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ORIGINAL ARTICLE

Absolute basophil count is associated with time to recurrence in patients with high-grade T1 bladder cancer receiving bacillus Calmette–Guérin after transurethral resection of the bladder tumor

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Abstract

Background Basophils, eosinophils and monocytes may be involved in BCG-induced immune responses and be associated with outcomes of bladder cancer patients receiving intravesical BCG. Our objective was to explore the association of baseline counts of basophils, eosinophils and monocytes with outcomes of patients with high-grade T1 bladder cancer receiving a standard course of intravesical BCG.

Methods We retrospectively reviewed medical records of patients with primary T1 HG/G3 bladder cancer. After re-TURBT, patients were treated with a 6-week course of intravesical BCG induction followed by intravesical BCG every week for 3 weeks given at 3, 6, 12, 18, 24, 30 and 36 months from initiation of therapy The analysis of potential risk factors for recurrence, muscle invasion and cancer-specific and overall survival was performed using univariable Cox regression models. Those factors that presented, at univariate analysis, an association with the event at a liberal $p < 0.1$, have been selected for the development of a multivariable model.

Results A total of 1045 patients with primary T1 HG/G3 were included. A total of 678 (64.9%) recurrences, 303 (29.0%) progressions and 150 (14.3%) deaths were observed during follow-up. Multivariate analysis showed that logarithmic transformation of basophils count was associated with a 30% increment in the hazard of recurrence per unit increase of logarithmic basophils count (HR 1.30; 95% confidence interval 1.09–1.54; $p = 0.0026$). Basophil count modeled by quartiles was also significantly associated with time to recurrence [second vs. lower quartile HR 1.42 (1.12–1.79); $p = 0.003$, third vs. lower quartile HR 1.26 (1.01–1.57); $p = 0.041$; upper vs. lower quartile HR 1.36 (1.1–1.68); $p = 0.005$]. The limitations of a retrospective study are applicable.

Conclusion Baseline basophil count may predict recurrence in BCG-treated HG/G3 T1 bladder cancer patients. External validation is warranted.

Keywords Bladder cancer · Basophils · BCG

Introduction

In 2012, bladder cancer was diagnosed in 430,000 people and caused 165,000 deaths worldwide [1]. The incidence has increased in some eastern European and developing countries, while it has decreased in most Western countries [2], although some areas in Western Europe (e.g. province of Naples, Italy [3]) present an unusually high incidence and prevalence. Patients with non-muscle invasive disease receiving an adequate course of intravesical Bacillus Calmette–Guérin (BCG) show a 1-and 5-year recurrence rate of approximately 25 and 40%, respectively [4]. After BCG approval by the FDA in 1990, no other intravesical treatment has proved to be superior to BCG, but a large body of evidence have been collected about the immunological

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mechanisms underlying its effectiveness in this setting [5]. In patients with high-grade T1 tumors receiving adjuvant BCG, the risk of recurrence can be modeled using multiple clinical and pathologic variables, which include tumor size, age and presence of carcinoma in situ [4], peripheral blood neutrophil-to-lymphocyte ratio and presence of high-grade T1 on re-Transurethral Resection of bladder tumor (TURBT) [6], obesity [7], as well as lymph-vascular invasion [8]. Importantly, while the predictive value of neutrophil to lymphocyte ratio has been widely confirmed in bladder cancer patients treated with BCG [9], the putative predictive value of other white blood cells assessed in the peripheral blood, which may be involved in BCG-induced immune responses, is yet to be adequately investigated.

On the basis of the alleged mechanisms of action of BCG [5], we hypothesized that baseline levels of monocytes, basophils and eosinophils measured in the peripheral blood at the time of first TURB could be associated with clinical outcomes in G3/HG (grade 3/high grade) T1 bladder cancer patients receiving intravesical BCG. Data of a large, multi-institutional, retrospective cohort that had been assessed elsewhere for a different purpose [6] were re-analyzed to explore this hypothesis.

