Pereira *et al.*
Surgical and Experimental Pathology (2023) 6:17
https://doi.org/10.1186/s42047-023-00142-0

Surgical and Experimental Pathology

RESEARCH

Open Access

Molecular classification of high-grade, muscle-invasive urothelial carcinomas and the relationship with CTLA-4 and PD-L1 expression

Fabiana T. B. Pereira[1*], Tatiana V. A. Mattos[2], Dino Martini Filho[1], Maria Antonieta L. G. da Silva[1], Roni C. Fernandes[3] and Wagner R. Montor[1]

Abstract

Purpose the aim of the current study was to molecularly classify high-grade, muscle-invasive, urothelial carcinoma of basal, luminal, and p53-like subtypes and to assess their relationship with CTLA-4 and PD-L1 expression.

Methods fifty-seven samples from transurethral resections were tested immunohistochemically for CK5/6, CD44, CK20, p63, p53, and CTLA-4/PD-L1.

Results about half of the cases presented a basal molecular profile (50.9%), the luminal profile was found in 29.8% and the *p53*-like profile in 15.8%. Positivity for CTLA-4 occurred in 24 samples (42.1%), with cytoplasmic and membrane expression reported only in neoplastic cells. Of these, 11 were of the luminal subtype, ten were of the basal subtype, and three were of the *p53*-like subtype. There was an association between CTLA-4 expression and the luminal classification, and an absence of CTLA-4 expression in the basal profile ($p=0.047$). PD-L1 expression was found in 12 samples, ten of them classified as the basal subtype ($p=0.036$). CTLA-4 and PD-L1 expression were not identified in the same subtypes ($p=0.08$), since only one sample was positive for both markers.

Conclusions A molecular classification into the subtypes was possible in 96.5% of cases of high-grade, muscle-invasive, urothelial carcinoma using immunohistochemistry. Thus, this type of classification is viable in most surgical pathology laboratories, including those in Brazil's Unified Health System (known as *SUS*, the acronym for *Sistema Único de Saúde*). The expression of CTLA-4 is related to the luminal molecular subtype, while the expression of PD-L1 is related to the basal molecular subtype. CTLA-4 and PD-L1 positivity are mutually exclusive.

Keywords Immunotherapy, Pathology, Medical oncology, CTLA-4 Antigen, Carcinoma

Introduction

Worldwide, bladder cancer is the tenth most incident type of cancer. In men, it ranks seventh, while in women, it is the 19th most frequent (INCA, 2022). According to Brazil's National Cancer Institute (*INCA*, *Instituto Nacional do Câncer*), 11,370 new cases were estimated for the year 2023 in Brazil, with about 4,500 deaths (INCA, 2022). Urothelial carcinoma is the most common malignant neoplasm of the urinary tract,

*Correspondence:
Fabiana T. B. Pereira
fabianatbueno@gmail.com
[1] Faculdade de Ciências Médicas da Santa Casa de São Paulo, Rua Dr Cesário Mota Júnior, 61, São Paulo 01221-020, Brazil
[2] Serviço de Anatomia Patológica da Irmandade da Santa Casa de Misericórdia de São Paulo, São Paulo, Brazil
[3] Serviço de Urologia da Irmandade da Santa Casa de Misericórdia de São Paulo, São Paulo, Brazil

corresponding to about 90% of bladder neoplasms. In 70–80% of cases, the disease is non-invasive or invasive but non-muscle-invasive, corresponding to the Ta or T1 stages of anatomic pathological staging according to American Joint Committee on Cancer (AJCC). The remaining 20–30% of the cases, on the other hand, correspond to stage T2 or higher (muscle-invasive disease). Currently, these cases are treated with neoadjuvant chemotherapy using cisplatin and gemcitabine, when possible, followed by radical or partial cystectomy (Aydh et al. 2023; Williamson et al. 2022).

Many studies have been conducted in search of further clarification of the molecular changes present in these neoplasms, focusing on possible target therapies and more accurate prognostic criteria (Williamson et al. 2022). The study carried out by Lindgren et al., in 2010 was the first to describe the association between a worse prognosis and gene expression in high-grade urothelial carcinomas with a squamous/keratinized phenotype and immunohistochemical expression of cytokeratin 5/6 (CK5/6). Four years later, Choi et al. (2014) proposed a molecular classification divided into three subtypes, namely: basal, luminal, and non-mutant p53 (wild-type *p53* or *p53*-like). The basal subtype is characterized by frequent squamous differentiation and is associated with the immunohistochemical expression of CK5/6 and cluster of differentiation 44 (CD44), proteins related to cell anchoring and adhesion, in addition to p63, which is positive in transitional cells and in squamous differentiation while it is negative for CK20, an epithelial marker, normally expressed only in the superficial cells of the urothelium. These tumors are clinically more aggressive, but potentially sensitive to neoadjuvant chemotherapy. The luminal subtype is CK20 positive and generally presents a papillary configuration. There are reports of positivity for HER2, which is a potential target for therapy, with some clinical trials in progress (clinicaltrials.gov, 2023). Finally, the *p53*-like subtype is characterized by the strong expression of p53, without, however, fitting the profiles of the two aforementioned subtypes. In these cases, high resistance to chemotherapy is described and this procedure can result in unnecessary toxic exposure and delays in surgical procedures for the patient (Choi et al. 2014; Sanli et al. 2017).

