European Journal of Cancer 108 (2019) 111–119

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Stromal lymphocyte infiltration is associated with tumour invasion depth but is not prognostic in high-grade T1 bladder cancer

Mathieu Rouanne [a,b,*], Reem Betari [b], Camélia Radulescu [c], Aïcha Goubar [d], Nicolas Signolle [e], Yann Neuzillet [b], Yves Allory [c,f], Aurélien Marabelle [a,g], Julien Adam [d,h,1], Thierry Lebret [b,1]

[a] *INSERM Unit U1015, Laboratoire de Recherche Translationnelle en Immunothérapie, Gustave Roussy, Villejuif, France*
[b] *Department of Urology, Hôpital Foch, Université Versailles-Saint-Quentin-en-Yvelines, Université Paris-Saclay, Suresnes, France*
[c] *Department of Pathology, Hôpital Foch, Université Versailles-Saint-Quentin-en-Yvelines, Université Paris-Saclay, Suresnes, France*
[d] *Department of Biostatistics, INSERM Unit U981, Gustave Roussy, Université Paris-Sud, Université Paris-Saclay, Villejuif, France*
[e] *Department of Experimental Pathology, INSERM Unit U981, Gustave Roussy, Université Paris-Sud, Université Paris-Saclay, Villejuif, France*
[f] *Institut Curie, CNRS, UMR144, Paris, France*
[g] *Drug Development Department (DITEP), Gustave Roussy, Université Paris-Sud, Université Paris-Saclay, Villejuif, France*
[h] *Department of Pathology, Gustave Roussy, Université Paris-Sud, Université Paris-Saclay, Villejuif, France*

Received 3 October 2018; received in revised form 23 November 2018; accepted 5 December 2018
Available online 14 January 2019

KEYWORDS
T1G3;
Bladder cancer;
Tumour lymphocyte infiltration;
Prognosis

Abstract ***Introduction:*** Assessment of tumour-infiltrating lymphocytes (TILs) can provide important prognostic information in various cancers and may be of value in predicting response to immunotherapy. The objective of the present study was to investigate the association of stromal lymphocytic infiltration with clinicopathological parameters and their correlation with outcomes in patients with high-grade pT1 non–muscle-invasive bladder cancer (NMIBC).

Abbreviations: NMIBC, non–muscle-invasive bladder cancer; MIBC, muscle-invasive bladder cancer; HGT1, high-grade T1; TILs, tumour-infiltrating lymphocytes; H&E, haematoxylin and eosin; BCG, Bacillus Calmette–Guérin; CD3, cluster of differentiation 3; PD-L1, programmed death ligand 1; EORTC, European Organization for Research and Treatment of Cancer; WHO, World Health Organization; CSS, cancer-specific survival; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival; TUR, transurethral resection.

* *Corresponding author*: INSERM U1015, Laboratoire de Recherche Translationnelle en Immunothérapie, Université Paris-Saclay, Gustave Roussy, Villejuif, France.
E-mail address: mathieu.rouanne@gustaveroussy.fr (M. Rouanne).
[1] Co-senior authors.

https://doi.org/10.1016/j.ejca.2018.12.010

Materials and methods: We retrospectively analysed clinical data and formalin-fixed paraffin-embedded (FFPE) tissues of 147 patients with primary high-grade pT1 NMIBC who underwent transurethral resection of the bladder. The stromal TIL density was scored as percentage of the stromal area infiltrated by mononuclear inflammatory cells over the total intratumoural stromal area. The main end-point was correlation with cancer-specific survival (CSS).
Results: Median follow-up was 8.2 years (6.1–9.5). Induction Bacillus Calmette–Guérin therapy was undergone by 126 patients (86%). Stromal TILs were high (≥10%) in 82 tumours (56%) and were positively associated with the tumour invasion depth ($p = 0.01$) and cancers with variant histology ($p = 0.01$). For the CSS analysis, high (≥10%) versus. low (<10%) stromal TIL hazard ratio (95% confidence interval) was 1.70 (0.7–3.9, $p = 0.2$).
Conclusions: A higher density of stromal TILs was associated with the tumour invasion depth in pT1 NMIBC. The level of TILs was not associated with survival outcomes. These data suggest that tumour aggressiveness is associated with an increased adaptive immune response in pT1 NMIBC. Characterisation of T-cell subtypes along with B-cells may be critical to enhance our knowledge of the host immune response in patients with high-risk NMIBC.

1. Introduction

High-grade T1 (HGT1) bladder cancer is the highest risk subgroup of non–muscle-invasive bladder cancer (NMIBC). It represents approximately 25% of bladder cancer at diagnosis [1]. These tumours have a variable but potentially lethal prognosis as they can exhibit biological behaviour of muscle-invasive bladder cancer (MIBC) [2]. Despite adjuvant Bacillus Calmette–Guérin (BCG) therapy, half of the patients will experience tumour recurrence within 5 years, and 20%–30% of the patients will progress to MIBC [3]. Ultimately, 10%–15% of patients will die of bladder cancer. Although the European Organization for Research and Treatment of Cancer (EORTC) risk score represents a major improvement for scoring the prognosis of NMIBC, it does not fully capture tumour heterogeneity [4]. Currently, one of the most important unmet needs is to identify potentially lethal HGT1 bladder cancer so that they can be managed aggressively before they become life threatening for the patient [5].

