Original Article

Evaluation of PD-L1 Expression in Colorectal Carcinoma and its Correlation with Different Clinical-pathological Parameters among Iraqi Patients

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Abstract

Background: PD-L1 is a key immune checkpoint transmembrane physiological ligand for programmed-death1 (PD1), the interaction of programmed cell death receptor 1 (PD-1) and programmed death ligand 1 (PD-L1) plays an important role in inhibiting the immune mechanism by which cancer cells can escape antitumor immunity. Immunotherapy using checkpoint inhibitors is a growing treatment modality in many cancers; one such is anti PD1/PD-L1. **Objectives:** To evaluate the expression of Programmed Death-Ligand 1 (PD-L1) in colorectal carcinoma and determine its association with clinicopathological characteristics, in order to explore its potential role as a predictive marker for immunotherapy. **Materials and Methods:** PD-L1 antibody retrospectively analyzed immunohistochemically in formalin-fixed paraffin-embedded tissue blocks of 60 specimens with colorectal carcinomas operated between December 2023 - September 2024. A comparison performed between PD-L1 expression in tumor cells (TCs) as well as tumor-infiltrating immune cells (TIICs) for age, sex, size of tumor, histological differentiation, Lymphovascular invasion, the primary tumor location, number of involved lymph nodes, Distant metastasis and AJCC stage. **Results:** Of the 60 patients, the median age was 62.5 (range: 42–80) years. Sixteen samples were PDL1 positive in TCs, increased to 35% in TIICs. A significant expression of PD-L1 in TCs was correlated with lymphovascular invasion (P=0.002), lymph node metastasis (P= 0.001) and AJCC staging (P=0.001). The PD-L1 status in TIICs was again connected with adverse clinical and pathological parameters. **Conclusions:** The expression of PD-L1 in TCs and TIICs is associated significantly with advanced cancer or lymphatic invasion in patients who underwent surgery after a diagnosis of CRC. The research designates the significance of estimation of TCs and TIICs in correlation to clinical-pathological characteristics of patients a finding that could produce a piece of evidence for precise electing immunotherapy.

Keyword: PD-L1, tumor cells (TCs), tumor-infiltrating immune cells (TIICs), cancer, Immunotherapy.

Introduction

Colorectal cancer (C.R.C.) is the most frequent gastrointestinal malignancy, causing morbidity and mortality around the world. In the United States, it is the third most common cancer among men and women and the second leading cause of death from cancer. It is more common in older people, with males being slightly more affected than females [1]. More than 90% of colorectal carcinomas are adenocarcinomas [2]. In Iraq, colorectal cancer was the second most common cancer in males and the third most common cancer in females [3]. The first-line treatment for colon cancer involves a combination of surgery and adjuvant chemotherapy; however, recurrence and metastasis are major causes of treatment failure. Although some recent progress has been made in both diagnosis and treatment, colorectal cancer continues to have a huge impact on human lives and health [4]. Therefore, new

targeted therapeutic interventions are greatly needed. The evolution of cancer is affected by the sequence of events, including both epigenetic and inherited epithelial cell changes, and is also directed by tumour–host interaction [5]. However, certain types of malignancies have the ability to escape immune response [6]. Programmed cell death ligand 1 (PD-L1, CD274) is a key immune checkpoint physiological ligand for programmed-death 1 (PD1), expressed by lymphocytes, macrophages, and dendritic cells. The PD-1/PD-L1 interaction plays an important role in the inhibition of T cell-mediated immune response, leading to the exhaustion of effector T cells [7]. It is also expressed in various malignancies and serves as a receptor transferring an anti-apoptotic signal to guard tumour cells against apoptosis and immune escape of tumour cells [5]. The PD1-PDL1 pathway, therefore, has been involved in cancer progression [8]. This observation leads to the production of PD1–PDL1 pathway inhibitors to counteract tumour cells from evading host immune responses and to intensify antitumour immunity, thus providing a promising approach in oncology toward immunotherapy [9]. This study aimed to evaluate the expression of Programmed Death-Ligand 1 (PD-L1) in colorectal carcinoma and determine its association with clinicopathological characteristics, in order to explore its potential role as a predictive marker for immunotherapy.