Over the past few years, several other studies have been published with the aim of examining possible ways of molecularly classifying urothelial carcinomas and the repercussions this might have in clinical practice. Several techniques have been used in these studies, including immunohistochemical and genetic testing. Some authors advocated a division into only two subtypes: basal and luminal. Others reported the possibility of a division into as many as six distinct molecular groups, with the inclusion of the neuronal, mesenchymal, and neuroendocrine subtypes (Hurst et al., 2014; Cancer Genome Atlas Research Network 2014; Sanli et al. 2017; Tan et al. 2019). However, in 2022, the latest issue of the WHO Classification of Tumors: urinary and male genital tumors, presented the consensus on molecular classification of urothelial carcinomas. This classification, published by Kamoun et al. in 2020, requires genetic and molecular tests and divides urothelial carcinomas into six categories: basal/squamous (Ba/Sq), luminal papillary (LumP), luminal unstable (LumU), Luminal non-Specified (LumNS), stroma-rich, and neuroendocrine (NE)-like (Williamson et al. 2022).

Mutations that occur during carcinogenesis lead to new antigenic loci being exposed by tumor cells. These antigenic targets are often not recognized by the adaptive immune system, which is inhibited because they are self-proteins. The pathways through which this inhibition occurs have been the object of intensive study over recent years. Researchers have increasingly been using this knowledge to produce potential blockers of specific targets, decreasing the immune downregulation in the tumor's environment (Ghasemzadeh et al. 2016; Cathomas et al. 2022). Recent clinical studies have suggested that bladder cancer patients are among the best responders to this category of therapy, especially those presenting metastatic disease who are unfit for platinum therapy which positions these drugs as new weapons to potentially overcame cancer (Alifrangis et al. 2019; Tang et al. 2023).

The best studied pathway is the PD-1/PD-L1 pathway, for which there already exists specific diagnostic and therapeutic proposals. Recently, the scientific community has also started to study the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) pathway, a homodimeric glycoprotein originally described on the surface of activated T-cells. This protein has an inhibitory function over the immune response due to its interaction with the CD80 (B7.1) and CD86 (B7.2) ligands, which are expressed in antigen-presenting cells (Contardi et al. 2005). The pioneer neoplasm that was successfully treated with anti-CTLA-4 was melanoma (U.S. Food and Drug Administration, 2023). Thanks to the promising results in respect of this neoplasm, this branch of immunotherapy has been tested as a neoadjuvant or adjuvant option and for treating advanced metastatic disease in numerous other solid tumors (Keung and Wargo, 2019). There are currently two drugs being tested in anti-CTLA-4 clinical trials for urothelial carcinoma: ipilimumab and tremelimumab.

The current study aimed to molecularly classify the high-grade, muscle-invasive, urothelial carcinoma cases of the basal, luminal, and *p53-like* subtypes diagnosed

between 2016 and 2018 in our institution and to describe their relationship with CTLA-4 and PD-L1 expression. The classification proposed by Choi et al. (2014) was chosen because it uses immunohistochemistry, a technique that is widely available in surgical pathology laboratories, making it feasible even in the context of Brazil's public health service, the Unified Health System (*SUS*, *Sistema Único de Saúde*).

Materials and methods

From 01/01/2016 to 12/31/2018, 302 transurethral resections (TUR) of the bladder were performed at the *Hospital Central da Irmandade da Santa Casa de Misericórdia de São Paulo*. For this study, the eligibility criteria was first diagnosis of high-grade, muscle-invasive urothelial carcinomas with no prior treatment, totalizing 60 samples. Recurrent cases were not included. Three cases were excluded from the study due to patients having sample specimens being withdrawn from the laboratory or a lack of representative samples. Therefore, 57 TUR specimens were included. In the histological slides, two areas of each sample that were the most representative were demarcated for making blocks using the tissue microarray (TMA) technique. Tumors were sampled in duplicates to minimize the possibility of loss. A 36-sample silicone mold was used (Tissue Microarray Mold, 3 mm mold, 36 cores – TED PELLA®). To include all samples, four paraffin blocks were made (Fig. 1). The immunohistochemical reactions were then conducted for CK5/6 (D5/16 B4 Dako®), CK20 (Ks20.8 Dako®), CD44 (MRQ-13 Dako®), p53 (Dako® Omnis) and p63 (Dako® Omnis), in addition to CTLA-4 (CAL49 Leica®) and PD-L1 (Ventana® PD-L1-SP142), for molecular classification purposes. The reactions were performed according to the manufacturer's instructions, using EnVision Flex target retrieval solution high pH (50x).

