i> -value	HR	95% CI	<i>p</i> -value
Age	≥ 75 years	0.896	0.546–1.470	0.664	-	-	-
Sex	Male	1.650	0.815–3.340	0.164	-	-	-
Etiology	Viral	0.723	0.442–1.182	0.196	-	-	-
BCLC stage	C	1.312	0.802–2.148	0.279	-	-	-
1st-line therapy	Yes	0.603	0.351–1.035	0.066	-	-	-
mALBI grade	2b or 3	1.956	1.155–3.311	0.013	1.972	1.147–3.389	0.014
NLR	≥ 3.03	1.643	1.006–2.682	0.047	1.711	1.038–2.820	0.035
AFP	≥ 100 ng/mL	2.460	1.477–4.099	< 0.001	2.211	1.303–3.751	0.003
AFP-L3	≥ 10%	1.638	1.001–2.680	0.050	-	-	-
DCP	≥ 186 mAU/mL	1.606	0.973–2.651	0.064	-	-	-
FL-GPC3	≥ 21.7 pg/mL	1.976	1.201–3.249	0.007	1.807	1.084–3.012	0.023
Abbreviations: AFP, alpha-fetoprotein; AFP-L3, alpha-fetoprotein bound to <i>Lens culinaris</i> agglutinin; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; DCP, des-gamma-carboxy prothrombin; FL-GPC3, full-length glypican-3; HR, hazard ratio; mALBI, modified albumin–bilirubin; NLR, neutrophil–lymphocyte ratio.							

Discussion

In this study, we investigated the usefulness of circulating FL-GPC3 levels in patients treated with Atezo/Bev for unresectable HCC. We analyzed its correlation with the concentrations of other HCC biomarkers, confirming a significant correlation between the FL-GPC3 and AFP concentrations. The

baseline serum concentration of FL-GPC3 was significantly higher in patients who experienced disease progression during Atezo/Bev therapy. Furthermore, a higher baseline FL-GPC3 concentration was associated with poorer PFS and OS among patients treated with Atezo/Bev. These findings suggest that serum FL-GPC3 levels predict the therapeutic effectiveness of Atezo/Bev for HCC and the outcome of patients receiving such treatment.

Previous studies suggested that GPC3 is involved in HCC progression by stimulating Wnt signaling, interacting with growth factors, stimulating macrophage recruitment, and promoting epithelial–mesenchymal transition^[16, 22, 23]. Cell-surface GPC3 forms a complex with Wnt, thereby stimulating Wnt/β-catenin signaling in HCC cells^[24]. Although the prognostic value of GPC3 expression in HCC remains inconclusive, its overexpression in the tissues of patients with HCC is associated with a poor prognosis^[17]. Patients with GPC3-positive HCC who received Atezo/Bev reportedly have a significantly worse OS and lower ORR than those with GPC3-negative HCC^[18].

In addition to tissue expression of GPC3, the prognostic value of serum GPC3 was previously investigated. However, its pathophysiological significance also remains unclear. To the best of our knowledge, this is the first study in which the relationship between the serum FL-GPC3 concentration and the outcome of Atezo/Bev therapy was examined. A recent study revealed similar findings to those of our study, that is, a higher plasma GPC3 concentration was associated with shorter PFS and OS and lower ORRs in patients treated with Atezo/Bev for HCC^[25]. The researchers assessed GPC3 expression in tumor tissue and plasma via IHC and an ELISA, respectively. The ELISA used in that study primarily recognized the C-terminus of GPC3. However, C-terminal GPC3 can be affected by proteolytic cleavage, GPC3 shedding, and post-translational modifications that regulate fragment release. This raises concerns that liver inflammation and fibrosis may influence the concentration of C-terminal GPC3 in the blood^[20]. Therefore, FL-GPC3 dynamics should be assessed for an accurate understanding of GPC3 biology and its clinical implications in HCC. Our findings confirmed that a higher circulating FL-GPC3 concentration is a poor prognostic indicator of Atezo/Bev treatment effectiveness, PFS, and OS for HCC.

AFP, AFP-L3, and DCP are commonly used as tumor markers in HCC in some countries, and they have also been reported as prognostic factors in HCC^[10]. In line with previous studies^[19, 20], this study found that baseline serum concentrations of FL-GPC3 in patients with HCC were significantly correlated with AFP concentrations. As AFP and GPC3 are both known as fetal cancer antigens, they may behave similarly^[19]. However, no significant correlation was found between FL-GPC3 and AFP-L3 in our results. As AFP-L3 is a core-fucosylated glycoform of AFP^[26], it may be affected not only by the tumor volume but also by post-translational modification of in tumor cells.

