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Multicenter evaluation of a novel ROS1 immunohistochemistry assay (SP384) for detection of ROS1 rearrangements in a large cohort of lung adenocarcinoma patients

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Running title: ROS1 immunohistochemistry and lung adenocarcinoma

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Abstract

INTRODUCTION: The detection of a ROS1 rearrangement in advanced and metastatic lung adenocarcinoma (LUAD) lead to a targeted treatment with tyrosine kinase inhibitors, with favorable progression-free survival and overall survival of the patients. Thus, it is mandatory to screen for the ROS1 rearrangement in all these patients. ROS1 rearrangements can be detected using break-apart fluorescence in situ hybridization (FISH), which is the “gold standard”; however, ROS1 immunohistochemistry (IHC) can be used as a screening test since it is widely available, easy and rapid to perform, and cost-effective.

METHODS: We evaluated the diagnostic accuracy and inter-pathologist agreement of two anti-ROS1 IHC clones, SP384 (Ventana, Tucson, AZ) and D4D6 (Cell Signaling, Danvers, MA), in a training cohort of 51 positive ROS1 FISH LUAD cases, and then in a large validation cohort of 714 consecutive cases of LUAD from six routine molecular pathology platforms.

RESULTS: In the two cohorts, the SP384 and D4D6 clones demonstrate variable sensitivity and specificity rates on the basis of two cutoff points ≥1+ (all % tumor cells and ≥2+ (>30% stained tumor cells). In the validation cohort, the D4D6 yielded the best accuracy for the presence of a ROS1 rearrangement by FISH. Inter-pathologist agreement was moderate to good (ICC, 0.722–0.874) for the D4D6 clone and good to excellent (ICC, 0.830–0.956) for the SP384 clone.

CONCLUSIONS: ROS1 IHC is an effective screening tool for the presence of ROS1 rearrangements. However, users must be acutely aware of the variable diagnostic performance of different anti-ROS1 antibodies prior to implementation into routine clinical practice.

Keywords: ROS1; SP384; D4D6; immunohistochemistry; FISH; lung adenocarcinoma.
INTRODUCTION

Patients with advanced and metastatic lung adenocarcinoma (LUAD) harboring a \textit{ROS1} rearrangement respond effectively to crizotinib, as well as to the other tyrosine kinase inhibitors that are currently used in clinical trials.\textsuperscript{1-5} \textit{ROS1} screening is thus recommended in most guidelines. \textit{ROS1} rearrangement detection using fluorescence in situ hybridization (FISH) is the typical approach that is consistently performed to identify patients with LUAD who would be eligible for these treatments. Based on its high sensitivity and specificity, this method is considered to be the “gold standard” for determining \textit{ROS1} positivity.\textsuperscript{6, 7} However, given the low incidence of \textit{ROS1} rearrangements in lung adenocarcinoma (1% to 2%),\textsuperscript{6} detection of \textit{ROS1} protein expression by immunohistochemistry (IHC) can be used as a surrogate, cost-effective screening test that is preferable to FISH analysis for screening the \textit{ROS1} status.\textsuperscript{8} Furthermore, the expression of the \textit{ROS1} protein, as detected by IHC, may indicate a functional gene product, which is necessary for effective drug targeting.\textsuperscript{9} Moreover, FISH testing requires high-level expertise for technical and diagnostic issues, which is not widely available in all laboratories. Finally, the turnaround time for getting the final result can sometimes delay an urgent treatment decision.\textsuperscript{10}

Considering these latter constraints, \textit{ROS1} IHC was recommended as a frontline screening test prior to confirmatory FISH analysis in \textit{ROS1}-positive cases and to avoid unnecessary FISH analysis in \textit{ROS1}-negative cases.\textsuperscript{8} To date, a single commercially available antibody (D4D6 clone, Cell Signaling Technology, Danvers, MA, USA) has been used in several studies and is currently used worldwide in routine clinical practice.\textsuperscript{11-13} Given the high sensitivity of IHC relative to FISH or other molecular approaches, such as next generation sequencing (NGS) technologies, 97-98% of tumors that clearly lack \textit{ROS1} staining can be safely interpreted as negative for \textit{ROS1} rearrangement.\textsuperscript{6, 11-13}