Assessment of tumour-infiltrating lymphocytes (TILs) can provide important prognostic information in various cancers and may also be of value in predicting response to treatments. High lymphocytic infiltration, detected using standard haematoxylin and eosin (H&E) staining, has been associated with a favourable prognosis in many different tumours types [6]. Recent efforts have been taken to standardise the assessment of TILs by morphology in routine pathology practice, resulting in guidelines for scoring TILs in solid tumours [7,8]. To our knowledge, large studies investigating the prognostic value of TILs in a homogeneous cohort of HGT1 bladder cancer have not been reported [9].

In the present study, we analysed the density of stromal TILs before BCG therapy, in a homogeneous cohort of patients with primary HGT1 bladder cancer. The primary objective of this study was to evaluate the association between the density of stromal TILs and the cancer-specific survival.

2. Methods

2.1. Patients

We analysed a cohort of 147 primary high-grade cT1N0M0 patients (CNIL declaration number 2059719) treated at the Hôpital Foch between January 2000 and May 2015. We solely included newly diagnosed, treatment-naive, non-metastatic HGT1 bladder tumours, excluding muscle-invasive (≥T2), recurrent, intradiverticular and upper tract urothelial carcinoma (Supplementary data Fig. S1). All patients underwent clinical examination, radiographic tests and transurethral resection (TUR) by an urologist at our institution. BCG therapy, radical cystectomy and management of metastatic disease were performed according to the national guidelines. Clinical follow-up included cystoscopy and urine cytology every 3 months for the first 2 years, then every 6 months. The study was approved by our institutional review board and was conducted according to ethical rules regarding research on tissue specimens and patients. Updated follow-up data were obtained from the patient records (up until a close out date of August 31, 2017, patient death or the last follow-up visit, whichever came last).

2.2. Tumour samples and pathology review

All surgical specimens were initially reviewed by a pathologist specialised in uropathology (C.R.). Tumours were graded according to the World Health Organization (WHO) 2016 system [10] and staged

according to the 2017 TNM classification, 8th edition [11]. Only initial high-grade tumours with a visible, clearly identifiable and disease-free muscularis propria were included in this study. The characteristics of lamina propria invasion were assessed to distinguish superficial invasion, defined as invasion of the lamina propria to the level of the muscularis mucosa (T1a); all other types of invasions into or beyond the muscularis mucosa were categorised as non-superficial (T1b). Progression and recurrence risk scores were calculated using the EORTC risk model (high score $\geq$ 14 and low score <14 for progression; high score $\geq$ 10 and low score <10 for recurrence).

2.3. Scoring of TILs

Representative slides from the initial TUR were reviewed for the purpose of the study and were evaluated independently for the presence of TILs, defined as mononuclear cells with lymphocytes and plasma cell's morphology, excluding granulocytes, following the recommendations of the international TILs Working Group [12]. TILs were reviewed by two pathologists (C.R. and J.A.) blinded to outcome data, using whole slides H&E-stained sections. TILs were evaluated in the stroma of the areas with infiltration of the lamina propria, excepted in areas with necrosis and artefacts secondary to coagulation in TUR samples. The percentage of the stromal area occupied by TILs was reported as a semicontinuous variable (1% increment between 1 and 10% and 5% increments above 10%). A binary scoring system was used to describe non-intense (stromal TILs $\geq$10%) versus intense lymphocytic infiltration (stromal TILs <10%). The overall agreement was good between the two readers (κ = 0.75). (see Fig. 1)

2.4. Study end-points

Recurrence was defined as reappearance on TUR of histologically proven high-risk disease after the start of therapy (any high grade and/or T1 and/or CIS). Progression was defined as development of muscle-invasive tumour, nodal or distant metastasis. Time to recurrence was defined as time from the first TUR to the first local recurrence or distant recurrence or death due to bladder cancer whatever occurs first. In the absence of recurrence event or death due to other cause, patients were censored at the date when last seen or dead. Time to progression was defined as the time from the first TUR to progression or death due to any cause. Time to bladder cancer–related death was defined as the time between the first TUR and death attributable to bladder cancer. Patients alive or died from other cause than bladder cancer were censored at the date of the last follow-up or date of death. Overall survival (OS) was defined as the time from the first TUR to the date of the last follow-up or death. Patients for whom none of these events were recorded were censored at the date of their last known follow-up.

2.5. Statistical analyses

As no formal recommendation for a clinically relevant TIL threshold has been identified for urothelial carcinoma of the bladder, we focussed on stromal TILs as a continuous parameter. We first investigated the prognostic performance of percentage of TILs as a continuous variable regarding the association with recurrence, progression, cancer-specific and non-specific related deaths. Then, we performed the same analyses using 10% as the cut-off to define a high level of stromal lymphocyte infiltration. The association of clinicopathological factors with the percentage of TILs as a continuous or a categorical variable was respectively performed using the Mann–Whitney test and the Chi-square or Fisher's exact test when it was appropriate. For each time to event endpoint, time to event will be presented graphically using Kaplan–Meier methods, and comparisons between groups will be made using the log-rank test. Association of each known clinical prognostic factor was investigated and analysed, and an unadjusted Cox proportional hazards model was used to calculate hazard ratios (HRs) to estimate the variable effect. The assumption of proportionality used by the Cox model was tested using log–log plots and Schoenfeld residuals. If the assumptions were found not to hold, methods for non-proportional hazards were considered. If the hazard is proportional between groups, but not other covariates, this was resolved by fitting non-proportional covariates as strata. Factors with a p-value $\leq$0.20 in univariate analysis were included in a multivariate analysis in addition to TILs. Forward selection was used to establish the final multivariate model. The significance threshold was 5%. Analyses were performed with R software, version 3.4.3.

