PD-L1 Testing In Metastatic Triple Negative Breast Cancer: Results Of An Italian Survey

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Research Article

Keywords: Triple-negative breast cancers, immunotherapy, PD-L1 evaluation, Italian survey, real life setting

Posted Date: March 15th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1432267/v1

PD-L1 testing in metastatic triple negative breast cancer: results of an Italian survey

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Abstract

Background

Immunotherapy has revolutionized the approach to metastatic triple-negative breast cancers (mTNBC). Atezolizumab was approved for patients with mTNBC whose tumours express PD-L1, determined by SP 142 assay. To assess the availability and practice of SP142 test we administered a survey to all the 15 Pathology Departments of the Lazio Region during a 6-months period.

Methods

The survey comprised 12 questions regarding the availability of SP142 in the Pathology Departments, the percentage of positive tests, the difficulties of pathologists in case close to cut-off value and the tested samples.

Results

The SP142 assay was available only in 8 Centers. In case of positive result, most Centers (5/8, 62.5%) reported values of PD-L1 expression ranging from > 1 to <= 5%, with values close to the cut-off point (≥ 1% or < 1%) being the greatest challenge.

Most of Centers (6/8, 75%) tested material from both their own and other Hospitals. In most Centers, the evaluations were performed either on primary tumors or metastasis, in particular lymph nodes (5/8, 62.5%), followed by lung (3/8, 37.5%) and liver (1/8, 12.5%) metastasis.

Conclusion

Our results raise some important issues concerning the evaluation of PD-L1 in the "real-life" setting, providing strategies for its implementation.

Keywords: Triple-negative breast cancers; immunotherapy; PD-L1 evaluation; Italian survey; real life setting

Introduction

Triple-negative breast cancers (TNBC) represent a group of clinically heterogeneous breast cancers that share a characteristic immunohistochemical definition: the lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 HER2. These tumours represent about 10-20% of breast cancers (BCs) and have always represented a challenge for the oncologist, as they lack effective targeted drugs [1,2]. In the last few years, immunotherapy based on blockade of the programmed death-ligand 1 (PD-L1) / programmed cell death protein 1 (PD-1) has revolutionized the approach to TNBC both in the metastatic and neoadjuvant setting [3]. PD-L1, expressed in both tumour cells and tumour-infiltrating immune cells, can bind PD-1, present on effector cells of the immune system, blocking the elicitation of an effective tumour-specific immune response. TNBC may benefit from immunotheraphy because of the presence of high mutation levels, tumor-infiltrating lymphocytes, and elevated levels of PD-L1 expression [4]. PD-L1 is expressed approximately in 50% of TNBCs [5] and the determination is based on immunohistochemistry. Several assays have been developed and approved for specific drugs, with different cut-off points for PD-L1 positivity on tumor cells (TC), immune cells (IC) or both [6].

The most used monoclonal antibodies for determination of PD-L1 expression are 22C3, SP142 and SP263. They show different affinity for TC and IC [7].

A recent phase III study, IMPASSION 130, compared atezolizumab with nab-paclitaxel versus nab-paclitaxel with placebo, showing a significantly higher progression free survival (PFS) in the combination strategy. Overall survival was significantly better in PD-L1 positive patients treated with atezolizumab and nab-paclitaxel compared to nab-paclitaxel + placebo (25 vs. 18 months, HR >0.0001) in the final exploratory analysis [8]. In a post-hoc exploratory biomarker sub-study of the Impassion130 trial the SP142 at IC≥1% was the only assay able to identify patients with mTNBC most likely to benefit from the addition of atezolizumab to nab-paclitaxel.

Based on these findings on March 8, 2019, the Food and Drug Administration granted accelerated approval to atezolizumab (TECENTRIQ, Genentech Inc.) in combination with nab-paclitaxel for patients with unresectable locally advanced or metastatic TNBC whose tumours express PD-L1 (tumour-infiltrating immune cells [ICs] stained with PD-L1 covering ≥ 1% of the tumour area), as determined by the FDA-approved VENTANA PD-L1 (SP142) assay [9].

