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Expression and Prognostic Significance of PD-L1 and NY-ESO1 in Gallbladder Carcinoma

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Abstract. *Background/Aim: Gallbladder cancer is a rare malignancy with a very high mortality, usually due to diagnosis in an advanced stage of the disease. Therefore, the aim of this study was to evaluate the clinical significance of cancer/testis antigen 1A (CTAG1A, NY-ESO1) and CD274 molecule (PD-L1, the ligand for programmed cell death protein 1) and their impact on the overall survival of patients with gallbladder cancer. Patients and Methods: Using immunohistochemical staining, we determined the expression of NY-ESO1 in tumor cells (positivity: cytoplasmic/nuclear staining of any intensity in ≥50%) and PD-L1 in tumor cells and intratumoral immune cells (positivity: cytoplasmic/membranous staining of any intensity in ≥1%). Results: The median overall survival (OS) of 58 patients with gallbladder cancer in our cohort was 7 months, and depended on the clinical stage of the disease; the 5-year OS rate was 10%. NY-ESO1 was expressed in 69.1% of cases. Immune cells were PD-L1-positive in 36.4% of cases, while tumor cells expressed PD-L1 in only 10.9% of cases. In six cases (10.9%), neither of the studied proteins were expressed. NY-ESO1 expression was negatively correlated with PD-L1 expression in immune cells (p=0.021). NY-ESO1 showed no correlation with any clinicopathological parameters or OS. PD-L1 expression in immune cells was significantly higher in tumors with perineural invasion (r_s=0.318; p=0.018) and higher clinical disease stage (r_s=0.339; p=0.013) but showed no correlation with OS. Conclusion: Patients whose gallbladder cancer expresses NY-ESO1 or PD-L1 might be candidates for immunotherapy.*

Gallbladder cancer is the most common primary malignant tumor of the biliary tract (1). GLOBOCAN data for 2020 show an overall incidence of gallbladder cancer of 0.6%, with accounting for 0.9% of all cancer deaths (2, 3). The incidence varies among different geographical regions of the world. High incidences are found in eastern Asia, parts of India and Chile, and in some eastern and central European countries (4, 5). According to the Croatian Cancer Registry, in 2019, the incidence rate of gallbladder cancer was 3/100,000, and 122 patients (81 women) were diagnosed with this disease (6). Unfortunately, fewer than 20% of gallbladder cancers were diagnosed at an early clinical stage, while 61.5% had disease of unknown clinical stage (6).

The most important risk factors associated with the development of gallbladder cancer include gallstones, polyps, obesity and biliary tract anomalies, but clinical symptoms are nonspecific (3, 7, 8). Patients most often present with upper quadrant abdominal pain. Other symptoms may include nausea, weight loss and jaundice. The clinical presentation of the disease most often occurs when the disease is already at an advanced stage and accurate preoperative diagnosis is reported in fewer than 10% of cases (9-11). Histologically, the most common type of gallbladder cancer is adenocarcinoma (GBC). There are several histological subtypes of gallbladder adenocarcinoma with biliary-type being the most common (1). Mainly due to late diagnosis, GBCs confer a very poor prognosis, which is a major obstacle for successful systemic antineoplastic treatment.

Recently, several drugs have been developed that act as monoclonal antibodies by binding to programmed cell death protein 1 (PDCD1 or PD1) or its ligand CD274 molecule (PD-L1). They block the interactions of the PD1 receptor with the PD-L1 and PD-L2 ligands, thus reactivating the antitumor

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immune response. The efficacy of this form of therapy is not related to the origin of the tumor, but rather to its wider antitumor effect. Therefore, it is used for different types of solid tumor (melanoma, lung, breast, pancreatic, urothelial carcinomas and others) (12-19). Several studies have focused on the expression of PD-L1 in carcinomas of the biliary system, some of which included GBC in combination with or without the study of PD-L1 expression on intratumoral lymphocytes. Results of these studies indicate the potential prognostic importance of determining the PD-L1 status in GBC (20-22).

In normal tissues, the expression of cancer/testis antigen 1A (CTAG1A; also known as NY-ESO1) is limited to the testis, trophoblast and placenta, but expression has also been described in different types of tumors, including breast cancer, lung cancer and melanoma (23-27). Very few studies have investigated the expression of NY-ESO1 in gallbladder carcinomas; Reiner *et al*. found a prevalence of only 3% (26). NY-ESO1 is considered one of the most immunogenic cancer/testis antigens. NY-ESO1 elicits a strong, integrated immune response in a large percentage of patients whose tumors express NY-ESO1 (28).

