Avoiding “False Positive” and “False Negative” Immunohistochemical Results in Breast Pathology

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Keywords

Biomarker · Breast cancer · Breast pathology · Immunohistochemistry · Pitfalls

Abstract

Immunohistochemistry (IHC) plays an important role in the evaluation of breast pathology specimens to provide both diagnostic and prognostic/therapeutic information. Although most IHCs used in breast pathology can be easily interpreted, pitfalls do exist, especially in some uncommon scenarios. This review intends to focus on the challenging areas such as the interpretation of myoepithelial cell markers in differentiating benign proliferation and in situ carcinoma from invasive carcinoma, lobular cell markers in differentiating lobular from ductal carcinoma, cytokeratin and other markers in diagnosing metaplastic carcinoma, and breast tissue origin markers in diagnosing breast primary carcinoma. The challenges in interpreting prognostic and predictive markers will be also discussed.

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Introduction

Although examination of hematoxylin and eosin (H&E)-stained slides can routinely diagnose the majority of breast lesions, immunohistochemistry (IHC) remains

an integral part of breast pathology to confirm diagnoses and/or resolve diagnostic dilemmas. Prognostic and predictive markers including estrogen receptor (ER), progesterone receptor (PR), and HER2/neu studies are routinely used in the evaluation of breast carcinoma specimens using IHC. Additionally, new therapeutic markers such as PD-L1 and other are emerging. In this review, we will discuss some commonly encountered pitfalls in interpreting IHC stains in breast pathology in order to avoid false-negative results.

The most commonly used markers in breast specimens are myoepithelial cell (MEC) markers such as smooth muscle myosin heavy chain (SMMHC), p63/p40, calponin, and CD10 used to differentiate benign or in situ from invasive carcinoma, and aid in the evaluation of papillary lesions and sclerosing lesions. Other important diagnostic markers commonly used in practice include cytokeratin (e.g., CK5 and CK14) and ER for differentiating usual ductal hyperplasia (UDH) from atypical ductal hyperplasia (ADH) and low/intermediate grade ductal carcinoma in situ (DCIS), and E-cadherin for differentiating lobular from ductal neoplasms. However, pitfalls in the interpretation of these markers exist and false results can result in misdiagnosis of the breast lesions. These will be discussed in this review. In addition, pitfalls in interpreting predictive and prognostic markers such as ER, PR, and HER2/neu, and PD-L1 will be discussed as well.

Table 1. Commonly used immunostains in breast pathology with their clinical utility and interpretation pitfalls