GPC3 is considered an attractive target for HCC therapy owing to its selective expression in this disease. Codrituzumab, a humanized monoclonal antibody against GPC3, was studied in a randomized phase II trial in patients with advanced HCC for whom prior systemic therapy had failed. Although it failed to demonstrate a clinical benefit, the investigators suggested that a higher dose of codrituzumab or the

selection of patients with high levels of GPC3 might improve the outcome^[27]. Recently, chimeric antigen receptor T-cells, which have shown promising curative effects in patients with hematological tumors, have been explored for the development of novel GPC3-targeted therapies in HCC^[28]. A phase I clinical trial has demonstrated its effectiveness and safety^[29], and further developments are ongoing. In GPC3-targeted therapies, the development of biomarkers reflecting the status of GPC3 expression in tumors or therapeutic efficacy will be important for appropriate patient selection. Despite discrepancies among some patients, plasma GPC3 levels have exhibited moderate concordance with tumor GPC3 levels^[20, 25]. Therefore, serum GPC3 has the potential to serve as a novel biomarker to select patients for and monitor the therapeutic effectiveness of immunotherapy and promising GPC3-targeted therapies. As mentioned above, circulating FL-GPC3 may be useful for these purposes.

This study has several limitations. First, it was a single-center, single-arm, retrospective study conducted in a real-world setting with a limited number of patients. Because only Japanese patients treated with Atezo/Bev therapy were included, the generalizability of the results to patients of other ethnicities or those treated with other immunotherapies cannot be guaranteed and requires further verification. Second, although the cutoff value of FL-GPC3 to predict disease control was originally derived from ROC analysis, the same threshold was applied to analyze PFS and OS. The optimal cutoff values to predict these different measures may differ. Nevertheless, a cutoff value for the prediction of disease control reflects biologically meaningful differences in tumor aggressiveness and may have prognostic relevance beyond merely the treatment response. Third, as not all patients underwent surgery or biopsy prior to Atezo/Bev therapy, we could analyze the correlation between serum FL-GPC3 levels and GPC3 expression in tissues for only a subset (10/102 patients). Further investigation in patients with both tissue samples and blood specimens taken prior to treatment is necessary.

In conclusion, we demonstrated that the baseline serum FL-GPC3 concentration is associated with disease control, PFS, and OS in patients treated with Atezo/Bev for HCC. Our results suggest that serum FL-GPC3 has the potential to serve as a noninvasive serum biomarker to predict therapeutic responses or survival in patients treated with immunotherapy for HCC.

Methods

Patients

In this retrospective cohort study, we included 102 patients who received Atezo/Bev as systemic therapy for HCC at our institute from October 2020 to July 2025. Atezo/Bev therapy was primarily indicated for patients with a good performance status and compensated liver disease, for whom locoregional therapies were not indicated owing to the presence of vascular invasion, extrahepatic spread, or a transarterial chemoembolization (TACE)-refractory or TACE-unsuitable state, in accordance with guidelines^[30]. Patients received 1200 mg of atezolizumab and 15 mg/kg of bevacizumab (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) intravenously every 3 weeks. If serious adverse events (AEs)

were observed, including grade 3 AEs or unacceptable grade 2 AEs, the Atezo/Bev therapy was discontinued until the patient recovered from the AE or the AE became less severe. Serum samples were collected at the start of Atezo/Bev therapy. All samples were cryopreserved at -80°C until measurement.

Written informed consent was obtained from all patients prior to their inclusion in this study. The study was conducted in accordance with the 2013 revision of the 1964 Declaration of Helsinki and with the approval of the Ethical Committee of Osaka Metropolitan University Graduate School of Medicine (approval nos. 1646 and 2023 – 140).

Assessment of treatment response and survival

The treatment response was assessed using dynamic computed tomography or gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid-enhanced magnetic resonance imaging, according to the RECIST version 1.1^[31]. The ORR and DCR were calculated based on the best radiological response. An objective response was defined as a complete response (CR) or partial response (PR), and disease control was defined as a CR, PR, or stable disease (SD). OS was defined as the time from the date of Atezo/Bev treatment initiation to the date of death or the last visit. PFS was defined as the time from Atezo/Bev treatment initiation to the observation of clinical disease progression, death, or the last visit. Patients who were lost to follow-up were censored on the most recent day of confirmed survival.