The aim of this study was to assess the potential clinical significance of PD-L1 and NY-ESO1 in GBCs. According to the available literature, no research has been conducted comparing the expression of PD-L1 in combination with NY-ESO1 in gallbladder carcinoma.

Patients and Methods

Patients. This retrospective study included 58 patients treated with cholecystectomy at Department of Surgery, Sestre Milosrdnice University Hospital Center, Zagreb, between 1st January 2008 and 31st December 2018, with histologically established diagnosis of GBC. Retrieval of archival tissue blocks was conducted under Institutional Review Board approval. Each patient received a unique identification number, and, in order to protect the patient's personal data, their identity is known only to the researchers.

The median age of patients at the time of surgery was 72 years (range=46-91 years), and most of them were women (70.7%), who were slightly older than the men (75 *vs*. 68.5 years). The median size of tumors measured at gross examination after surgery was 25 mm (range=10-105 mm). Most of the tumors were diagnosed as pathological T2 (44.8%) and T3 (41.4%) (pT) stage, mainly with unknown N-stage. Histologically, almost all were of pancreatobiliary type (56/58), mostly moderately differentiated (60.3%) (1). At the time of the study, all patients had died of GBC. Clinicopathological data of patients are summarized in Table I.

Table I. *Clinicopathological data of patients with gallbladder adenocarcinoma*.

Characteristic	Subgroup	Frequency, n (%)
Sex	Male	17 (29.3)
	Female	41 (70.7)
Clinical stage	I	7 (12.1)
	IIA	15 (25.9)
	IIB	3 (5.1)
	IIIA	7 (12.1)
	IIIB	8 (13.8)
	IV	18 (31.0)
Tumor size	≤20 mm	19 (32.7)
	>20 mm	19 (32.7)
	Unknown	20 (34.6)
Histological subtype	Pancreatobiliary	56 (96.6)
	Intestinal	1 (1.7)
	Poorly cohesive	1 (1.7)
Histological grade	I	8 (13.8)
	II	35 (60.3)
	III	15 (25.9)
pT stage	1	7 (12.1)
	2	26 (44.8)
	3	24 (41.4)
	4	1 (1.7)
pN stage (n=29)	0	7 (12.1)
	1/2	22 (38)
	Unknown	29 (50)
Perineural invasion	No	16 (27.6)
	Yes	42 (72.4)
Lymphovascular invasion	No	32 (55.2)
	Yes	26 (44.8)
Death	≤12 Months	39 (67.2)
	>12 Months	19 (32.8)
NY-ESO1	Negative	17 (30.9)
	Positive	38 (69.1)
PD-L1		
Tumor cells	Negative	49 (89.1)
	Positive	6 (10.9)
Immune cells	Negative	35 (63.4)
	Positive	20 (36.4)

NY-ESO1: Cancer/testis antigen 1A; PD-L1: programmed cell death 1 ligand; pT: pathological T stage, depth of tumor invasion; pN: pathological lymph node metastasis.

Immunohistochemistry. Formalin-fixed paraffin-embedded specimens were cut at 5-mm thickness, and routinely stained with hematoxylin and eosin. The diagnosis of adenocarcinoma was histologically confirmed in all cases. For each patient, all hematoxylin-eosin-stained sections were analyzed in order to select a representative paraffin block for additional immunohistochemical analysis.

Tissue deparaffinization, antigen unmasking and immunohistochemical staining were carried out on a Ventana BenchMark® GX (Ventana Medical Systems, Tucson, AZ, USA) automated immunohistochemical staining device. To detect PD-L1 protein, a rabbit monoclonal ready-to-use antibody was used (clone SP142; Ventana, Mannheim, Germany). Tonsil tissue was used as a positive control, as recommended by the manufacturer. To detect NY-ESO1 protein, we used a mouse monoclonal antibody to NY-ESO1 we used (clone E978, 1:200; Thermo Fisher Scientific, Inc.). Testicular tissue was used as a positive control, as recommended by the manufacturer. The replacement of the primary antibodies with isotype-matched immunoglobulin served as negative controls.

Two independent pathologists, who were blinded to the clinical data and overall survival (OS) of the patients, and who resolved minor differences in interpretations by joint review, performed evaluation of PD-L1 and NY-ESO1 expression in GBC.

Immunohistochemistry was performed on 58 specimens but three specimens were excluded from further statistical analysis due to extensive technical issues with tissue staining.

For further statistical analysis, depending on the results, the immunohistochemical expression of PD-L1 and NY-ESO1 was classified as a negative or positive reaction. PD-L1 was assessed in intratumoral and peritumoral immune cells and were considered PD-L1-positive when cytoplasmic or membranous staining of any intensity was observed in ≥1% of cells. PD-L1 was also evaluated in tumor cells, and positive status was determined by tumor cell staining in ≥5% (20). Cytoplasmic or nuclear staining of any intensity in ≥50% of tumor cells was considered a positive reaction for NY-ESO1 (29, 30).

Statistical analysis. The obtained data were statistically processed using the statistical program Statistica 13.5.0.17 (StatSoft Inc., Tulsa, OK, USA) with a statistical significance level of $p<0.05$. Differences in the qualitative variables of PD-L1 and NY-ESO1 expression (positive/negative) were tested with chi-squared and Fisher exact tests in relation to the stage of the disease. Using Spearman's correlation analysis, we determined the relations between the clinical and pathological characteristics of the GBC and the expression of NY-ESO1.

Kaplan–Meier curve with log-rank test were used to analyze OS according to PD-L1 and NY-ESO1 status in the tumor. OS was defined as the period from the date of GBC diagnosis until the death of the patient. At the time of the study, all patients had died of GBC, so no patients were censored (alive). Relative risk of death was calculated using MedCalc, available at https://www.medcalc.org/calc/relative_risk.php.

Results

In a period of 10 years, 58 patients with GBC were surgically treated at our Institution, and at the time of the study, all patients had died. The mean OS was 19±35.4 months, but the median OS was 7 months (range=0-170 months), and 54.3% of patients died during that period (Figure 1). Although 70% of patients were women, no statistically significant difference in the duration of OS was observed between women and men (log-rank test=1.43; test statistics=0.44; $p=0.661$).

The longest OS, with a median of 61 months was achieved in patients with pT1 stage (Kaplan–Meier chi-squared=18.3; $p<0.001$) and clinical stage I of the disease (Kaplan–Meier chi-squared=18.2; $p=0.006$), compared to other stages. Prolonged OS of over 12 months depended on the degree of tumor differentiation, *i.e.*, histological grade ($r_s=-0.312$; $p=0.0183$). Among classic histological characteristics, Spearman's correlation analysis confirmed that poorly differentiated tumor (histological grade III) was associated with a higher depth of infiltration (higher pT stage) ($r_s=0.414$; $p=0.001$), and thus a higher clinical stage ($r_s=0.533$; $p<0.001$).

Immunohistochemical expression. PD-L1 expression in tumor cells was recorded in only six cases (10.9%) (Figure 2A), while the expression of PD-L1 in immune cells was recorded in 20 cases (36.4%) (Figure 2B). Expression of NY-ESO1 was recorded in 38 cases (69.1%) (Figure 2C). Only six tumors (10.9%) did not express either of the investigated proteins.

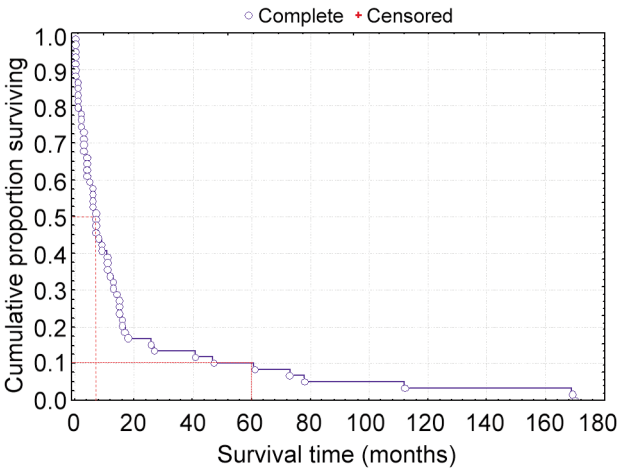

Figure 1. *Kaplan–Meier curve of overall survival for 58 patients with gallbladder carcinoma. The median survival was 7 months, with a 5-year overall survival rate of 10%.*