Materials and Methods

This retrospective cross-sectional study was carried out in the Babylon Training Center for Histopathology during the period from December 2023 through September 2024. The study group comprises formalin-fixed paraffin-embedded tissue blocks collected from 60 patients diagnosed with colorectal carcinoma. The selected cases were obtained from the archives of the histopathology laboratory of Al-Hillah Teaching Hospital, Al-Imam Al-Sadiq Teaching Hospital, and private laboratories based in Hillah City. The sampling of cases includes the following: Sixty patients with colorectal carcinoma, confirmed by hematoxylin and eosin stain, were included in this study. An expert pathologist did the re-evaluation of all the slides to confirm the histopathological diagnosis. The pathologic staging based on the 8th edition of the American Joint Committee on Cancer staging manual [10].

Immunohistochemistry technique

Immunohistochemical (IHC) staining was conducted on TMA sections using the Dako automated Autostainer Link 48 and the ZytoChem Plus HRP Polymer Kit detection system. Briefly, 3 μm thick TMA sections were baked overnight at 58 °C, deparaffinized in xylene, and rehydrated through a series of graded ethanol solutions. Tissue sections then underwent heat-induced epitope retrieval (HIER) and were treated with a 3% hydrogen peroxide solution at 37 °C for 10 minutes to block endogenous peroxidase activity. This was followed by antigen retrieval using high-pressure cooking in citrate buffer (pH 6.0) for 10 minutes for PD-L1 detection. The sections were incubated at 37 °C for 60 minutes with rabbit IgG monoclonal antibodies against PD-L1 (1:100, Cat. No. RBK063-05, Zytomed Systems, Berlin, Germany). Immunostaining was carried out using the ZytoChem Plus HRP Polymer (DAB) (POLHRP-006, Zytomed Systems, Berlin, Germany), resulting in the formation of a brown precipitate at the antigen site. Finally, the slides were counterstained with hematoxylin (Sigma Aldrich, St. Louis, MO, USA), followed

by blueing and mounting in a non-aqueous medium [11].

Evaluation of immunostaining

In this current study, we evaluate PD-L1 immunoreactivity in tumor cell and tumor infiltrating immune cells separately. The evaluation of positive immunohistochemical reaction for PD-L1 antibody is by complete circumferential or partial cell membranous staining of viable tumor cells or membranous and/or cytoplasmic staining of TIICs of any intensity on ≥1% of all TCs or TIICs. Since there is no standardized scoring system for CRC, we used Tumor Proportion Score of PD-L1 staining in NSCLC (non-small cell lung cancer) [12,13] and the guidelines from the PD-L1 Kit (Dako PD-L1 IHC 22C3 pharmDx) as a reference.

Statistical analyses

Statistical analysis was carried out using SPSS version 27. Categorical variables were presented as numbers and percentages. Pearson Chi-Square test and Fisher's Exact test were used to find the association between categorical variables. P value ≤ 0.05 was considered as significant.

Ethical considerations

All patients received verbal information explaining the aims of the study. Verbal consent was obtained from all the patients participating in the study. Ethical approval for the study was obtained from the ethical committee of the Department of Pathology and Council of the College of Medicine, University of Babylon.

Results

Clinical characteristics of patients

The baseline clinicopathological characteristics of 60 primary CRC samples are listed within Table 1. Briefly, the median age was 62.5 years (range: 42–80 years old), with a predominance of males (56.7%), resulting in a male-to-female ratio of 1.31:1. Histologically, 91.7% moderate to poorly differentiated adenocarcinoma. Most of the tumours invaded through the muscularis propria into the pericolorectal tissues (T3 = 73.3%). Only eleven cases presented with lymphovascular invasion. Twenty-five cases presented with regional lymph node involvement with tumour spread. Distant metastases were observed in 8.3% of cases and approximately 8.3% of cases were classified as stage IV.

