



On the relevance of a testing algorithm for the detection of *ROS1*-rearranged lung adenocarcinomas

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ABSTRACT

Objectives: *ROS1* proto-oncogene translocations define a new molecular subgroup in non-small cell lung cancers (NSCLC) and are associated with a response to the MET/ALK inhibitor, crizotinib. These rearrangements are described in 0.9–1.7% NSCLC, in wild-type *EGFR*, *KRAS* and *ALK* (“triple negative”) lung adenocarcinomas. Rapid and efficient identification of these alterations is thus becoming increasingly important.

Materials and methods: In this study, 121 triple negative lung adenocarcinomas were screened by both IHC with the *ROS1* D4D6 antibody, and FISH using two commercially available *ROS1* break-apart probes. To address a possible cross-reactivity of the *ROS1* antibody with other protein kinase receptors, we screened 80 additional cases with known *EGFR*, *KRAS*, *PI3KCA*, *BRAF*, *HER2* mutations or *ALK*-rearrangement.

Results: We diagnosed 9 *ROS1*-rearranged adenocarcinomas, with both a positive FISH result (51–87% rearranged nuclei) and a positive IHC staining (2+/3+ cytoplasmic staining). Only one of the *ROS1*-positive FISH cases was characterized by a classical split pattern, the others showed a variant pattern, most commonly involving a loss of the 5' telomeric probe. Considering a positivity threshold of 2+ stained cells, the sensitivity of the *ROS1* D4D6 antibody compared to FISH was 100% and the specificity 96.9%, as two *HER2*-mutated tumors were positive with D4D6 antibody, without any translocation in FISH. All the *ROS1*-positive cases were at an advanced stage, arising in never or light smokers. They were mainly solid cribriform and acinar adenocarcinomas, with signet ring cells noted in 5 cases, and calcifications in 3 cases. One positive case was an invasive mucinous carcinoma.

Conclusion: Our results show that a screening algorithm based on an IHC detection of *ROS1* fusion proteins, confirmed if positive or doubtful by a *ROS1* break-apart FISH assay, is pertinent in advanced “triple negative” lung adenocarcinomas, since the prevalence of *ROS1*-positive cases in this selected population reaches 7.4% in our series.

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1. Introduction

Lung cancer remains the leading cause of death by cancer worldwide. However, in addition to the most common mutations involving the *EGFR* and *KRAS* genes, driver mutations/rearrangements involving more than 10 other genes have been found in 60% of non-small cell lung carcinomas (NSCLCs) [1], allowing a further subdivision of NSCLCs into clinically relevant molecular subgroups. Rapid and efficient identification of genetic

Abbreviations: *ROS1*, c-ros oncogene-1; *ALK*, anaplastic lymphoma kinase; *EGFR*, epidermal growth factor receptor; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; FISH, fluorescent in situ hybridization; IHC, immunohistochemistry.

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alterations, and in particular of druggable ones, is thus becoming increasingly important for the treatment of NSCLC patients. Out of these are the recently described *ROS1* (c-ros oncogene 1, located at 6q22) gene rearrangements. The *ROS1* gene encodes a receptor tyrosine kinase of the insulin receptor family. No *ROS1* ligand has been identified to date but cellular attachment via its extracellular domain could trigger *ROS1* kinase activation [2,3]. Although *ROS1* protein expression has been evidenced in various tissues, normal lung tissue seems devoid of *ROS1* expression [4]. In a similar way to ALK aberrant kinase activity, *ROS1* kinase alterations lead to activated downstream signaling of several oncogenic pathways controlling cell proliferation, survival and cell cycling (STAT3, PI3K/AKT/mTOR, RAS-MAPK/ERK pathways) [5,6]. Historically, *ROS1* rearrangements were identified in glioblastomas [7,8], and more recently in NSCLC cell lines and patients, where *ROS1* fusions lead to a constitutive activation of the kinase [9–17]. The first two major papers [9,15] described *ROS1* rearrangements in 0.9–1.7% NSCLC cases and, remarkably, only in wild-type *EGFR*, *KRAS* and *ALK* (“triple negative”) lung adenocarcinomas (ADC). Promising results show *ROS1* fusions in NSCLC are associated with sensitivity to the ALK/MET inhibitor crizotinib, as well as to other small-molecule *ROS1* inhibitors [6,18–20].

In this study, we used our previously described testing algorithm for the screening of potential *ALK*-rearranged tumors [21] to test “triple-negative” (wild-type *EGFR*, *KRAS* and *ALK*) adenocarcinomas for *ROS1* rearrangements. We diagnosed by break-apart FISH using commercial probes and by IHC nine *ROS1*-positive tumors out of 121 patients, tested by both *ROS1* IHC and FISH, leading to a proportion of 7.4% *ROS1*-positive patients in this selected population [4,9,15].

2. Materials and methods

From June 2012 to June 2013, 121 specimens of primary lung adenocarcinomas, classified according to the 2004 WHO classification of lung tumors [22] were sent to the Grenoble University Hospital Genetics and Molecular Platform. These specimens were formalin-fixed and paraffin-embedded. They included small biopsies (bronchial, transthoracic) and surgical specimens (lung resections, lymph node, pleural surgical biopsies). All of them were *EGFR* and *KRAS* wild-type and showed no *ALK* rearrangement, as assessed by IHC and FISH. When needed, IHC stainings for p63 (clone 4A4, Dako, Glostrup, Denmark) and TTF-1 (clone 8G7G3/1 Neomarkers, Fremont, CA, USA) were performed. Clinical data from patients with *ROS1*-rearranged tumors were collected, including their smoking status, TNM/tumor staging, therapy, and tumor response to therapy. In addition, in order to address a possible cross-reactivity of the *ROS1* antibody with other protein kinase receptors, 80 additional cases were investigated by D4D6 *ROS1* IHC: 17 *ALK*-rearranged, 6 *HER2*-, 7 *BRAF*-, 6 *PI3KCA*-, 20 *KRAS*-, and 24 *EGFR*-mutated lung adenocarcinomas.

2.1. *ROS1* FISH

FISH was performed on unstained 3–4 μ m formalin-fixed paraffin embedded (FFPE) tumor tissue sections with the use of a *ROS1* break-apart probe set using a paraffin pretreatment reagent kit (Vysis, