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EP1: a novel rabbit monoclonal antibody for detection of oestrogen receptor α

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ABSTRACT

Aims Assessment of hormone receptor expression is part of routine examination of every breast cancer. In this study, we report the characterisation of a novel rabbit monoclonal antibody, clone EP1, directed against oestrogen receptor (ER) α . Additionally, its immunohistochemical performance characteristics in archival tissues are evaluated in normal tissues and two distinct cohorts of breast cancer patients.

Methods Comparative analyses between EP1 and the anti-ER α component of the ER/PR pharmDx kit (cocktail of mouse monoclonal antibody clones 1D5 and ER-2-123) and between EP1 and another commercially available rabbit monoclonal antibody, clone SP1, are described.

Results Clone EP1 specifically detects nuclear ER in all tissues examined; cytoplasmic staining was not observed. The analysis shows a high degree of concordance (~95%) between EP1 and both the ER α component of the Dako ER/PR pharmDx kit and Ventana clone SP1. However, the use of EP1 antibody together with Dako EnVision FLEX detection system resulted in a stronger staining intensity as compared with SP1 antibody using the Ventana ultraView DAB detection system resulting in better 'ease of use.'

Conclusions The use of EPI can result in better interpretation of the results of the ER analysis.

formulation of specific guidelines for their use and interpretation.^{8–11}

In recent years, a number of significant improvements have been made to IHC methods. These include the introduction of polymer-based detection methods that improve the quality of staining by decreasing the non-specific binding of endogenous biotin and enhanced sensitivity.¹² At the same time, advances in the generation and production of primary antibodies have resulted in the replacement of rabbit polyclonal antibodies by mouse monoclonal and, most recently, by rabbit monoclonal antibodies.¹³ During the past few years, the use of rabbit monoclonal antibodies in ER IHC assays has been implemented in an effort to continuously improve assay quality by introducing new highly sensitive, specific and robust reagents. These ongoing efforts have resulted in the rigorous evaluation of a novel rabbit monoclonal antibody, clone EP1.

In this study, we describe the characterisation of this antibody and demonstrate its utility in detecting ER α in breast cancer tissue specimens. The performance characteristics of this rabbit antibody were compared with the anti-ER α component of the ER/PR pharmDx kit (Dako) (cocktail of mouse monoclonal antibody clones 1D5 and ER-2-123) and with another commercially available rabbit monoclonal antibody to ER α , clone SP1 (Ventana).

INTRODUCTION

The oestrogen receptor (ER) is a regulator of normal breast development and function. It also plays an important role in the development and progression of breast cancer, with approximately 80% of invasive breast cancers expressing ER. In the clinical setting, ER-positive patients with both metastatic and nonmetastatic disease have been shown to respond to hormonal therapies.^{1–5}

As ER status is a critically important variable for prediction of response to hormonal therapies, a great deal of attention has been focused on the laboratory methods that are employed to assess ER expression. Immunohistochemistry (IHC) has been a well-accepted technique for the detection of ER in formalin-fixed, paraffin-embedded (FFPE) tissues of breast cancer specimens for the last few decades. This technique is preferred by pathologists because it also enables the simultaneous evaluation of morphologic characteristics.^{6–7} With the general adoption of this method, there has been particular emphasis on identifying new ER antibodies for use in IHC with appropriate sensitivity and specificity, and supported by clinical and technical validation of their performance. These efforts have led to the standardisation of these ER IHC assays and

MATERIALS AND METHODS

Epitope mapping

In order to identify the epitope of human ER α to which monoclonal rabbit anti-ER α , clone EP1 binds, a large number of overlapping 15-mer peptides were synthesised, covering an amino acid sequence corresponding to 1–300 of the human protein (UniProt ID: P03372), which is equal to the recombinant protein fragment used for generating the EP1 clone. Binding of the antibody to each linear peptide was tested in a PEPSCAN-based ELISA.¹⁴ In brief, the 455-well miniPEPSCAN card, containing the covalently linked peptides, was incubated with the ER α antibody, clone EP1 (1 μ g/mL). After washing, the wells were incubated with peroxidase-conjugated antirabbit antibody at a dilution of 1/1000 for 1 h at 25°C. The wells were then repeatedly washed, and a peroxidase substrate was added. After 1 h, the colour development of the ELISA was quantified with a charge-coupled device camera and an image-processing system.