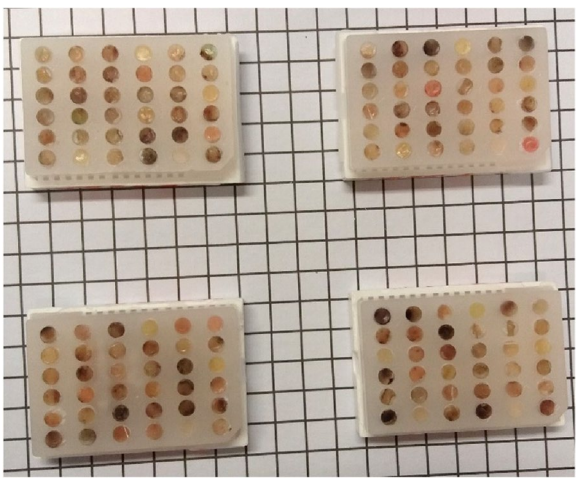

Fig. 1 Paraffin blocks built for tissue microarrays

Table 1 Expression distribution of immunohistochemical markers according to molecular subtypes

	CK5/6	CD44	p63	CK20	p53
BASAL	+	+	+	-	-/+
LUMINAL	-	-	+/-	+	+/-
p53-like	+/-	+/-	+/-	+/-	+

Legend: *CK* Cytokeratin 5/6, *CD* Cluster of differentiation 44, *CK20* Cytokeratin 20

The classification into the basal, luminal and *p53*-like subtypes were performed as shown in Table 1. A basal subtype was considered in those cases where there was positivity for CK5/6 and CD44, but with a negative result for CK20. The luminal subtype was characterized by CK20 expression, in the absence of CK5/6 and CD44 expressions. Finally, the p53-like subtype was found in cases where there was a strong p53 expression, in the absence of a specific pattern for the other reagents.

Due to the absence of a protocol for interpreting the CTLA-4 results in urothelial carcinomas, its expression in tumor cells and immune cells from the tumor's microenvironment were considered, similarly to the criteria established at Ventana® PD-L1 (SP142) Assay Interpretation Guide for Urothelial Carcinoma. Thus, in order for the result to be considered positive, there needed to be membrane detection of PD-L1 in over 5% of viable tumor cells (TPS score) or any expression pattern in over 5% of immune cells from the tumor's microenvironment (IC score).

For the statistical analysis, the Pearson´s chi-squared test and Fisher´s Exact test were used (Software SPSS 25.0) A significance level of $p < 0.05$ was considered. This project was submitted to and approved by the institution's Research Ethics Committee (*CEP*, *Comitê de Ética em Pesquisa*), under registration number 3,180,218 in the national *Plataforma Brasil* ("Brazil Platform").

Results

About half of the cases (29 samples) had a basal molecular profile (50.9%). The luminal profile was found in 17 specimens (29.8%) and the *p53*-like profile in nine (15.8%) (Figs. 2, 3 and 4). Two samples were negative for all reagents, not belonging to any of these groups and therefore being classified as an "undefined" profile (3.5%). Squamous differentiation was found in 11 samples, eight of them showing basal molecular profile and three belonging to p53-like group. None of the luminal samplespresented squamous areas, establishing a statistically significant relationship between the basal molecular profile and the squamous morphology ($p = 0{,}003$).

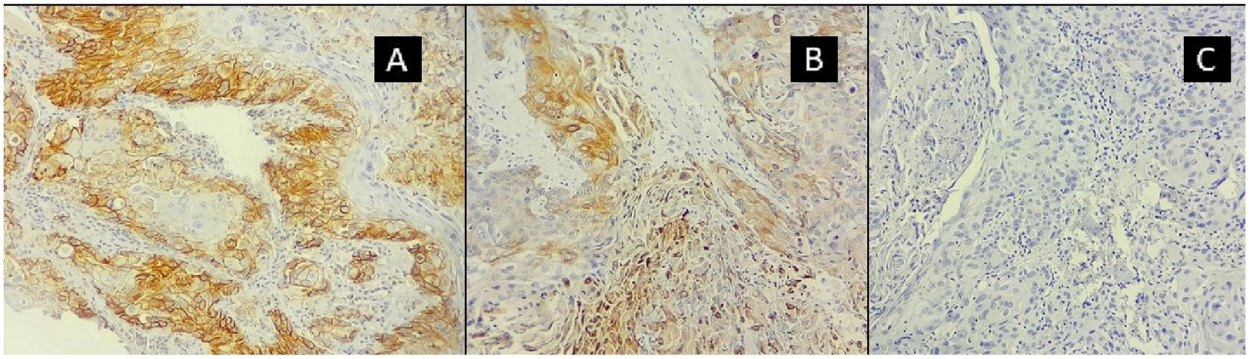

Fig. 2 Basal molecular profile. Expression of CK5/6 (**A**) and focal expression of CD44 (**B**); lack of expression of CK20 (**C**). Technique: immunohistochemistry. Magnification: 200x

