Surgical and Experimental Pathology

REVIEW Open Access

Applicability of PD-L1 tests to tailor triple-negative breast cancer treatment in Brazil

Kátia Ramos Moreira Leite[1*], Carlos Henrique Barrios[2], Antônio Carlos Buzaid[3,4], Débora Gagliato[3], Helenice Gobbi[5] and Fernando Soares[6,7]

Abstract

Background: Triple-negative breast cancer (TNBC) is a heterogeneous disease that represents 10–20% of breast cancer cases. The prognosis for advanced TNBC is usually poor, with a median overall survival of approximately 18 months or less.

Main text: New targeted therapies such as anti-PD-L1 agents are emerging as an option to treat advanced TNBC. A panel of 6 national experts with an active interest in breast cancer convened online. Panel members had either clinical or pathology experience in breast cancer. The experts pre-defined critical questions in the management of PD-L1 in TNBC, and a literature review was performed for selected topics before the online meeting.

Conclusion: The experts led active discussions involving a multidisciplinary team comprising pathologists and clinical oncologists. The meeting served to discuss the most relevant issues. A total of 10 critical questions for PD-L1+ TNBC were debated and are presented in this review. This article discusses the current landscape for PD-L1 tests in TNBC in Brazil.

Keywords: Triple-negative breast cancer, Target therapy, Immunohistochemistry, PD-L1, Immune checkpoint inhibitor

Background

Triple-negative breast cancer (TNBC) is a heterogeneous disease representing 10–20% of breast cancer cases. In Brazil, data from the AMAZONA study demonstrated that 21% of breast cancer patients in Brazil are triple-negative (Simon et al. 2019). The TNBC definition includes the absence of estrogen and progesterone receptors and no overexpression of the human epidermal growth factor receptor two genes (Kumar and Aggarwal 2016). The prognosis for advanced TNBC is usually poor, with a median overall survival of approximately 18 months or less. Given the suboptimal outcomes with chemotherapy, new targeted therapies for advanced TNBC are urgently needed (Khosravi-Shahi et al. 2019).

Currently, atezolizumab is approved for metastatic TNBC in Brazil and other parts of the world. Immunohistochemistry (IHC)–based detection of PD-L1 expression has been proposed as the predictive biomarker for selecting candidate patients who can benefit from cancer immunotherapy in metastatic cases (Gonzalez-Ericsson et al. 2020). Four main commercial primary antibody clones have been used in a number of clinical trials, paired with a specific staining platform (Gonzalez-Ericsson et al. 2020): SP142 (Roche Tissue Diagnostics, Tucson, AZ, USA), SP263 (Roche Tissue Diagnostics), 22C3 (Agilent Technologies Inc., Santa Clara, CA, USA), and 28–8 (Agilent Technologies Inc.). Parallel to the multiple assays, multiple scoring systems also exist (Gonzalez-Ericsson et al. 2020). Table 1 shows technical details and defines the scoring methods used for each antibody. Furthermore, different cutoffs and definitions are employed to determine PD-L1 positivity for various tumor types.

* Correspondence: katiaramos@usp.br
[1]Faculdade de Medicina da Universidade de São Paulo, Av. Dr. Arnaldo, 455, Pacaembu, São Paulo, SP CEP 01246-903, Brazil
Full list of author information is available at the end of the article

Table 1 Technical, regulatory and score cell type for PD-L1 clones

Commercial diagnostic assays used in clinical studies				
Clone	**SP142**	**SP263**	**22C3**	**28–8**
Binding epitope	Cytoplasmic domain		Discontinuous segments in the extracellular domain	
Platform	Ventana BenchMark ULTRA		Agilent Link 48	
Partner drug	Atezolizumab	Durvalumab	Pembrolizumab	Nivolumab
Drug regulatory situation[a]	Approved	Not approved	Not approved	Not approved

[a]For metastatic TNBC in Brazil on December 10, 2020

The reliability of IHC-based detection for PD-L1 in accurately identifying the best candidates for immune checkpoint inhibitor (ICI) has been a topic of scientific debate for some years. At present, the PD-L1 assay is the only approved biomarker for ICI agents. It is a complex matter, and the assay's clinical use remains controversial due to variability in assay performance of the PD-L1 IHC antibodies. Issues include tumor histology, tumor heterogeneity, absence of a unified scoring system, and concerns related to inter-reader reproducibility for scoring PD-L1 on immune cell (IC) or combined positive score (CPS) (Gonzalez-Ericsson et al. 2020). Due to these concerns, implementing PD-L1 in real-world practice remains a challenge (Gonzalez-Ericsson et al. 2020).

An ideal biomarker should be analytically valid, robust, reproducible, and clinically meaningful. For a biomarker to be deployed in daily practice, it must also be affordable and accessible to pathologists in community-laboratory practices (Gonzalez-Ericsson et al. 2020). In this expert panel, we conducted a review and discussion of the challenges of PD-L1 tests for TNBC in Brazil and offer mitigation approaches within a risk-management framework as previously described (Hall et al. 2014).