IHC protocols and reagents

Tissue pretreatment and IHC staining were performed using the protocols, reagents and instrumentation platforms shown in table 1.



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Table 1 Protocols, reagents, and instrumentation used in the analysis

Anti-ER α	Pretreatment protocol	Pretreatment instrument	Immunohistochemical staining reagents and protocol	Automated staining instrument
Dako monoclonal rabbit anti-ER α , clone EP1, FLEX ready-to-use	20 min @ 97°C in Dako EnVision FLEX target retrieval solution, high pH	Dako PT link pretreatment module	<i>Dako EnVision FLEX</i> 20 min primary antibody 20 min EnVision <i>FLEX/HRP</i> 10 min DAB+	Dako autostainer Link 48
ER α component of the Dako ER/PR pharmDx kit (monoclonal mouse anti-ER α , clones 1D5 and ER-2-123)	5 min @ 125°C in Dako EnVision FLEX target retrieval solution, low pH	Dako Pascal pressurised heating chamber	<i>Dako EnVision+ /HRP, mouse</i> 30 min primary antibody 30 min EnVision+/HRP 10 min DAB+	Dako autostainer Link 48
Ventana monoclonal rabbit anti-ER α , clone SP1, ready-to-use (CONFIRM)	32 min mild CC1	Ventana BenchMark XT	XT ultraView DAB kit	Ventana BenchMark XT

ER, oestrogen receptor.

Specimens

Specimens for the studies were procured and used as shown in table 2. Some of the tissue samples from the Dako Tissue Bank were provided by the Cooperative Human Tissue Network, which is funded by the National Cancer Institute. For the concordance testing between EP1 and the ER component of the pharmDx kit, both ER positive and negative specimens were used. For the comparative analysis between EP1 and SP1, two different sets of tissue microarrays (TMAs) were used. The first set consisted of 200 ER+ cases with known *Oncotype DX* recurrence scores, and the second set was generated from a large series of cases (n=400) with known long-term outcomes. Multiples of 1 mm cores were used to generate the TMA. The latter TMA was generated from patients who had received adjuvant hormonal and chemotherapy.

Scoring

The data captured for the analytical specificity study on 30 normal tissue types included cell type, staining intensity (0–3 scale), percentage of positively stained cells and staining pattern (nuclear, cytoplasmic or membranous).

For scoring the results of the EP1 and the ER α component of the ER/PR pharmDx Kit concordance study, nuclear staining intensity and proportion of positive tumour cells were recorded and were combined to formulate a diagnostic score (Allred score) for the ER pharmDx results. Cutoff for positivity was according to the Allred score for the ER pharmDx and 1% for EP1.^{8 15}

For the comparative analysis between clones EP1 and SP1, both Allred and H-score systems were used. The TMAs were analysed in a blinded fashion by two pathologists using the ASCO/CAP 1% cutoff for positivity, and differences in scores

were resolved by consensus achieved through simultaneous viewing using a dual-headed microscope.

Statistical analysis

Graph pad programme was used to perform χ^2 test analysis to observe correlations between the different parameters. Additionally, paired t test was performed to analyse the correlations between H-scores of SP1 and EP1 expression. κ analysis was performed to assess the degree of agreement between the two reagents.

RESULTS

Epitope mapping

The presumptive epitope of human ER α that is recognised by clone EP1 was identified by assessing the binding of the antibody to a series of overlapping 15-mer peptides that spanned the amino acid sequence of the human protein. As shown in figure 1, the results of these epitope mapping studies clearly indicate that rabbit monoclonal antibody to ER α , clone EP1 recognises the amino acid sequence RPLGEV, which corresponds to amino acid residues 37–42 of human ER α (figure 1). This linear sequence is unique to the α form of the ER protein, and is located in the unstructured, N-terminal A/B domain (AF1).