Laboratory assays

Complete blood counts and prothrombin times were determined and biochemical tests performed using standard procedures. Serum AFP and DCP levels were determined via chemiluminescent enzyme immunoassays. Serum AFP-L3 levels were determined using a liquid-phase binding assay and an electrokinetic analyte transport assay. Serum FL-GPC3 concentrations were measured by Sysmex Corporation (Kobe, Japan) using an enzyme-linked immunosorbent assay (ELISA) and an HISCL™ automated immunoassay. It consists of a sandwich assay with an antibody that recognizes the N-terminus of GPC3 for capture and another that recognizes the C-terminus for detection^[19].

Immunohistochemistry

Liver specimens were fixed in 10% formalin, embedded in paraffin, and subjected to IHC staining. In brief, the deparaffinized sections were treated with 3% H₂O₂ in 100% methanol for 10 min at room temperature to block endogenous peroxidase activity. Antigen retrieval was performed via autoclave treatment in citrate buffer. The sections were preincubated in a serum-free protein blocking solution for 30 min at room temperature and subsequently incubated overnight with an anti-GPC3 (GC33) mouse monoclonal primary antibody (Ventana Medical Systems, Inc., Tucson, AZ; #790–4564) at 4°C. After washing, the sections were incubated with Histofine® Simple Stain Rat MAX-PO (M) (Nichirei Biosciences, Tokyo,

Japan; #414171) for 30 min at room temperature. Signals were developed using diaminobenzene (Agilent Technologies, Santa Clara, CA, USA; #K3468). ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to quantify the percentage of immunopositive cells. The mean value from four high-power fields was used for analysis. A cutoff value of 1% was used to define GPC3 positivity.

Statistical analysis

Continuous variables were expressed as medians and the first to third quartiles. Statistical analyses were performed using Fisher's exact test and the Mann–Whitney U-test. The Pearson correlation coefficient with common logarithmic transformation was used to analyze correlations between baseline serum concentrations of FL-GPC3 and known tumor markers for HCC. We evaluated the predictive value of the serum FL-GPC3 concentration for disease control by using the area under the receiver operating characteristic (ROC) curve. Cutoff levels were determined using the Youden index. The predictive performance of FL-GPC3 was internally validated using bootstrap resampling with 1,000 iterations. Univariate and multivariable logistic regression analyses were performed to evaluate the association between serum FL-GPC3 concentrations and therapeutic responses to Atezo/Bev therapy, as well as survival. We used Kaplan–Meier analysis and log-rank tests to estimate and compare PFS and OS between the FL-GPC3-high and FL-GPC3-low groups, divided according to the cutoff value obtained via ROC analysis. Cox proportional-hazards models were used to analyze factors associated with PFS and OS. Variables that exhibited significant differences in the univariate Cox regression analysis were subjected to stepwise multivariable Cox regression analysis. A p -value < 0.05 was considered to indicate statistical significance. All statistical analyses were performed using R software (version 4.5.2; R Foundation for Statistical Computing, Vienna, Austria), Easy R (EZR) version 1.70 (Saitama Medical Center, Jichi Medical University, Saitama, Japan)^[32], and GraphPad Prism 10.4.1 (GraphPad Inc, USA).

Declarations

Competing Interests Statement

The authors declare no competing interests.

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Author Contribution

Naoshi Odagiri and Masaru Enomoto conceived and designed the study. Naoshi Odagiri, Masaru Enomoto, Hiroko Ikenaga, Kanako Yoshida, Kohei Kotani, Ritsuzo Kozuka, Etsushi Kawamura, Atsushi Hagihara, Hideki Fujii, and Sawako Uchida-Kobayashi collected clinical data and participated in patient management. Naoshi Odagiri and Hideto Yuasa performed immunohistochemical analyses. Naoshi Odagiri and Jumpei Okamura performed statistical analyses. Naoshi Odagiri and Masaru Enomoto drafted the manuscript. All authors critically revised the manuscript for important intellectual content and approved the final version.

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Data Availability

The datasets generated and/or analyzed during the current study are not publicly available due to institutional restrictions and protection of patient privacy but are available from the corresponding author on reasonable request.