Main text

Methods

A panel of 6 national experts with an active interest in breast cancer convened online on May 21, 2020, by videoconference. Panel members had either clinical or pathology experience in breast cancer. The experts predefined critical questions in the management of PD-L1 in triple-negative breast cancer, and a literature review was performed for selected topics before the online meeting. The experts led active discussions involving a multidisciplinary team comprising pathologists and clinical oncologists. Each panelist consolidated a theme and presented this to the other participants during the virtual meeting. The meeting served to discuss the most relevant issues. The panel addressed two major TNBC areas concerning PD-L1: 1) metastatic TNBC; 2) early TNBC. A total of 10 critical questions for PD-L1+ TNBC were debated. This article discusses the current landscape for PD-L1 tests in TNBC in Brazil.

PD-L1 antibody - current landscape in Brazil

We currently have four commercially available assays in Brazil, two by Ventana (SP142 and SP263) and two by Dako (22-C3 and 28–8). These assays differ structurally and analytically and are discordant, producing different clinical results (Rugo et al. 2019). Studies comparing PD-L1 assay concordance in TNBC have shown analytical discrepancies among SP142, SP263, and 22C3 clones. For instance, PD-L1 positivity (IC ≥1%) using SP142 showed between 10 and 35% fewer PD-L1+ cases than 22C3 CPS ≥1% in different studies (Downes et al. 2020; Noske et al. 2019; Scott et al. 2019). More important than analytical concordance studies are studies assessing clinical outcomes. In this regard, Rugo et al. presented a post-hoc analysis of IMpassion130 (Rugo et al. 2019). The post-hoc analysis at prespecified cutoffs revealed that 22C3 and SP263 assays identified more patients with PD-L1 positivity tumors than SP142. However, the most significant clinical benefits of using atezolizumab combined with nab-paclitaxel were derived from SP142 PD-L1 positivity nested within SP263+ or 22C3+ (Rugo et al. 2019). Technical and regulatory details, together with score cell type for each antibody is given in Table 1.

Metastatic breast cancer scenario

Q1. What tests would be requested to target treatment for triple-negative breast cancer in the first-line advanced setting? Consider the diagnosis in a metastatic setting and the available evidence using cancer immunotherapy.

Answer: faced with a scenario of limited therapeutic options, with rapid disease relapses, it is recommended that every patient with advanced triple-negative breast cancer undergo a PD-L1 test using clone SP142 to determine PDL-1 expression in IC (Gonzalez-Ericsson et al. 2020; Schmid et al. 2018). In addition, a genetic panel for germinative BRCA1 and BRCA2/PALB2 mutations, irrespective of age (National Comprehensive Cancer Network 2020), should be performed. It is important to recognize that as a biomarker to identify patients for immunotherapy, PD-L1 does not have perfect performance

and has limitations. So, while it is not the best biomarker, it is the best we have at present.

Q2. Regarding therapeutic conduct:

a) **For patients with a history of previous treatment in the neoadjuvant or adjuvant disease setting: what is the approach for patients who have had prior therapy with taxane?**

Answer: in this situation, the recommendation is to perform a test for PD-L1 using clone SP142, provided eligibility criteria are met (i.e., expression of PD-L1 in IC ≥1%), the patient can receive a treatment regimen of nab-paclitaxel and atezolizumab, as long as the neoadjuvant/ adjuvant treatment has ceased for 12 months or longer (Schmid et al. 2018). However, a shorter interval (disease-free interval greater than 6 months) may be considered, as used in Keynote 355 (Cortes et al. 2020).

Patients who recur with a disease-free interval shorter than 6 months should be treated with second-line chemotherapy agents. Alternatively, PD-L1 positivity can be evaluated using clone 22C3 with CPS ≥20, a situation in which the patient can benefit from pembrolizumab (Cortes et al. 2020). However, it is important to note that neither of the above indications has yet been approved in Brazil, and overall survival data from the clinical trials were not reported (October 2020).

Q3. For patients with gBRCAm (or gPALB2) and PD-L1+, what would be the best therapeutic approach?

Answer: we recommend a combination of nab-paclitaxel and atezolizumab as the first-line approach as the analysis of the Impassion 130 for this subset of patients showed an overall survival benefit with the combination (Schmid et al. 2018). In the event of progression, the physician can use a PARP inhibitor, as there is an indication for this drug class in subsequent therapy lines (Robson et al. 2017).

Alternatively, PARP inhibitors, such as olaparib or talazoparib, have shown improved progression-free survival compared with chemotherapy when given to gBRCAm (or gPALB2) patients (Robson et al. 2017; Litton et al. 2018). These agents can be less toxic and easier to use the first-line option than chemotherapy (Hurvitz et al. 2019). However, it is important to point out that PARP inhibitors have not yet demonstrated overall survival benefit, and most clinical trial patients received PARP inhibitors as a second-line option or greater (Litton et al. 2018; Robson et al. 2019).

Q4. Which sample type is the most appropriate for evaluating PD-L1 in the metastatic scenario: primary breast tissue or metastatic tissue?