Antigen/antibody	Description	Clinical use	Pitfalls
CK5	High molecular weight cytokeratin; MEC marker	Stains MECs; stains proliferative components in UDH, negative in ADH/DCIS (low/intermediate grade)	Also positive in high-grade DCIS, metaplastic carcinoma, and basal-like carcinoma
E-cadherin	Cell adhesion protein; membranous staining in normal breast epithelial cells	Membranous staining in ductal carcinoma; negative in lobular carcinoma	Some lobular carcinomas may show decreased/aberrant staining
ER	ER, patchy nuclear staining in normal breast ducts/lobules	Prognostic and predictive marker; diffusely positive in ADH/low-grade DCIS	Can be affected by many preanalytic, analytic, and postanalytic factors such as tissue processing, fixation, decalcification, different antibodies
GATA3	GATA-binding protein-3; nuclear staining in normal breast luminal epithelial cells	Breast origin marker (>95% of breast carcinoma)	Positive in only up to 70% of TNBCs, and positive in other primary carcinomas including urothelial carcinoma, squamous cell carcinoma, lung adenocarcinoma, pancreatobiliary carcinoma, etc.
GCDFP-15	Gross cystic disease fluid protein-15, stains normal breast epithelial cells	Specific marker for breast carcinoma	Low sensitivity, only positive in up to 25% of TNBCs
HER2	Epidermal growth factor receptor 2, oncoprotein not overexpressed in normal breast epithelium	Prognostic and predictive marker, overexpression in 15%–20% of breast carcinoma	Can be affected by many preanalytic, analytic, and postanalytic factors such as tissue processing, fixation, decalcification, etc.
Mammaglobin	Stains normal breast epithelium	Specific marker for breast carcinoma	Low sensitivity, only positive in up to 31% of TNBCs
p120	Catenin that is bound to E-cadherin in normal ductal epithelial cells	Membranous staining in ductal carcinoma; cytoplasmic staining in lobular carcinoma	
p40	Truncated form of p63; MEC marker	Nuclear staining in MECs to help differentiate benign/in situ carcinoma from invasive carcinoma	Also stains some high-grade carcinoma (in situ and invasive), metaplastic carcinoma (invasive), squamous cell carcinoma, etc.
p63	MEC marker	Nuclear staining in MECs to help differentiate benign/in situ carcinoma from invasive carcinoma	Also stains some high-grade carcinoma (in situ and invasive), metaplastic carcinoma (invasive), squamous cell carcinoma, etc.
PR	PR; patchy nuclear staining in normal breast ducts/lobules	Prognostic and predictive marker	Can be affected by many preanalytic, analytic, and postanalytic factors such as tissue processing, fixation, decalcification, and different antibodies; shows more heterogeneous staining than ER
S100	Marker of neuromelanocytic differentiation	Stains positive in MGA; positive for melanoma; occasionally used for MECs	Stains occasionally positive in some invasive carcinomas
SMMHC	SMMHC; MEC marker	Cytoplasmic staining in MECs to help differentiate benign/in situ carcinoma from invasive carcinoma	Cross-reactivity with myofibroblasts in up to 8% of cases
SOX-10	Marker of neuromelanocytic differentiation	Stains positive in up to 60% of TNBCs, can be used as complementary marker to GATA3	Stains positive in many other tumor cells (melanoma, tumors with neural origin); the sensitivity is low
TRPS1	TRPS1; stains positive in normal breast normal epithelial cells	Highly sensitive marker for breast carcinoma, even for TNBC and metaplastic carcinoma (>90%)	New marker, need more studies

Commonly used immunostains in breast pathology with their clinical utility and interpretation pitfalls were briefly summarized in Table 1. Tissue processing, fixation, and decalcification in addition to the analytical variables such

as antibody validation and kits used play important roles in interpreting breast IHC markers, especially in those predictive/therapeutic biomarkers such as ER, PR, and HER2. In addition, appropriate IHC controls (both posi-

Fig. 1. MGA. H&E stain, $\times 200$ (a); immunostain of SMMHC, $\times 200$ (b); immunostain of ER, $\times 200$ (c); immunostain of S100, $\times 200$ (d).

tive and negative controls) should be included in all IHCs, and this is mostly important when interpreting false-negative results.

Diagnostic IHC Markers

“False” Negative Myoepithelial Markers in Noninvasive Breast Lesions

Both benign breast lesions and in situ carcinomas are contained within the ducts and lobules and are surrounded by MECs and basement membrane [1]. Invasion is defined as breaching the MEC layer and basement membrane into the surrounding stroma. The presence of MECs around proliferating or nonproliferating epithelial

cells of breast lesions determines the difference between in situ and invasive carcinoma, and between benign pseudo-invasive lesions and invasive carcinoma [1–5]. MECs cells can be visualized easily in normal breast ducts and acini. When these structures are compressed, dilated, or distorted, it is difficult to detect MECs on H&E-stained sections, but IHC can be very helpful.

Microglandular adenosis (MGA) is an exception to the abovementioned statement, since it lacks an outer MEC layer, but is still considered as a benign adenosis lesion [6–9]. Histologically, MGA consists of a benign poorly circumscribed nonorganoid proliferation of small, discrete epithelial-lined tubular structures, which permeate into the surrounding stroma and adipose tissue. Tubules are relatively uniformly small and usually

Fig. 2. EPC. H&E stain, ×200 (a); immunostain of SMMHC, ×200 (b); immunostain of p40, ×200 (c); side by side view of H&E and immunostain of SMMHC to demonstrate microinvasion, ×200 (d).

have open lumens. Amorphous eosinophilic material is often present within the lumens. Although MGA lacks an MEC layer, it does have a thick layer of basement membrane around the glands (Fig. 1). Immunohistochemically, MGA is negative for all MEC markers including p63/p40 and calponin, etc., and also negative for ER, PR, and HER2. MGA is characteristically positive for S100 (Fig. 1) [6, 7].

