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Liquid Biopsy for Predicting Bacillus Calmette-Guérin Unresponsiveness in Non–muscle-invasive Bladder Cancer

Intravesical immunotherapy with bacillus Calmette-Guérin (BCG) is the gold-standard treatment for non–muscle-invasive bladder cancer (NMIBC) with a high risk of recurrence or progression. Unfortunately, despite treatment, 25–45% of patients will not benefit from therapy, and an additional 40% will ultimately require more aggressive treatment [1]. BCG-induced overexpression of PD-L1 has been described as one mechanism of acquired resistance to BCG in patients with NMIBC, and the US Food and Drug Administration (FDA) recently approved an anti–PD-1 agent as monotherapy in patients with BCG-unresponsive NMIBC [2]. Although baseline PD-L1 expression in tumor tissue predicts an unfavorable response to BCG [3], spatial and temporal tumor heterogeneity are major biological issues to overcome when using tumor tissues for predictive biomarker analysis, since PD-L1 might be inadequately represented in the biopsy specimen. To overcome these limitations, liquid biopsy for assessment of PD-L1 was identified as suitable for guiding therapeutic choice for patients who were candidates for PD-L1 inhibitors [4]. A role for enumeration of circulating tumor cells (CTCs) in improving current risk stratification models in high-risk NMIBC was recently proved in a study demonstrating that CTCs are detectable in almost 20% of NMIBC cases, who represent a subgroup of "super-high risk" patients requiring closer monitoring for local recurrence and/or progression of disease [5].

Here we explored PD-L1 expression in CTCs isolated from 20 selected CTC-positive patients (CTC cutoff $\geq$1) prospectively enrolled before starting adjuvant BCG therapy for NMIBC. PD-L1 analysis was not performed for tumor tissues because of the scarcity of tissue sample available. Two different methods for CTC isolation were compared: the antigen-dependent FDA-approved CellSearch system and a size-based CTC isolation method (ScreenCell). A blood sample from a healthy donor and spiked SKBR3 cells were used as negative and positive controls, respectively. Patients were followed for median follow-up of 18 mo (range 3–20).

Table 1 – Clinicopathological features of the study cohort.

Pt	Age (yr)	Gender	Tumor		CIS	CTC number				TTRP (mo)	BCG response
			Stage	Grade		CellSearch		ScreenCell			
						Total	PD-L1$^+$	Total	PD-L1$^+$		
1	77	M	T1	G3	Yes	3	3	4	4	6	Unresponsive
2	65	M	T1	G3	No	4	4	5	4	8	Unresponsive
3	71	M	T1	G3	No	3	3	4	3	5	Unresponsive
4	59	F	T1	G3	Yes	2	2	3	3	3	Unresponsive
5	70	M	T1	G3	No	2	2	3	2	12	Unresponsive
6	64	F	T1	G3	No	2	2	3	2	9	Unresponsive
7	56	F	T1	G3	No	2	1	5	2	12	Unresponsive
8	66	M	T1	G3	Yes	3	3	4	3	8	Unresponsive
9	79	M	T1	G3	No	0	0	0	0	15	Unresponsive
10	75	M	T1	G3	Yes	1	1	2	2	9	Unresponsive
11	71	M	T1	G3	No	2	2	4	2	3	Unresponsive
12	52	F	T1	G3	No	2	2	4	4	6	Unresponsive
13	66	M	T1	G3	No	0	0	0	0	NA	Responsive
14	68	M	T1	G3	Yes	0	0	0	0	NA	Responsive
15	70	M	T1	G3	No	0	0	0	0	NA	Responsive
16	64	M	T1	G3	No	0	0	0	0	NA	Responsive
17	59	M	T1	G3	No	0	0	0	0	NA	Responsive
18	77	F	T1	G3	No	0	0	0	0	NA	Responsive
19	71	M	T1	G3	No	0	0	0	0	NA	Responsive
20	60	F	T1	G3	No	0	0	0	0	NA	Responsive

BCG = bacillus Calmette-Guérin; CIS = carcinoma in situ; CTC = circulating tumor cell; F = female; M = male; NA = not applicable; Pt = patient; TTRP = time from BCG to recurrence/progression.

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Patients with recurrence or progression during therapy were included in a BCG-unresponsive group. During follow-up, no recurrence or tumor progression was observed in eight patients (40%), while 12 patients were defined as BCG-unresponsive (60%). Among the latter, eight patients (66.7%) had local recurrence and four (33.3%) experienced progression to muscle-invasive disease. PD-L1–positive CTCs were found in 11/12 of the BCG-unresponsive group (92%), while CTCs from the eight BCG-responsive patients stained negative for PD-L1, with a concordance rate of 100% between the two assays (Table 1). The slight difference in CTC number detected by the two assays might be explained by the low ability of CellSearch to detect CTCs in partial epithelial-mesenchymal transition.

This proof-of-concept study demonstrates for the first time the feasibility of a liquid biopsy test for PD-L1 assessment in high-risk NMIBC using two different assays. Although the standardized pre-analytical preparation of CTCs and inclusion of a positive control render CellSearch more suitable for clinical practice, ScreenCell is an easy technique with the advantage that it does not require complex instruments or adapted staff training. We suggest that baseline and serial assessment of PD-L1 using liquid biopsies might be a suitable tool for guiding therapeutic choice for patients who are candidates for PD-L1 inhibitors after BCG failure.

Conflicts of interest: The authors have nothing to disclose.

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