More recently, the phase 3 KEYNOTE 522 study evaluated the efficacy of combining pembrolizumab with chemotherapy in metastatic TNBC. Patients were stratified according to PD-L1 status (combined positive score [CPS] < 1 or ≥1), and it was found that in patients with CPS of 10 or more, the median PFS was significantly higher in the pembrolizumab treatment group compared to the control group. In the group of patients with CPS ≥ 1 there were no statistically significant differences in median PFS; however, the 12-month PFS rate in patients with CPS ≥ 1 was significantly higher in the pembrolizumab group than in the control group (31.7% versus 19.4%). In contrast, no difference in PFS was achieved in patients with PD-L1 CPS < 1 (PFS 6.3 months vs. 6.2 months; HR 1.08) [10].

Overall, these results confirm the role of PD-L1 as a predictive biomarker in metastatic TNBC.

However, there are several questions still open, regarding PD-L1 evaluation in the pathology laboratories. These include pre-analitical issues, the choice of the most representative samples (either primary tumor or metastatic site) and factors that could affect the inter-observer reproducibility, especially when close to the cut-off point. To verify the impact of these variables on PD-L1 interpretation in the “real life” setting, a survey was carried out to assess the experience with the SP 142 assay in the Pathology Deparments of the Lazio region in central Italy. The Lazio region is 17 227 km^2 wide and has a population of 5 730 399 inhabitants. We focused on the SP 142 assay, since Atezolizumab plus nab-paclitaxel is currently the only treatment approved by the Italian Medicines

Agency. We administered a survey to all the 15 Pathology Departments of the Lazio Region during a 6-months period to assess the availability and practice of SP 142 test in metastatic TNBC.

Materials and Methods

The survey comprised twelve questions administered to all the 15 Pathology Departments of the Lazio Region during a 6-months period. Questions were focused on the availability and frequency of SP 142 test in the Pathology Departments, the percentage of positive tests and the difficulty of pathologists either in case close to the cut-off value or with technical artifacts. Questions investigated also were the tested material come from and which are the most frequent type of sample and metastatic sites assessed.

Results

The SP 142 assay was available only in 8 of these Centers, which filled the survey. The results are listed in Table 1.

Results Briefly, the frequency of PD-L1 evaluation was variable among the different Centers, with the majority performing from 5 to 10 test in the selected timeframe. Only 2 Centers had evaluated more than 15 cases. Most of the interviewed pathologists performed PD-L1 evaluation weekly or once every two weeks. The percentage of PD-L1 positivity (i.e., IC $\geq$ 1%) differed among the participating Departments, with 3/8 recording less than 10% of positive results.

In case of positive result, the majority of Centers (5/8, 62.5%) reported values of PD-L1 expression ranging from > 1 to <= 5%, with values close to the cut-off point ($\geq$ 1% or < 1%) being the greatest challenge. Importantly, 5/8 (63%) Centers reported some difficulties in the evaluation of the immunostainings due to suboptimal pre-analytical conditions.

Most of Centers (6/8, 75%) reported that they tested material from both their own and other Hospitals. In the majority of cases (83%) the material coming from other Institutions consisted of paraffin blocks. In most Centers, the evaluations were performed either on primary tumors or metastasis, in particular lymph nodes (5/8, 62.5%), followed by lung (3/8, 37.5%) and liver (1/8, 12.5%) metastasis. The choice of the most suitable sample for PD-L1 assessment was shared between Pathologist and Oncologist in 7/8 Centers, underlying the importance of the multidisciplinary approach to improve the diagnostic pathway for TNBC.

Table 1 Survey questions and results

QUESTION	ANSWER (N, %)			
How many evaluations have you performed in the last 6 months on triple negative breast cancers with the SP142 test?	**<5** (1/8, 12%)	**5-10** (5/8, 63%)	**>10 <15** 0 %	**>15** (2/8, 25%)
How often is the SP142 test for PD-L1 expression performed in your laboratory?	**Weekly** (3/8, 37%)	**Every 15 days** (2/8, 25%)	**Monthly** (1/8, 13%)	**≥30 days** (2/8, 25%)
Which is the percentage of positive tests?	**<10%** (3/8, 37%)	**10-20%** (1/8, 13%)	**20-50%** (3/8, 37%)	**>50%** (1/8, 13%)
If positive, how are your cases divided in percentage between the listed values?	**1%** (2/8, 25%)	**≤5%** (6/8, 75%)	**>5 ≤10%** 0 %	**>10%** 0 %
Did you have difficulty with cases close to the cut-off (≥ 1% or < 1%)?	**Yes** (8/8, 100%)		**Not** 0 %	
Have you had cases that were difficult to interpret due to technical artefacts?	**Yes** (3/8, 37%)		**Not** (5/8, 63%)	
Where do you get the sample from for SP142 evaluation?	**Only from my Center** (2/8, 25%)		**Only from external Centers** (0 %)	**Both** (6/8, 75%)
What type of sample do you receive most frequently from external Centers?	**Unstained slides** (2/8, 25%)		**Paraffin block** (6/8, 75%)	
What are the samples most frequently evaluated?	**Primary tumour only** (2/8, 25%)		**Either primary tumour or metastasis** (6/8, 75 %)	
Which metastatic sites are most frequently assessed in your Center?	**Lymph nodes** (5/8, 63%)		**Lung** (2/8, 25%)	**Liver** (1/8, 12%)
Do you think it is appropriate to share the choice of sample for PD-L1 assessment with the oncologist?	**Yes** (7/8, 88%)		**No** (1/8, 12%)	

