Lung Cancer 120 (2018) 108–112

Contents lists available at ScienceDirect

Lung Cancer

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

Short communication

PD-L1 expression in circulating tumor cells of advanced non-small cell lung cancer patients treated with nivolumab☆

Nicolas Guibert[a,b,d,*], Myriam Delaunay[a,b,d], Amélie Lusque[e], Nadia Boubekeur[b,c], Isabelle Rouquette[f], Estelle Clermont[f], Jean Mourlanette[a], Sandrine Gouin[a], Inge Dormoy[a], Gilles Favre[b,c,d], Julien Mazieres[a,b,d], Anne Pradines[b,c]

[a] Thoracic Oncology Department, Larrey Hospital, University Hospital of Toulouse, France
[b] Inserm, Centre de Recherche en Cancérologie de Toulouse, CRCT UMR-1037, Toulouse, France
[c] Laboratoire de Biologie Médicale Oncologique, Institut Universitaire du Cancer de Toulouse, France
[d] University of Toulouse III (Paul Sabatier), Toulouse, France
[e] Cellule Biostatistique, Bureau des Essais Cliniques, Institut Claudius Regaud, IUCT-Oncopole, Toulouse, France
[f] Pathology Department, IUCT-Oncopole, Toulouse, France

ARTICLE INFO

Keywords:
Non-small-cell lung cancer
PD-L1 expression
Circulating tumor cells
Monitoring of response
Immunotherapy
Anti-PD1

ABSTRACT

Background: Inhibitors of the PD-1/PD-L1 immune checkpoint have become a standard of care in non-small cell lung cancer (NSCLC). Patient selection, currently based on PD-L1 expression on tumor tissue, is limited by its temporal and spatial heterogeneity. We hypothesized that liquid biopsy with PD-L1 analysis on circulating tumor cells (CTCs) might overcome this limitation.

Methods: Blood samples were prospectively collected from patients with advanced NSCLC before nivolumab treatment and at the time of progression. CTCs were isolated using a cell size-based technology. PD-L1 expression was assessed by immunofluorescence on CTCs and immunohistochemistry on tissue biopsies.

Results: 113 specimens from 96 patients were collected. Baseline PD-L1 expression could be assessed on 72% and 93% of tissue and CTC, respectively. CTCs were more frequently found to be PD-L1 positive than tissue (83% vs. 41%) and no correlation was observed between tissue and CTC PD-L1 expression ($r = 0.04$, $p = 0.77$). Pre-treatment high CTC number was associated with increased risk of death and progression (HR1.06, $p = 0.03$ for OS; HR1.05, $p = 0.02$ for PFS). The presence of pre-treatment PD-L1$^+$CTC was not significantly correlated with outcomes but a higher baseline PD-L1$^+$ CTC number ($\geq 1\%$) was observed in the "non-responders" group (PFS < 6 months) ($p = 0.04$) and PD-L1$^+$CTC were seen in all patients at progression.

Conclusion: Assessment of PD-L1 expression in CTCs is feasible and CTCs are more often positive than in tissue. Pre-treatment PD-L1$^+$CTCs are associated with bad prognosis in patients treated with PD-1 inhibitors.

1. Introduction

Immunotherapy is proving to be an effective approach in NSCLC. US and EU regulatory agencies have approved antibodies targeting the PD-L1/PD-1 axis in second and, more recently, front-line settings [1]. However, only a subset of patients exhibits durable responses, underlying the need for biomarkers.

PD-L1 tumor expression is, along with tumor mutational burden [2], the most established predictive biomarker of response to these drugs [1,3]. However, nearly 10% of patients whose tumor does not express PD-L1 will eventually benefit from immune checkpoint inhibitors (ICI), while a large number of tumors that express PD-L1 do not respond [3]. PD-L1 expression is usually assessed on archived tissue, which could explain part of these discrepancies since PD-L1 is a dynamic biomarker that can be induced by targeted therapy, chemotherapy or radiation therapy [4–6]. Re-biopsy would expose patients to the risk of complications and delayed results. Moreover, these small samples are sometimes inadequate for PD-L1 analysis due to tumor heterogeneity [7]. While tissue only offers a snapshot of PD-L1 expression at a given time and location, liquid biopsies have the ability to dynamically and non-invasively interrogate the whole molecular landscape of tumors. More specifically, circulating tumor cells (CTCs) might represent a substrate for analysis of PD-L1 expression. This approach has been reported in NSCLC [9–11], but the concordance with tissue and the correlation

☆ Portions of these data have been presented as a poster at the WCLC Annual Meeting 2017.

* Corresponding author at: Thoracic Oncology Unit, Respiratory Disease Department, Hôpital Larrey, CHU Toulouse, Chemin d Pouvourville, 31059, Toulouse Cedex, France.
E-mail address: guibert.n@chu-toulouse.fr (N. Guibert).

https://doi.org/10.1016/j.lungcan.2018.04.001
Received 16 February 2018; Received in revised form 28 March 2018; Accepted 1 April 2018

with response to PD-1 inhibitors need further investigation.

2. Material and methods

2.1. Patients and samples

All consecutive patients with advanced metastatic NSCLC who relapsed after chemotherapy and planned to receive nivolumab were included. All patients gave their informed consent to participate in this study (NCT02827344).