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Figures

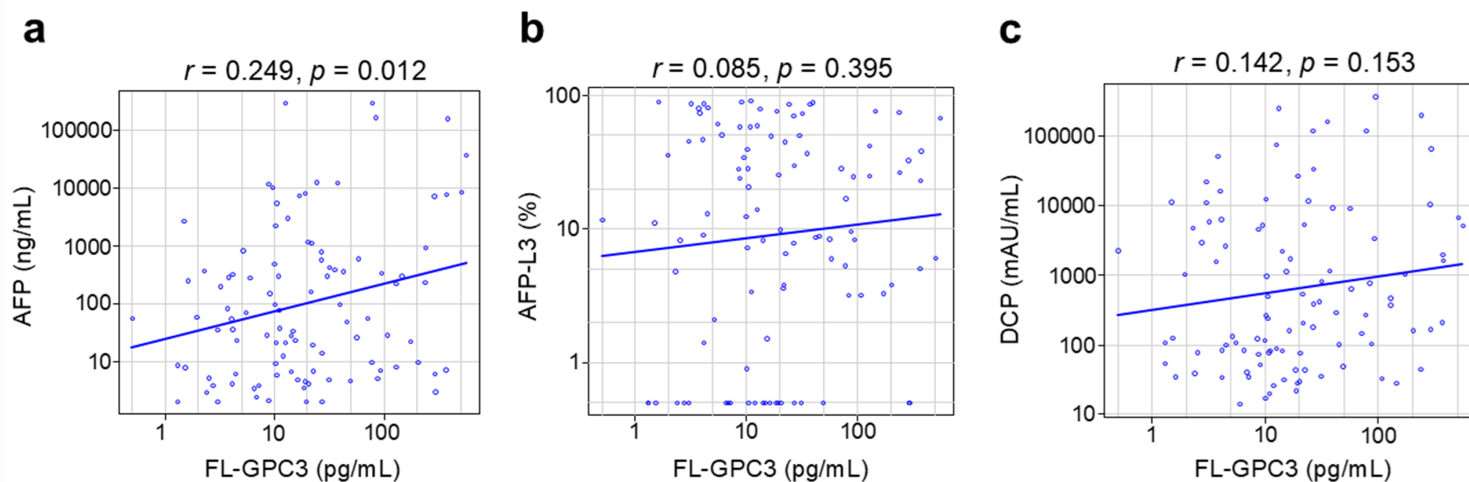


Figure 1

Figure 1

Correlations between baseline concentrations of FL-GPC3 and the three available tumor markers in patients with HCC

a: Correlation between baseline concentrations of FL-GPC3 and AFP.

b: Correlation between baseline concentrations of FL-GPC3 and AFP-L3.

c: Correlation between baseline concentration of FL-GPC3 and DCP.

Significance was determined via the Pearson correlation coefficient with common logarithmic transformation. A p -value < 0.05 was considered statistically significant. Abbreviations: AFP, alpha-fetoprotein; AFP-L3, alpha-fetoprotein bound to *Lens culinaris* agglutinin; DCP, des-gamma-carboxy prothrombin; FL-GPC3, full-length glypican-3.

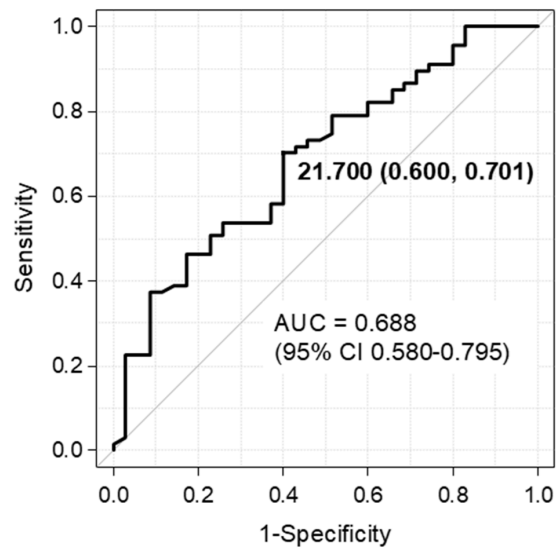


Figure 2

Figure 2

Receiver operating characteristic curve analysis for the ability of serum FL-GPC3 concentrations to predict disease control

The area under the curve was 0.688 (95% confidence interval, 0.580–0.795), with a sensitivity of 70.1% and specificity of 60.0%.