3. Results

3.1. Patient characteristics

The median follow-up was 8.2 years (range 6.1–9.5 years). The median age was 71.1 years, and 130/147 (88%) were male. Associated CIS was found in 65 patients (44%). Primary treatment strategies included primary BCG therapy in 121 patients, up-front radical cystectomy in five patients and only TUR in 21 patients. Seventy-one patients were BCG non-responders (Fig. 2). Radical cystectomy was performed in 45 patients (31%) due to BCG resistance (n = 16) or muscle-invasive disease (n = 29). At the time of analysis, deaths and distant metastasis occurred in 41 (28%) and 25 (14%) patients, respectively. Clinicopathological characteristics of the cohort are reported in Table 1.

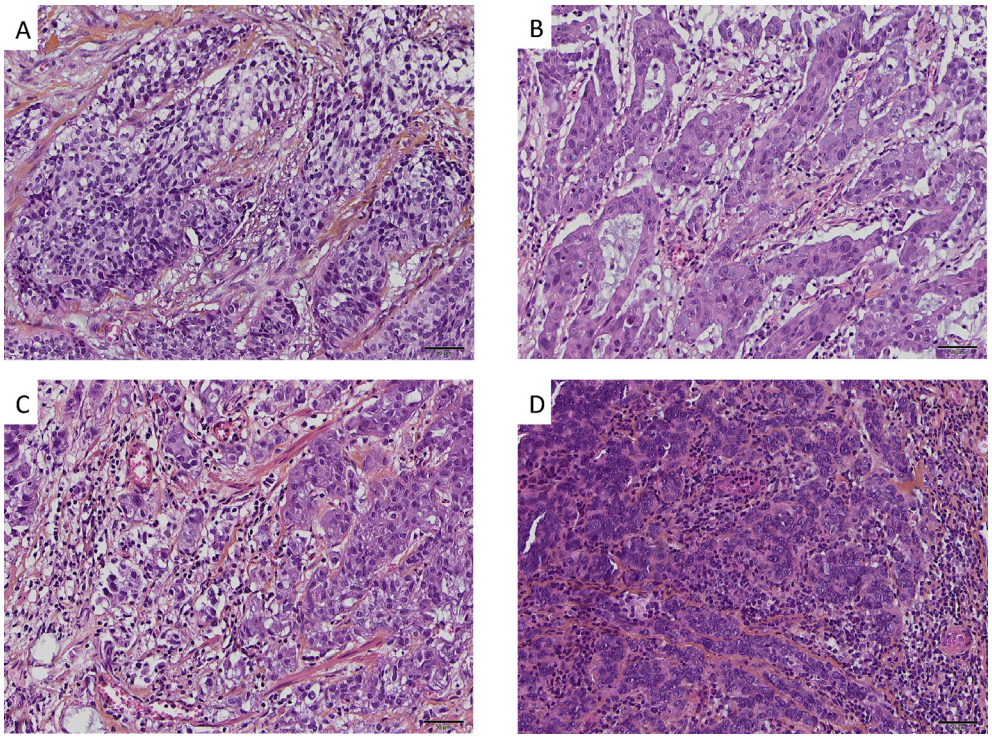

Fig. 1. Histopathologic examples of stromal lymphocytic infiltration in high-grade T1 urothelial carcinoma. (A) Non-intense lymphocytic infiltration (TIL density 0%) B. Non-intense lymphocytic infiltration (TIL density 5%) C. Intense lymphocytic infiltration (TIL density 20%) D. Intense lymphocytic infiltration (TIL density 60%).

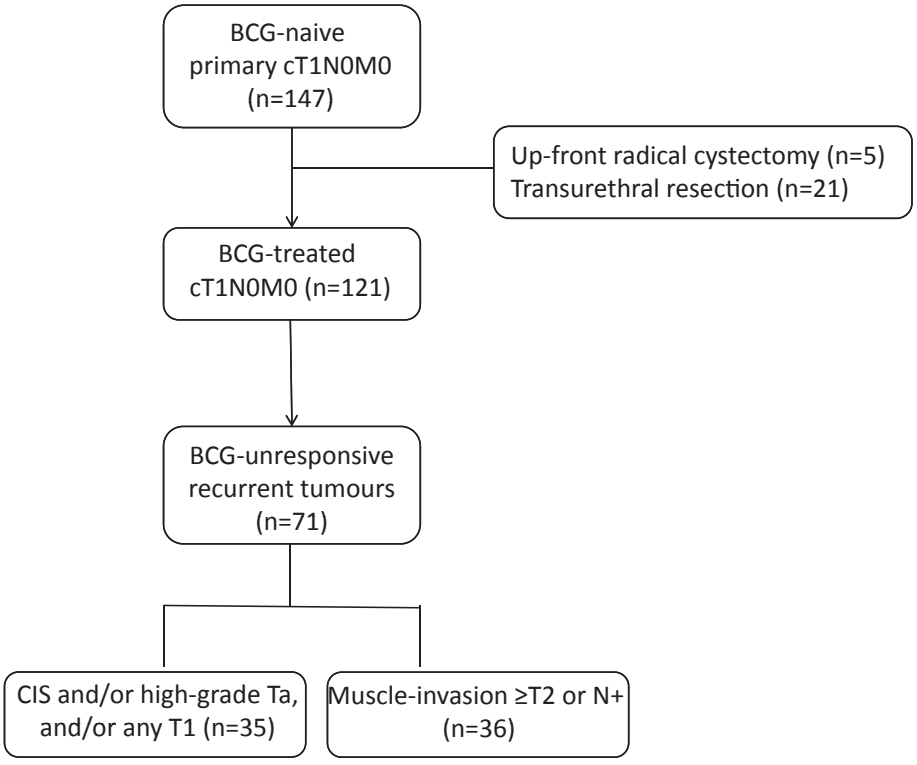

Fig. 2. Flowchart of the primary treatment strategies. BCG, Bacillus Calmette–Guérin.