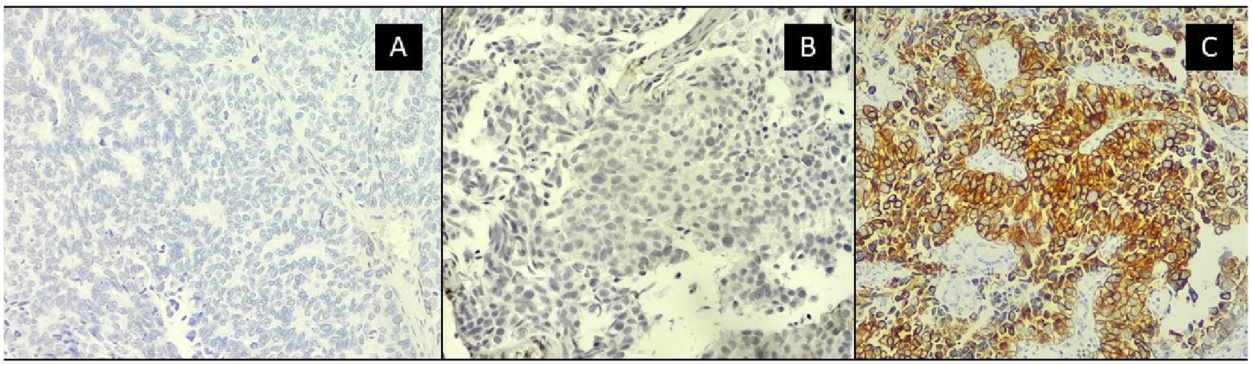

Fig. 3 Luminal molecular profile. Lack of expression of CK5/6 (**A**) and CD44 (**B**); marked expression of CK20 (**C**). Technique: immunohistochemistry. Magnification: 200x

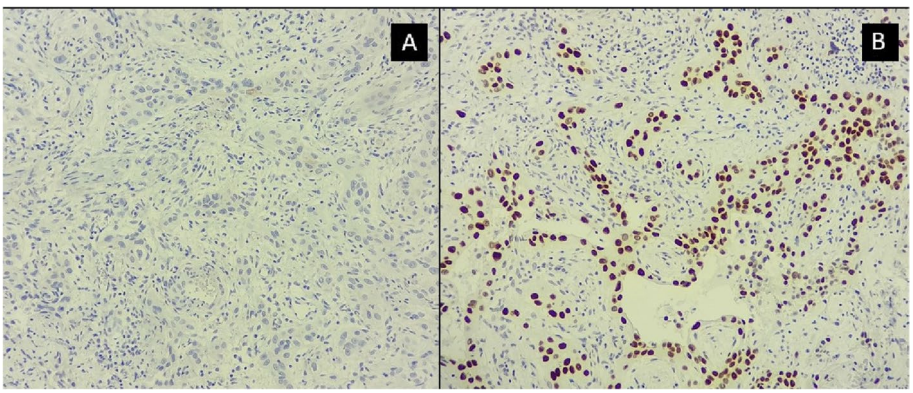

Fig. 4 p53-like molecular profile. Lack of expression of CK5/6 (**A**), CD44 and CK20; strong and diffuse expression of p53 (**B**). Technique: immunohistochemistry. Magnification: 200x

Positivity for CTLA-4 occurred in 24 samples (42.1%). Cytoplasmic and membrane expression was observed only in neoplastic cells (Fig. 5). There was no significant expression of this reagent in immune cells from the tumor's microenvironment in our specimens.

Of the 24 samples expressing CTLA-4, 11 were of the luminal subtype, ten were of the basal subtype, and three were of the *p53*-like subtype. No case classified as an undefined molecular subtype was positive for this reagent (Table 2). When comparing the basal

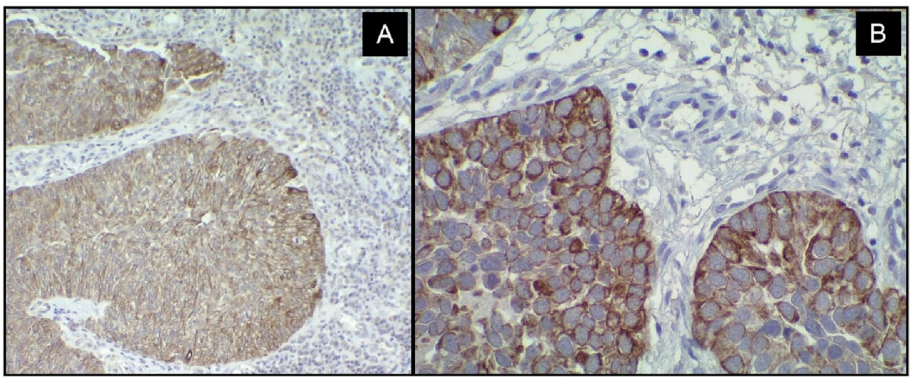

Fig. 5 CTLA-4 expression in neoplastic cells, showing no positive immune cells from the tumor's microenvironment. Technique: immunohistochemistry. Magnification: 200x (**A**), 400x (**B**)