Discussion

Our results raise some important issues concerning the evaluation of PD-L1 on IC in TNBC in the "real-life" setting. The first concerns the frequency of this practice: in fact, most participating Centers had performed a low number of evaluations (from 5 to 10) in six months. These figures, possibly related to the stringent selection criteria for Atezolizumab plus nab-paclitaxel administration, could affect the diagnostic performance of pathologists. This hypothesis is supported by the difficulties reported in the interpretation of PD-L1 expression around the cut-off value, and points to the need of a constant re-training of involved pathologists. Another important factor potentially affecting the PD-L1 test interpretation is the presence of tissue artifacts related to sub-optimal pre-analytical

conditions. This is particularly important, considering that most Centers involved in the survey (6/8) received material from external laboratories. The impact of specimen handling (i.e., time of cold ischaemia and duration of formalin fixation) on PD-L1 interpretation is well known in literature [11-13] and strict quality assurance procedures need to be shared between different Pathology laboratories. Finally, the choice of the sample for evaluation is a crucial step in the diagnostic pathway. Recent literature has reported significant heterogeneity in PD-L1 expression between primary tumours and different metastatic sites [14]. According to the results of the IMpassion-130 study, PD-L1 expression is higher in primary tumors compared with metastatic sites (44% vs 36, p=0.014). In addition, PD-L1 expression is higher in lymph nodes compared with other metastatic sites [15]. These results have been confirmed by additional studies [16].

Decalcified bone tissue is considered unsuitable for PD-L1 evaluation [17,18]. Given the possibility of discordance in PD-L1 expression between the primary tumor and the site of metastasis, in case of PD-L1 negativity on the primary tumor, it may be desirable to evaluate PD-L1 also on the metastatic site if it is suitable for evaluation [19].

Immunohistochemical evaluation of PD-L1 expression is expected to increase in Italy with the pending approval of Pembrolizumab for TNBC in the metastatic setting by the National Medicines Agency. Patients' stratification will be based on a cut-off ≥ 10 by the CPS. This will represent a further challenge for Pathologists, requiring a specific training and quality controls.

Conclusion

In conclusion, our survey proved to be a useful tool to outline the current practice of PD-L1 evaluation in a large Italian region and to provide strategies for its implementation.

Acknowledgements

None

Authors' contributions

Conception and design: BC, AB and GD. Manuscript writing: BC, AC and GP. Data acquisition: AC. Analysed the data: BC and AC. Discussion of the results and implications of findings: BC, AC, GP, AB and GD. Data interpretation: BC, AB and BC. Drafting of the manuscript: BC, AB nd GP. Revising the content: All the authours. All authors read and approved the final manuscript.

Funding

This research was funded by Fondazione per la Medicina Personalizzata (FMP)

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All patients provided a written informed consent, and protocol approval of the

Local Ethics Committee was obtained [CE 5618].

Consent for publication

Not applicable.

Competing interests

Paolo Marchetti (PM) has a consultant/advisory role for BMS, Roche-Genentech, MSD, Novartis, Amgen, Merck Serono, Pierre Fabre, and Incyte. The other Authors declare that the research was conducted in the absence of any commercial or financial relationship which could be construed as a potential conflict of interest.

Abbreviations

TNBC Triple-negative breast cancers

ER Estrogen receptor

PR Progesterone receptor

HER 2 Human epidermal growth factor receptor 2

BCs Breast cancers

PD-L1 Programmed death-ligand 1

PD-1 programmed cell death protein 1

TC tumor cells

IC immune cells

PFS progression free survival

OS Overall Survival

CPS Combined positive score

ICs tumour-infiltrating immune cells

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