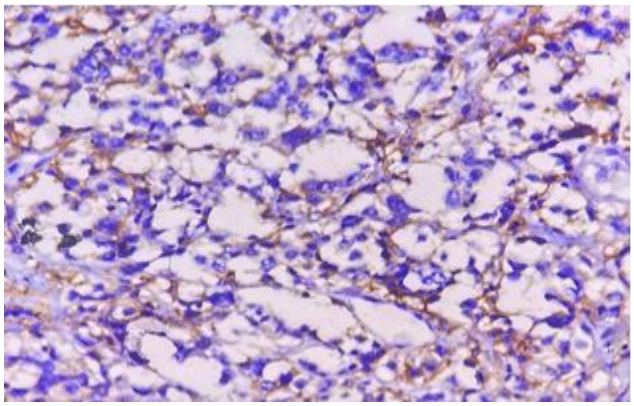
Table 1: Distribution of patients with CRC according to the clinical-pathological features

Parameters	No.	Percentage%
Age(years)		
<60	26	43.3%
≥60	34	56.7%
Total	60	100.0%
Sex		
Male	34	56.7%
Female	26	43.3%
Total	60	100.0%
Size of tumor		
<5cm	25	41.7%
≥5cm	35	58.3%
Total	**60**	**100.0%**
Differentiation of tumor		
Well differentiated	5	8.3%
Moderately differentiated	47	78.4%
Poorly differentiated	8	13.3%
Total	60	100%
Lymphovascular invasion		
Present	11	18.3%
Absent	49	81.7%
Total	60	100%
AJCC Staging		
Stage I	6	10.0%
Stage II	28	46.7%
Stage III	21	35.0%
Stage IV	5	8.3%
Total	60	100.0%
Primary tumor (pT)		
T1	0	0.0%
T2	6	10.0%
T3	44	73.3%
T4	10	16.7%
Total	60	100.0%

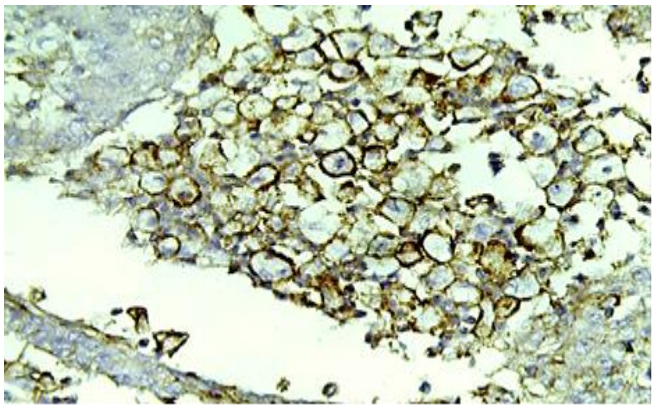
Regional lymph node metastasis		
N0	35	58.3%
N1	22	36.7%
N2	3	5.0%
Total	60	100.0%
Distant metastasis		
M0	55	91.7%
M1	5	8.3%
Total	60	100.0%

Immunohistochemical expression of PD-L1

The PD-L1 staining was at various frequencies, both within TCs and TIICs. With a ≥1% cut-off, tumor cells were positive in 27% of specimens, while in 35% of TIICs. The normal colonic mucosa did not show any staining. Membranous staining was observed in positive TCs cells and cytoplasmic and/or membranous staining in TIICs. In TCs, the focal PD-L1 positive pattern was prevailing. However, in TIICs, the diffuse positivity was the most common. The expression of PD-L1 is shown in Figures 1 and 2. The correlation between the expression of PD-L1 within TCs and the clinicopathological parameters is shown in Table 2. On TCs, expression of PD-L1 was significantly linked with lymphovascular invasion (P=0.002), lymph node metastasis (P=0.001) and AJCC staging (P=0.001). On the contrary, other variables such as age, gender, size, differentiation, primary tumour (pT) and distant metastasis were of no significance.

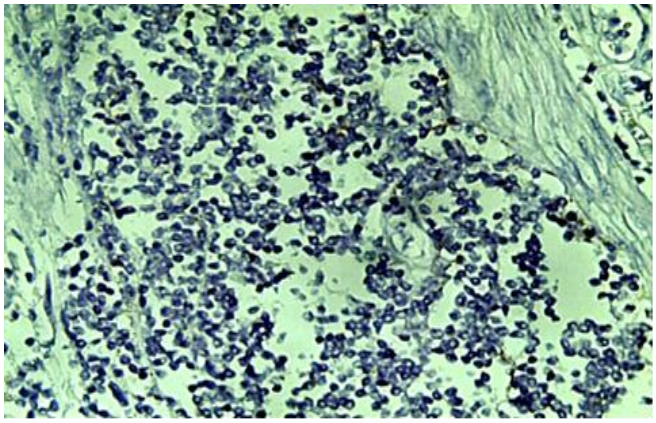


A



B

Figure 1: Programmed cell death ligand-1 immuno-histochemical expression in CRC neoplastic cells (original magnification, ×400). (A) positive membranous PD-L1 expression (≥1% of stained cells)**.** (B) positive PD-L1 expression (≥50% of stained cells)**.**