Answer: use the primary tumor and metastasis samples. If available, test both samples. There are, however, a few points to consider:

a) Primary sample freshness (more recent samples are superior).
b) Specimen storage time (degradation typically occurs after 3 years).
c) Metastasis site:
   i) If liver, analyze the evidence carefully and compare this to findings for the primary sample.
   ii) A positive result is confirmatory for bone marrow or bone samples, but a negative result is not.
   iii) PD-L1 expression at different metastasis sites can be associated with varied predictive responses to ICI. For instance, higher PD-L1 in lymph nodes was not related to clinical response or survival in non-small-cell lung cancer (NSCLC) (Hong et al. 2020; Schoenfeld et al. 2020). PD-L1 expression differed significantly between primary lung tumors and brain metastases (Mansfield et al. 2016). Further studies are needed, specifically in breast cancer, to clarify the relevance of PD-L1 in this clinical setting.
d) Pre-analytical and post-analytical sample handling:
   i) Pre-analytical care of tissue samples is increasingly demanding for laboratory and pathologists owing to more stringent requirements of new clinical methodologies employed in clinical practice, such as genomic and immunohistochemical tests for selecting target therapies (Bussolati et al. 2015). In the pre-analytical phase, four points warrant special attention to optimize the quality of reports.
      1) Fixation is the most critical step in the pre-analytical phase (Groenen et al. 2011). Tissue fixation must be done in 10% buffered formalin. The effects of different buffering agents used in 10% formalin, such as phosphate buffers, improve RNA preservation compared to other agents, consequently enhancing the quality of immunohistochemical assays (Chung et al. 2008);
      2) Fixation time must be no shorter than 6 h and no longer than 72 h (*US Food and Drug Administration. Summary of Safety and Effectiveness Data. VENTANA PD-L1 (SP142) Assay.* [n.d.]).

3) In the case of surgical specimens: controlling the time between surgery (time of specimen removal/cold ischemia, cleavage) and removal of samples is important to prevent autolysis and degradation of tissue antigens. Also, the immediate transport of specimens from the operating room to the pathology lab is crucial (Groenen et al. 2011). Delays in transportation, gross examination, sectioning, and sampling surgical specimens may speed up DNA and RNA degradation (Medeiros et al. 2007). Antigen stability detected by immunohistochemistry decreases with increasing warm ischemia time (Groenen et al. 2011).
4) It is important to use the criteria for assessing PD-L1 according to standards recommended by the assay provider (*US Food and Drug Administration. Summary of Safety and Effectiveness Data. VENTANA PD-L1 (SP142) Assay.* [n.d.]; *Dako. PD-L1 IHC 22C3 pharmDx*).

ii) Post-Analytical phase: pathologists' training should take place under ideal conditions. Ideal training conditions entail learning programs and case discussions intended to teach pathologists how to report the findings according to the cutoffs recommended by each clone's manufacturers. Despite being important in pathologists' training, we believe that continuing training programs that simulate the pathologist's daily challenges are vital for these professionals' quality continuing medical education. In a real-world setting, analytical performance and standardization are often time-consuming and challenging (Medeiros et al. 2007). Regular training/audits performed after initiating the pathological assessment program under real-world conditions ensure proper standardization of procedures to achieve optimal clinical practice results. A recent publication involving 19 pathologists from 14 academic centers in the USA showed poor reproducibility across multiple pathologists, where more than half of pathologists had discordant IC scores (Reisenbichler et al. 2020). This poor reproducibility can lead to error in either direction, i.e., overdetection or underdetection (Reisenbichler et al. 2020). Also crucial in the post-analytical phase is to consider that most molecular biology variables are continuous, which raises the problem of concordance with clinically validated cut-off points for diagnostic testing (De Gramont et al. 2015). These limitations and precautions are applicable in the immunohistochemical analysis of PD-L1 in breast cancer samples. The immunohistochemical report should also state which assay (SP 142 or 22C3) was used and which system was employed to classify the case as positive or negative (IC or CPS).

e) Primary sample with a negative result: in these cases assess the need for a repeat biopsy for PD-L1 testing.
   i) There are inherent challenges in capturing phenotypic heterogeneity through a single individual biopsy (Jamal-Hanjani et al. 2017; Turajlic et al. 2018; Warrick et al. 2019). According to current practice for either clinical or research settings, the problem seems to arise primarily from dependence on using a single site to evaluate tumor biology or evolution (Litchfield et al. 2020). Available evidence from clinical trials in metastatic breast cancer so far indicates that benefit from therapy is seen with a positive PD-L1 result independent from which site the biopsy was obtained.
f) Risk of missing the main tumor subclones: there is a risk of overlooking some of the primary tumor subclones in sites not covered by the sample, such as distal tumors, given the existence of intratumoral heterogeneity (Warrick et al. 2019), generating a bias that cannot be mitigated in the same tissue sample (Jamal-Hanjani et al. 2017).
   i) A positive result for metastasis or primary tumor automatically renders the patient eligible for immunotherapy. Patients whose results exhibit discrepancy (primary versus metastasis sites) had similar outcomes to those who tested positive in both samples in the IMpassion 130 study (Schmid et al. 2018).
g) The PD-L1 test is available in all regions of Brazil. Most Brazilian breast cancer pathologists are well trained to evaluate PD-L1 in breast cancer. Although technological advances are a game-changer for developing robust therapeutic plans, the experts recognize that there is still a learning curve. We believe there is a case for training general pathologists to interpret PD-L1 in breast cancer. Concerted efforts focused on the standardization of methodologies, quality control, and quality assurance of these new technologies can translate to benefits in real-world practice (Groenen et al. 2011). Table 2 summarizes the challenges and risks of implementing PD-L1 testing in clinical practice and proposed mitigation strategies.