Patients and methods

Patients and methods

The patient population and the methods used to collect the data analyzed here have already been described [6]. Briefly, patients were included in this cohort if they had pathologically proven T1 HG/G3 at first TURBT. Patients underwent re-TURBT performed within 4–6 weeks after the first TURBT to maximally resect residual tumor. Patients included in this cohort had no residual tumor or non-muscle invasive residual tumor at re-TURBT and were treated with a 6-week course of intravesical BCG induction followed by intravesical BCG every week for 3 weeks given at 3, 6, 12, 18, 24, 30 and 36 months from initiation of therapy. Patients with signs of acute infection or incomplete data were excluded from this study cohort. In the analysis performed here, only patients with full blood count data performed before TURBT, including absolute while blood cell, absolute neutrophil, absolute lymphocyte, absolute eosinophil, absolute basophil and absolute monocyte counts were included.

The following data observed within 2 weeks of first TURBT were retrieved: age, gender, smoking history (categorized as current smoker, non-smoker or former smoker), SED (sedimentation) rate, C reactive protein (CRP), serum albumin, body mass index, full blood count. The following pathology findings related to TURBT and re-TURBT were retrieved: pathology grade and stage, maximum tumor size, presence of multiple tumors of the bladder, presence of concomitant carcinoma in situ, presence of HG/G3 disease at re-TURBT, lympho-vascular invasion (LVI). The following data about cancer history were retrieved: date of first TURBT, date of re-TURB, date of first progression (if patient had progressed), date of first recurrence (if patient had recurred), date and cause (Author query: Please consider rephrasing the following sentence: date of first TURBT, date of re-TURB, date of first progression (if patient had progressed), date of first recurrence (if patient had recurred), date and cause of (if patient had died), date of last follow-up.)of (if patient had died), date of last follow-up. Maximum tumor size was defined as the longest diameter of the largest lesion. The TNM system of the Union for International Cancer Control and the 1973 World Health Organization grading classification were followed by the local pathologist for the pathology analysis. Recurrent disease was defined as any disease recurrence, both non muscle-invasive and muscle-invasive, diagnosed during follow-up. Progressive disease was defined as muscle-invasive disease diagnosed during follow-up. The endpoints were time to recurrent disease (TTR), time to progressive disease (TTP), overall- and cancer-specific survival (OS and CSS).

Statistical analysis

Descriptive statistics have been used to characterize the overall cohort with respect to the main demographical and clinical characteristics. Median follow-up was computed according to the reverse Kaplan–Meier. Survival curves were estimated with the Kaplan–Meier method. The analysis of potential risk factors for recurrence, muscle invasion and overall survival was performed using univariable Cox regression models. Those factors that presented, at univariate analysis, an association with the event at a liberal $p < 0.1$, have been selected for the development of a multivariable model. The proportional hazard (PH) assumption was tested using the Schoenfeld residuals. When evidence of violation of PH assumption was found a weighted Cox regression model was estimated using the method described in literature [10], which provides unbiased average HR estimates irrespective of proportionality of hazards. A supportive analysis was conducted considering the overall recurrence rate at the landmark time points of 24, 36 and 48 months. Patients with a shorter follow-up were excluded from this analysis if they had not recurred during follow-up. Basophiles count was modeled using both the observed quartiles and logarithmic transformation, thus allowing for its nonlinear association with time-to-event variables. For all statistical comparisons, a level of p value < 0.05 was accepted as statistically significant. All analyses were performed using r version 3.5.1 (http://www.r-project.org).

Results

Patient population

A total of 1045 patients with primary T1 HG/G3 treated between January 1, 2002 and December 31, 2012 at 13 academic institutions were eligible to be included in this analysis. Main characteristics of the study population are depicted in Table 1. Median follow-up was 26 months [interquartile range (IQR) 10–47 months]. Three hundred and three (29%) patients received the full 36-month BCG treatment. A total of 678 (64.9%) exhibited recurrence, 303 exhibited (29.0%) progressions, and 150 (14.3%) all-cause deaths and 77 bladder cancer-related deaths (7.3%) were observed during follow-up.