Conversely, the interpretation of a positive \textit{ROS1} D4D6 IHC test may be challenging and can lead to false-positive results (related to a patchy pattern and weak intensity of the staining) that do not harbor an underlying \textit{ROS1} rearrangement as detected by FISH.\textsuperscript{14-16} Therefore, the variability of specificity reported for the \textit{ROS1} D4D6 IHC assay requires caution and subsequent confirmation of the \textit{ROS1} status by a validated method, irrespective of the staining intensity and the percentage
of positive tumor cells. Only the confirmation of a ROS1 rearrangement by a molecular or cytogenetic method (e.g., FISH, NGS) can be used prior to considering a patient with LUAD for ROS1-targeted therapy.

In this context, the development of a new anti-ROS1 antibody (SP384 clone, Ventana, Tucson, AZ, USA) prompted the evaluation of its diagnostic performance to predict the presence of a ROS1 rearrangement in a routine clinical setting.

This study aimed to investigate the correlation between two ROS1 IHC assays and paired ROS1 FISH analysis as well as inter-pathologist agreement for IHC interpretation in two large-scale multicenter cohorts of LUAD cases.

PATIENTS AND METHODS

Case selection

The training cohort was comprised of retrospective archival formalin-fixed paraffin-embedded (FFPE) tumor samples from 51 patients with stage IIIIB/IV LUAD and ROS1 FISH positive. None of the ROS1 positive cases harbored concurrent ALK rearrangements nor EGFR or BRAF activating mutations. In ROS1 positive patients, tumor response was evaluated per RECIST 1.1 on target lesions, 3 months after the initiation of crizotinib and was compared with baseline results.

In the validation cohort, 714 cases of consecutive LUAD diagnosed in the Laboratory of Clinical and Experimental Pathology (Pasteur Hospital, Université Côte d’Azur, Nice, France) between 2006 and 2017 were integrated in tissue microarray (TMA) FFPE blocks. Three tissue cores of 600 µm in diameter were taken from different tumor areas within the same FFPE block for each case.

A signed informed consent was obtained from all patients. The study was approved by the local ethics committee (Human Research Ethics Committee, Nice University Hospital Center/hospital-related Biobank BB-0033-00025) and was performed in accordance with the guidelines of the Declaration of Helsinki. ROS1 FISH positive tissue samples were provided by 6 pathology laboratory centers in France (University Hospitals of Bordeaux, Caen, Nancy, Nice, Rouen and Toulouse) and centralized for IHC and FISH analysis in the Laboratory of Clinical and Experimental Pathology (Nice, France), which is accredited by COFRAC (French National Authority for Accreditation delivery, n°8-3034; www.cofrac.fr) for FISH and IHC testing according to the ISO 15189 norm.
**ROS1 FISH analysis**

Tumor samples were fixed in formaldehyde 10% and paraffin, embedded at each participating center before centralization. Tissue sections (4 µm thick) were stained with hematoxylin eosin and the percentage of tumor cells was assessed independently by three senior thoracic pathologists (VH, MI, PH). Briefly, FISH analysis was conducted using the Abbott Molecular FISH probe (Abbott Molecular Inc., Des Plaines, IL, USA), LSI ROS1 (Tel) SpectrumOrange Probe and LS1 ROS1 (Cen) SpectrumGreen Probe. Additionally, some tumors were assessed for ROS1 rearrangement using the ZytoLight SPEC ROS1 Dual Colour Break Apart Probe (ZytoVision, Bremerhaven, Germany). FISH was performed according to the manufacturer's recommendations. Interphase signals were manually counted in at least 100 tumor nuclei per case using an epi-fluorescence microscope (Eclipse 80i, Nikon, Champigny-sur-Marne, France) supported in parallel by the PathScan® Viewer software (Excilone, Elancourt, France).

A positive case was defined if more than 15% of tumor cells in more than 100 tumor cells demonstrated a split signal at least 2 signal distances apart or an isolated centromeric 3' (green signal) pattern.