Q5. What information "on medical request" is essential for the proper performance of tests defining the treatment plan (metastatic setting)?

Table 2 Risks associated with PD-L1 biomarkers for clinical practice

Risk	Details	Recommendation
Patient safety		
False-positive or false-negative	Incorrect results lead to inappropriate therapy and put patient safety at risk.	When both primary and metastatic samples are available, test both.
Tumor heterogeneity	Heterogeneity of PD-L1 expression between primary and metastatic lesions in TNBC can lead to erroneous classification.	
Logistical risks		
Sample collection and processing issues	Poor quality tissues can result in unclear test results.	Ensure correct sample fixation for 6 to 72 h and processing. Determine tissue adequacy on H&E: the presence of TC and tumor-associated IC. Cut 4um-thick slices for PD-L1 IHC testing and sections for other IHC to preserve tissue in biopsy samples. Use within 2 months of cutting.
Biomarker risks		
PD-L1 expression prevalence among clones	Clones differ in quantitative expression for TC and IC, therefore analytical comparison does not reflect clinical performance.	An assay must identify patients who will most likely respond based on clinical trial data, thereby identifying a more significant proportion of PD-L1 positive patients.
Multiple scoring systems	Multiple scoring systems for PD-L1 clones complicate reproducibility.	For BC, PD-L1 expressed in IC and not in TC is predictive of response in the SP142 assay.
Inter-pathologist variability for report results	PD-L1 on IC is not reproducible.	Ensure real-world training on the expected staining profile and cut-off for pathologists in clinical practice. Assess interobserver variability with a sufficiently large and statistically powered number of pathologists to guarantee reproducibility.

Adapted from (Gonzalez-Ericsson et al. 2020)
BC Breast cancer, *IC* Immune cells, *TC* Tumor cells, *IHC* Immunohistochemistry

Answer: there was no consensus on this point. The authors mentioned informing the pathologist for which line of treatment and drug the patient is being considered. Importantly, multidisciplinary care should include the pathologist's active involvement, particularly at the time of analysis of any biopsy.

Early breast cancer scenario

Q6. Is it necessary to test PDL-1 expression in patients considered for CIT in the neoadjuvant setting?

Answer: in the scenario of neoadjuvant therapy, it is not necessary to order PD-L1 tests. The KEYNOTE 522 study analyzed 602 patients with untreated stage II and III triple-negative breast cancer, comparing the use of pembrolizumab to placebo, both in association with chemotherapy (carboplatin and paclitaxel) in the neoadjuvant scenario. The results document that both PD-L1 positive and negative patients derived clinical benefit from CIT (Schmid et al. 2020). Similar results were observed in the IMpassion 031, which evaluated atezolizumab or placebo combined with nab-paclitaxel followed by ddAC (doxorubicin combined with cyclophosphamide). After surgery, patients in the atezolizumab arm received 11 additional cycles of therapy. The study demonstrated that the addition of the atezolizumab led to an improvement in pathologic complete response of about 17%. Akin to the KEYNOTE 522, the benefit was observed regardless of PD-L1 expression (Mittendorf et al. 2020). PD-L1-positive patients derived more benefit, but all patients had higher pCR rates with the combination of chemotherapy and immunotherapy.

Q7. Is it necessary to retest for PD-L1 in the surgical specimen of patients who have a PD-L1+ result on initial biopsy and underwent neoadjuvant immunotherapy?

Answer: a positive result suffices for the patient to receive immunotherapy, precluding a retest's need.

Q8. If the patient has undergone upfront surgery, is it advisable to perform immunotherapy in the adjuvant setting?

Answer: the consensus was not to consider using immunotherapy in the adjuvant disease setting. Clinical studies are ongoing.

Q9. What is the ideal immunotherapy-free interval before re-exposing the patient to immuno-oncology?

Answer: the consensus determined a period of no exposure of at least 6 months, but there is insufficient evidence to establish an ideal rechallenge period. A recent

retrospective observational multicenter national study in NSCLC explored the efficacy of anti-PD-1/PD-L1 rechallenge in advanced NSCLC patients. The authors sought to identify potential clinical features associated with more significant outcomes. One hundred forty-four patients with advanced NSCLC were rechallenged with an immune checkpoint inhibitor (ICI) after ≥12 weeks of discontinuation. Patients were discontinuing first ICI due to toxicity or clinical decision, cases able to maintain a treatment-free period, and those with good performance status may be potential candidates for rechallenge (Gobbini et al. 2020). Other small studies found similar results, mainly in NSCLC (Kitagawa et al. 2020; Simonaggio et al. 2019). Data on breast cancer rechallenge cases are anecdotal (Simonaggio et al. 2019).

Q10. According to the previous question, is changing the strategy in the event of the disease progression recommended?

Answer: there is no ideal period to indicate rechallenge with anti-PD-L1 therapy after disease progression following previous treatment. The resistance mechanisms involved remain unclear. Data are available for other agents (Nivolumab rechallenge), suggesting some degree of benefit, but this evidence needs further confirmation.