Table 1 Characteristics of the patient population

	Overall cohort ($n=1045$)[a]
Age (years)	70 [64; 77] (29–91)
Gender; female	182 (17.4)
BMI (kg/m^2)	27 [23.8; 29] (16–38.1)
Smoking status	
Yes	484 (46.3)
No	295 (28.2)
Former	266 (25.5)
Multifocality	460 (44)
Tumor size > 3 cm	673 (64.5)
Concomitant Cis	149 (14.3)
LVI	165 (15.8)
LVI at re-turb	59 (5.6)
Residual disease at re-turb	257 (24.6)
SED (mm/h)	13 [8; 22] (1–109)
CRP (mg/dL)	2 [0.7; 4.6] (0–33.8)
Albumin (g/dL)	4.2 [3.9; 4.5] (1.1–7.1)
Neutrophils/lymphocytes ratio	2.9 [1.9; 4.6] (0.5 to 22.2)
Monocytes count ($\times 10^3$/μL)	0.53 [0.4; 0.76] (0.01–6.7)
Eosinophils count (10^3/μL)	0.18 [0.1; 0.29] (0–2)
Basophils count (10^3/μL)	
1° quartile [min to 0.01]	278 (26.6)
2° quartile [0.01–0.03]	202 (19.3)
3° quartile [0.03–0.05[	266 (25.5)
4° quartile [0.05 to max]	299 (28.6)
Platelets count (×10^3/μL)	232 [193; 284.5] (40–486)

BMI body mass index, *Cis* carcinoma in situ, *LVI* lymph vascular invasion, *SED* sedimentation rate, *CRP* C reactive protein

[a]Data are expressed as either median [25th; 75th percentile] (min to max) or absolute frequencies (percentages)

Results of univariate and multivariate analysis

At univariate analysis, absolute monocytes and eosinophil counts were not associated with either time to recurrence, time to progression or overall survival, while logarithmic transformation of basophils count showed a 30% increment in the hazard of recurrence per unit increase (HR 1.30; 95% confidence interval 1.10–1.53; $p=0.0018$). A significant association between high basophil count and shorter time to recurrence was also found for the second vs. the first and the fourth vs. the first quartile of the baseline basophil count distribution (Table 2). The median recurrence-free survival time in the four baseline basophil count quartiles were the following: 43 months (95% CI 33–52 months), 22 months (95% CI 16–35 months), 31 months (95% CI 24–46 months) and 22 months (95% CI 17–27 months) (Fig. 1). No association of baseline basophil count was reported with overall- and cancer-specific survival (data not shown). Baseline basophil count was associated with time to progression at univariate analysis when analyzed as a continuous, log-transformed variable (HR 1.32; 95% confidence interval 1.02–1.71; $p=0.0296$), but not as a categorial variable defined by quartiles (data not shown).

Multivariate analysis confirmed that logarithmic transformation of basophils count was associated with a 30% increment in the hazard of recurrence per unit increase (HR 1.30; 95% confidence interval 1.09–1.54; $p=0.0026$). Based on this finding, we modeled basophil count using quartiles and explored the association with recurrence using the lower quartile as reference for the others. The three upper quartiles were all significantly and consistently associated with increased risk of recurrence at multivariate analysis when compared to the lower quartile [second vs. lower quartile HR 1.42 (1.12–1.79); $p=0.003$; third vs. lower quartile HR 1.26 (1.01–1.57); $p=0.041$; upper vs. lower quartile HR 1.36 (1.1–1.68); $p=0.005$) (Table 2), but it was not associated with time to progression or overall survival (data not shown). Univariate and multivariate logistic-regression analysis showed that the odds of having a recurrence within 36 months were more than double in patients in the second, third and fourth quartiles vs. the first (see Table 3). Similar results were obtained considering the overall recurrence rate at 24 and 48 months (data not shown).