Immunohistochemical staining of normal tissues using clone EP1

The specificity of clone EP1 was evaluated by examining the immunoreactivity pattern on a set of 90 (89 evaluable) FFPE normal tissue specimens composed of three patient cases from 30 different tissue types. When tested with these normal tissues, EP1 demonstrated nuclear positivity only in tissue types known

Table 2 Details of the specimens used in the study and the source from where they were obtained

ER α study	FFPE specimen type	Source/description	Number of specimens
Analytical specificity	30 normal tissue types	Dako Tissue Bank/tissue arrays	90 (89 evaluable)
Concordance of clone EP1 and ER α component of the ER/PR pharmDx kit	Breast carcinoma (TMAs)	BioChain (Z7020004, Z7020005, T8235721), LifeSpan (LS-BRCA32)	274
	Breast carcinoma (single blocks)	Dako Tissue Bank/single specimens	40
Comparison study of EP1 and SP1	<i>Oncotype DX</i> (TMA)	Indiana University/ER+ with <i>Oncotype DX</i>	311 cores/176 cases
	Breast carcinoma (TMA)	Indiana University/long-term follow-up data available	617 cores/390 cases

ER, oestrogen receptor; FFPE, formalin-fixed, paraffin-embedded.

Figure 1 Summary of epitope mapping results, antioestrogen receptor α , clone EP1.

Amino Acid Position	Amino Acid Sequence	Reactivity
a) 27-41	NRPQLKIPLERPLGE	–
b) 28-42	RPQLKIPLERPLGEV	+
c) 29-43	PQLKIPLERPLGEVY	+
d) 30-44	QLKIPLERPLGEVYL	+
e) 31-45	LKIPLERPLGEVYLD	+
f) 32-46	KIPLERPLGEVYLDS	+
g) 33-47	IPLERPLGEVYLDSS	+
h) 34-48	PLERPLGEVYLDSSK	+
i) 35-49	LERPLGEVYLDSSKP	+
j) 36-50	ERPLGEVYLDSSKPA	+
k) 37-51	RPLGEVYLDSSKPAV	+
l) 38-52	PLGEVYLDSSKPAVY	–

Clone EP1 recognizes R37-P38-L39-G40-E41-V42 of the Estrogen Receptor protein

to express ER α . These included epithelial cells and/or stromal cells from breast, cervix, oesophagus, ovary, prostate, tonsil and uterus.

Comparison of clone EP1 with the ER α component of the ER/PR pharmDx kit

As recommended in the current ASCO/CAP guidelines, a concordance study was performed to compare the monoclonal rabbit anti-ER α , clone EP1 with the anti-ER α component of the clinically validated ER/PR pharmDx kit as the predicate device. As shown in table 3 and figure 2, the staining results obtained on breast carcinoma specimens with clone EP1 were found to be highly concordant with those produced by the ER α

component of the ER/PR pharmDx kit. Among the 314 cases analysed (table 3), 183 cases were found to be positive with both antibody assays, and 119 cases were found to be negative. There were 10 cases that were scored as negative with ER α pharmDx kit, but positive with clone EP1. Two cases were scored as positive with the predicate test, but negative with clone EP1. Values for the positive, negative and overall percent agreement were 98.9%, 92.2% and 96.2%, respectively.

Comparison studies between clones EP1 and SP1

Following the demonstration of concordance with the ER/PR pharmDx Kit, clone EP1 was also tested on two different TMAs containing breast cancer tissues (Oncotype DX TMA and a TMA with long-term follow-up), and the IHC staining results were compared with those produced by rabbit monoclonal anti-ER α , clone SP1 (figure 3).

Oncotype DX TMA

Staining results for both clones EP1 and SP1 were assessed on 311 breast cancer cores, representing 176 unique cases in the Oncotype DX ER-positive TMA. Tables 4 and 5 summarise the positive and negative comparisons by cores (311 cores; table 4) and by cases (176 cases; table 5). Both antibodies identified 277 (89.1%) cores as positive and five (1.6%) cores as negative. Seventeen (5.5%) cores were characterised as positive by SP1 alone and 12 (3.9%) cores as positive by EP1 alone. When the same analysis was performed by cases, 165 (93.8%) cases were characterised as positive by both antibodies and two (1.1%) cases as negative. Five (2.8%) cases were classified as positive by SP1 alone and four (2.3%) cases by EP1 alone. For the positive cases, the distribution of the intensity and percentages

Table 3 Concordance of clone EP1 and the ER α component of the ER/PR pharmDx kit

ER α component of ER/PR pharmDx	EP1	#	Per cent
Positive/negative comparison			
Positive	Positive	183	58.3
Positive	Negative	2	0.6
Negative	Positive	10	3.2
Negative	Negative	119	37.9
Agreement			
Positive agreement		183/185=98.9%	
Negative agreement		119/129=92.2%	
Overall agreement		302/314=96.2%	
κ (95% CI)		0.920 (0.876 to 0.965)	

ER, oestrogen receptor.