Meanwhile, the authors agreed that the combination of chemotherapy with anti-PD-L1 is an option to be considered on a case-by-case basis. The most appropriate strategy for the patient should be compatible with their profile. While recognizing that clinical practice does not always strictly reflect clinical trials, a discussion on the use of immunotherapy after having received it in the early setting can be a consideration. Also, for BRCA mutated patients, the use of PARP inhibitors is an alternative (M. Robson et al. 2017). A clinical study should ideally answer this last question.

Conclusion

Implementation of a validated biomarker assay in daily practice and changes in treatment decisions for a given disease usually requires level 1 evidence. With IMpassion130 - a randomized phase III trial - results led to the approval of atezolizumab and nab-paclitaxel as the standard of care for PD-L1+ ($IC_A \geq 1\%$) metastatic TNBC in Brazil and many other countries (Schmid et al. 2018).

For an assay to be incorporated into clinical guidelines, a biomarker must be available in pathology labs. Pathologists' training is essential for the reliability of IHC reports of PD-L1 to select patients who benefit most from ICI. To date, PD-L1 is the only approved biomarker for ICI, but due to variability in test performance of the different PD-L1 clones, heterogeneity, and absence of a standardized score system, concerns regarding inter-reader reproducibility for scoring PD-L1 must be addressed. Guidelines such as NCCN include PD-L1 diagnostic testing for recurrent or metastatic TNBC. Nevertheless, the PD-L1 assay is not routinely performed for metastatic TNBC in Brazil unless requested by the oncologist.

It is important to point out that PD-L1 assay concordance in TNBC has shown differences among SP142, SP263, and 22C3 clones (Rugo et al. 2019). The Observers Needed to Evaluate Subjective Tests (ONEST) is a new method for determining the minimum number of evaluators needed to estimate concordance between large numbers of readers, as occurs in the real-world setting. The intraclass correlation coefficient (ICC) for the scoring of SP142 was 0.560 (Reisenbichler et al. 2020). ONEST plots showed a decreasing overall percent agreement (OPA) as the observer number increased, reaching a low of 0.41 at eight observers for SP142 (Reisenbichler et al. 2020). This low concordance among pathologists could lead to many patients receiving atezolizumab when they are unlikely to benefit or to not receiving atezolizumab when it may provide clinical benefit (Reisenbichler et al. 2020). To minimize this negative impact on patients, labs and industry should deploy robust training programs simulating real-world practice. The need for more tissue sample representativeness for molecular assays is also a vital point to address in training programs, given that most pathologists are unaware of this issue. Incorporating pathologist in the multidisciplinary discussions is mandatory to improve the flow and optimize management strategies for these patients.

Particular care with pre-analytical factors is also fundamental to ensure test quality. For instance, a fixation time exceeding 96 h increases suboptimal results for the SP142 clone by 16% (Barberà et al. 2020). Each pathology laboratory has different issues to overcome, such as sample selection, sample processing, quality control, and interpretation to guarantee proper patient selection.

Patient selection is the most crucial aspect for the future management of TNBC. We need to recognize that we are dealing with patients with different diseases under the same name. The entire discussion will evolve according to our ability to identify who responds to which treatment. As a corollary to this discussion and at the same time, an exciting challenge is when a patient has two or more predictive markers (i.e., BRCAm and PD-L1) and how to manage this situation (calling for specific clinical studies).

Abbreviations

CIT: Chemoimmunotherapy; CPS: Combined positive score; IC: Immune cell; ICI: Immune checkpoint inhibitor; IHC: Immunohistochemistry; TNBC: Triple-negative breast cancer

Acknowledgments
CoreBox Medical Communications provided medical writing assistance.

Authors' contributions
Roche Diagnostics supported this paper. The data were collected by the sponsor and analyzed in conjunction with the authors. All of them contributed to writing the article and were approved to submit for publication.

Funding
Roche Diagnostics supported this paper.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The authors consented to the publication.

Competing interests
Antônio Carlos Buzaid, MD
Advisor Roche, MSD, BMS, Lilly, Pfizer, Blau, AstraZeneca
Carlos H. Barrios, MD
Grants/research support: (to the institution) Pfizer, Novartis, Amgen, AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Roche/Genentech, Lilly, Sanofi, Taiho Pharmaceutical, Mylan, Merrimack, Merck, AbbVie, Astellas Pharma, BioMarin, Bristol-Myers Squibb, Daiichi Sankyo, Abraxis Biosciences, AB Science, Asana Biosciences, Medivation, Exelixis, ImClone Systems, LEO Pharma, Millennium
Advisory Boards and Consulting: Boehringer-Ingelheim, GSK, Novartis, Pfizer, Roche/Genentech, Eisai, Bayer, MSD, Astra Zeneca, Lilly.
Débora de Melo Gagliato, MD
Speaker fees: Roche, AstraZeneca, Pfizer, Lilly, Amgen, United Medical, and Libbs.
Consulting: Libbs.
The other authors declare that they have no competing interests.

Author details
[1]Faculdade de Medicina da Universidade de São Paulo, Av. Dr. Arnaldo, 455, Pacaembu, São Paulo, SP CEP 01246-903, Brazil. [2]Latin American Cooperative Oncology Group – LACOG, Porto Alegre, RS, Brazil. [3]Hospital Beneficência Portuguesa, São Paulo, SP, Brazil. [4]Hospital Israelita Albert Einstein, São Paulo, SP, Brazil. [5]Disciplina de Patologia Especial, Universidade Federal do Triângulo Mineiro, Uberaba, MG, Brazil. [6]Instituto de Anatomia Patológica, Rede D'Or São Luiz, São Paulo, SP, Brazil. [7]Instituto de Pesquisa e Ensino D'Or, São Paulo, SP, Brazil.