Encapsulated papillary carcinoma (EPC) is considered as an in situ tumor although it lacks MECs at the periphery. It is a well circumscribed papillary neoplasm with expansile growth pattern within a cystic space, and surrounded by a thick fibrous capsule [10, 11]. (Fig. 2) MEC markers usually stain negatively within papillae and at the periphery (Fig. 2). The new World Health Organization classification of tumors of the breast recommends staging EPC as in situ disease in the absence of frank invasion due to its indolent behavior, in order to avoid overtreatment [12, 13]. A diagnosis of invasion should only be considered when tumor cells are infiltrating into the surrounding stroma outside fibrous capsule and must be distinguished from entrapped epithelial cell clusters within the fibrous capsule or stroma caused by sclerosis or biopsy artifacts. It is not uncommon that a true invasive carcinoma is present together with EPC (Fig. 2). In this scenario, only the true invasive component is measured and staged accordingly [13].

Similar to EPC, solid papillary carcinoma (SPC) can be problematic regarding its absence of MECs at the periphery. SPC is also a papillary neoplasm with a solid growth pattern with interspersed delicate fibrovascular cores [14–16]. SPC can be either in situ or invasive [13]. When SPC tumor nodule(s) are rounded and well circumscribed with pushing borders, it is considered as an in situ tumor. Invasive SPC is considered when solid tumor nodules are irregular with jagged contours and surrounding desmoplastic stroma [17, 18]. MECs are often absent within tumor nodule and at the periphery for both SPC in situ and invasive SPC, but focal or attenuated staining of MECs may be present (Fig. 3).

“False” Positive Myoepithelial Markers in Invasive Breast Carcinoma

Many MEC markers are available with varying sensitivity and specificity and the most commonly used markers include SMMHC, p63 or p40, calponin, high molecular weight keratin (HMWK) such as CK5/6 and CK14, and smooth muscle actin. It should be noted these markers are not specific for MECs, and they may be expressed in certain types of invasive breast carcinoma or other cells, resulting in a “false positive MECs” and thus false-negative diagnosis of invasive carcinoma.

P63/p40, a member of the p53 gene family, is both a sensitive and specific MEC marker, being expressed ex-

Fig. 3. SPC. H&E stain, $\times 100$ (a); H&E stain, $\times 200$ (b); immunostain of SMMHC, $\times 100$ (c); immunostain of p40, $\times 100$ (d); SPC with invasion, H&E $\times 200$ (e); invasive SPC, $\times 100$ (f).

clusively in the nuclei of MECs [19–21]. P40 is a truncated isoform of p63 and is highly specific for squamous and basal cells [22]. It has been shown that p40 is as specific as p63 in highlighting MECs with less staining intensity in occasional cases, and both antibodies can be used interchangeably for labeling MECs in breast pathology practice [21]. However, p63/p40 is also expressed in cells with squamous differentiation or basal differentiation, such as low-grade adenosquamous cell carcinoma, squamous cell carcinoma, metaplastic carcinoma with squamous differentiation, adenoid cystic carcinomas, and basal-like triple negative breast carcinoma, etc. [20, 23, 24] (Fig. 4) Besides metaplastic carcinomas of the breast, p63 expression was identified in up to 7.2% of IDCs with highest percentage in TNBCs (11%), particularly those with basal-like features, suggesting p63 may play a role in basal-like invasive carcinoma development [25, 26].

Anti-smooth muscle protein antibodies can detect MECs due to their partial smooth muscle phenotype, including smooth muscle actin, calponin, and SMMHC [27–29]. These markers share the common trait of staining myofibroblasts, sometimes causing problems in interpretation since myofibroblasts can closely surround invasive carcinoma. As compared with the other two markers, SMMHC is significantly easier to interpret due to its

highest specificity with cross-reactivity with myofibroblasts in only 8% of cases [30, 31].