Spearman's correlation analysis showed statistically significant negative correlation between the expression of NY-ESO1 and PD-L1 in immune cells ($r_s=-0.313$; $p=0.021$), and in tumor cells ($r_s=-0.271$; $p=0.045$). Co-expression of PD-L1 and NY-ESO1 is presented in Figure 3. Of the 38 cases in which tumor cells expressed NY-ESO1, simultaneous expression of PD-L1 in immune cells was recorded in 10/38 cases, and in tumor cells in only 2/38 cases. On the other hand, in NY-ESO1-negative tumors, PD-L1 was expressed in tumor cells in 4/17 cases and in immune cells in 10/17 cases. Overall, 49.1% of cases showed only NY-ESO1 protein expression in tumor cells, while only 10 GBCs showed simultaneous expression of NY-ESO1 in tumor cells with PD-L1 expression in immune cells (Fisher's exact test $p=0.033$) (Figure 3).

According to Spearman's analysis, higher histological grade significantly correlated with PD-L1 expression in tumor cells ($r_s=0.303$, $p=0.024$) but not with PD-L1 expression in immune cells ($r_s=0.240$, $p=0.077$). PD-L1 expression in immune cells correlated significantly with perineural invasion ($r_s=0.318$, $p=0.018$), while the correlation with positive lymph nodes was not significant ($r_s=0.359$, $p=0.066$). Overall, only the expression of PD-L1 in immune cells significantly correlated with higher clinical stage of the disease ($r_s=0.339$, $p=0.013$). Thus, in patients with clinical stage III and IV, PD-L1-positivity in immune cells was recorded in 14 out of 31 (45.2%) cases. However, Cox regression analysis showed no significant difference in OS time after surgery depending on PD-L1 expression in tumor or immune cells, and NY-ESO1 expression (Table II)

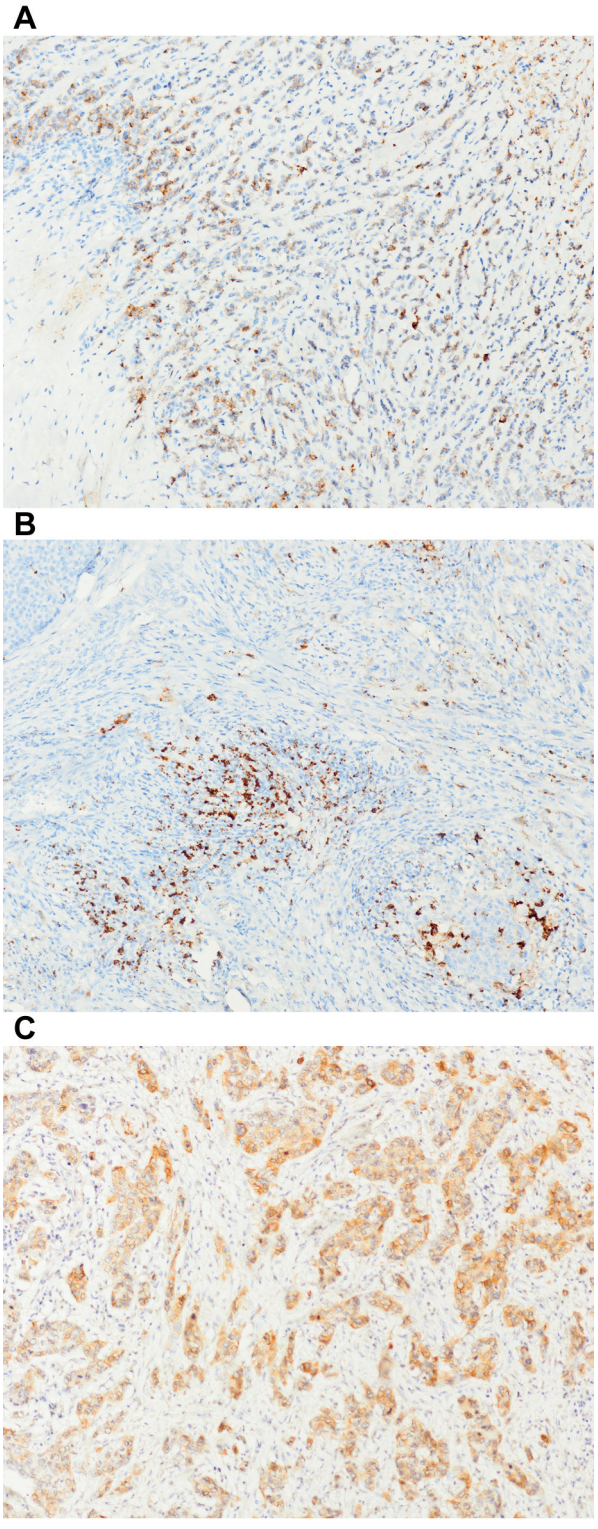

Figure 2. *Immunohistochemical analysis of programmed cell death 1 ligand (PD-L1) and cancer/testis antigen 1A (CTAG1A; also known as NY-ESO1) in patients with gallbladder carcinoma. A: Positive immunohistochemical reaction for PD-L1 in tumor cells, ×200. B: Positive immunohistochemical reaction for PD-L1 in immune cells, ×200. C: Positive immunohistochemical NY-ESO1 reaction in tumor cells, ×200.*