Received: 16 January 2021 Accepted: 31 March 2021
Published online: 20 April 2021

References

Barberà A, Martin M, Mate JL, Oriol A, Martínez-Soler F, Santalucia T, Fernández PL (2020) The immunohistochemical expression of programmed death ligand 1 (PD-L1) is affected by sample overfixation. Appl Immunohistochem Mol Morphol 29(1):76–81

Bussolati G, Annaratone L, Maletta F (2015) The pre-analytical phase in surgical pathology. In: Pre-analytics of pathological specimens in oncology. Switzerland: © Springer International Publishing, pp 1–13

Chung J-Y, Braunschweig T, Williams R, Guerrero N, Hoffmann KM, Kwon M, Song YK, Libutti SK, Hewitt SM (2008) Factors in tissue handling and processing that impact RNA obtained from formalin-fixed, paraffin-embedded tissue. J Histochem Cytochem 56(11):1033–1042. https://doi.org/10.1369/jhc.2008.951863

Cortes J, Cescon DW, Rugo HS, Nowecki Z, Im S-A, Yusof MM, Gallardo C, Lipatov O, Barrios CH, Holgado E, Iwata H, Masuda N, Torregroza Otero M, Gokmen E, Loi S, Guo Z, Zhao J, Aktan G, Karantza V, Schmid P (2020) KEYNOTE-355: randomized, double-blind, phase III study of pembrolizumab + chemotherapy versus placebo + chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer. J Clin Oncol 38(15_suppl):1000. https://doi.org/10.1200/JCO.2020.38.15_suppl.1000

De Gramont A, Watson S, Ellis LM, Rodón J, Tabernero J, De Gramont A, Hamilton SR (2015) Pragmatic issues in biomarker evaluation for targeted therapies in cancer. Nat Rev Clin Oncol 12(4):197

Downes MR, Slodkowska E, Katabi N, Jungbluth AA, Xu B (2020) Inter-and intraobserver agreement of programmed death ligand 1 scoring in head and neck squamous cell carcinoma, urothelial carcinoma and breast carcinoma. Histopathology 76(2):191–200. https://doi.org/10.1111/his.13946

Gobbini E, Toffart AC, Pérol M, Assié J-B, Duruisseaux M, Coupez D, Dubos C, Westeel V, Delaunay M, Guisier F, Veillon R, Gounant V, Giroux Leprieur E, Vanel F-R, Chaabane N, Dansin E, Babey H, Decroisette C, Barlesi F, Daniel C, Fournel P, Mezquita L, Oulkhouir Y, Canellas A, Duchemann B, Molinier O, Alcazer V, Moro-Sibilot D, Levra MG (2020) Immune checkpoint inhibitors rechallenge efficacy in non-small-cell lung cancer patients. Clin Lung Cancer 21(5):e497–e510. https://doi.org/10.1016/j.cllc.2020.04.013

Gonzalez-Ericsson PI, Stovgaard ES, Sua LF, Reisenbichler E, Kos Z, Carter JM, Michiels S, Le Quesne J, Nielsen TO, Lænkholm A, Fox SB, Adam J, Bartlett JM, Rimm DL, Quinn C, Peeters D, Dieci MV, Vincent-Salomon A, Cree I et al (2020) The path to a better biomarker: application of a risk management framework for the implementation of PD-L1 and TILs as immuno-oncology biomarkers in breast cancer clinical trials and daily practice. J Pathol 250(5): 667–684. https://doi.org/10.1002/path.5406

Groenen PJ, Blokx WA, Diepenbroek C, Burgers L, Visinoni F, Wesseling P, van Krieken JH (2011) Preparing pathology for personalized medicine: possibilities for improvement of the pre-analytical phase. Histopathology 59(1):1–7. https://doi.org/10.1111/j.1365-2559.2010.03711.x

Hall JA, Salgado R, Lively T, Sweep F, Schuh A (2014) A risk-management approach for effective integration of biomarkers in clinical trials: perspectives of an NCI, NCRI, and EORTC working group. Lancet Oncol 15(4):e184–e193. https://doi.org/10.1016/S1470-2045(13)70607-7

Hong L, Negrao MV, Dibaj SS, Chen R, Reuben A, Bohac JM, Liu X, Skoulidis F, Gay CM, Cascone T (2020) Programmed death-ligand 1 heterogeneity and its impact on benefit from immune checkpoint inhibitors in NSCLC. J Thorac Oncol 15(9):1449–1459. https://doi.org/10.1016/j.jtho.2020.04.026

Hurvitz SA, Gonçalves A, Rugo HS, Lee K-H, Fehrenbacher L, Mina LA, Diab S, Blum JL, Chakrabarti J, Elmeliegy M, DeAnnuntis L, Gauthier E, Czibere A, Tudor IC, Quek RGW, Litton JK, Ettl J (2019) Talazoparib in patients with a Germline BRCA-mutated advanced breast cancer: detailed safety analyses from the phase III EMBRACA trial. Oncologist. https://doi.org/10.1634/theoncologist.2019-0493