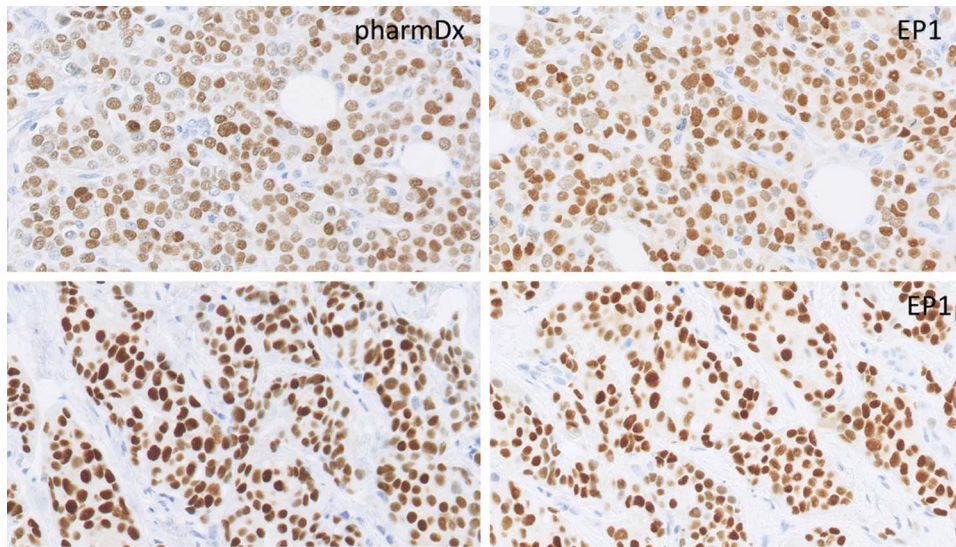


Figure 2 Comparison of rabbit monoclonal antibody EP1 with mouse monoclonal oestrogen receptor α (ER α) component of the ER/PR pharmDx kit. Matched images from cases showing low and high expression of pharmDx and EP1 are demonstrated in these panels.

(proportion of positive staining cells) by cores and cases is detailed in online supplementary tables S1 and S2. Both antibodies showed a strong correlation with ER α mRNA levels in the Oncotype DX assay (figure 4). Correlation of SP1 (Log2) and EP1 (Log2) with ER α mRNA levels were 0.44 ($p < 0.0001$) and 0.37 ($p < 0.0001$), respectively.

In brief, when compared with SP1, EP1 stained a greater number of cores and cases with a staining intensity of 3+ (185 vs 139, χ^2 test $p = 0.0003$; 114 vs 91, $p = 0.0173$), and a nearly identical number of cores and cases with greater than 10% of tumour cell positivity (281 vs 284; 167 vs 165), with overall a

similar number of negative cases (22 vs 17; 7 vs 6). Paired t test was performed between the H-score for EP1 (Log2) and SP1 (Log2), the mean difference was 0.147 (SD (diff)=0.598; $p = 0.0059$; 95% CI (0.0431 to 0.2507).

TMA with long-term follow-up

In the long-term follow-up TMA, EP1, and SP1 immunostaining was assessed in 617 cores representing 390 cases. Tables 6 and 7 show the positive and negative comparisons by cores (617 cores; table 6), and by cases (390 cases; table 7). Both antibodies identified 416 (67.4%) cores as positive and 173