Table 1
Patient characteristics.

Characteristics		All patients	Stromal TILs		p value
			<10%	≥10%	
		n (%)	n (%)	n (%)	
		147 (100)	65 (44)	82 (56)	
Age	<70 yr.	69 (47)	30 (46)	39 (47)	0.99
	≥70 yr.	78 (53)	35 (54)	43 (53)	
Sex	Female	17 (12)	6 (9)	11 (13)	0.59
	Male	130 (88)	59 (91)	71 (87)	
Smoking	Never	24 (16)	6 (9)	18 (22)	0.18
	Former	62 (42)	29 (45)	33 (40)	
	Current	29 (20)	12 (18)	17 (21)	
	Unknown	32 (22)	18 (28)	14 (17)	
Tumour size	<3 cm	31 (21)	14 (22)	17 (21)	1
	≥3 cm	54 (37)	25 (38)	29 (35)	
	Unknown	62 (42)	26 (40)	36 (44)	
Multifocality	Single	52 (36)	23 (35)	29 (35)	0.87
	Multiple	73 (50)	35 (54)	39 (48)	
	Unknown	21 (14)	7 (11)	14 (17)	
Growth pattern	Papillary	67 (45)	30 (46)	37 (45)	0.92
	Solid	36 (25)	15 (23)	21 (26)	
	Unknown	44 (30)	20 (31)	24 (29)	
Concomitant CIS	Yes	65 (44)	29 (45)	36 (44)	0.99
	No	82 (56)	36 (65)	46 (56)	
Lymphovascular invasion	Yes	15 (10)	06 (9)	9 (11)	0.94
	No	132 (90)	59 (91)	73 (89)	
Invasion depth	T1a	102 (70)	51 (79)	51 (62)	0.05
	T1b	45 (30)	14 (21)	31 (38)	
Histological variants	Yes	40 (27)	11 (17)	29 (35)	0.02
	No	107 (73)	54 (83)	53 (65)	
EORTC risk score	Low	16 (10)	6 (9)	10 (12)	0.67
	High	85 (58)	40 (62)	45 (55)	
	Unknown	46 (32)	19 (29)	27 (33)	
Second look performed	Yes	67 (46)	30 (46)	37 (45)	1
	No	80 (54)	35 (54)	45 (55)	
Primary BCG treatment	Yes	126 (86)	56 (91)	70 (85)	0.99
	No	21 (14)	9 (9)	12 (15)	

TILs, tumour-infiltrating lymphocytes; EORTC, European Organization for Research and Treatment of Cancer; BCG, Bacillus Calmette–Guérin.

3.2. TILs positively associated with the tumour invasion depth and variant histologies

At baseline, the median level of TILs was 20% (range 5%–60%). Stromal lymphocytic infiltration was ≥10% (intense) in 82 tumours (56%) and <10% (non-intense) in 65 tumours (44%). Heterogeneity was observed between the intense (≥10% TILs) versus the non-intense (<10% TILs) subgroups, for tumour invasion depth ($p = 0.05$) and variant histologies ($p = 0.03$) (Table 1). The distribution of lymphocytic infiltration among tumours with variant histologies is reported in Supplementary data Table S1. None of the other clinicopathological characteristics of the patients were associated with the baseline TILs levels. Particularly, no association was found between the density of TILs and concomitant CIS ($p = 0.78$) or lymphovascular invasion ($p = 0.53$). The baseline TIL level was significantly higher ($p = 0.009$) in patients with T1b substage (median, interquartile range [IQR]; 22.8, 10–30) compared with T1a substage (median, IQR; 17.7, 10–28.8) (Fig. 3A). Similarly, the density of TILs significantly increased ($p = 0.01$) in patients with variant histologies (median, IQR; 20, 10–30 compared with patients with pure urothelial carcinoma (median, IQR; 10, 10–27.5) (Fig. 3B).

3.3. Survival analysis

Tumour multiplicity and a high EORTC score were significantly associated with high-risk recurrence in the univariate analysis. Similarly, the tumour size (≥3 cm) was significantly associated with progression-free survival in the univariate analysis (Supplementary data Table S2). Age ≥70 years and lymphovascular invasion remained independent prognostic factors, respectively, for OS and bladder cancer-specific survival in the multivariate models (Table 2). Kaplan–Meier analysis of cancer-specific survival according to the TIL density showed an HR of 1.70 with 95% CI (0.7–3.9; $p = 0.2$). No statistical difference was found when adjusting on other prognostic factors (adjusted HR = 1.8; 95% CI 0.8–4.2; $p = 0.18$). Similar results were observed for OS (unadjusted and adjusted HR = 1.2; 95% CI 0.6–2.2, $p = 0.6$); PFS (unadjusted HR = 1.91; 0.6–6.1, $p = 0.3$; adjusted HR = 2.10; 0.7–6.7, $p = 0.22$) and recurrence-free survival (RFS) analyses (unadjusted HR = 0.8; 95% CI 0.4–1.5, $p = 0.5$; adjusted HR = 0.7; 95% CI 0.4–1.4), $p = 0.33$) (Fig. 4).

4. Discussion

Our study encompasses a homogenous population of 147 treatment-naive, primary cT1N0M0 bladder cancer with a long-term median follow-up of 8.2 years. We demonstrate a correlation between stromal TILs and clinical outcomes. The major finding is that the density of stromal TILs was not associated with a better clinical outcome including cancer-specific survival and OS as opposed to numerous neoplasms for other organs. Second, we found that the level of TILs significantly increased with the tumour invasion depth. Third, we also identified a statistically significant difference between the density of stromal TILs in tumours with variant histologies, listed on the WHO 2016 classification of bladder tumours, compared with pure urothelial carcinoma [11]. Altogether, these data further suggest that tumour aggressiveness may be associated with an increased adaptive immune response to the invasion depth and variant histologies in T1 bladder cancer.