Jamal-Hanjani M, Wilson GA, McGranahan N, Birkbak NJ, Watkins TBK, Veeriah S, Shafi S, Johnson DH, Mitter R, Rosenthal R, Salm M, Horswell S, Escudero M, Matthews N, Rowan A, Chambers T, Moore DA, Turajlic S, Xu H, Lee SM, Forster MD, Ahmad T, Hiley CT, Abbosh C, Falzon M, Borg E, Marafioti T, Lawrence D, Hayward M, Kolvekar S, Panagiotopoulos N, Janes SM, Thakrar R, Ahmed A, Blackhall F, Summers Y, Shah R, Joseph L, Quinn AM, Crosbie PA, Naidu B, Middleton G, Langman G, Trotter S, Nicolson M, Remmen H, Kerr K, Chetty M, Gomersall L, Fennell DA, Nakas A, Rathinam S, Anand G, Khan S, Russell P, Ezhil V, Ismail B, Irvin-Sellers M, Prakash V, Lester JF, Kornaszewska M, Attanoos R, Adams H, Davies H, Dentro S, Taniere P, O'Sullivan B, Lowe HL, Hartley JA, Iles N, Bell H, Ngai Y, Shaw JA, Herrero J, Szallasi Z, Schwarz RF, Stewart A, Quezada SA, le Quesne J, van Loo P, Dive C, Hackshaw A, Swanton C (2017) Tracking the evolution of non–small-cell lung cancer. N Engl J Med 376(22):2109–2121. https://doi.org/10.1056/NEJMoa1616288

Khosravi-Shahi P, Cabezón-Gutiérrez L, Aparicio Salcedo MI (2019) State of art of advanced triple negative breast cancer. Breast J 25(5):967–970. https://doi.org/10.1111/tbj.13369

Kitagawa S, Hakozaki T, Kitadai R, Hosomi Y (2020) Switching administration of anti-PD-1 and anti-PD-L1 antibodies as immune checkpoint inhibitor rechallenge in individuals with advanced non-small cell lung cancer: case series and literature review. Thorac Cancer 11(7):1927–1933. https://doi.org/10.1111/1759-7714.13483

Kumar P, Aggarwal R (2016) An overview of triple-negative breast cancer. Arch Gynecol Obstet 293(2):247–269. https://doi.org/10.1007/s00404-015-3859-y

Litchfield K, Stanislaw S, Spain L, Gallegos LL, Rowan A, Schnidrig D, Rosenbaum H, Harle A, Au L, Hill SM, Tippu Z, Thomas J, Thompson L, Xu H, Horswell S, Barhoumi A, Jones C, Leith KF, Burgess DL et al (2020) Representative sequencing: unbiased sampling of solid tumor tissue. Cell Rep 31(5):107550. https://doi.org/10.1016/j.celrep.2020.107550

Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee K-H, Fehrenbacher L, Yerushalmi R, Mina LA, Martin M, Roché H, Im Y-H, Quek RGW, Markova D, Tudor IC, Hannah AL, Eiermann W, Blum JL (2018) Talazoparib in patients with advanced breast cancer and a Germline BRCA mutation. N Engl J Med 379(8):753–763. https://doi.org/10.1056/NEJMoa1802905

Mansfield AS, Aubry MC, Moser JC, Harrington SM, Dronca RS, Park SS, Dong H (2016) Temporal and spatial discordance of programmed cell death-ligand 1 expression and lymphocyte tumor infiltration between paired primary lesions and brain metastases in lung cancer. Ann Oncol 27(10):1953–1958. https://doi.org/10.1093/annonc/mdw289

Medeiros F, Rigl CT, Anderson GG, Becker SH, Halling KC (2007) Tissue handling for genome-wide expression analysis: a review of the issues, evidence, and opportunities. Arch Pathol Lab Med 131(12):1805–1816. https://doi.org/10.5858/2007-131-1805-THFGEA

Mittendorf EA, Zhang H, Barrios CH, Saji S, Jung KH, Hegg R, Koehler A, Sohn J, Iwata H, Telli ML, Ferrario C, Punie K, Penault-Llorca F, Patel S, Duc AN, Liste-Hermoso M, Maiya V, Molinero L, Chui SY, Harbeck N (2020) Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): a randomised, double-blind, phase 3 trial. Lancet (London, England). https://doi.org/10.1016/S0140-6736(20)31953-X

National Comprehensive Cancer Network (2020) NCCN guidelines: breast cancer, version 6.2020—September 8, 2020. Recuperado 29 de setembro de 2020, de https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf

Noske A, Ammann J, Wagner D-C, Denkert C, Lebeau A, Sinn P, Kreipe H-H, Baretton G, Steiger K, Kiechle M (2019) Reproducibility and concordance of 4 clinically developed programmed death-ligand 1 (PD-L1) immunohistochemistry (IHC) assays in triple negative breast cancer (TNBC). Ann Oncol 30:v130–v131. https://doi.org/10.1093/annonc/mdz242.054

Reisenbichler ES, Han G, Bellizzi A, Bossuyt V, Brock J, Cole K, Fadare O, Hameed O, Hanley K, Harrison BT (2020) Prospective multi-institutional evaluation of pathologist assessment of PD-L1 assays for patient selection in triple negative breast cancer. Mod Pathol 33:1746–1752