Discussion

Adaptive T-cell mediated immune responses have been classically divided in type 1 (Th1) and type 2 (Th2) T helper cell responses [11]. Th1 vs. Th2 polarization has been related to different cytokine patterns, with the former being associated with secretion of interferon (IFN)-gamma, interleukin (IL)-2 and tumor necrosis factor (TNF)-beta,

Table 2 Univariate and multivariate analysis of predictors of time to recurrence

Time to recurrence				
	Univariate		Multivariate	
	HR [95% CI]	*p* value	HR [95% CI]	*p* value
Age (years)	0.99 [0.99–1]	0.161		
Gender; female	**1.24 [1.03–1.5]**	**0.023**	1.06 [0.88–1.29]	0.53
BMI (kg/m^2)	**1.17 [1.15–1.2]**	**< 0.001**	**1.13 [1.1–1.15]**	**< 0.001**
Smoking status				
Yes	Ref	–	Ref	–
No	0.95 [0.8–1.13]	0.572	1 [0.83–1.2]	0.964
Former	**0.56 [0.46–0.68]**	**< 0.001**	**0.62 [0.5–0.77]**	**< 0.001**
Multifocality	**1.32 [1.13–1.53]**	**< 0.001**	1.1 [0.95–1.29]	0.209
Tumor size > 3 cm	**1.3 [1.11–1.53]**	**0.001**	1.16 [0.98–1.37]	0.083
Concomitant Cis	1.19 [0.97–1.47]	0.095	1.08 [0.87–1.33]	0.501
LVI at TURB	**1.53 [1.26–1.85]**	**< 0.001**	**1.36 [1.12–1.66]**	**0.002**
LVI at re-TURB	**2.9 [2.21–3.8]**	**< 0.001**	**2.07 [1.56–2.76]**	**< 0.001**
Pathologic grade at re-turb G3	**1.74 [1.48–2.06]**	**< 0.001**	**1.36 [1.14–1.62]**	**< 0.001**
SED (mm/h)	**1.01 [1–1.01]**	**0.002**	1 [0.99–1]	0.24
CRP (mg/dL)	**1.03 [1.01–1.04]**	**0.001**	1.01 [0.99–1.03]	0.22
Albumin (g/dL)	1.07 [0.93–1.23]	0.335		
Neutrophils/lymphocytes ratio	**1.16 [1.14–1.19]**	**< 0.001**	**1.12 [1.09–1.15]**	**< 0.001**
Monocytes count (× 10^3/μL)	0.89 [0.77–1.03]	0.112		
Eosinophils count (× 10^3/μL)	1 [0.71–1.41]	0.999		
Basophils count (× 10^3/μL)				
1° quartile [min to 0.01]	**Ref**	**–**	**Ref**	**–**
2° quartile [0.01–0.03]	**1.38 [1.1–1.72]**	**0.005**	**1.42 [1.12–1.79]**	**0.003**
3° quartile [0.03–0.05]	1.11 [0.9–1.38]	0.334	**1.26 [1.01–1.57]**	**0.041**
4° quartile [0.05 to max]	**1.33 [1.09–1.64]**	**0.006**	**1.36 [1.1–1.68]**	**0.005**
Platelets count (× 10^3/μL)	**1.13 [1.02–1.25]**	**0.017**	1.11 [0.99–1.23]	0.069

Bold values indicate significant correlations at the level of 0.05

BMI body mass index, *Cis* carcinoma in situ, *LVI* lymph vascular invasion, *SED* sedimentation rate, *CRP* C reactive protein