HMWKs, such as CK5/6 or CK5, can be used as markers for MECs [32]. These markers show no cross-reactivity with myofibroblasts [33], but they are positive in up to 38% of high-grade invasive carcinomas [34], especially in basal-like triple-negative breast carcinomas, low-grade adenosquamous carcinoma, and squamous cell carcinoma of the breast [35, 36]. CK14, another HMWK and a marker for MECs, shows mosaic staining pattern in UDH, while mostly negative in ADH/DCIS [37, 38].

“False Positive” of HMWKs in DCIS

HMWKs, such as CK5 or CK5/6, have been extensively used to distinguish ADH or DCIS from UDH due to their distinct staining patterns between ADH/DCIS and UDH. UDH usually has a mosaic pattern, whereas ADH/DCIS tend to be negative or have only scattered positivity caused by clonal proliferation of luminal cells [39–42]. This is very useful in breast core biopsy specimens, since distinguishing UDH from ADH/DCIS can be challenging on H&E stain sections with limited materials and this distinction bears significant difference in clinical management. However, HMWKs can be positive in up to 32% of high-grade DCISs [43]. Therefore, it is

Fig. 4. Low-grade adenosquamous cell carcinoma. H&E stain, $\times 200$ (a); immunostain of AE1/3, $\times 200$ (b); immunostain of CK5, $\times 200$ (c); immunostain of p40, $\times 200$ (d); immunostain of SMMHC, $\times 200$ (e); immunostain of ER, $\times 200$ (f).

necessary to pay attention to nuclear pleomorphism and other morphologic features such as comedonecrosis and architectural changes, not only rely on CK5/6 staining (Fig. 5).

“False Positive” of E-Cadherin Membranous Staining (Aberrant Staining) in Lobular Lesions

Breast lobular lesions including invasive lobular carcinoma (ILC), atypical lobular hyperplasia and lobular carcinoma in situ (LCIS), and ILCs are characterized by the loss of membranous E-cadherin expression, which is useful for distinction from ductal lesions [44–51]. E-cadherin, a transmembrane protein encoded by *CDH1* gene (16q22.1), is a member of calcium-dependent adhesion family and mediates epithelial cell-cell adhesions [52]. E-cadherin is composed of an extracellular domain, a transmembrane domain, and an intracytoplasmic domain,

which binds p120 catenin [53]. In normal or abnormal ductal cells, both E-cadherin and p120 catenin are present at the cell membrane and can be identified there by IHC. If E-cadherin is absent or nonfunctional such as in lobular lesions, p120 translocate to and accumulates in the cytoplasm [54–57]. Loss of E-cadherin expression can be caused by mutations, homozygous deletion of *CDH1* gene, loss of heterozygosity (LOH) of *CDH1* locus, or promoter hypermethylation [58]. ILCs and LCIS often show inactivating mutations of E-cadherin in combination with LOH of *CDH1* locus [59, 60].

It should be noted that the presence of E-cadherin membranous staining does not preclude a diagnosis of lobular lesion. Some ILCs and LCIS show the so-called aberrant membranous staining, characterized by fragmented, focal, or beaded expression patterns, which are usually obviously different from a continuous strong

Fig. 5. High-grade DCIS. H&E stain, ×200 (a); immunostain of CK5, ×200 (b); immunostain of p40, ×200 (c); immunostain of SMMHC, ×200 (d).

membrane staining seen in normal epithelial cells or abnormal ductal lesions [51, 61, 62] (Fig. 6). Besides aberrant staining pattern, ILC and LCIS may show a complete, weak membranous E-cadherin staining [61], which can be easily distinguished from the continuous strong membranous staining in the surrounding normal epithelial cells if present (Fig. 6). IHC of p120 catenin can be useful in these cases by showing cytoplasmic staining due to dysfunctional E-cadherin which can no longer be capable of binding with other molecules including p120 catenin in the cadherin-catenin complex [36, 44, 47, 51, 61] (Fig. 6).