Robson M, Im S-A, Senkus E, Xu B, Domchek SM, Masuda N, Delaloge S, Li W, Tung N, Armstrong A (2017) Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. N Engl J Med 377(6):523–533. https://doi.org/10.1056/NEJMoa1706450

Robson ME, Tung N, Conte P, Im S-A, Senkus E, Xu B, Masuda N, Delaloge S, Li W, Armstrong A, Wu W, Goessl C, Runswick S, Domchek SM (2019) OlympiAD final overall survival and tolerability results: Olaparib versus chemotherapy treatment of physician's choice in patients with a germline BRCA mutation and HER2-negative metastatic breast cancer. Ann Oncol 30(4):558–566. https://doi.org/10.1093/annonc/mdz012

Rugo HS, Loi S, Adams S, Schmid P, Schneeweiss A, Barrios CH, Iwata H, Dieras VC, Winer EP, Kockx M (2019) Performance of PD-L1 immunohistochemistry (IHC) assays in unresectable locally advanced or metastatic triple-negative breast cancer (mTNBC): post-hoc analysis of IMpassion130. Ann Oncol 30:v858–v859. https://doi.org/10.1093/annonc/mdz394.009

Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, Diéras V, Hegg R, Im S-A, Shaw Wright G (2018) Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 379(22):2108–2121. https://doi.org/10.1056/NEJMoa1809615

Schmid P, Cortes J, Pusztai L, McArthur H, Kümmel S, Bergh J, Denkert C, Park YH, Hui R, Harbeck N, Takahashi M, Foukakis T, Fasching PA, Cardoso F, Untch M, Jia L, Karantza V, Zhao J, Aktan G, Dent R, O'Shaughnessy J (2020) Pembrolizumab for early triple-negative breast cancer. N Engl J Med 382(9):810–821. https://doi.org/10.1056/NEJMoa1910549

Schoenfeld AJ, Rizvi H, Bandlamudi C, Sauter JL, Travis WD, Rekhtman N, Plodkowski AJ, Perez-Johnston R, Sawan P, Beras A, Egger JV, Ladanyi M, Arbour KC, Rudin CM, Riely GJ, Taylor BS, Donoghue MTA, Hellmann MD (2020) Clinical and molecular correlates of PD-L1 expression in patients with lung adenocarcinomas☆. Ann Oncol 31(5):599–608. https://doi.org/10.1016/j.annonc.2020.01.065

Scott M, Scorer P, Barker C, Al-Masri H (2019) Comparison of patient populations identified by different PD-L1 assays in in triple-negative breast cancer (TNBC). Ann Oncol 30(Supplement_3):mdz095–mdz009

Simon SD, Bines J, Werutsky G, Nunes JS, Pacheco FC, Segalla JG, Gomes AJS, Adam Van Eyll BMHR, Gimenes DL, Crocamo S, Freitas-Junior R, Lago LD, Queiroz GS, Jobim de Azevedo S, Rosa DD, Delgado G, Borges GS, Verônica do Nascimento Y, Zaffaroni F et al (2019) Characteristics and prognosis of stage I-III breast cancer subtypes in Brazil: the AMAZONA retrospective cohort study. Breast (Edinburgh, Scotland) 44:113–119. https://doi.org/10.1016/j.breast.2019.01.008

Simonaggio A, Michot JM, Voisin AL, Le Pavec J, Collins M, Lallart A, Cengizalp G, Vozy A, Laparra A, Varga A, Hollebecque A, Champiat S, Marabelle A, Massard C, Lambotte O (2019) Evaluation of readministration of immune checkpoint inhibitors after immune-related adverse events in patients with cancer. JAMA Oncol 5(9):1310. https://doi.org/10.1001/jamaoncol.2019.1022

Turajlic S, Xu H, Litchfield K, Rowan A, Horswell S, Chambers T, O'Brien T, Lopez JI, Watkins TBK, Nicol D, Stares M, Challacombe B, Hazell S, Chandra A, Mitchell TJ, Au L, Eichler-Jonsson C, Jabbar F, Soultati A et al (2018) Deterministic evolutionary trajectories influence primary tumor growth: TRACERx renal. Cell 173(3):595–610.e11. https://doi.org/10.1016/j.cell.2018.03.043

US Food and Drug Administration (n.d.) Approval order. PD-L1 IHC 22C3 pharmDx Assay. Available from: https://www.accessdata.fda.gov/cdrh_docs/pdf15/P150013S014A.pdf. Accessed 24 Mar 2021

US Food and Drug Administration (n.d.) Summary of Saftey and Effectiveness Data. VENTANA PD-L1 (SP142) Assay. Available from: https://www.accessdata.fda.gov/cdrh_docs/pdf16/p160002s009b.pdf. Accessed 24 Mar 2021

Warrick JI, Sjödahl G, Kaag M, Raman JD, Merrill S, Shuman L, Chen G, Walter V, DeGraff DJ (2019) Intratumoral heterogeneity of bladder cancer by molecular subtypes and histologic variants. Eur Urol 75(1):18–22. https://doi.org/10.1016/j.eururo.2018.09.003

Publisher's Note