**ROS1 IHC analysis**

ROS1 IHC analysis was performed using the D4D6 clone (Cell Signaling Technology, Danvers, MA, USA; CC1 buffer for 64 min, 1:50 dilution for 32 min, ultraView Universal DAB Detection Kit, Ventana, Tucson, AZ, USA) and the SP384 clone (Ventana, Tucson, AZ, USA; CC1 buffer for 64 min, 1:50 dilution for 16 min, OptiView DAB IHC Detection Kit, Ventana) and processed on the Ultra Benchmark autostainer (Ventana) according to the manufacturer's recommendations. Immunostainings were interpreted independently by three senior lung pathologists (VH, MI, PH), blinded to FISH results, for the following criteria: signal intensity (0; no staining, 1+; weak, 2+; moderate; and 3+; strong) and percentage of stained cells, topography and characteristics of the signal (cytoplasmic – diffuse or focal and/or granular; cytoplasmic and membranous; membranous). Results were quantified by multiplying the percentage of ROS1 positive tumor cells by the intensity (H-score, range, 0–300). For each case included on the TMA FFPE blocks, the mean H-score of the three cores was recorded. Non-specific staining of macrophages and type II
pneumocytes was disregarded. ROS1 negative and positive controls were put on each IHC slide (HCL0024, HistoCyte Laboratories Ltd, Newcastle University, UK).

**Statistical analysis**

Statistical analyses assessing agreement between the D4D6 and SP384 antibodies with the ROS1 FISH analysis (gold standard) were performed on dichotomized variables using two positivity cutoff points: IHC ROS1 positive (intensity ≥1+, any percentage of tumor cells) and IHC ROS1 strongly positive (intensity ≥2+, >30% tumor cells), as recently described.\textsuperscript{17, 19} Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated for the comparison between the dichotomized results using each of the two antibodies relative to the FISH results. The Spearman’s rank correlation test (\( \rho \)) was used for a correlation analysis between the H-scores as continuous variables of each clone. Intra-class correlation coefficients were calculated for the comparison of H-scores as continuous variables from each of the ROS1 clones, and \( \kappa \) scores were calculated for dichotomized results based on two positivity cutoff points, as described above, to assess the inter-pathologist agreement for each of the clone. The paired Student’s t-test was used to assess the correlation between the ROS1 IHC status and RECIST 1.1. All statistical analyses were carried out using R software (version 3.2.2, R Core Team, Vienna, Austria). Any \( P \)-value <0.05 was assumed to indicate a statistically significant difference.

**RESULTS**

**Training cohort**

In a clinically selected cohort enriched for tumors with FISH-identified ROS1 rearrangements, the frequency of split or isolated centromeric signals averaged 64% and ranged from 15% to 100%. IHC SP384 showed strong 3+ expression in 40/51 (78%), 2+ moderate in 10/51 (20%) and 1+ weak in 1/51 (2%) of cases (Table 1, Figure 1). IHC D4D6 showed a wider range of intensity in 15/51 (30%) with 3+ staining, 16/51 (31%) with 2+ staining and 20/51 (39%) with 1+ staining (Table 1, Figure 1). Inter-pathologist agreement was high in interpreting both ROS1 IHC clones (Supplementary Table S1).

On the basis of the ≥1+ expression cutoff, both ROS1 IHC clones are 100% sensitive, whereas if ≥2+ tumors are considered positive, the SP384 clone is 98%
sensitive and D4D6 is only 61% sensitive for the detection of a ROS1 rearrangement by FISH (Table 1).

H-scores ranged from 5 to 300 and from 50 to 300 when using the D4D6 and SP384 clones, respectively. However, the Spearman’s rank correlation was not linear (rho=0.508; 95% CI, 0.19–0.72) between the two clones (Figure 1, Supplementary Figure S1).

Neither the intensity nor the percentage of ROS1 protein expression detected with both SP384 and D4D6 antibodies correlated with the percentage of ROS1 split signals by FISH (Supplementary Figure S2).