Morphological assessment of TILs has been shown to provide prognostic and potentially predictive significance in many different tumour types [6]. A dense T-cell infiltrate is a common characteristic of tumours that have a favourable prognosis [13,14]. However, an

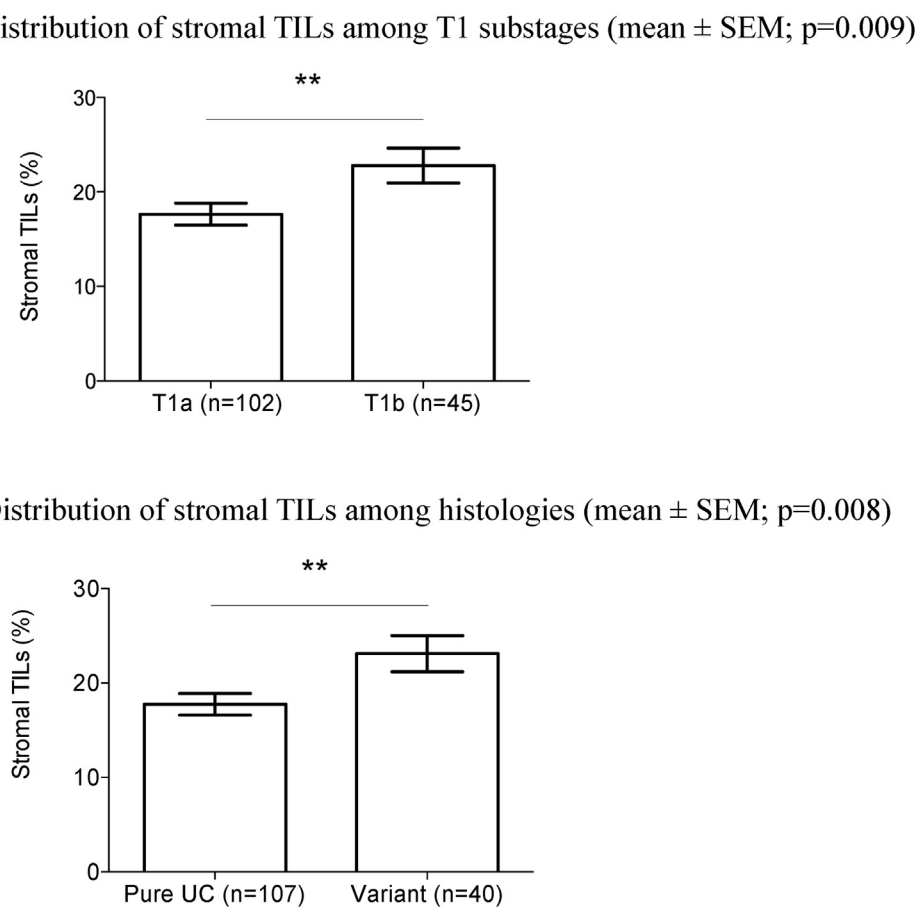

Fig. 3. Association among the density of stromal TILs, tumour invasion depth (A) and variant histologies (B). TILs, tumour-infiltrating lymphocytes; SEM, standard error of the mean.

association between high densities of CD8+ cytotoxic T-cells and short PFS and OS durations has also been reported in several solid tumours, including clear cell renal cell carcinoma and prostate cancer [15–17]. Large studies investigating the pattern of T-cell infiltrate and its potential prognostic value among various stages of urothelial carcinoma are lacking [9].

Sharma *et al.* [18] reported one of the first analyses evaluating the prognostic value of T-cell infiltration in urothelial carcinoma of the bladder (n = 38 pTa or pT1; n = 31 pT2-T4). Indeed, the authors reported that the presence of CD8+ TILs did not influence disease-free survival time among patients with NMIBC ($p = 0.693$) but had a substantial influence among patients with muscle-invasive disease ($p < 0.001$). This finding also suggests that TIL density may be a marker of disease aggressiveness rather than a sign of an ongoing effective antitumour immune response. To enhance the statistical power of our study, we focussed only our analysis on pT1 bladder tumours rather than a mixed population of high-risk NMIBC tumours.

In a previous study, Patschan *et al.* [19] assessed CD3+ T-cells infiltration in a cohort of primary T1 bladder cancer (n = 156) using a tissue microarray technique. The authors showed that high levels of CD3+ T-cells were significantly associated with poor prognosis and progressive disease. In agreement with our findings, they also reported a strong association between high levels of CD3+ T-cells and invasive ≥ pT1b tumours. The authors hypothesised that pT1b disease with high CD3 scores represented tumours that have reached an epithelial depth, which could allow a better immune recognition and therefore trigger an antitumour immune response. Whether the intense lymphocytic infiltration is directly linked to the tumour invasion depth remains difficult to demonstrate. Also, the use of tissue microarray (TMA) calls for great caution regarding the heterogeneity of the *in situ* antitumour immune response. In our study, the pathological assessment of stromal TILs was independently performed by two pathologists using a standardised, reference methodology on whole tissue sections using H&E staining [8,12]. This analysis allowed a specific characterisation of the immune response in the stromal infiltration (pT1) as the immune infiltrate in pTa and CIS is limited to the urothelial layer.

