Pathology - Research and Practice xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Potential impact of PD-L1 (SP-142) immunohistochemical heterogeneity in clear cell renal cell carcinoma immunotherapy

José I. López[a,b,c,*], Rafael Pulido[b,d], Jesús M. Cortés[d,e,f], Javier C. Angulo[g,h], Charles H. Lawrie[d,i,j,k]

[a] Department of Pathology, Cruces University Hospital, Barakaldo, Spain
[b] Biomarkers in Cancer Unit, Biocruces Research Institute, Barakaldo, Spain
[c] Department of Medical-Surgical Specialties, University of the Basque Country (UPV/EHU), Leioa, Spain
[d] IKERBASQUE, The Basque Foundation for Science, Bilbao, Spain
[e] Quantitative Biomedicine Unit, Biocruces Research Institute, Barakaldo, Spain
[f] Department of Cell Biology and Histology, University of the Basque Country (UPV/EHU), Leioa, Spain
[g] Department of Urology, University Hospital of Getafe, Getafe, Madrid, Spain
[h] Clinical Department, European University of Madrid, Laureate Universities, Madrid, Spain
[i] Molecular Oncology, Biodonostia Research Institute, Donostia-San Sebastián, Spain
[j] Department of Physiology, University of the Basque Country (UPV/EHU), Leioa, Spain
[k] Radcliffe Department of Medicine, University of Oxford, UK

ARTICLE INFO

Keywords:
Clear cell renal cell carcinoma
Immune checkpoint
PD-L1
Immunohistochemistry
Intratumor heterogeneity
Multisite tumor sampling
Targeted therapy

ABSTRACT

Intratumor heterogeneity (ITH) detection remains a challenge in modern oncology because it can have a direct impact on the success of new therapies. Anti-PD-1/PD-L1 immunotherapy is an emerging treatment modality that is showing great promise for clear cell renal cell carcinoma (CCRCC) patients with advanced disease. Patient selection for such therapy relies upon the immunohistochemical detection of PD-1/PD-L1, however the degree of ITH for these markers among tumor cells and/or inflammatory mononuclear infiltrates remains unknown. Therefore, we analyzed PD-L1 (SP-142) expression in the tumor inflammatory cells of 22 CCRCC cases with the aim to define the pattern of PD-L1 expression, and to compare the reliability of current tumor sampling protocols (RS) with a multisite tumor sampling strategy (MSTS). While the RS protocol identified 5/22 (22.7%) of cases that were positive for PD-L1 expression, MSTS identified 10/22 (45.45%) of cases. This suggests that RS may miss a proportion of CCRCC patients that might benefit from immunotherapy. In addition, MSTS demonstrated that positive and negative regions of PD-L1 expression are very variable within each tumor.

1. Introduction

Renal cell carcinoma is included in the top-ten list of the most common malignancies in Western countries [1]. Clear cell renal cell carcinoma (CCRCC) is the most frequent renal malignancy, accounting for roughly 70%–80% of the cases [2]. CCRCC is an aggressive neoplasm with different molecular profiles influencing treatment response [3]. Despite all therapeutic efforts, however, only radical surgery and early diagnosis have had a significant influence on survival [4].

CCRCC is a paradigmatic example of intratumor heterogeneity (ITH) typically displaying both temporal and spatial differences at the morphological, immunohistochemical and molecular levels [5]. Importantly ITH is the cornerstone of many therapeutic failures, and many efforts are being made to achieve a full characterization of tumors that may eventually allow better personalized approaches [6]. Immune checkpoint inhibitors, alone or in combination with anti-angiogenic drugs, have emerged in recent years as promising new therapeutic options for advanced CCRCC [7]. As a consequence, the influence of the cancer immune microenvironment is attracting great interest [8].

The expression of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), and programmed death-1 (PD-1) on activated T-cells inhibits the immune-mediated attack on tumor cells. Checkpoint inhibitors, in particular anti-CTLA-4 and anti-PD-1 (and its ligand anti-PD-L1), show great promise for renal cancer patients, however not all patients receive a benefit from these therapies, and as a consequence there is great interest in finding effective predictive biomarkers [9,10].

Currently patient selection for anti-PD-L1 treatments relies on the identification of PD-1/PD-L1 by routine immunohistochemical protocols, and several anti PD-1/PD-L1 clones have been developed for this purpose. However, up to 17% of patients that respond to PD-L1 therapy

* Corresponding author at: Department of Pathology, Hospital Universitario Cruces, Plaza de Cruces s/n, 48903, Barakaldo, Bizkaia, Spain.
E-mail address: joseignacio.lopez@osakidetza.eus (J.I. López).

https://doi.org/10.1016/j.prp.2018.06.003
Received 7 May 2018; Received in revised form 7 June 2018; Accepted 8 June 2018

appear not to express PD-L1 when tested using current methodologies [9]. This apparent contradiction suggests that either factors other than PD-L1 are involved in the therapeutic action of anti-PD-L1 treatment, or more likely that current protocols are suboptimal for detection of PD-L1 expression [10].

Recent evidence has shown that routine protocols may be insufficient to reliably detect ITH in large tumors [11,12]. We have developed a multi-site tumor sampling (MSTS) protocol that out-performs routine sampling (RS) protocols in detecting histological ITH, and prognostic biomarkers [13]. MSTS follows the rationale *the more you sample the more you find*, and it is based in the divide-and-conquer algorithm [14], a successful strategy to solve complex problems in physics [15] that has been successfully applied also in biology [16] and in medicine [17]. Here, we have applied this strategy to analyze the expression of PD-L1 in the microenvironment of CCRCC tumors.