Table 2 Distribution of cases according to molecular profile and CTLA-4 expression. $p = 0.047$

Profile	Positive CTLA-4		Negative CTLA-4		Total	
	No.	%	No.	%	No.	%
Undefined	0	0.0	2	6.06	2	3.51
Basal	10	41.67	19	57.58	29	50.88
Luminal	11	45.83	6	18.18	17	29.82
p53-like	3	12.50	6	18.18	9	15.79
Total	24	100.0	33	100.0	57	100.0

Legend: *CTLA* Cytotoxic T-lymphocyte-associated antigen-4

and luminal subgroups, the result was statistically significant ($p = 0.047$), hence an association between CTLA-4 expression and luminal classification could be established, as well as an association between the absence of CTLA-4 expression and the basal profile. The association between the CTLA-4 result and the p53-like or undefined profiles was not statistically significant.

Positivity for PD-L1 occurred in 12 samples (21%). Despite the 5% cuttoff, all of them presented more than 25% of positivity and 8 of them (66% of positive samples) were high expressors with more than 50% of membrane detection (Figs. 6, 7 and 8). The 45 patients left were completely negative. Ten of them were classified as basal subtype (83,33%), one was luminal (8,33%) and the last was p53-like (8,33%). No case classified as an undefined molecular subtype was positive for this reagent either (Table 3).

When comparing CTLA-4 and PD-L1 expressions, an excluding pattern was observed (Table 4). Only one sample was positive for both markers, while 23 were positive only for CTLA-4 and 11 were positive only for PD-L1 ($p = 0{,}008$).

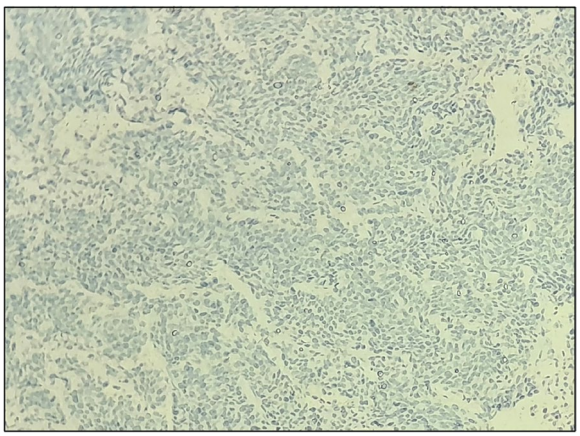

Fig. 6 PD-L1 negative. Technique: immunohistochemistry. Magnification: 200x

Discussion

To date, there is no consensus on either how the molecular classification of this neoplasm should be carried out or its repercussion for therapy and clinical use. Research in recent years has involved gene studies by molecular methods or by means of immunohistochemical expression (Williamson et al. 2022) Despite the recently

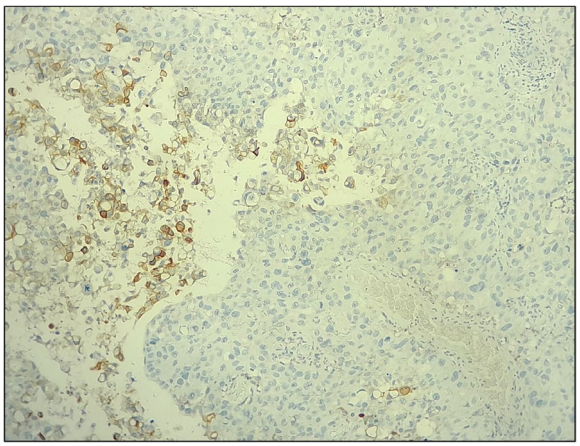

Fig. 7 PD-L1 positive in neoplastic cells. Technique: immunohistochemistry. Magnification: 200x

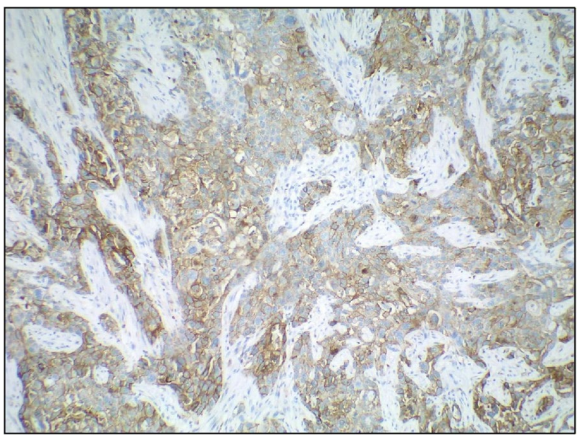

Fig. 8 PD-L1 high expression in neoplastic cells. Technique: immunohistochemistry. Magnification: 200x

published consensus on molecular classification of urothelial carcinomas, we have adopted the classification by Choi et al. (2014), i.e. subdividing samples into the basal/luminal/*p53*-like subtypes, because of its reproducibility by immunohistochemistry, which is currently a widely available technique in surgical pathology laboratories used by the public health service in Brazil.