In most of the cases analyzed with the SP384 clone, a diffuse cytoplasmic staining was observed with or without membranous staining (Figure 2). Less frequently, a granular cytoplasmic or a membranous staining alone was noted (Figure 2). Corresponding D4D6 IHC staining was weaker and in some cases the percentage of positive tumor cells was lower than those noted in the corresponding tumors stained with the SP384 clone (Figure 1, Supplementary Figure S1). ROS1 protein expression was also seen with both D4D6 and SP384 antibodies in non-neoplastic alveolar epithelium at the tumor periphery, in sub-pleural pneumocytes or in areas of type II pneumocyte hyperplasia and bronchiolar metaplasia. However, the expression in this epithelium was weak in intensity in most cases.

In this retrospective study, at the time of clinical care, the vast majority of ROS1 positive patients, as confirmed by FISH, were treated with crizotinib in the second and third line settings. After 3 months, 18% of patients had a complete response, 19% demonstrated a partial response, 19% had stable disease, and 44% had progressive disease (Supplementary Figure S3). In total, 56% of patients had disease control after 3 months on crizotinib. However, no significant difference existed between the two IHC clones (Supplementary Figure S3) nor between the % of rearranged tumor cells by FISH and the response by RECIST (t-test, P-value=0.306). In order to identify putative resistance mechanisms, an NGS analysis (Ion AmpliSeq Cancer Hotspot Panel, ThermoFisher Scientific, San Francisco, CA, USA) was performed in two patients with progressive disease and available tumor material. In one patient, we found a mutation in the gene encoding for β-catenin (CTNNB1 p.S45F, VAF 43%). The NGS analysis detected no copy-number
variations. Moreover, these cases had no ROS1 copy-number gain, as determined by FISH.20

Validation cohort

A total of 714 cases of LUAD with an unknown ROS1 rearrangement status were screened on TMA blocks using paired a ROS1 IHC and FISH assay. ROS1 rearrangements were detected in this screening cohort by FISH at a rate of 9 of 714 cases (1.3%).

On the basis of the ≥1+ expression cutoff, both ROS1 IHC clones are 100% sensitive for the presence of a ROS1 translocation detected by FISH, while the D4D6 clone was deemed more specific and accurate (Table 1, Figure 3). Conversely, on the basis of the ≥2+ expression cutoff, the SP384 clone was more sensitive than the D4D6 clone, while the D4D6 was more specific and accurate than SP384 (Table 1, Figure 3).

Inter-pathologist agreement ranged from moderate to good (ICC, 0.722–0.874) in interpreting the D4D6 IHC assay and from good to excellent (ICC, 0.830–0.956) for the SP384 IHC assay (Supplementary Table S1).
DISCUSSION

This is the first study to assess the diagnostic performance of a newly
developed anti-ROS1 antibody (SP384 clone) by comparison to two diagnostic ROS1
assays that are validated for a clinical setting (e.g., D4D6 IHC and ROS1 break-apart
FISH assays) in two large-scale multicenter cohorts of LUAD cases.

We considered two cutoffs for positivity and showed that none of the ROS1
IHC assays demonstrated 100% accuracy in detection of ROS1 rearrangements as
identified by FISH. In the validation cohort of ROS1-positive LUAD cases, while these
two clones demonstrate similar sensitivity around the \( \geq 1+ \) cutoff, the sensitivity rate
dropped at 61% for the D4D6 clone when the \( \geq 2+ \) cutoff was applied. In addition, the
intensity and the percentage of stained tumor cells were higher when using the
SP384 clone than with the D4D6 clone. Inter-reader agreement varied from moderate
to good when interpreting the D4D6 assay, while it was deemed good to excellent for
the SP384 clone, as recently reported.\(^{19}\) Most of the cases analyzed with the SP384
cloned showed diffuse cytoplasmic staining, with or without membranous labeling.

It is noteworthy that the percentage and the intensity of SP384 and D4D6 IHC
did not correlate with the percentage of ROS1 FISH split signals, as had previously
been reported.\(^{12}\) No significant difference was noted between the different ROS1 IHC
assays and the RECIST 1.1 subgroups in patients treated with crizotinib.