2. Material and methods

The authors declare that all the analyses carried out in this study comply with current Spanish and European Union legal regulations. Samples from patients included in this study were obtained retrospectively from the archive of the Pathology Lab, Cruces University Hospital, Barakaldo, Spain. All patients gave written consent for the use of their samples in this study as approved by the Ethical and Scientific Committees of the Basque Health Service (Osakidetza) (CEIC-E PI2016096).

Twenty-two CCRCC were selected between November 2015 and February 2016. All cases were simultaneously sampled following two different protocols: RS [18], one large tumor sample per cassette for each centimeter of the tumor diameter (i.e., 22 tumors, 22 samples, 22 cassettes); and MSTS [13], six to eight small samples per cassette (22 tumors, 160 samples, 22 cassettes), as illustrated in Fig. 1. Note that both methods use the same number of cassettes.

Immunostaining was performed in a BenchMark Ultra (Ventana, Roche, AZ, USA) immunostainer following routine protocols and specific instructions of the manufacturer. Prediluted PD-L1 antibody (clone SP-142, Ventana, Roche, AZ, USA) was used for the analysis.

Microscopic evaluation of all samples in both sampling protocols was performed in a blind way by the same observer to guarantee objectivity. As suggested by the manufacturer, only immunostaining of the inflammatory mononuclear cells present in the tumor itself, or within the inner side of the tumor capsule, were considered positive (Fig. 2). Mimickers of PD-L1 immunostaining (namely, formaldehyde precipitation and hemosiderin deposition) were identified as such (Fig. 3). A cut-off of 1% positive tumor-associated inflammatory cells [19] was used as this cut-off has previously been associated with increased progression free survival in patients treated with atezolizumab, both alone or in association with bevacizumab [7].

Concordant positive and negative results between the two sampling protocols (RS and MSTS) were considered above and below 1% of positive inflammatory cells, respectively.

3. Results

The main clinicopathological data of the patients included in this study are depicted in Table 1. Most cases were male (16 M/6 F), and the average age of patients was 60 years (range 15–82). Radical nephrectomy was carried out on 20 patients, and partial nephrectomy in two patients. The average tumor diameter was 8.5 cm with a range between 3.5 and 15 cm. Eight cases were low-grade (G1/2) and fourteen cases high-grade (G3/4). Pathological staging revealed an equal distribution of organ-confined and non-organ-confined disease (11/11).

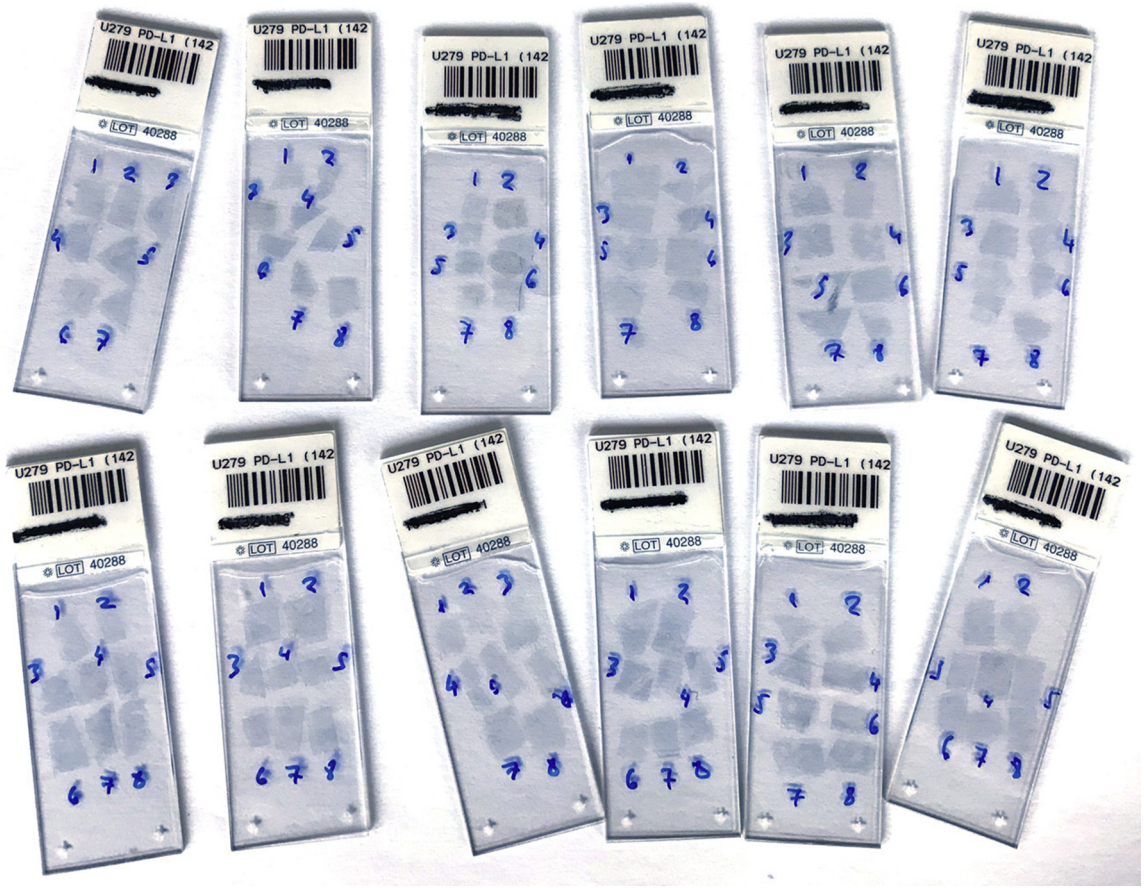

Fig. 1. Selection of the multisite tumor sampling protocol (6–8 samples per cassette) in 12 clear cell renal cell carcinomas ready for microscopic analysis.