2.2. Tissue analysis

PD-L1 expression on tissue was assessed by immunohistochemistry (IHC) using an anti-PD-L1 rabbit monoclonal antibody (clone E1L3N, Cell signalling Technology) blinded to CTCs analysis.

2.3. CTCs analysis

Median time between tissue biopsy and pre-treatment blood collection was 7.8 months (n = 69). Blood specimen were obtained less than one month after tissue biopsy in 3 patients (5.7%); 1–12 months after in 37 patients (69.8%) and more than one year after in 13 patients (24.5%). Blood samples (10 ml) were collected and CTC were captured by ISET technology within the next 4 h. Five spots (1 ml of blood per spot) for each patient were subjected to IF staining for PD-L1 with a rabbit mAb (clone D8T4X, Cell signalling Technology) and CD45 (clone MEM-28, Abcam); blinded to tissue analysis. CTCs were defined as DAPI+/CD45- cells with cytomorphometric malignant features (size, shape, mononuclear, nucleus to cytoplasm ratio) [8] (Fig. 1**A**). H827 and H23 cell lines were selected as positive and negative controls, respectively, among several lung cancer cell lines, based upon their PD-L1 expression analysed by western blot (Sup. Fig. 1A). The specificity of the D8T4X rabbit mAb was evaluated on H23 and H827 cells spiked into healthy volunteer blood and enriched on ISET filters (Sup. Fig. 1B).

2.4. Statistical analysis

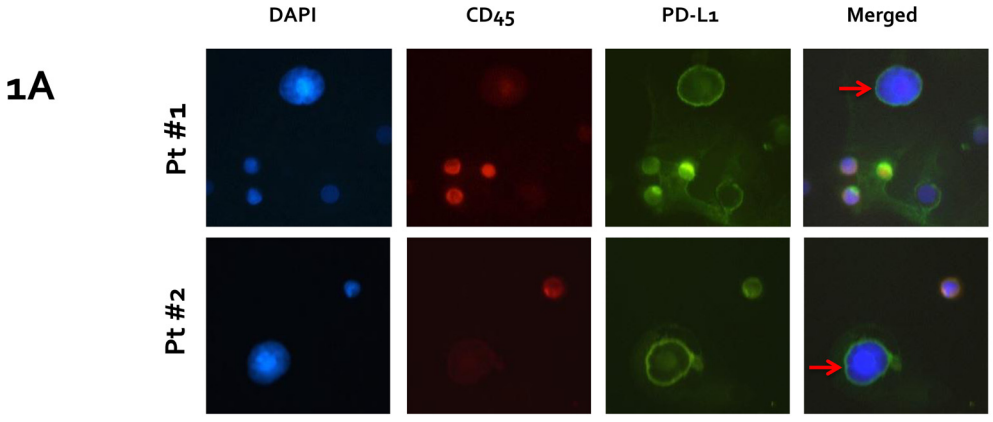
The data were summarized by frequency and percentage for qualitative variables and by median and range for continuous variables. Comparisons were assessed using chi-squared test and Fisher exact test for qualitative variables and Kruskal-Wallis test for continuous variables. Correlation was calculated using Spearman coefficient. All survival times were calculated from the initiation of immunotherapy and estimated by the Kaplan-Meier method with 95% confidence intervals (95%CI). Univariate analysis was performed using the logrank test for qualitative variables and Cox proportional hazards model for continuous variables.

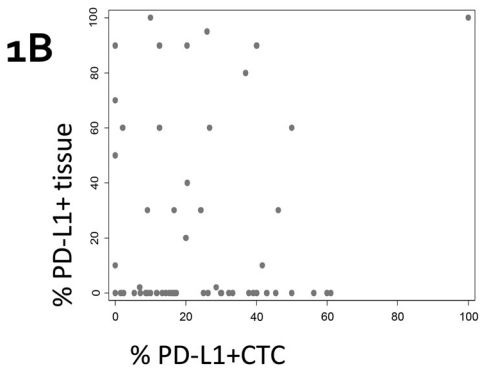
Tests were two-sided and p-values < 0.05 were considered significant. Statistical analyses were conducted using Stata®, version 13.

3. Results

3.1. Patient cohort characteristics

The clinical and pathological characteristics of the patient population are detailed in Sup. Table 1. Blood specimens were collected pre-treatment (n = 96, all treated with nivolumab) and at progression (n = 24).





1C

Baseline PD-L1 expression on Tissue (IHC) (n=69)			Baseline PD-L1 expression on CTCs (IF) (n=89)		
% PD-L1+ tissue	n	%	% PD-L1+ CTC	n	%
0%	41	59%	0%	15	17%
≥1%	28	41%	≥1%	74	83%
≥5%	25	36%	≥5%	71	80%
≥50%	16	23%	≥10%	61	68%

Correlation Tissues/CTCs				
	PD-L1 tissue (%)			
PD-L1+CTC (%)	0%	≥1%	ND	Total
0%	8	5	2	15
≥1%	31	22	21	74
ND	2	1	4	7
Total	41	28	27	96

Fig. 1. 1A: Analysis of PD-L1 on CTCs. Example of 2 cases with strong membranous PD-L1 staining on CTC (red arrow). **1B**: Correlation study between PD-L1 expression on tissue and CTC. **1C**: Correlation between tissue and CTCs PD-L1 expression. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)