which stimulate phagocytosis, and the latter being associated with IL-4, IL-5, IL-6, IL-10 and IL-13, which stimulate antibody production (including antibodies belonging to the IgE class), but inhibit several functions of phagocytic cells and can exert an anti-inflammatory effect. Although type 2 immune reactions have been discovered to be useful not only in fighting helminthic worm infections, but also in suppressing type 1-driven autoimmune disease, neutralizing toxins and maintaining metabolic homeostasis, they are also associated with a variety of morbid conditions related to atopic reactions, including asthma, anaphylaxis and rhinitis [11]. In the particular case of intravesical BCG therapy in early bladder cancer, a large body of evidence suggests that a Th2 cell-polarized response is associated with BCG ineffectiveness, while type 1 helper T cells induce BCG-mediated immune responses capable of anti-tumor activity [12] [13] [14] [15]. Urine levels of selected Th1 and Th2 cytokines have been successfully assessed as biomarkers of response to BCG treatment. Increased urine levels of IL-2 [16], a Th1 cytokine secreted by CD4+ T cells that exerts its main effects by stimulating cytotoxic CD8+ lymphocyte and macrophage activity, have been reported in BCG responders vs. non-responders. A higher interleukin-6/10 ratio has been associated with increased risk of recurrence of BCG-treated patients, while another study found that IL-2 urine levels showed an earlier peak than IL-10 levels in responders vs. non responders.

Basophils may have a role in BCG-treated bladder cancer patients because of their ability to facilitate Th2 polarization by secreting IL-4 and skewing antigen-presenting cells towards a type 2 response [17]. In fact, studies in mouse models have shown that depletion of basophils can impair Th2 polarization [18] and that Th2 responses to cysteine protease are mediated by a complex interplay between migratory dermal dendritic cells (DCs) and basophils positive for interleukin 4 (IL-4) via ROS-mediated signaling [19].

Although literature data support the non-redundant role of basophils in Th2 responses [20], their significance in solid