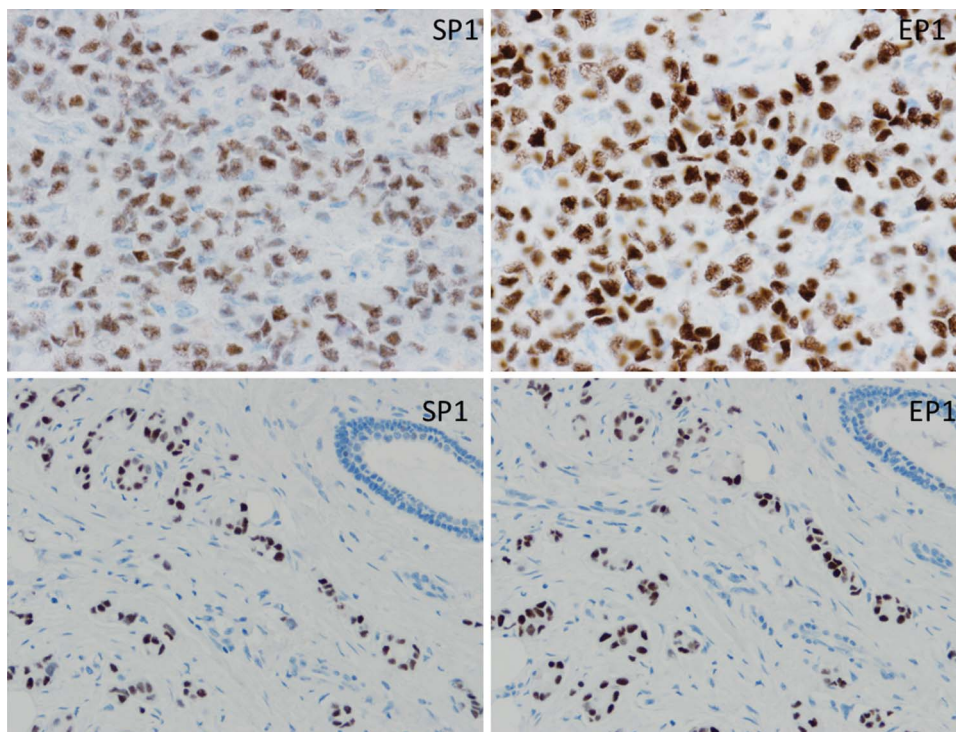


Figure 3 Comparison of clone EP1 performed using Dako reagents and kits with SP1 using Ventana BenchMark reagents and kits. Representative images of SP1 and matched EP1 expression in breast tumours are shown in these panels.

Table 4 Comparison of clone EP1 and the SP1 in the *Oncotype* DX ER+ TMA set by cores

SP1	EP1	#	Per cent
Positive/negative comparison by cores			
Positive	Positive	277	89.1
Positive	Negative	17	5.5
Negative	Positive	12	3.9
Negative	Negative	5	1.6
Agreement			
Positive agreement		277/294=94.2%	
Negative agreement		5/17=29.4%	
Overall agreement		282/311=90.7%	
κ (95% CI)		0.208 (0.020 to 0.395)	

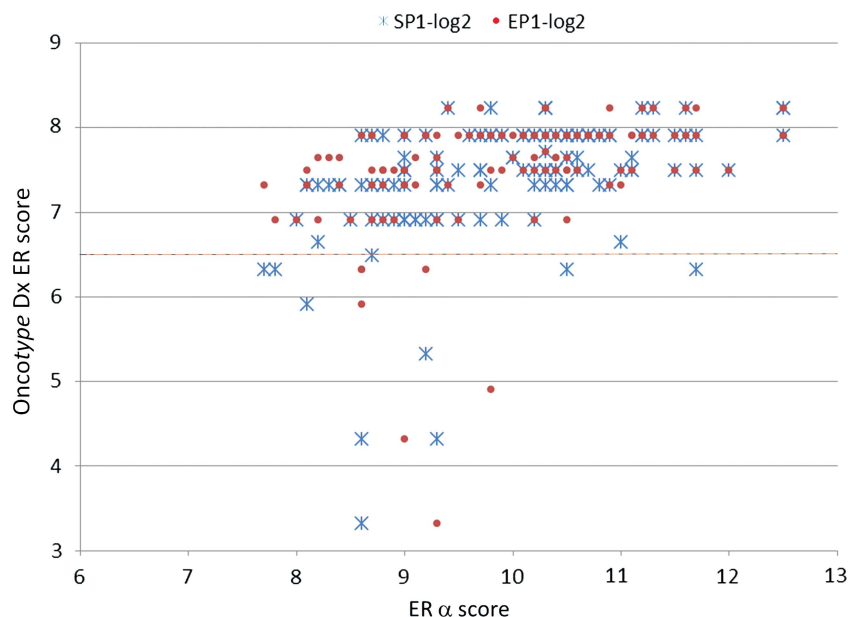
ER, oestrogen receptor.