Recently, Wang *et al.* [20] investigated the clinical significance of CD103+ T-cells, a marker of tissue

Table 2
Multivariate analysis of clinicopathological factors for recurrence, progression, cancer-specific and overall survival.

Clinicopathological factors		No. events/ No. Patients	Univariate		Multivariate	
			HR (95% CI)	*p*	HR (95% CI)	*p*
Overall survival						
TILs	<10%	17/65	1		1	
	≥10%	24/82	1.2 (0.6–2.2)	0.6	1.2 (0.6–2.2)	0.6
Age (years)	<70	16/69	1		1	
	≥70	25/78	2.4 (1.3–4.5)	0.01	2.2 (1.2–4.2)	**0.02**
Variant	No	36/107	1		1	
	Yes	5/40	0.4 (0.2–1.1)	0.06	0.4 (0.2–1.1)	0.09
Cancer-specific free survival						
TILs	<10%	8/65	1		1	
	≥10%	17/82	1.7 (0.7–3.9)	0.2	1.8 (0.8–4.2)	0.18
Age (years)	<70	10/69	1		1	
	≥70	15/78	2.1 (0.9–4.8)	0.07	2.1 (0.9–4.7)	0.08
Lymphovascular invasion	No	20/132	1		1	
	Yes	5/15	2.7 (1.0–7.3)	0.04	3.1 (1.2–8.4)	**0.03**
Variant	No	22/107	1		1	
	Yes	3/40	0.4 (0.1–1.3)	0.1	0.40 (0.1–1.3)	0.14
Progression-free survival[b]						
TILs	<10%	4/26	1		1	
	≥10%	10/33	1.9 (0.6–6.1)	0.3	2.10 (0.7–6.7)	0.22
Growth pattern	Papillary	4/34	1		1	
	Solid	10/25	3.9 (1.2–12.5)	0.02	2.50 (0.7–9.8)	0.18
EORTC score	Low	1/15	1		1	
	High	13/44	5.2 (0.7–40.0)	0.1	3.19 (0.3–31.4)	0.32
Tumour size	<3 cm	4/29	1		1	
	≥3 cm	10/30	3.19 (1–10.2)	0.05	1.27 (0.3–5.3)	0.75
Recurrence-free survival [a]						
TILs	<10	18/33	1		1	
	≥10	21/41	0.8 (0.4–1.5)	0.5	0.7 (0.37–1.39)	0.33
No. tumours	Unique	19/44	1		1	
	Multiple	20/30	1.9 (1–3.5)	0.05	1.8 (0.9–3.6)	0.12
Growth pattern	Papillary	5/15	1		1	
	Solid	34/59	2.3 (0.9–5.9)	0.08	1.8 (0.7–5.1)	0.25
EORTC score	Low	11/25	1		1	
	High	28/49	1.5 (0.8–3.1)	0.2	1.0 (0.5–2.3)	0.95

TILs, tumour-infiltrating lymphocytes; EORTC, European Organization for Research and Treatment of Cancer; HR, hazard ratio; CI, confidence interval; Bold values $p<0.05$.

[a] Seventy-four.

[b] Fifty-nine patients with all the risk factors available were included in the multivariable analyses.

resident memory CD8+ T-cells, in a cohort of Ta-T4 urothelial carcinoma of the bladder (n = 302). Interestingly, the authors reported that the density of intratumour CD103 + TILs could represent a favourable prognostic of overall and RFS. As the dual role of the host's immunity in promoting or suppressing tumour growth has been well established [21], in depth analysis of inhibitory mechanisms negatively regulating T-cell activation is required.

Several studies analysed the impact of the tumour immune microenvironment on BCG therapy, without reaching formal and definitive conclusions [22–24]. Anti-PD1/PD-L1 immune checkpoint inhibitors have become the gold standard treatment in the second-line setting of metastatic urothelial carcinoma [25,26]. Interestingly, data from a large retrospective study (n = 296) showed that high mRNA expression of PD-L1 was associated with better outcomes in pT1 NMIBC [27]. These antibodies are now evaluated in monotherapies or combination therapies in the HGT1 setting [28]. From a research standpoint, extensive characterisation of the immune infiltrate using longitudinal samples during immunotherapy may further improve our understanding of immune escape.

Limitations of this study include the retrospective nature of the analysis and the absence of validated scoring system to assess stromal TILs. Therefore, external validation of the results is required. Furthermore, not all patients had a second-look TUR performed. Thus, understaging may not be formally excluded even if only HGT1 tumours with a visible, clearly identifiable and disease-free muscularis propria were included. Unquestionably, constitution of NMIBC cohorts with sufficient amount of tumour tissue available and high-quality clinical annotations is a critical step to further allow in-depth molecular and immune profiling of such tumours.