Distinguishing lobular lesions from ductal lesions is important in several circumstances [63, 64]. First, microscopic margin evaluation is required in cases of DCIS treated with lumpectomy, but not required for classic LCIS. Second, the distinction of ILC from invasive ductal carcinoma (IDC) may have prognostic implications since ILCs tend to have a poorer response to neoadjuvant chemotherapy compared with IDCs [65, 66], although ILCs have similar overall survival outcome with IDCs [67, 68]. Third, ILC has different metastatic pattern from IDC and is more likely to spread to unusual sites such as gastrointestinal tract and ovary [69–71]. Lastly, knowledge of the primary breast tumor type can be very helpful for pathologists to evaluate metastatic tumor suspicious for breast primary.

“Negative” Breast Origin Markers in Breast Carcinoma

Several markers are available to confirm breast origin, including gross cystic disease fluid protein-15 (GCD-FP-15), mammaglobin, and GATA3. GCD-FP-15 and mammaglobin are specific, but they suffer from low sensitivity, especially in TNBCs with only 31% positive for mammaglobin and 25% positive for GCD-FP-15 [72, 73]. Therefore, their negativity is not informative. A positive staining of GCD-FP-15 or mammaglobin supports a breast primary tumor although up to 15% of lung carcinomas can express GCD-FP-15 [74–76]. Both GCD-FP-15 and mammaglobin are cytoplasmic proteins and often show variable or focal staining, necessitating careful examination of entire stained slide. GATA3 is a transcription factor with nuclear staining and is most widely used for clinical diagnosis due to its better sensitivity than GCD-FP-15 and mammaglobin. The sensitivity of GATA3 in ER + breast carcinomas is over 90% [77–80]; however, GATA3 positivity is significantly lower in TNBC than in ER + breast carcinomas with reported ranges from 50% to 70% [73, 78, 79, 81, 82]. GATA3 is also positive in lymphocytes, urothelial carcinomas, squamous cell carcinomas, and mesotheliomas, with labeling in a few percentage of pancreatobiliary adenocarcinomas (37%) and lung adenocarcinomas (8%) [79, 82].

SOX-10 has been emerging as a complementary marker to GATA3 in diagnosing metastatic breast cancer, es-

Fig. 6. ILC with different E-cadherin and p120 staining. ILC case #1 (grade 1) (a), H&E stain, $\times 200$; ILC case #1 with complete loss of E-cadherin membranous staining, immunostain of E-cadherin, $\times 200$ (b); ILC case #1 with p120 cytoplasmic staining, immunostain of p120, $\times 200$ (c); ILC case #2 (grade 2) (d), H&E stain, $\times 100$; ILC case #2 with decreased E-cadherin membranous staining and benign duct with strong membranous staining, immunostain of E-cadherin, $\times 100$ (e); ILC case #2 with p120 cytoplasmic staining

and benign duct with strong membranous staining, immunostain of p120, $\times 100$ (f); ILC case #3 (grade 3) (g), H&E stain, $\times 200$; ILC case #3 with fragmented and decreased E-cadherin membranous staining, immunostain of E-cadherin, $\times 200$ (h); invasive mixed ductal and lobular carcinoma with decreased E-cadherin membranous staining in lobular component (left) and strong E-cadherin membranous staining in ductal component (right), immunostain of E-cadherin, $\times 200$ (i).

pecially for TNBC [83–85]. One study found that 82% of TNBCs was GATA3 positive and 58% was SOX-10 positive, with 95% being positive for at least one marker [86]. Another study demonstrated that SOX-10 had sensitivity of 62.3% and specificity of 100% as a marker in favor of TNBC compared with lung adenocarcinoma, while GATA3 only had a moderate sensitivity of 30.4% and a specificity of 98.7% in TNBCs [87]. A recent study found that SOX-10 exhibited the greatest utility in diagnosing poorly differentiated, GATA3 negative TNBC cases as most SOX-10 positive TNBCs were GATA3 negative or focal/weak (5/6, 83.3%) [88].