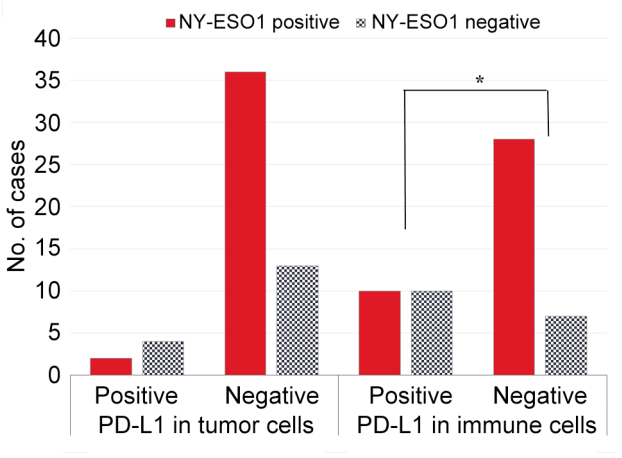

Figure 3. *Distribution of immunohistochemical expression of programmed cell death 1 ligand (PD-L1) in tumor cells (Fisher's exact test, p=0.066), and intratumoral immune cells according to expression of cancer/testis antigen 1A (CTAG1A; also known as NY-ESO1) in tumor cells (Fisher exact test, *p=0.033).*

which is evident from the Kaplan–Meier curves shown in Figure 4 and Figure 5. Although we observed a slightly better mean OS for cases which were negative for both PD-L1 (in tumor and immune cells) and NY-ESO1, the median OS was 7 months in each subgroup, regardless of the expression of the studied proteins.

Univariate Cox regression analysis showed that pT1 stage (p=0.001), histological grade I (p=0.021), and clinical stage I (p=0.005) were associated with a longer survival but after multivariate analysis, none of the included pathohistological characteristics was determined to be an independent prognostic factor (Table II).

The forest plot in Figure 6 presents the pathohistological parameters included in the estimation of the relative risk of death within 12 months of the diagnosis of GBC. As can be seen, among standard clinical parameters, greater tumor depth of invasion (pT3/4) was associated with a 5.15 times higher risk of death within 12 months than pT1 (p=0.077). The risk was also almost two-fold higher for higher histological grade (grade II/III), and 2.45 times higher if the disease were diagnosed in clinical stages higher than stage I. In our study, only a small proportion of patients were diagnosed with such early stages of the disease. The relative risk of death within 12 months from diagnosis did not depend on the immunohistochemical expression of the studied proteins.

Discussion

By molecular profiling, GBCs have been characterized with homologous recombination repair deficiency (19.1%), with human epidermal growth factor receptor 2 (*HER2/ERBB2*)

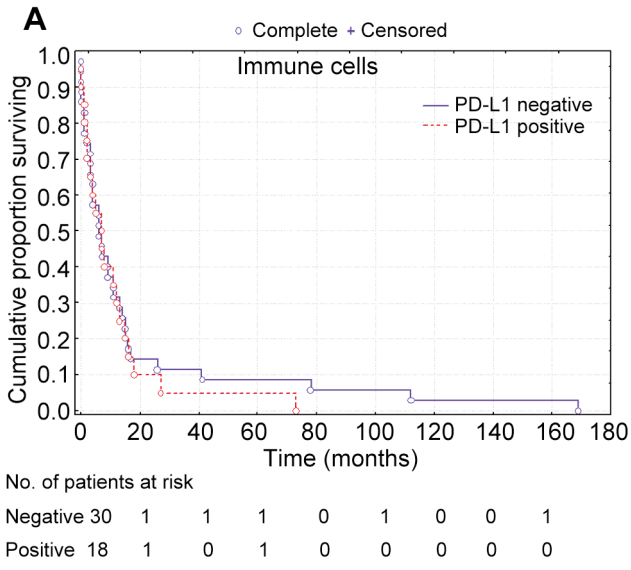

Figure 4. *Kaplan–Meier overall survival curve for patients with gallbladder carcinoma according to programmed cell death 1 ligand (PD-L1) expression in: A tumor cells; B peritumoral immune cells.*