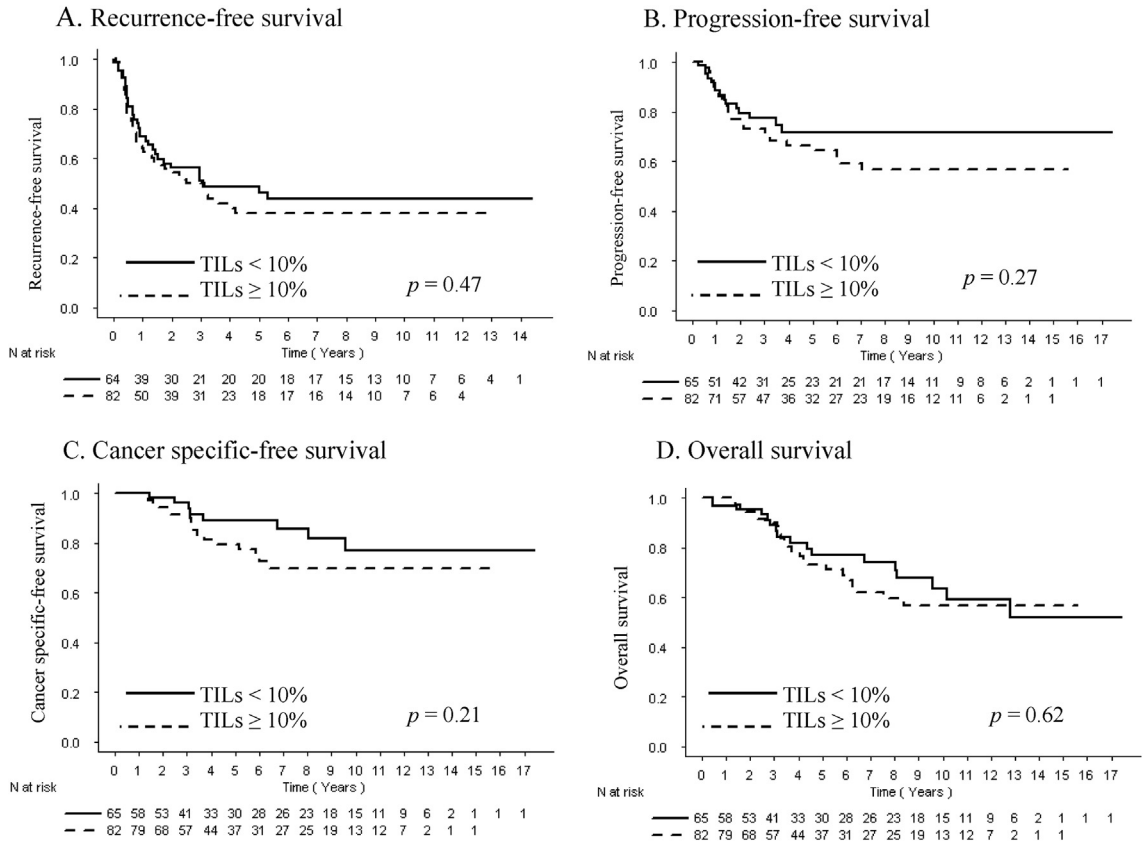

Fig. 4. Kaplan–Meier survival curves according to stromal TILs percentage for recurrence-free survival (A), progression-free survival (B) cancer-specific survival (C) and overall survival (D). The P value of the log-rank test is reported. TILs, tumour-infiltrating lymphocytes.

5. Conclusions

In summary, the present study showed that the density of stromal TILs is associated with tumour invasion depth and aggressive variant histologies in patients with completely resected primary high-grade T1 bladder cancer. A lack of correlation with clinical outcomes suggests a different prognostic value of TILs between NMIBC and MIBC. These data suggest that tumour progression is associated with an increased adaptive immune response in HGT1 bladder cancer. The role of other parameters including myeloid cells, B-cells and tumour stroma cells may be critical to enhance our knowledge of the host immune response in HG T1 bladder cancer. Therefore, the assessment of the immune infiltrate in longitudinal samples may be a cornerstone to unravel the mechanisms of BCG refractory tumours.

Conflict of interest statement

All the authors have no conflict of interest to declare.

Acknowledgements

The authors would like to thank Alice Perchenet for technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2018.12.010.

Funding

This work was supported by a grant for research into cancer immunotherapy from the French Association of Urology (AFU).

References

[1] Hurst C, Rosenberg J, Knowles M. SnapShot: bladder cancer. Cancer Cell 2018 13;34(2). https://doi.org/10.1016/j.ccell.2018.07.013. 350-350.
[2] Hedegaard J, Lamy P, Nordentoft I, et al. Comprehensive transcriptional analysis of early-stage urothelial carcinoma. Cancer Cell 2016;30(1):27–42. https://doi.org/10.1016/j.ccell.2016.05.004.
[3] Gontero P, Sylvester R, Pisano F, et al. Prognostic factors and risk groups in T1G3 non-muscle-invasive bladder cancer patients initially treated with Bacillus Calmette-Guérin: results of a retrospective multicenter study of 2451 patients. Eur Urol 2015;67(1):74–82. https://doi.org/10.1016/j.eururo.2014.06.040.
[4] Kakiashvili DM, van Rhijn BW, Trottier G, et al. Long-term follow-up of T1 high-grade bladder cancer after intravesical bacille Calmette-Guérin treatment. BJU Int 2011;107(4):540–6. https://doi.org/10.1111/j.1464-410X.2010.09572.x.

[5] Tan TZ, Rouanne M, Tan KT, Huang RY, Thiery TP. Molecular subtypes of urothelial bladder cancer: results from a meta-cohort analysis of 2411 tumors. Eur Urol 2018 Sep 10. https://doi.org/10.1016/j.eururo.2018.08.027. pii: S0302-2838(18)30619-5, [Epub ahead of print] PMID: 30213523.

[6] Fridman WH, Zitvogel L, Sautès-Fridman C, et al. The immune contexture in cancer prognosis and treatment. Nat Rev Clin Oncol 2017;14(12):717–34. https://doi.org/10.1038/nrclinonc.2017.101.

[7] Pruneri G, Lazzeroni M, Bagnardi V, et al. The prevalence and clinical relevance of tumor-infiltrating lymphocytes (TILs) in ductal carcinoma in situ of the breast. Ann Oncol 2017;28(2):321–8. https://doi.org/10.1093/annonc/mdw623.