In our screening cohort, which was drawn from a clinical molecular diagnostics
practice, ROS1 rearrangement was detected in 1.3% of tumors, compared to 1–2%
of unselected LUAD cases, as previously reported.\(^{6}\) Around the \( \geq 1+ \) cutoff, sensitivity
was similar for both IHC assays; however, the specificity of the D4D6 clone (97.87%)
was higher than that of the SP384 clone (87.38%). We also explored the recently
described \( \geq 2+ \) cutoff in \( >30\% \) tumor cells.\(^{17, 19}\) Whereas the sensitivity of the D4D6
cloned declined to 66.67% under these criteria, and was 88.89% for the SP384
antibody, the specificity was still higher for the D4D6 assay (99.57%) than the SP384
cloned (91.06%).

The average H-score of the SP384 clone was higher that the D4D6 antibody,
which led to easier interpretation. However, if an algorithm for ROS1 testing that is
similar to that established for ALK status testing were used and if the \( \geq 1+ \) cutoff were
to be used for both IHC clones, this could lead to an increase of nearly 10% in
unnecessary FISH testing based on screening with the SP384 clone. Moreover, in
our study, the ≥2+ cutoff in >30% tumor cells did not improve the sensitivity and specificity rates of either IHC assay.

When newly developed diagnostic assays for the detection of rare events such as ROS1 rearrangements are considered for implementation in a routine clinical setting, our findings highlight the importance of their evaluation in large-scale cohorts, and the necessity of both positive and negative cases. As accurate detection of ROS1 rearrangements in clinical tumor samples is mandatory for optimal targeted treatment, investigation of new testing modalities should not be limited to positive samples only.\(^\text{18}\)

Previous studies reported ROS1 D4D6 IHC as being highly sensitive, but less specific compared with FISH for the detection of ROS1 rearrangements. ROS1 D4D6-positive tumors, especially when the tumor is stained with moderate to strong intensity or a diffuse pattern, are recommended to undergo FISH to confirm the presence of ROS1 rearrangement.\(^\text{12-14}\) Besides the assessment of the ROS1 rearrangement status, it is mandatory to systematically detect other genomic alterations in advanced and metastatic lung adenocarcinoma. This can be performed by molecular biology (e.g., EGFR mutation) and by molecular and/or IHC approaches (e.g., BRAFV600 mutation, ALK rearrangement). Moreover, PD-L1 IHC is now required in this population. Additionally, the evaluation of other genomic alterations (e.g., RET rearrangement, MET mutations, HER2 mutations, NTRK rearrangement) can be required if the tumors are EGFR, ALK, ROS1 and BRAF wild-type and with less than 50% of PD-L1 positive tumor cells. Moreover, recent clinical trials for immunotherapy associated with the assessment of the tumor mutation burden (TMB) have indicated some promising results for the systemic use of this predictive biomarker in the near future.\(^\text{21, 22}\) In this context, one challenge is to be able to perform these analyses on smaller tissue biopsies and/or samples with low proportions of tumor cells (less than 20%). Considering that a minimum number of tissue sections are required for the diagnosis of NSCLC, it is critical to conserve tissue as much as possible for ancillary analyses (different predictive IHC, targeted sequencing analyses or NGS panels – potentially including TMB assessment). In this context, it is of interest to economize and only use tissue sections for ROS1 FISH analysis in IHC positive cases. More importantly, the feasibility of using ROS1 IHC for ROS1 status assessment has many advantages in comparison with the ROS1 FISH.
IHC is available in all pathology laboratories and can easily be conducted. The turnaround time to get the results is much shorter than the FISH approach. IHC is a cost-effective technology. Finally, shipment and archiving of stained slides can be done at room temperature.

Different testing approaches, such as DNA-based hybrid capture library preparation followed by NGS, RNA-based anchored multiplex polymerase chain reaction library preparation followed by NGS or nCounter technologies could allow the simultaneous detection of gene rearrangements such as ALK, ROS1, RET, or NTRK. Thus, it might be questionable whether the development of IHC assays for the detection of these alterations should continue, in particular for ROS1 rearrangement. However, some difficulties can be encountered for the NGS approach in comparison with the IHC method in a clinical setting. Depending on the quantity and quality of nucleic acids present in the FFPE tissue biopsy, NGS can give false negative results, in particular in small specimens and/or when a low percentage of tumor cells are present. Moreover, the technology is not available in all laboratories and requires specific and high-level expertise. The time lag for results is also much longer than for IHC testing. Finally, the accreditation is certainly easier to obtain for an IHC test than for a test developed on an NGS platform.