Table 5 Comparison of clone EP1 and the SP1 in the *Oncotype* DX ER+ TMA set by cases

SP1	EP1	#	Per cent
Positive/negative comparison by cases			
Positive	Positive	165	93.8
Positive	Negative	5	2.8
Negative	Positive	4	2.3
Negative	Negative	2	1.1
Agreement			
Positive agreement		165/170=97%	
Negative agreement		2/6=33%	
Overall agreement		167/176=94.9	
κ (95% CI)		0.281 (-0.053 to 0.615)	

ER, oestrogen receptor.

(28.0%) cases as negative. Six (1.0%) cores were characterised as positive by SP1 alone and 22 (3.6%) cores as positive by EP1 alone. When the same analysis was performed by cases, 284 (73.0%) cases were characterised as positive by both antibodies, and 93 (23.9%) cases as negative. Four (1.0%) cases were

Figure 4 Comparison of clones EP1 and SP1 with oestrogen receptor α score from *Oncotype* DX assay. The dotted line at 6.5 units indicates the cutoff for positivity.

classified as positive by SP1 alone and nine (2.3%) cases by EP1 alone. The distribution of the intensity and percentages (proportion of positively staining cells) by cores and cases is detailed in online supplementary tables S3 and S4. Survival analysis was not performed as the two antibodies detected similar numbers of positive cases, and the dataset was considered too small for doing survival analysis. Additionally, all patients in the long-term follow-up cohort had received adjuvant chemotherapy.

Briefly, when EP1 was compared with SP1, EP1 was observed to immunostain a larger number of cores and cases with a staining intensity of 3+ (186 vs 111, χ^2 test $p=0.0001$; 132 vs 84, $p=0.0002$) and a similar number of cases with greater than 10% tumour cell staining (391 vs 379; 268 vs 259), with an overall smaller number of negative cases (179 vs 195; 97 vs 102). However, the differences were not statistically significant.

DISCUSSION

Accurate assessment of ER status is critical to ensure that breast cancer patients receive appropriate therapy. IHC is currently the most commonly used method for determining ER status because of its relatively low cost, its general applicability to routinely processed tissue samples, and importantly, its utility in evaluating small cancers and ensuring that only invasive tumour cells are assessed.⁹ Because it is especially important to minimise false-negative and false-positive test results, guidance has been provided for analytical (technical) validation procedures, and recommendations have been made for ER testing methods and test interpretation.^{9 10}

Previously, the mouse monoclonal antibody, clone 1D5, had been one of the most routinely used antibodies for the detection of ER α in FFPE tissue. More recently, the clinically validated ER/PR pharmDx kit using a mouse monoclonal antibody cocktail has been introduced. The continued need to improve reagent quality through generation of highly sensitive, specific and robust reagents led to the development of a new rabbit monoclonal antihuman ER α , designated clone EP1. In this manuscript, we describe the specificity and sensitivity of this novel antibody and demonstrate its utility in detecting ER α in FFPE breast cancer tissues. In keeping with recommended practices, the performance characteristics of EP1 were compared with those of other anti-ER antibodies, particularly to the

Table 6 Comparison of clone EP1 and the SP1 in the long-term follow-up TMA set by cores

SP1	EP1	#	Per cent
Positive/negative comparison by cores			
Positive	Positive	416	67.4
Positive	Negative	6	1.0
Negative	Positive	22	3.6
Negative	Negative	173	28.0
Agreement			
Positive agreement		416/422=98.6%	
Negative agreement		173/195=88.7%	
Overall agreement		589/617=95.5%	
κ (95% CI)		0.893 (0.854 to 0.931)	

predicate ER α component of the ER/PR pharmDx kit and another rabbit monoclonal antibody, clone SP1.