Of the 57 cases analyzed, only two (3.5%) could not be classified as per Choi et al. (2014), since all reactions were negative. This may have occurred either due to poor specimen fixation, thereby technically impairing the success of the reaction, or because these are distinct, less frequent molecular subtypes. Accordingly, we found that 96.5% of the TUR samples could be classified molecularly in the laboratory routine.

Just over half of our samples (50.9%) corresponded to the basal subtype. The luminal profile was found in 29.8% of the cases, and the *p53*-like profile in 15.8%. This distribution pattern was to be expected, since there are reports that the luminal subtype is related to non-invasive urothelial carcinoma and, to a lesser extent, to invasive neoplasm. In contrast, the basal subtype is described as being more aggressive, usually seen in muscle-invasive disease and rarely occurring in samples where the lamina propria is not affected (Warrick et al. 2016; Williamson et al. 2022). Since our samples were limited to muscle-invasive cases, the predominance of the basal pattern corroborates these findings.

There are no guideline or manuals for interpreting CTLA-4 expression in urothelial carcinoma, as is the case with PD-1 and PD-L1. Studies involving this reagent usually address its expression in immune cells from the tumor's microenvironment or in neoplastic cells a hematopoietic origin (Dhariwal et al. 2019; Signorelli et al.

Table 4 Comparison between CTLA-4 and PD-L1 expressions. $p = 0.008$

	PD-L1 Positive	PD-L1 Negative	Total
CTLA-4 Positive	1	23	24
CTLA-4 Negative	11	22	33
Total	12	45	57

Legend: *CTLA* Cytotoxic T-lymphocyte-associated antigen-4, *PD-L1* Programmed death ligand 1

Table 3 Distribution of cases according to molecular profile and PD-L1 expression. $p = 0{,}036$

Profile	PD-L1 Positive		PD-L1 Negative		Total	
	Nº	%	Nº	%	Nº	%
Undefined	0	0.0	2	4.44	2	3.51
Basal	10	83.33	19	42.22	29	50.88
Luminal	1	8.33	16	35.56	17	29.82
p53-like	1	8.33	8	17.78	9	15.79
Total	12	100.0	45	100.0	57	100.0

Legend: *PD-L1* Programmed death ligand 1

2019). Dum et al. (2022), for example, report urothelial carcinoma as being one of the solid tumors with higher density of CTLA-4 positive in immune cells. Contardi et al. (2005) reported, however, CTLA-4 expression by immunohistochemistry in osteosarcoma and breast carcinoma cells, while the non-neoplastic population in these same samples was negative. Their study discusses the importance of evaluating CTLA-4 expression in solid neoplasms, which suggests that these cells can bind to CD80 and CD86, culminating in the inhibition of the immune system (Contardi et al. 2005). In our sample series, no case was positive for CTLA-4 in at least 5% of the immune cells from the tumor's microenvironment; however, 42.1% of the samples had over 5% of their neoplastic cells testing positive, from moderate to strong intensity. Thus, we emphasize the importance of also identifying this protein in tumor cells.

We found a statistically significant relation associating CTLA-4 positivity to the luminal subgroup of Choi et al. (2014) ($p=0.047$). There is a lack of studies in the literature in respect of this association. The possibility of a treatment with anti-CTLA-4 drugs is a recent discovery, especially in the context of urothelial carcinoma. Clinical trials focusing on this therapy, albeit with partial results, usually recruit patients with advanced or metastatic neoplasms, without discriminating molecular subgroups (Felsenstein and Theodorescu 2018; Andrews et al. 2019). More recently studies have been investigating if there is room for immunotherapy in the treatment of localized muscle-invasive urothelial carcinomas (Peyrottes et al. 2021). In a context in which these drugs are released, the relation between CTLA-4 expression and molecular classification, as herein more specifically associated to the luminal subtype, will provide fundamental data for establishing more effective diagnostic and therapeutic protocols.

PD-L1 expression showed a statistically significant relationshipwith the basal subtype ($p=0.036$). Some authors have reported PD-L1 expression in urothelial carcinomas with squamous differentiation (Reis et al. 2019). Since this morphology is associated with basal molecular subtype, these variables can be linked. In 2020, Kim et al. published an article that, for the first time, analyzed the association of immunohistochemically defined basal-squamous-like and non-basal-squamous-like subtypes with PD-L1 expression, for both SP142 and SP263. In his study, the expression of PD-L1 was significantly associated with the basal-squamous-like profile ($p<0{,}001$), as is described here. As PD-L1 blockades are used as standard therapy for patients with advanced stage and both squamous differentiation and basal molecular profile are related to more aggressive disease, these findings may be important for immunotherapy.