Assuming that the implementation of the SP384 IHC assay in the testing algorithm of advanced and metastatic LUAD patients can be considered, following the results showed in the present work and probably other forthcoming studies, independent validation studies need to be performed. In addition, it will be of interest to further assess the performance of the SP384 clone on cytology samples such as cellblocks. Finally, as a perspective, the ROS1 IHC assay could be a good candidate to be associated with other “predictive” antibodies such as the anti-ALK, anti-BRAFV600E and anti-PD-L1 in a chromogenic multiplex IHC assay for small tissue biopsies.

In conclusion, ROS1 IHC testing is an effective screening tool for the presence of ROS1 rearrangements. However, users must be aware of the variable diagnostic performance of different commercialized anti-ROS1 antibodies before incorporation into the diagnostic molecular pathology workup of LUAD cases.
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**Conflict of interests:**

I.R. has received honoraria from AstraZeneca, MSD, Bristol-Myers Squibb, Boehringer-Ingeheim, Roche and Takeda.

J.M has received research grant and honoraria from Roche, AstraZeneca and Bristol-Myers Squibb, and honoraria from Boehringer-Ingeheim, MSD, PharmaMar, Pfizer, Takeda.

F.B. has received honoraria from Bristol-Myers Squibb, MSD, Amgen, Merck & Co., Sanofi, Haliodx and AstraZeneca, and grants and travel expenses from Bristol-Myers Squibb and MSD.

J-C.S. has received honoraria from Amgen, Roche, Pfizer, MSD, Bristol-Myers Squibb, Merck & Co., AstraZeneca and Boehringer-Ingeheim.

M.I. has received honoraria and travel grants from AstraZeneca, Bristol-Myers Squibb, Roche, Boehringer-Ingeheim and Merck & Co.

P.H. has received honoraria and travel grants from AstraZeneca, Roche, Bristol-Myers Squibb, Novartis, MSD, Qiagen, Thermofisher, Biocartis, and Merck & Co.

The remaining authors have declared no conflict of interests.
Legend to Figures

**Figure 1.** Representative images of ROS1 IHC using the D4D6 and the SP384 antibodies in the training cohort. (A) Strong diffuse staining with the SP384 antibody, and (B) weak to moderate diffuse staining with the D4D6 antibody in the same case as in (A). (C) Weak to moderate diffuse staining with the SP384 antibody, and (D) weak focal staining with the D4D6 antibody in the same case as in (C). A ROS1 rearrangement was identified in both cases (A-B, and C-D) by FISH. (Scale bars are shown on the figures).

**Figure 2.** Representative immunostaining localization in tumor cells using the SP384 antibody. (A) Diffuse cytoplasmic. (B) Membranous and cytoplasmic. (C) Membranous. (D) Cytoplasmic granular. Scale bars are shown on the figures.

**Figure 3.** Representative comparative images between the ROS1 IHC using the D4D6 and the SP384 antibodies and the ROS1 FISH analysis in the validation cohort.

(A) Strong diffuse (3+) staining with the D4D6 and SP384 antibodies in a LUAD case positive by ROS1 FISH.

(B) Weak diffuse staining (1+) with the D4D6 antibody but strong staining (3+) with the SP384 clone in a LUAD case positive by ROS1 FISH.

(C) Weak diffuse staining (1+) with the D4D6 antibody and moderate staining (2+) with the SP384 clone in a LUAD case positive by ROS1 FISH.

(D) Lack of staining with both D4D6 and SP384 clones in a LUAD negative by ROS1 FISH.

(E) No staining with the D4D6 clone in a LUAD case moderately stained (2+) with SP384 clone and positive by ROS1 FISH.