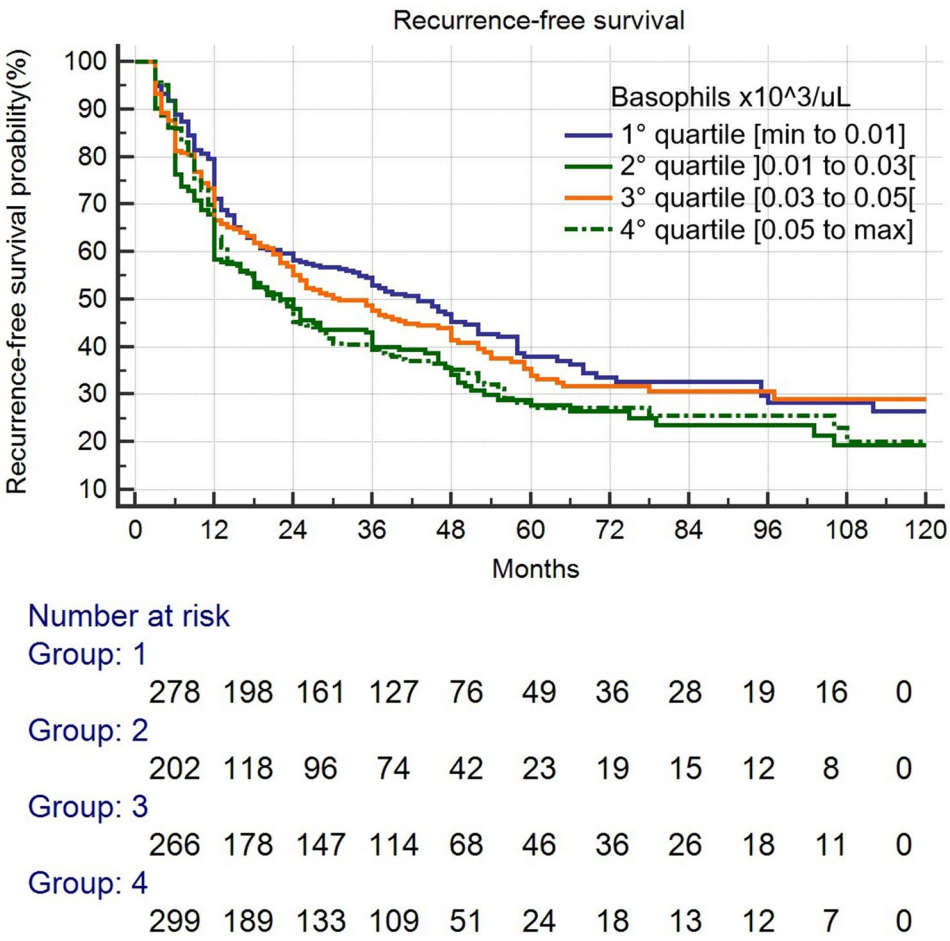

Fig. 1 Recurrence free survival by basophils quartiles

malignancies has been poorly investigated. One preclinical/clinical study conducted in 85 pancreatic ductal adenocarcinoma patients and in a mouse model [21] reported three main findings that clarify the role of basophils identified in tumor-draining lymph nodes in inducing Th-2 responses. First, IL-4 levels were higher in tumor-draining vs. non-tumor draining lymphnodes and the lymph node fraction containing basophils also had higher IL-4 levels. Furthermore, IL-4 mRNA expression correlated with basophil presence demonstrated at immunohistochemistry, supporting the role of basophils as source of IL-4 in tumor tumor-draining lymphnodes. Second, the percentage of basophils in tumor-draining lymphnodes positively correlated with the ratio of GATA-3+/T-bet+ lymphoid infiltrates, which is indicative of Th2 polarization [22]. Third, multivariate analysis in 36 patients with available survival data stratified for known prognostic variables showed that the percentage of basophils in tumor-draining lymphnodes was independently predictive of disease-free survival ($p = 0.02$, HR = 11.07, range 1.38–88.60) and overall survival ($p = 0.04$, HR = 8.51, range 1.04–69.33). All the limitations of a retrospective study are applicable to our work. Specifically, the study design could have been improved by collecting basophil count at various time points and by using more stringent exclusion criteria, e.g. by excluding patients affected by parasitic infections or other conditions that may affect basophil count [23]. The use of different laboratory equipment and methods among the participating centers may also constitute a bias. We noted that basophil count analyzed both as a logarithmic-transformed continuous variable and when modeled by using quartiles was associated with time to recurrence at multivariate analysis, although the third quartile appeared to have better time to recurrence than the second quartile, as shown in Fig. 1. This finding does not emerge when all the available predictive variables are included in the multivariate analysis (Table 2) and is probably the result of confounding factors. Nevertheless, as the underlying biology of the relationship between basophils and BCG effectiveness—if it does exist—is unknown, we cannot exclude that; further studies may identify separate basophil count intervals that are associated with worse time to recurrence. We hypothesize that the reported findings are consistent with the known role of basophils in mediating Th2 polarization, which gives biological plausibility to the statistically significant association reported. In fact, patients with high basophils before receiving BCG(e.g. > 10 basophils/ml) may benefit

Table 3 Univariate and multivariate analysis of predictors of recurrence within 36 months of follow-up