There is a lack of information in the literature about the expressions of CTLA-4 and PD-L1 simultaneously. Our results evidenced that urothelial carcinomas may express only one of them ($p=0.008$). There are a few studies testing the use of both anti-PD-L1 and anti-CTLA-4 in advanced bladder cancer, with more promising results than the monotherapy, such as described by Stockem et al. 2023. Simultaneous expressions were rare in our sample collection and given the many ongoing studies for the clinical use of anti-PD-L1 and anti-CTLA-4 drugs, these findings become immediately relevant.

Conclusions

It was possible to molecularly classify 96.5% of the cases of high-grade, muscle-invasive, urothelial carcinomas into the subtypes defined by Choi et al. (2014), using immunohistochemistry. Thus, this type of classification is viable in most surgical pathology laboratories, including those used by SUS, Brazil's public health system. The basal subtype is the most prevalent, accounting for 50.9% of our samples. The luminal subtype ranks second, with 29.8% of the cases, and the *p53*-like subtype, less frequent, corresponding to 15.8%. CTLA-4 positivity was related to the luminal molecular subtype, while PD-L1 positivity was related to the basal molecular subtype. CTLA-4 and PD-L1 positivity seem to be mutually exclusive and were not identified in the same samples or same subtypes, considering our sample collection.

Abbreviations

AJCC	American Joint Committee on Cancer
CD44	Cluster of Differentiation 44
CEP	*Comitê de Ética em Pesquisa* / Research Ethics Committee
CK5/6	Cytokeratin 5/6
CK20	Cytokeratin 20
CTLA-4	Cytotoxic T-lymphocyte-associated antigen-4
INCA	*Instituto nacional do Câncer*/Brazil's National Cancer Institute
SUS	Sistema Único de Saúde/Brazil's Unified Health System
TMA	Tissue Microarray
TUR	Transurethral resections

Authors' contributions

FTB Pereira: Protocol/project development, Data collection or management, Data analysis, Manuscript writing/editing. TVA Mattos: Protocol/project development, Data collection or management. D Martini Filho: Protocol/project development, Manuscript writing/editing. MAL Galvão: Protocol/project development, Data collection or management. RC Fernandes: Data collection or management. WR Montor: Protocol/project development, Data analysis, Manuscript writing/editing.

Funding

None.

Availability of data and materials

All data relevant to the study are included in the article or uploaded as supplementary information.

Declarations

Ethics approval and consent to participate

This project was submitted to and approved by the institution's Research Ethics Committee (CEP, Comitê de Ética em Pesquisa), under registration number 3,180,218 in the national Plataforma Brasil ("Brazil Platform").

Consent for publication

Not applicable.

Competing interests

None.

Received: 31 March 2023 Accepted: 15 October 2023
Published online: 10 December 2023

References

Alifrangis C, McGovern U, Freeman A, et al. Molecular and histopathology directed therapy for advanced Bladder cancer. Nat Rev Urol. 2019;16(8):465–83. https://doi.org/10.1038/s41585-019-0208-0.

Andrews LP, Yano H, Vignali DAA. Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups. Nat Immunol. 2019;20(11):1425–34. https://doi.org/10.1038/s41590-019-0512-0.

Aydh A, Motlagh RS, Alamri A, et al. Comparison between different neoadjuvant chemotherapy regimens and local therapy alone for Bladder cancer: a systematic review and network meta-analysis of oncologic outcomes. World J Urol. 2023;41:2185–94. https://doi.org/10.1007/s00345-023-04478-w.

Brasil. Instituto Nacional do Câncer (INCA). Câncer De Bexiga - versão para profissionais de saúde. Brasília: Ministério da Saúde; 2022. (Accessed June 2023).

Cancer Genome Atlas Research Network. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature. 2014;507:315–22. https://doi.org/10.1038/nature12965.

Cathomas R, Lorch A, Bruins HM, et al. The 2021 Updated European Association of Urology Guidelines on Metastatic Urothelial Carcinoma. Eur Urol. 2022;81(1):95–103. https://doi.org/10.1016/j.eururo.2021.09.026.

Choi W, Porten S, Kim S, et al. Identification of distinct basal and luminal subtypes of muscle-invasive Bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell. 2014;25(2):152–65. https://doi.org/10.1016/j.ccr.2014.01.009.

Contardi E, Palmisano GL, Tazzari PL, et al. CTLA-4 is constitutively expressed on Tumor cells and can trigger apoptosis upon ligand interaction. Int J Cancer. 2005;117(4):538–50. https://doi.org/10.1002/ijc.21155.

Dhariwal R, Pindoria N, Dasgupta P, et al. #Checkmate: could checkpoint inhibitors be the game changer in the fight against metastatic urothelial carcinoma? BJU Int. 2019;123(2):203–7. https://doi.org/10.1111/bju.14463.