The specificity of this new antibody was established by epitope mapping and an assessment of the IHC staining of normal tissues. Epitope mapping clearly demonstrated that clone EP1 recognises an antigenic domain defined by the linear sequence RPLGEV, which corresponds to amino acid residues 37–42 of human ER α .¹⁶ The specificity of clone EP1 for an epitope in the N-terminal A/B domain is consistent with that of the ER α antibody cocktail in the ER/PR pharmDx kit, but distinct from clone SP1 which is derived from the C-terminal domain of the ER α molecule.¹⁷ When assessed with normal tissues, the IHC staining patterns produced by this antibody are consistent with previous observations, with epithelial cells and/or stromal cells from breast, cervix, oesophagus, ovary, prostate, tonsil and uterus exhibiting nuclear staining.^{18–23}

In a comparative analysis of 314 cases, clone EP1 demonstrated 96.2% overall concordance with the ER α component of the ER/PR pharmDx kit. In additional comparisons with clone SP1, a very strong overall agreement (94.9% and 96.9% for Oncotype Dx and follow-up TMA, respectively) was observed.

Disagreement in reading of ER IHC stains often occurs at the lower limit of the staining intensity range, as it may be difficult to classify a nucleus as weakly positive or negative when contrasted against a blue nuclear counterstain. A recent study documented the importance of staining intensity as the major source of discordance between IHC and fluorescence-based AQUA analysis of ER.²⁴ To analyse this issue, we also evaluated the intensity of staining in the comparative testing between clones EP1

Table 7 Comparison of clone EP1 and the SP1 in the long-term follow-up TMA set by cases

SP1	EP1	#	Per cent
Positive/negative comparison by cases			
Positive	Positive	284	73.0
Positive	Negative	4	1.0
Negative	Positive	9	2.3
Negative	Negative	93	23.9
Agreement			
Positive agreement		284/288=98.6%	
Negative agreement		93/102=91.2%	
Overall agreement		377/390=96.7%	
κ (95% CI)		0.912 (0.866 to 0.959)	

and SP1 using cases that showed strong intensity or had greater than 10% of tumour cells expressing ER. This analysis clearly demonstrated the better intensity of staining with EP1 antibody when compared with SP1, the p value being significant for both the analyses.

In summary, we document that rabbit monoclonal antihuman ER α , clone EP1 is a highly sensitive and specific antibody for use in the detection of ER α by IHC. In multiple comparisons on a large series of breast cancer cases, clone EP1, when used with the Dako Autostainer Link system and FLEX protocol, identifies a highly comparable number of cases as ER-positive or ER-negative when compared with other established ER antibody assays. Ease of interpretation is also facilitated by the use of EP1, with a greater number of low positive cases demonstrating a stronger intensity and higher proportion of positive tumour cells.

Take-home messages

Immunohistochemical (IHC) assessment of hormone receptor expression is important in breast cancer diagnosis. It is essential that optimal reagents and assays are used for accurate performance of this assessment. Rabbit monoclonal antibodies have been shown to be superior to mouse monoclonal antibodies in IHC assays, and a new rabbit monoclonal antibody to ER α , clone EP1, was evaluated in the studies described in this manuscript. It was demonstrated that clone EP1, used with the Dako EnVision FLEX detection system, is comparable to the anti-ER α component of the Dako ER/PR pharmDx kit, and shows a high degree of concordance to the Ventana CONFIRM ER α assay which uses rabbit monoclonal antibody clone SP1. The EP1/EnVision FLEX assay resulted in a stronger staining intensity compared to SP1/BenchMark ultraView DAB, allowing improved interpretation of the ER IHC results.

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REFERENCES

- Manni A, Arafah B, Pearson OH. Estrogen and progesterone receptors in the prediction of response of breast cancer to endocrine therapy. *Cancer* 1980;46:2838–41.
- Osborne CK, Yochmowitz MG, Knight WA III, *et al.* The value of estrogen and progesterone receptors in the treatment of breast cancer. *Cancer* 1980;46:2884–8.
- Harvey JM, Clark GM, Osborne CK, *et al.* Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. *J Clin Oncol* 1999;17:1474–81.
- Abe O, Abe R, Enomoto K, *et al.* Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005;365:1687–717.