Recurrence within 36 months of follow-up				
	Univariate		Multivariate	
	HR [95% CI]	*p* value	HR [95% CI]	*p* value
Age (years)	0.99 [0.98–1.01]	0.277		
Gender; female	**1.65 [1.11–2.47]**	**0.014**	**2.03 [1.12–3.67]**	**0.02**
BMI (kg/m^2)	**1.51 [1.43–1.6]**	**< 0.001**	**1.43 [1.33–1.53]**	**< 0.001**
Smoking status				
Yes	Ref	Ref		
No	0.97 [0.69–1.36]	0.867	1.14 [0.68–1.91]	0.619
Former	**0.45 [0.32–0.63]**	**< 0.001**	0.74 [0.44–1.25]	0.267
Multifocality	**1.54 [1.17–2.04]**	**0.002**	1.42 [0.92–2.17]	0.112
Tumor size > 3 cm	**1.54 [1.16–2.05]**	**0.003**	**1.65 [1.05–2.6]**	**0.031**
Concomitant Cis	1.3 [0.87–1.93]	0.206		
LVI	**1.98 [1.31–3.01]**	**0.001**	1.84 [1–3.4]	0.052
LVI at re-TURB	n.a.	n.a		
Pathologic grade at re-turb G3	**2.85 [1.98–4.1]**	**< 0.001**	**2.12 [1.23–3.65]**	**0.007**
SED (mm/h)	**1.02 [1.01–1.04]**	**< 0.001**	1 [0.99–1.02]	0.748
CRP (mg/dL)	**1.06 [1.02–1.09]**	**0.002**	1.01 [0.96–1.06]	0.737
Albumin (g/dL)	1.13 [0.88–1.46]	0.332		
Neutrophils/lymphocytes ratio	**3.46 [2.9–4.14]**	**< 0.001**	**3.13 [2.54–3.85]**	**< 0.001**
Monocytes count (× 10^3/μL)	**0.74 [0.58–0.94]**	**0.014**	1.15 [0.77–1.73]	0.487
Eosinophils count (× 10^3/μL)	0.93 [0.48–1.8]	0.826		
Basophils count (× 10^3/μL)				
1° quartile [min to 0.01]	Ref	Ref		
2° quartile [0.01–0.03]	**1.56 [1.03–2.36]**	**0.038**	**2.87 [1.51–5.45]**	**0.001**
3° quartile [0.03–0.05]	1.14 [0.78–1.67]	0.487	**2.14 [1.19–3.85]**	**0.011**
4° quartile [0.05 to max]	**1.43 [0.99–2.07]**	**0.058**	**2.85 [1.52–5.36]**	**0.001**
Platelets count (× 10^3/μL)	**1.02 [1–1.04]**	**0.064**	1.01 [0.98–1.04]	0.433

Bold values indicate significant correlations at the level of 0.05

BMI body mass index, *Cis* carcinoma in situ, *LVI* lymph vascular invasion, *SED* sedimentation rate, *CRP* C reactive protein

from a personalized therapeutic strategy to increase BCG effectiveness. IL-4 may be a candidate druggable target in these patients. Anti IL-4 available agents include anti IL-4 monoclonal antibodies [24], a soluble recombinant human IL-4 receptor [25] or a variant form of IL-4 [26]. Even if obtained in a large data-set, we recognize that the findings reported here may simply be the result of some bias. If confirmed by independent investigators, our discovery may unveil novel therapeutic options for a neglected condition that has recorded little improvements in therapy over the past 30 years.

Author contributions MF: project development; data management; data analysis. GDL: project development; data management; data analysis. MDV: project development; manuscript editing. DB: data analysis. FC: Data collection. GL: Project development; manuscript editing. GM: project development; manuscript editing. SDS: project development; manuscript editing. RH: project development; manuscript editing. GG: project development; manuscript editing. GMB: project development; manuscript editing. AG: data management and collection. FDG: data management and collection. RD: data management and collection. FP: project development; manuscript editing. SP: project development; manuscript editing. PV: data management and collection. MB: data management and collection. RS: project development; manuscript editing. GLA: data management and collection. PB: data management and collection. EL: data management and collection. RA: project development; manuscript editing. NC: data management and collection. ARAF: data management and collection. MB: project development; manuscript editing. GIR: data management and collection. VI: project development; manuscript editing. GM: project development; manuscript editing. PDP: project development; manuscript editing. DT: project development; manuscript editing. AC: project development; manuscript editing. LS: project development; manuscript editing. VM: project development; manuscript editing. OdC: project development; manuscript editing. SS: project development; manuscript editing. GS: project development; manuscript editing. CB: conception of the original research hypothesis; project development; data management; data analysis: manuscript writing.

Compliance with ethical standards

Conflict of interest The authors declare they have no potential conflicts of interest to disclose.

Ethical approval This was a retrospective study involving Human Participants treated according to standard clinical practice. All permissions granted by competent Ethics Committees and other authorities were obtained and all informed consents required by the existing law and regulations were collected.

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