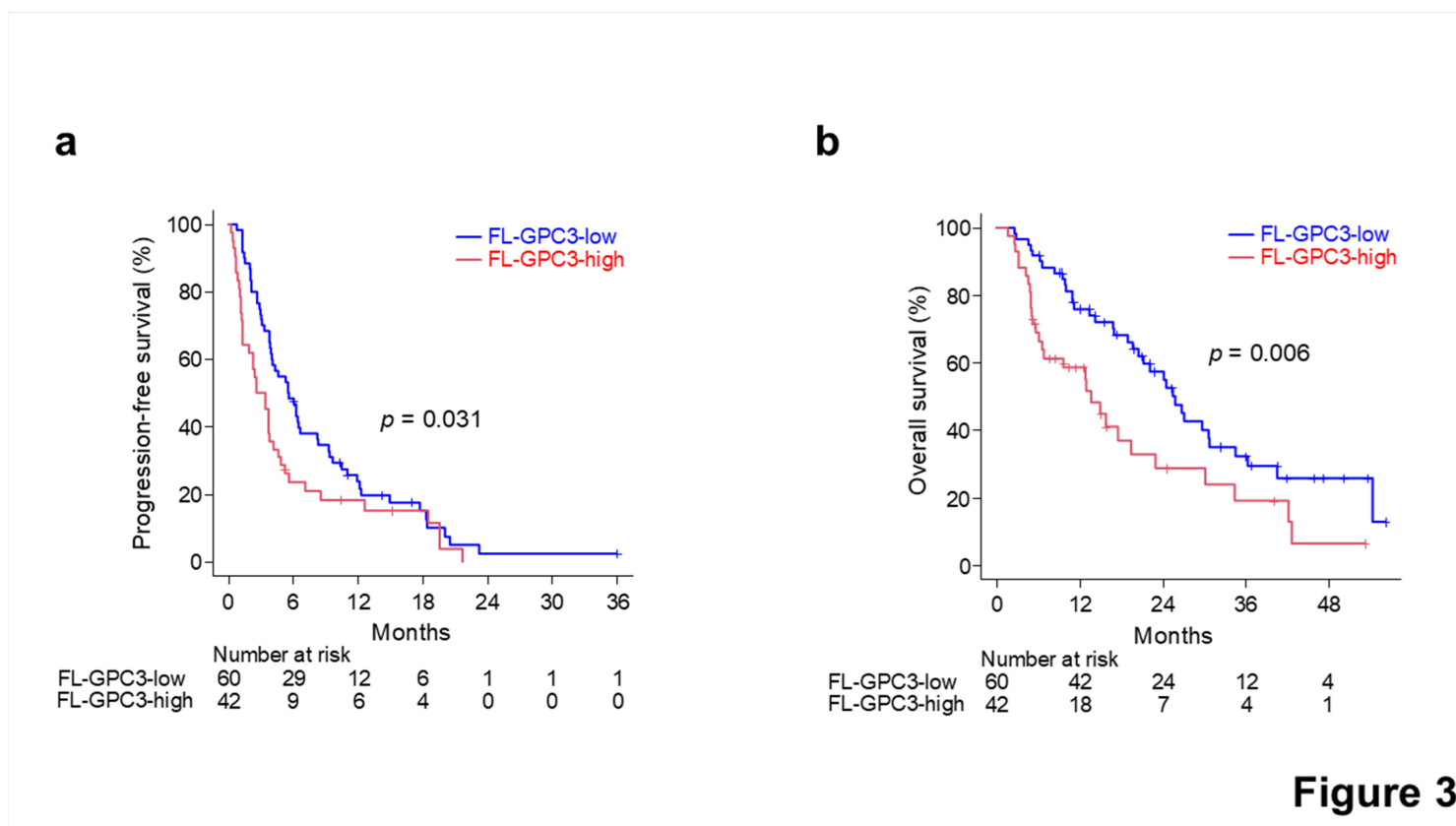


Figure 3

Figure 3

PFS and OS in patients treated with atezolizumab plus bevacizumab, stratified according to a cutoff serum FL-GPC3 concentration of 21.7 pg/mL

a: The median PFS was 3.0 months (95% CI, 1.3–3.8) in the FL-GPC3-high group and 5.6 months (95% CI, 3.9–8.3) in the FL-GPC3-low group ($p = 0.031$).

b: The median OS was 13.7 months (95% CI, 6.2–19.5) in the FL-GPC3-high group and 25.8 months (95% CI, 19.7–30.7) in the FL-GPC3-low group ($p = 0.006$).

Significance was determined via the log-rank test. A p -value < 0.05 was considered statistically significant. Abbreviations: CI, confidence interval; FL-GPC3, full-length glypican-3; PFS, progression-free survival; OS, overall survival.

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