(F) No staining with the D4D6 clone in a LUAD case strongly stained (3+) with the SP384 clone but revealed negative by ROS1 FISH.

Red circles; positive tumor cells by FISH; green circles, negative tumors cells by ROS1 FISH.
Legend to Supplementary Figures

Supplementary Figure S1. Distribution plot of H-scores for each anti-ROS1 antibody.

Supplementary Figure S2. Correlation analysis between the H-scores of (A) D4D6 or (B) SP384 clones and the percentage of tumor cells harboring ROS1 rearrangement by FISH. Note: the X-axis in (A) is manually set to have a maximum of 300 to match with SP384.

Supplementary Figure S3. Correlation analysis between response assessments by RECIST 1.1 after 3 months under crizotinib and the IHC status by D4D6 (paired Student’s t-test; P=0.002) and SP384 (paired Student’s t-test; P=0.006) clones. Abbreviations: Progressive Disease (PD), Stable Disease (SD), Partial Response (PR), Complete Response (CR).
Table 1. Comparative performance analyses between ROS1 FISH and SP384 and D4D6 IHC clones.

<table>
<thead>
<tr>
<th>Training cohort</th>
<th>SP384 IHC</th>
<th>D4D6 IHC</th>
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</thead>
<tbody>
<tr>
<td><strong>FISH ROS1</strong></td>
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<tr>
<td>Positive n = 51</td>
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<tr>
<td>Strong 3+</td>
<td>40 (78%)</td>
<td>15 (30%)</td>
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<tr>
<td>Moderate 2+</td>
<td>10 (20%)</td>
<td>16 (31%)</td>
</tr>
<tr>
<td>Weak 1+</td>
<td>1 (2%)</td>
<td>20 (39%)</td>
</tr>
<tr>
<td>Negative 0</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
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<tr>
<td><strong>Sensitivity</strong></td>
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<tr>
<td>Cutoff ≥1+</td>
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<td>100% (95% CI, 93.02%–100%)</td>
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<td>Cutoff ≥2+</td>
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<td><strong>Validation cohort</strong></td>
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<tr>
<td>Positive n = 9 (1.3%)</td>
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<td></td>
</tr>
<tr>
<td>Strong 3+</td>
<td>6 (67%)</td>
<td>3 (33%)</td>
</tr>
<tr>
<td>Moderate 2+</td>
<td>2 (22%)</td>
<td>3 (33%)</td>
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<tr>
<td>Weak 1+</td>
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<td>Negative 0</td>
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<td><strong>Cutoff ≥1+</strong></td>
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<td><strong>Cutoff ≥2+</strong></td>
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<td>97.87% (95% CI, 96.51–98.80%)</td>
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<td><strong>PPV</strong></td>
<td>9.18% (95% CI, 7.69%–10.94%)</td>
<td>37.50% (95% CI, 26.67%–49.75%)</td>
</tr>
<tr>
<td><strong>NPV</strong></td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>87.54% (95% CI, 84.89%–89.87%)</td>
<td>97.90% (95% CI, 96.56%–98.82%)</td>
</tr>
<tr>
<td><strong>Cutoff ≥2+</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>88.89% (95% CI, 51.75%–99.72%)</td>
<td>66.67% (95% CI, 29.93%–92.51%)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>91.06% (95% CI, 88.71%–93.06%)</td>
<td>99.57% (95% CI, 98.76%–99.91%)</td>
</tr>
<tr>
<td><strong>PPV</strong></td>
<td>11.27% (95% CI, 8.37%–15.01%)</td>
<td>66.67% (95% CI, 37.12%–87.14%)</td>
</tr>
<tr>
<td><strong>NPV</strong></td>
<td>99.84% (95% CI, 99.02%–99.98%)</td>
<td>99.57% (95% CI, 98.93%–99.83%)</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>91.04% (95% CI, 88.70%–93.03%)</td>
<td>99.16% (95% CI, 98.18%–99.69%)</td>
</tr>
</tbody>
</table>

Abbreviations: FISH, Fluorescence in situ hybridization; NPV, Negative Predictive Value; PPV, Positive Predictive Value.
References