[8] Hendry S, Salgado R, Gevaert T, et al. Assessing tumor-infiltrating lymphocytes in solid tumors: a practical review for pathologists and proposal for a standardized method from the international immuno-oncology biomarkers working group: Part 2: TILs in melanoma, gastrointestinal tract carcinomas, non-small cell lung carcinoma and mesothelioma, endometrial and ovarian carcinomas, squamous cell carcinoma of the head and neck, genitourinary carcinomas, and primary brain tumors. Adv Anat Pathol 2017;24(6):311–35. https://doi.org/10.1097/PAP.0000000000000161.

[9] Solinas C, Chanzá NM, Awada A, et al. The immune infiltrate in prostate, bladder and testicular tumors: an old friend for new challenges. Cancer Treat Rev 2017;53:138–45. https://doi.org/10.1016/j.ctrv.2016.12.004.

[10] Humphrey PA, Moch H, Cubilla AL, et al. The 2016 WHO classification of tumours of the urinary system and male genital organs-Part B: prostate and bladder tumours. Eur Urol 2016;70(1):106–19. https://doi.org/10.1016/j.eururo.2016.02.028.

[11] Paner GP, Stadler WM, Hansel DE, et al. Updates in the eighth edition of the tumor-node-metastasis staging classification for urologic cancers. Eur Urol 2018;73(4):560–9. https://doi.org/10.1016/j.eururo.2017.12.018.

[12] Salgado R, Denkert C, Demaria S, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol 2015;26(2):259–71. https://doi.org/10.1093/annonc/mdu450.

[13] Fridman WH, Pagès F, Sautès-Fridman C, et al. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Canc 2012;12(4):298–306. https://doi.org/10.1038/nrc3245.

[14] Fridman WH, Galon J, Pagès F, et al. Prognostic and predictive impact of intra- and peritumoral immune infiltrates. Cancer Res 2011;71(17):5601–5. https://doi.org/10.1158/0008-5472.CAN-11-1316.

[15] Giraldo NA, Becht E, Pagès F, et al. Orchestration and prognostic significance of immune checkpoints in the microenvironment of primary and metastatic renal cell cancer. Clin Canc Res 2015;21(13):3031–40. https://doi.org/10.1158/1078-0432.CCR-14-2926.

[16] Becht E, Giraldo NA, Beuselinck B, et al. Prognostic and theranostic impact of molecular subtypes and immune classifications in renal cell cancer (RCC) and colorectal cancer (CRC). OncoImmunology 2015;4(12). e1049804.

[17] Petitprez F, Fossati N, Vano Y, et al. PD-L1 expression and CD8+ T-cell infiltrate are associated with clinical progression in patients with node-positive prostate cancer. Eur Urol Focus 2017;(17):30151–7. https://doi.org/10.1016/j.euf.2017.05.013. pii: S2405-4569.

[18] Sharma P, Shen Y, Wen S, et al. CD8 tumor-infiltrating lymphocytes are predictive of survival in muscle-invasive urothelial carcinoma. Proc Natl Acad Sci U S A 2007;104(10):3967–72.

[19] Patschan O, Sjödahl G, Chebil G, et al. A molecular pathologic framework for risk stratification of stage T1 urothelial carcinoma. Eur Urol 2015;68(5):824–32. https://doi.org/10.1016/j.eururo.2015.02.021. discussion 835-6.

[20] Wang B, Wu S, Zeng H, et al. CD103+ tumor infiltrating lymphocytes predict a favorable prognosis in urothelial cell carcinoma of the bladder. J Urol 2015;194(2):556–62. https://doi.org/10.1016/j.juro.2015.02.2941.

[21] Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011;331(6024):1565–70. https://doi.org/10.1126/science.1203486.

[22] Ayari C, LaRue H, Hovington H, et al. Bladder tumor infiltrating mature dendritic cells and macrophages as predictors of response to bacillus Calmette-Guérin immunotherapy. Eur Urol 2009;55(6):1386–95. https://doi.org/10.1016/j.eururo.2009.01.040.

[23] Pichler R, Fritz J, Zavadil C, et al. Tumor-infiltrating immune cell subpopulations influence the oncologic outcome after intravesical Bacillus Calmette-Guérin therapy in bladder cancer. Oncotarget 2016;7(26):39916–30. https://doi.org/10.18632/oncotarget.9537.

[24] Pichler R, Gruenbacher G, Culig Z, et al. Intratumoral Th2 predisposition combines with an increased Th1 functional phenotype in clinical response to intravesical BCG in bladder cancer. Cancer Immunol Immunother 2017;66(4):427–40. https://doi.org/10.1007/s00262-016-1945-z.

[25] Siefker-Radtke AO, Apolo AB, Bivalacqua TJ, et al. Immunotherapy with checkpoint blockade in the treatment of urothelial carcinoma. J Urol 2018;199(5):1129–42. https://doi.org/10.1016/j.juro.2017.10.041.

[26] Rouanne M, Roumiguié M, Houédé N, et al. Development of immunotherapy in bladder cancer: present and future on targeting PD(L)1 and CTLA-4 pathways. World J Urol 2018. https://doi.org/10.1007/s00345-018-2332-5.

[27] Breyer J, Wirtz RM, Otto W, et al. High PDL1 mRNA expression predicts better survival of stage pT1 non-muscle-invasive bladder cancer (NMIBC) patients. Cancer Immunol Immunother 2018;67(3):403–12. https://doi.org/10.1007/s00262-017-2093-9.

[28] Wankowicz SAM, Werner L, Orsola A, et al. Differential expression of PD-L1 in high grade T1 vs muscle invasive bladder carcinoma and its prognostic implications. J Urol 2017;198(4):817–23. https://doi.org/10.1016/j.juro.2017.04.102.