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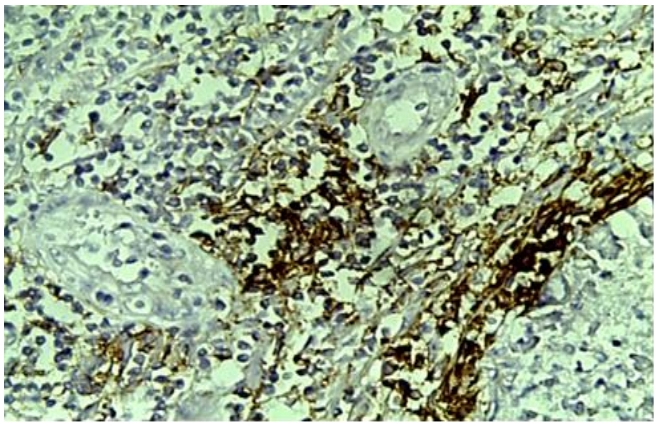

B

Figure 2: Programmed cell death ligand-1 immuno-histochemical expression in colorectal carcinoma immune cells (original magnification, ×100). (A) A lack of PD-L1 expression; (B) positive PD-L1 expression.

The PD-L1 status in TIICs was studied, and its association with the variable clinical and pathological features of CRC patients was analysed (Table 3). The TIICs exhibited higher PD-L1 immunoexpression and were significantly associated with tumour differentiation (P=0.01), AJCC staging (0.004), regional lymph node (pN) status (P=0.009) and distant metastasis (P=0.04), while other variables provided no significant association, although the primary tumour (pT) status approached borderline significance (P=0.05).

Table 2: IHC expression of PD-L1 in tumor cells (TC)

Study variables	IHC expression of PD-L1 in tumor cells (TC)			*P-value
	Negative (N=44)	Positive (N=16)	Total (N=60)	
Age (years)				
< 60 years	19 (43.2%)	7 (43.8%)	26 (43.3%)	0.96
≥ 60 years	25 (56.8%)	9 (56.2%)	34 (56.7%)	
Sex				
Male	25 (56.8%)	9 (56.2%)	34 (56.7%)	0.96
Female	19 (43.2%)	7 (43.8%)	26 (43.3%)	
Size of tumor				
< 5 cm	19 (43.2%)	6 (37.5%)	25 (41.7%)	0.69
≥ 5 cm	25 (56.8%)	10 (62.5%)	35 (58.3%)	
Differentiation of tumor				
Well differentiated	5 (11.4%)	0 (0.0)	5 (8.3%)	0.19
Moderately differentiated	32 (72.7%)	15 (93.7%)	47 (78.4%)	
Poorly differentiated	7 (15.9%)	1 (6.3%)	8 (13.3%)	
LVI				
Yes	4 (9.1%)	7 (43.8%)	11 (18.3%)	0.002**
No	40 (90.9%)	9 (56.2%)	49 (81.7%)	
AJCC				
Stage I	6 (13.6%)	0 (0.0)	6 (10.0%)	0.001**
Stage II	25 (56.8%)	3 (18,7%)	28 (46.7%)	
Stage III	9 (20.5%)	12 (75%)	21 (35.0%)	
Stage IV	4 (9.1%)	1 (6.3%)	5 (8.3%)	
Primary tumor (pT)				
T1	0 (0.0)	0 (0.0)	0 (0.0)	0.081
T2	6 (13.6%)	0 (0.0)	6 (10%)	
T3	33 (75%)	11 (68.7%)	44 (73.3%)	
T4	5 (11.4%)	5 (31.3%)	10 (16.7%)	
Regional lymph node metastasis				
N0	32 (72.7%)	3 (18.7%)	35 (58.3%)	0.001*
N1	10 (22.8%)	12 (75%)	22 (36.7%)	
N2	2 (4.5%)	1 (6.3%)	3 (5.0%)	
Distant metastasis				
M0	40 (90.9%)	15 (93.7%)	55 (91.7%)	0.72
M1	4 (9.1%)	1 (6.3%)	5 (8.3%)	