Dum D, Henke TLC, Mandelkow T, et al. Semi-automated validation and quantification of CTLA-4 in 90 different Tumor entities using multiple antibodies and artificial intelligence. Lab Invest. 2022;102(6):650–7.

Felsenstein KM, Theodorescu D. Precision medicine for urothelial Bladder cancer: update on tumour genomics and immunotherapy. Nat Rev Urol. 2018;15:92–111. https://doi.org/10.1038/nrurol.2017.179.

Ghasemzadeh A, Bivalacqua TJ, Hahn NM, et al. New strategies in Bladder cancer: a second coming for immunotherapy. Clin Cancer Res. 2016;22(4):793–801. https://doi.org/10.1158/1078-0432.CCR-15-1135.

Hurst CD, Knowles MA. Molecular subtyping of invasive Bladder cancer: time to divide and rule? Cancer Cell. 2014;25(2):135–6. https://doi.org/10.1016/j.ccr.2014.01.026.

Kamoun A, de Reyniès A, Allory Y, et al. A Consensus Molecular classification of muscle-invasive Bladder Cancer. Eur Urol. 2020;77(4):420–33.

Keung EZ, Wargo JA. The current landscape of immune checkpoint inhibition for solid malignancies. Surg Oncol Clin N Am. 2019;28(3):369–86. https://doi.org/10.1016/j.soc.2019.02.008.

Kim B, Lee C, Kim Y, et al. PD-L1 expression in muscle-invasive urinary bladder urothelial carcinoma according to basal/squamous-like phenotype. Front Oncol. 2020;10: 527385. https://doi.org/10.3389/fonc.2020.527385.

Lindgren D, Frigyesi A, Gudjonsson S, et al. Combined gene expression and genomic profiling define two intrinsic molecular subtypes of urothelial carcinoma and gene signatures for molecular grading and outcome. Cancer Res. 2010;70(9):3463–72. https://doi.org/10.1158/0008-5472.CAN-09-4213.

Peyrottes A, Ouzaid I, Califano G, et al. Neoadjuvant immunotherapy for muscle-invasive Bladder cancer. Med (Kaunas). 2021;29(8): 769. https://doi.org/10.3390/medicina57080769.

Reis H, Serrette R, Posada J, Lu V, Chen YB, Gopalan A, et al. PD-L1 expression in Urothelial Carcinoma with predominant or pure variant histology: Concordance among 3 commonly used and commercially available antibodies. Am J Surg Pathol. 2019;43:920–7.

Sanli O, Dobruch J, Knowles MA, et al. Bladder cancer. Nat Rev Dis Primers. 2017;3:17022. https://doi.org/10.1038/nrdp.2017.22.

Signorelli D, Giannatempo P, Grazia G, et al. Patients selection for immunotherapy in solid tumors: overcome the naïve vision of a single biomarker. Biomed Res Int. 2019;2019:9056417. https://doi.org/10.1155/2019/9056417.

Stockem CF, Galsky MD, van der Heijden MS. Turning up the heat: CTLA4 blockade in urothelial cancer. Nat Rev Urol. 2023. https://doi.org/10.1038/s41585-023-00801-7.

Tan TZ, Rouanne M, Tan KT, et al. Molecular subtypes of urothelial Bladder cancer: results from a meta-cohort analysis of 2411 tumors. Eur Urol. 2019;75(3):423–32. https://doi.org/10.1016/j.eururo.2018.08.027.

Tang Q, Li S, Huang G, Liu H. Research progress on PD-1 and PD-L1 inhibitors in the treatment of metastatic urothelial carcinoma. Int Immunopharmacol. 2023;119: 110158. https://doi.org/10.1016/j.intimp.2023.110158.

U.S. National Library of Medicine. ClinicalTrials.gov. U.S. National Library of Medicine. https://clinicaltrials.gov/ct2/results?cond=Urothelial+Carcinoma+Bladder&term=ERBB2%2C+HER2&cntry=&state=&city=&dist=. Accessed June 2023.

U.S. Food and Drug Administration. Drugs@FDA. FDA-Approved Drugs. 2023 June 26. Silver Spring, MD: Food and Drug Administration; 2023. https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=BasicSearch.process. Accessed June 2023.

Warrick JI, Walter V, Yamashita H, et al. FOXA1, GATA3 and PPARγ cooperate to drive luminal subtype in Bladder cancer: a molecular analysis of established human cell lines. Sci Rep. 2016;6: 38531. https://doi.org/10.1038/srep38531.

Williamson SR, McKenney JK, Raspollini MR, et al. Invasive urothelial carcinoma. In: Netto GJ, Tsuzuki T, Compérat EM, editors. WHO classification of tumours of the urinary and male genital organs. Volume 8, 5th ed. World Health Organization; 2022. pp. 150–64.

Publisher's Note