Recently, trichorhinophalangeal syndrome type 1 (TRPS1) was reported as a highly sensitive and specific marker for breast carcinomas with positive staining in 98% of ER+, 87% of HER2+, 86% of metaplastic breast carcinomas, and 86% of nonmetaplastic TNBCs [89, 90] (Fig. 7) In addition, TRPS1 showed no or little expression in urothelial carcinomas or in other tumor types including lung adenocarcinoma, pancreatic adenocarcinoma, and others [89]. Although TRPS1 shows very promising results in labeling breast carcinomas sensi-

tively and specifically, more studies are still necessary to explore its expression in both breast tumors and other primary tumors. In summary, no marker has shown 100% sensitive and specific for a breast origin, and interpretation of these breast markers should be correlated with clinical and radiologic findings, and histologic features.

Predictive and/or Therapeutic IHC Markers

False-Negative Hormone Receptor Testing

Assessment of hormone receptor (ER/PR) status is almost exclusively by IHC using a semiquantitative method to report percentage of carcinoma cells showing nuclear staining with anti-ER/PR antibody and staining intensity. Guidelines for ER/PR IHC assays recommend defining ER/PR positivity as 1% or more staining of tumor cells [91]. For reliable hormone receptor IHC testing, there are several preanalytic, analytic, and postanalytic variables that need to be standardized. One of the most important requirements actually begins with the

Fig. 7. TRPS1 staining in breast carcinoma. Invasive ductal carcinoma case #1 (a), H&E stain, $\times 200$; invasive ductal carcinoma case #1 with ER staining (b), immunostain of ER, $\times 200$; invasive ductal carcinoma case #1 with positive TRPS1 nuclear staining, immunostain of TRPS1 (c), $\times 200$; invasive ductal carcinoma case #2 (triple negative) (d), H&E stain, $\times 200$; invasive ductal carcinoma case #2 with negative GATA3 staining, immunostain of GATA3 (e),

$\times 200$; invasive ductal carcinoma case #2 with positive TRPS1 nuclear staining, immunostain of TRPS1 (f), $\times 200$; invasive metaplastic carcinoma (chondromyxoid) (g), H&E stain, $\times 200$; invasive metaplastic carcinoma (chondromyxoid) with negative GATA3 staining, immunostain of GATA3 (h), $\times 200$; invasive metaplastic carcinoma (chondromyxoid) with positive TRPS1 staining, immunostain of TRPS1, $\times 200$ (i).

tissue collection. The tumor specimen should be placed in 10% neutral phosphate-buffered formalin as soon as possible since prolonged tumor ischemia induced by delays in formalin fixation is known to cause decreased ER/PR expression [92–95]. The American Society of Clinical Oncology and College of American Pathologists (CAP) recommends a cold ischemic time of <1 h [91]. Other factors such tissue processing, fixation, decalcification, antibody validation, and kits used also play important roles in interpreting ER/PR IHCs. In addition, appropriate positive and negative controls should be included, and this is mostly important when interpreting false-negative results. For example, strong acid decalcification solutions containing hydrochloric acid can decrease ER/PR antigenicity [96, 97]. Similarly, decalcification using formic acid, a weak acid, also has mild but statistically significant negative effect on ER IHC [98]. PR expression is more heterogeneous than ER and may be a cause of false-negative results, especially in core needle biopsy specimens [99, 100]. Therefore, any suspicious negative PR IHC can be repeated on resected specimen if needed.

False Positive and Negative HER2 Testing

HER2 status can be determined by assessing HER2 protein expression with IHC or *HER2* gene amplification with in situ hybridization. Similar to hormone receptor IHC testing, the cold ischemic time for HER2 IHC testing should be as short as possible (less than 1 h) and the duration of tissue fixation should be 6–72 h according American Society of Clinical Oncology / College of American Pathologists HER2 guidelines [101]. False-positive HER2 IHC results (3+) are usually caused by postanalytic interpretation by pathologists due to unfamiliarity with the interpretation criteria. Therefore, the utmost care should be exercised in interpreting those cases with “strong 2 + or weak 3+” staining.