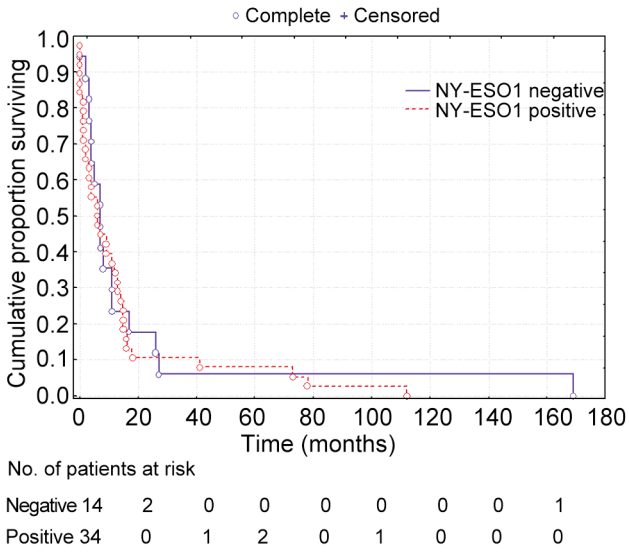

Figure 5. *Kaplan–Meier overall survival curve for patients with gallbladder carcinoma according to the expression of cancer/testis antigen 1A (CTAG1A; also known as NY-ESO1) in tumor cells.*

amplification (14.4%), topoisomerase 2 alpha (*TOP2A*) expression (78.3%), and B-Raf proto-oncogene serine/threonine kinase (*BRAF*) mutations (21, 31, 32). On the other hand, GBCs were not characterized by a high tumor mutational burden nor microsatellite instability-high status (31, 32). Although some of these markers are already a therapeutic option for other types of cancer, more selective markers that target only tumor cells are the focus of numerous research studies. Thus, the aim of our retrospective study was to evaluate the potential prognostic or predictive clinical significance of NY-ESO1 and PD-L1 in GBCs.

In our cohort, NY-ESO1 protein was expressed in two-thirds of GBCs (69.1%). Almost half of the cases expressed only NY-ESO1. Interestingly, NY-ESO1 expression did not show correlation with any clinicopathological parameters. Moreover, there was no correlation with OS depending on NY-ESO1 expression in our GBC cohort. However, the relative risk of death within 12 months was slightly in favor of positive status.

A meta-analysis by Wang *et al*. (33), conducted on 23 studies, suggested a shorter OS in the case of positive NY-ESO1 expression, with differences between various cancer types. The pooled hazard risk of OS (positive *vs*. negative NY-ESO1) was 1.41 (p=0.54), and was worst for serous ovarian cancer, non-small cell lung cancer, and esophageal cancer. However, the literature is still inconsistent. For example, the meta-analysis by Wang *et al*. (33) showed that in some studies of triple-negative breast cancer, a positive NY-ESO1 status had a favorable clinical significance but in others an unfavorable one. Given that the studies are from an older period, we can assume that immunotherapy was not used in the treatment.

One-third (36.4%) of GBCs in our cohort showed PD-L1 expression in immune cells, which correlated with a higher clinical stage of the disease. This result is higher than those already published in the literature, which showed PD-L1 expression in 8% to 24% of GBC cases (21, 31, 32). Interestingly, we did not find a correlation of PD-L1 with positive lymph node status (p=0.066). Studies on colorectal, gastric and gallbladder cancer also showed no correlation of

Table II. *Univariate and multivariate Cox regression analysis for overall survival of 58 patients with gallbladder adenocarcinoma.*