Table 3: IHC expression of PD-L1 in tumor infiltrating immune cells (TIICs)

Study variables	PD-L1 in TIICs		Total (N=60)	*P*-value
	Positive (N=21)	Negative (N=39)		
Age (years)				
≤ 60 years	8 (38.1%)	18 (46.2%)	26 (43.3%)	0.54
> 60 years	13 (61.9%)	21 (53.8%)	34 (56.7%)	
Total	21 (100%)	39 (100%)	60 (100%)	
Sex				
Male	12 (57.1%)	22 (56.4%)	34 (56.7%)	0.95
Female	9 (42.9%)	17 (43.6%)	26 (43.3%)	
Total	21 (100%)	39 (100%)	60 (100%)	
Size of tumor				
< 5 cm	9 (42.9%)	16 (41%)	25 (41.7%)	0.89
> 5 cm	12 (57.1%)	23 (59%)	35 (58.3%)	
Total	21 (100%)	39 (100%)	60 (100%)	
Differentiation of tumor				
Well differentiated	0 (0.0%)	5 (12.8%)	5 (8.3%)	0.015*
Moderately differentiated	15 (71.4%)	32 (82.1%)	47 (78.4%)	
Poorly differentiated	6 (28.6%)	2 (5.1%)	8 (13.3%)	
Total	21 (100%)	39 (100%)	60 (100%)	
Lymphovascular invasion				
Yes	5 (23.8%)	6 (15.4%)	11 (18.3%)	0.49
No	16 (76.2%)	33 (84.6%)	49 (81.7%)	
Total	21 (100%)	39 (100%)	60 (100%)	
AJCC				
Stage I	0 (0.0%)	6 (15.4%)	6 (10%)	0.004**
Stage II	6 (28.6%)	22 (56.4%)	28 (46.7%)	
Stage III	11 (52.4%)	10 (25.6%)	21 (35%)	
Stage IV	4 (19%)	1 (2.6%)	5 (8.3%)	
Total	21 (100%)	39 (100%)	60 (100%)	
primary tumor (pT)				
T1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.05
T2	0 (0.0%)	6 (15.4%)	6 (10%)	
T3	15 (71.4%)	29 (74.4%)	44 (73.3%)	
T4	6 (28.6%)	4 (10.3%)	10 (16.7%)	
Total	21 (100%)	39 (100%)	60 (100%)	
regional lymph node				
N0	7 (33.3%)	28 (71.8%)	35 (58.3%)	0.009**
N1	12 (57.1%)	10 (25.6%)	22 (36.7%)	
N2	2 (9.5%)	1 (2.6%)	3 (5%)	
Total	21 (100%)	39 (100%)	60 (100%)	
distant metastasis				
M0	17 (81%)	38 (97.4%)	55 (91.7%)	0.046*
M1	4 (19%)	1 (2.6%)	5 (8.3%)	
Total	21 (100%)	39 (100%)	60 (100%)	

Discussion

Colorectal cancer (CRC) is one of the most common cancers in men and women worldwide [14]. At present, we understand that CRC is a highly heterogeneous disease characterised by biological diversity [15]. That means CRC develops as a result of the successive