- 5 Early Breast Cancer Trialists' Collaborative G, Clarke M, Coates AS, *et al.* Adjuvant chemotherapy in oestrogen-receptor-poor breast cancer: patient-level meta-analysis of randomised trials. *Lancet* 2008;371:29–40.
- 6 Pertschuk LP, Feldman JG, Kim YD, *et al.* Estrogen receptor immunocytochemistry in paraffin embedded tissues with ER1D5 predicts breast cancer endocrine response more accurately than H222Sp gamma in frozen sections or cytosol-based ligand-binding assays. *Cancer* 1996;77:2514–19.
- 7 Taylor CR. Paraffin section immunocytochemistry for estrogen receptor—The time has come. *Cancer* 1996;77:2419–22.
- 8 Allred D, Harvey J, Berardo M, *et al.* Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol* 1998;11:155–68.
- 9 Collins LC, Botero ML, Schnitt SJ. Bimodal frequency distribution of estrogen receptor immunohistochemical staining results in breast cancer: an analysis of 825 cases. *Am J Clin Pathol* 2005;123:16–20.
- 10 Hammond M, Hayes D, Dowsett M, *et al.* American Society of Clinical Oncology/ College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol* 2010;28:2784–95.
- 11 Fitzgibbons PL, Murphy DA, Hammond ME, *et al.* Recommendations for validating estrogen and progesterone receptor immunohistochemistry assays. *Arch Pathol Lab Med* 2010;134:930–5.
- 12 Charbgo F, Mirshahi M, Sarikhani S, *et al.* Synthesis of a unique high-performance poly-horseradish peroxidase complex to enhance sensitivity of immunodetection systems. *Biotechnol Appl Biochem* 2012;59:45–9.
- 13 Rossi S, Laurino L, Furlanetto A, *et al.* Rabbit monoclonal antibodies: a comparative study between a novel category of immunoreagents and the corresponding mouse monoclonal antibodies. *Am J Clin Pathol* 2005;124:295–302.
- 14 Slootstra JW, Puijk WC, Ligtoet GJ, *et al.* Structural aspects of antibody-antigen interaction revealed through small random peptide libraries. *Mol Divers* 1996;1:87–96.
- 15 Hammond ME, Hayes DF, Dowsett M, *et al.* American Society of Clinical Oncology/ College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *Arch Pathol Lab Med* 2010;134:907–22.
- 16 Elledge RM, Fuqua SAW. Estrogen and progesterone receptors. In: Harris JR, Lippman ME, Morrow M, Osborne CK, eds *Diseases of the breast*. New York: Lippincott Williams & Wilkins, 2000:471–85.
- 17 Gomez-Fernandez C, Mejias A, Walker G, *et al.* Immunohistochemical expression of estrogen receptor in adenocarcinomas of the lung: the antibody factor. *Appl Immunohistochem Mol Morphol* 2010;18:137–41.
- 18 Nadjji M, Gomez-Fernandez C, Ganjei-Azar P, *et al.* Immunohistochemistry of estrogen and progesterone receptors reconsidered: experience with 5,993 breast cancers. *Am J Clin Path* 2005;123:21–7.
- 19 Fadare O, Yi X, Liang SX, *et al.* Variations of mitotic index in normal and dysplastic squamous epithelium of the uterine cervix as a function of endometrial maturation. *Mod Pathol* 2007;20:1000–8.
- 20 Brodowska A, Laszczynska M, Starczewski A, *et al.* Immunohistochemical analysis of steroid receptors in ovaries of postmenopausal women—effects of aging and hormone status. *Histol Histopathol* 2010;25:1009–16.
- 21 Deroo BJ, Korach KS. Estrogen receptors and human disease. *J Clin Invest* 2006;116:561–70.
- 22 Jones RK, Bulmer JN, Searle RF. Immunohistochemical characterization of proliferation, oestrogen receptor and progesterone receptor expression in endometriosis: comparison of eutopic and ectopic endometrium with normal cycling endometrium. *Hum Reprod* 1995;10:3272–9.
- 23 Shiraski H, Watanabe K, Sato J, *et al.* Expression of steroid receptors in palatine tonsils. *Int Congress Ser* 2003;1257:115–18.
- 24 Welsh AW, Moeder CB, Kumar S, *et al.* Standardization of estrogen receptor measurement in breast cancer suggests false-negative results are a function of threshold intensity rather than percentage of positive cells. *J Clin Oncol* 2011;29:2978–84.

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