		Univariate		Multivariate	
		HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Age	>72 Years	1	0.633		
	≤72 Years	1.142 (0.662-1.969)			
Sex	Female	1	0.737		
	Male	1.102 (0.625-1.944)			
pT	1	1	**0.001**	5.138 (0.427-61.884)	0.197
	Other	5.621 (1.950-16.206)			
LNI	Negative	1	0.169		
	Positive	1.908 (0.761-4.786)			
Histological grade	I	1	**0.021**	1.334 (0.465-3.826)	0.592
	Other	2.792 (1.168-6.673)			
PNI	Negative	1	0.100		
		1.717 (0.902-3.267)			
LVI	Positive	1	0.331		
		1.313 (0.758-2.274)			
Clinical stage	I	1	**0.005**	0.717 (0.097-5.301)	0.744
	Other	4.581 (1.591-13.190)			
PD-L1	Tumor-negative	1	0.858		
		1.081 (0.459-2.545)			
	Immune cell-positive	1	0.687		
		1.122 (0.641-1.964)			
NY-ESO1	Tumor-negative	1	0.618		
		1.161 (0.645-2.090)			

LVI: Lymphovascular invasion; NY-ESO1: cancer/testis antigen 1A; PD-L1: programmed cell death 1 ligand; PNI: perineural invasion; pT: pathological T stage, depth of tumor invasion. Statistically significant *p*-values are shown in bold.

PD-L1 expression with histopathological features such as age, sex, tumor size, pT and pN stage (34-38). In most, PD-L1 was correlated with tumor differentiation or a higher clinical stage of the disease. Inconsistencies in immunohistochemical results in the literature depend on the threshold value set, the antibody clone used, and the evaluation of PD-L1 in tumor-infiltrating lymphocytes or tumor cells (37, 38). There is also inconsistency in the literature regarding the correlation of PD-L1 and OS (37, 39), while the meta-analysis by Gang *et al*. (40) showed no significant correlation in cholangiocarcinomas.

In our study, the tumor cells themselves rarely expressed PD-L1 (only 10.9% of cases), mainly in those with a higher histological grade (p=0.024). However, we did not find a correlation of PD-L1 expression in immune or tumor cells with OS in our GBC cohort. Kong *et al*. (22) reported the case of a patient with recurrent metastatic GBC with PD-L1 expression who achieved a significant response to treatment combining radiotherapy with nivolumab. In late 2022, the PD-L1 inhibitor, darvolumab, plus chemotherapy was approved in the US as the first immunotherapy regimen for patients with advanced biliary tract cancer (41).

Immunotherapy targeting PD1/PD-L1 has been shown to enhance T-cell-mediated antitumor immunity (12). Merhi *et al*. (42) presented a case in which they correlated clinical response to anti-PD1 treatment (nivolumab) with immunity to NY-ESO1 in a patient with recurrent head and neck squamous cell carcinoma. PD1 expression restricted to the $CD8^+$ T-cell population was reduced 15-fold after treatment. At the same time, the NY-ESO1 antibody level decreased with the number of immunotherapy cycles received and was consistent with stable disease.

Our results show NY-ESO1 protein expression to be negatively correlated with PD-L1 expression in immune and tumor cells. This is consistent with the mechanism of action of PD-L1, which interacts with PD1 and results in tumor immune escape. That is to say, NY-ESO1 exhibits the ability to induce potent natural antibodies, *via* $CD4^+$ and $CD8^+$ T-cell responses. However, therapeutic targeting of tumors expressing NY-ESO1 may be compromised by the strong interaction of inhibitory immune checkpoint molecules such as PD1, PD-L1 or other cells. In these cases, the observed high titers of anti-NY-ESO1 are ineffective and lead to a limited objective clinical response (43). Blockade of the PD1/PD-L1 pathway can partially restore NY-ESO1-specific $CD8^+$ T-cells (44).

NY-ESO1 has potential for several clinical application (45). Antibodies to NY-ESO1 were observed in the blood of patients whose tumors express NY-ESO1 but were not