accumulation of multiple molecular factors such as mutation in K-RAS genes and MSI status [16]. It seems that molecular variability leads to a more complicated mechanism of CRC. On the other hand, some key molecular alterations can be used as biomarkers to predict prognosis and even therapeutic targets [17]. In recent years, targeted therapies using antibodies against immune checkpoints have shown promising results in the treatment of various malignancies, including melanoma, lung carcinoma, renal cell carcinoma, and urothelial carcinoma. Remarkable responses to these immune checkpoint inhibitors (ICPI) that interfere with the PD-1/PD-L1 interaction and act to restore the innate immune system's ability to control malignancies have been reported [18]. In this current study, the detected PD-L1 expression in tumour cells was 27%, with 12% of them showing a high level of PD-L1 expression. Researches that study PD-L1 expression in colorectal carcinoma show variable results. Aziz ZW et al. [19] showed that PD-L1 was expressed in 14.1% of 99 CRC cases. Elfishawy M, et al. [20] showed PD-L1 was positive in 25% of the cases. Shan T et al. [21]found that PD-L1 was positive in 57.5%. Tadachina S et al. [22] found that PD-L1 was expressed in 17.6% of cases of CRC involved in his study. These discrepancies might be due to the various scoring systems, positivity cut-offs applied, intratumoral staining heterogeneity, and the different antibodies used [23]. In terms of histopathological parameters, PD-L1 in TCs was significantly associated with lymphovascular invasion; these findings align with those reported by Enkhbat T. et al. [24] and Tadachina S. et al. [22]. However, these results are not consistent with the findings of Elfishawy M. et al. [20] For instance, the presence and extent of LVI could be influenced by tumour heterogeneity, and variations in how LVI is defined or quantified may lead to differing conclusions. Regarding tumour stage, the results of this study demonstrate a significant association between tumour stage and PD-L1 expression, with a p-value of 0.001. These findings support the association between more aggressive tumours and increased PD-L1 expression, aligning with the results of Aziz ZW et al. [19] and Shan T et al. [21]. However, this was not consistent with the findings of Enkhbat T. et al. [24], Tadachina S. et al. [22], and Elfishawy M. et al. [20]. The results of this study showed a significant association between regional lymph node (LN) metastasis and PD-L1 expression, with a p-value of 0.001. However, no significant association was found between PD-L1 expression and distant metastasis. These findings align with those of Shan T et al. [21] and Zhu H et al. [25]. On the contrary, other variables such as age, sex, tumour differentiation and tumour size were of no significance, compatible with Aziz ZW et al. [19] and Shan et al. [21]. According to this study, these findings indicate that PD-L1 expression in TCs is closely linked to aggressive histological features in CRC, in consistency with other studies that linked PD-L1 expression to an unfavourable prognosis [26]. Tumour-infiltrating immune cells (TIICs) are gaining attention in cancer immunotherapy research due to their potential role in inhibiting activated T-cell function when PD-L1 is expressed [27]. In this study, we also assessed PD-L1 expression in immune cells, a factor now incorporated into the scoring criteria for various malignancies, such as triple-negative breast cancer [28]. However, the prognostic significance of PD-L1 positivity in these immune cells remains under investigation [29]. To explore this further, we independently analysed PD-L1 immunoexpression in TIICs and examined its correlation with the clinic-pathological characteristics of the patients. The

current results identified PD-L1 positivity in 35% of the tumour infiltrating immune cells. These immunoexpressions show insignificant association with age, sex, tumour size, and LVI but are significantly associated with advanced stage, lymph node metastasis, distant metastasis, and tumour grade. So, again connected with adverse clinical and pathological parameters. These findings were very close to those obtained by Aziz ZW et al. [19], who were showing PD-L1 positivity in 32.3% of the infiltrating immune cells. However, these were not consistent with what Wang et al. [30] and Elfishawy M. et al. [20] found in their study. The metastatic progression and tumour stage advancement in CRC patients with PD-L1-positive tumour-infiltrating immune cells may be attributed to the inhibition of activated T lymphocytes, leading to immune evasion and, consequently, poorer outcomes. So, from our perspective, our findings support the hypothesis that PD-L1 expression is associated with poor clinical and pathological features in colorectal carcinoma. This is reinforced by our focus on the frequency of PD-L1 positivity in tumour-infiltrating immune cells (TIICs) in CRC, rather than in tumour cells (TCs) alone. Our results suggest that patients with advanced cancer or lymphatic invasion are more likely to exhibit PD-L1 expression.

Conclusion

In our study, we found a significant association between PD-L1 expression and key prognostic factors in colorectal carcinoma. High PD-L1 expression was notably linked to advanced tumor stage, regional lymph node (LN) metastasis, and lymphovascular invasion (LVI), all of which are indicators of aggressive tumor behavior and poor prognosis. Our findings suggest that PD-L1 expression could serve as a valuable biomarker for identifying colorectal carcinoma patients with poor prognostic features. This assist in early identification of high-risk patients who may benefit from more intensive monitoring or targeted therapeutic strategies aimed at immune modulation.

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