Similar to ER/PR IHCs, tissue processing, fixation, decalcification, antibody validation, and kits used also play important roles in interpreting HER2 IHCs. Similar to ER/PR IHCs, HER2 IHC can be negatively affected by acid decalcifying solutions, causing false-negative results [96, 97]. False-negative HER2 IHC results can also be caused by intratumoral heterogeneity (ITH), resulting in extreme variability in reporting (Fig. 8). HER2 ITH refers to coex-

Fig. 8. HER2 ITH. Case 1 with different morphology, H&E $\times 100$ (a); case 1 with HER2 ITH, immunostain of HER2, $\times 100$ (b); case 2 with similar morphology, H&E $\times 100$ (c); case 2 with HER2 ITH, immunostain of HER2, $\times 100$ (d).

Fig. 9. PD-L1 (SP142) staining in breast carcinoma. One case with negative SP142 staining (0%), $\times 200$ (a); One case with positive SP142 staining (1%), $\times 200$ (b); One case with positive SP142 staining (2%), $\times 200$ (c); One case with positive (SP142 staining (40%), $\times 200$ (d).

istence of multiple tumor cell subpopulations with varying HER2 status within the same tumor and is a major cause of discordance between HER2 IHC and FISH results [102]. HER2 ITH occurs in a wide range from 5% to 40% in breast cancers according to different studies [102–112].

PD-L1 (SP142 and 22C3) IHC Testing in Triple Negative Breast Carcinomas

PD-L1 IHC testing is an emerging predictive biomarker for TNBCs. The Food and Drug Administration has designated two PD-L1 assays as companion diagnostics to determine patient eligibility for use of specific checkpoint inhibitors in TNBC patients as accelerated approvals, including 22C3 testing for pembrolizumab (Dako) based on the results of KEYNOTE-522 clinical trial and SP142 testing (Ventana) based on the results of IMpassion130 clinical trial [113–117].

For 22C3 assay, a combined positive score (CPS) is calculated using the number of PD-L1-positive cells (tumor cells, lymphocytes, and macrophages) divided by the total number of tumor cells multiplied by 100, and a CPS of ≥ 1 is defined as positive [118]. The interim analysis of KEYNOTE-355 showed that first-line pembrolizumab plus chemotherapy significantly improved progression-free survival in patients with metastatic TNBC with PD-L1 (22C3) CPS ≥ 10 , but the boundary for a statistically significant benefit in patients with PD-L1 (22C3) CPS ≥ 1 tumors was not met [119]. Recent final analysis of KEYNOTE-355 revealed that pembrolizumab plus chemotherapy showed a statistically significant and clinically meaningful improvement in overall survival from patients with previously untreated locally recurrent inoperable or metastatic TNBC with PD-L1 (22C3) CPS ≥ 10 , but not in patients with CPS ≥ 1 tumors [120].

The SP142 assay uses an immune score (IC) to evaluate PD-L1 expression, and the positivity is defined as $\geq 1\%$.

IC is calculated as a percentage of tumor area covered by discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells (lymphocytes, macrophages, dendritic cells, and granulocytes) divided by tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma [121]. Recently, atezolizumab indication for TNBC was withdrawn based on IMpassion131 trial which failed to meet the primary end point of progression-free survival superiority in the frontline treatment of patients with PD-L1 (SP142) positivity (IC ≥ 1) [119, 120, 122].

False-positive or false-negative PD-L1 testing results are mostly caused by postanalytic interpretations because the reproducibility of PD-L1 scoring has been a concern due to the complexity of scoring systems and the cutoff value of 1%. Although scoring 22C3 labeling has been shown to have good inter-/intra-observer agreements, there is substantial debate on the reproducibility of scoring SP142 labeling [123–133] (Fig. 9).

Conflict of Interest Statement

Zaibo Li has nothing to disclose. David J. Dabbs is an independent contractor with PreludeDx.

Funding Sources

Both authors have no funding support to disclose.

Author Contributions

Z.L. and D.J.D. contributed to preparation of the manuscript and reviewed the manuscript.

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