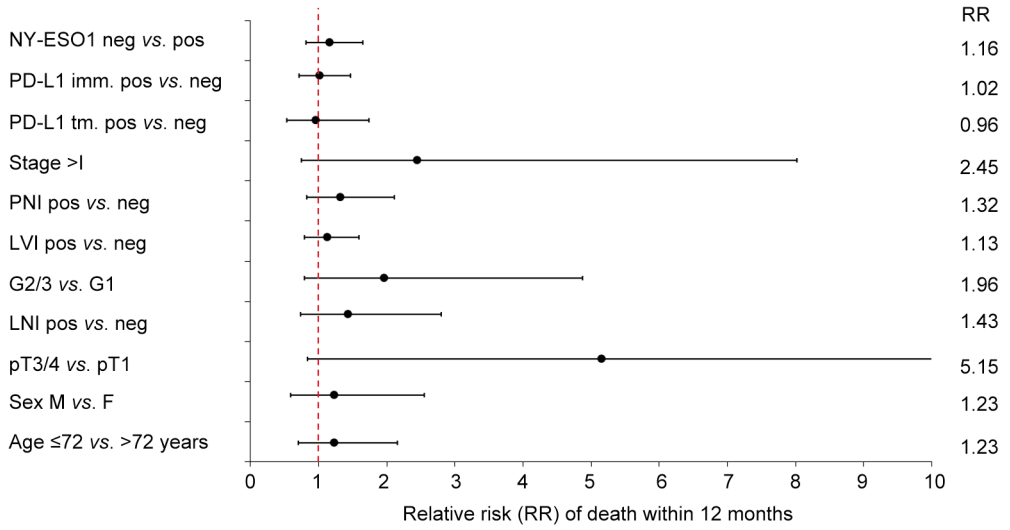

Figure 6. *Relative risk (RR) of death within 12 months of gallbladder cancer diagnosis, according to data from our study on 58 patients. The risk was 5.15 times higher with tumor depth of invasion pT3/4, and 2.45 times higher when the disease was diagnosed in clinical stages higher than stage I. F: Female; imm: immune cells; M: male; LNI: lymph node involvement; LVI: lymphovascular invasion; neg: negative; NY-ESO1: cancer/testis antigen 1A; PD-L1: programmed cell death 1 ligand; pos: positive; PNI: perineural invasion; pT: pathological T stage, depth of tumor invasion; tm: tumor.*

observed in healthy persons (42, 45, 46). Oshima *et al*. (46) showed measurable levels of NY-ESO1 antibodies in the serum of patients with different types of cancer, comparable to tumor markers used so far, such as carcinoembryonic antigen, and cytokeratin 19 soluble fragment. Jung *et al*. (47) found that NY-ESO1 expression was associated with a favorable prognosis in patients with advanced non-small cell lung cancer receiving immunotherapy. They concluded that NY-ESO1 might have potential as a surrogate marker for the treatment *via* PD1 blockade. This is supported by the fact that the expression of PD-L1 does not guarantee response to PD1/PD-L1 inhibitors and tumors with low PD-L1 expression also respond to such treatment. Two other reports found significant correlations between serum NY-ESO1 antibody levels and tumor response rates to anti-PD1 therapy (42, 48).

Camisaschi *et al*. (49) showed that vaccination with peptide NY-ESO-1157-167(V) was able to induce NY-ESO1-specific immunity in patiento with neuroblastoma.

Another potential future direction is NY-ESO1-specific T-cell receptor therapy, which has been investigated in a number of clinical trials in patients with various types of cancer [reviewed in (45)]. Such treatments activate pro-inflammatory cytokines, and patients with high expression of NY-ESO1 in their tumor benefit the most (49). A similar treatment method was used by Kawamoto *et al*. (50), who extended the survival of a patients with GBC to 9 years by cytokine-activated killer cell infusion.

Our research has some limitations such as the relatively small number of due to the low incidence of GBC. In addition, most patients were diagnosed at a later stage of the disease. Due to the accidental finding of GBC, part of the pathohistological data that might show correlations (*e.g*., tumor size or lymph node status) are missing. Moreover, all patients died within a short time of diagnosis, so the investigated markers did not show a significant effect on survival.

Conclusion

In our study, patients with GBC had very poor survival and OS did not show any correlation with the investigated proteins. However, a significant proportion of GBCs showed expression of NY-ESO1 and PD-L1, therefore patients with this type of tumor might be potential candidates for immunotherapy. In addition, NY-ESO1 might serve as a predictive marker of immunotherapy, as well as a serum marker in monitoring the response to it. Furthermore, detection of NY-ESO1 in the serum during the diagnosis of cholelithiasis may indicate the possibility of cancer. Moreover, a high percentage of patients with NY-ESO1-positive GBC might also benefit from the NY-ESO-peptide vaccine. Additional research is needed in this direction due to the low frequency but high mortality of this cancer type, as well as diagnosis in the late stages of the disease.

Conflicts of Interest

All Authors state they have no financial relationships or conflicts of interest related to this research to disclose.

Authors' Contributions

AI, MZ and ZB provided patient care and, generated and interpreted the data; AI and AD conceptualized and designed the analysis; DT and AD performed immunohistochemistry, evaluated the immunohistochemical expression and interpreted the data; SR, performed the formal data analysis; SR, DT and AD wrote the article with edits and revisions from all Authors.

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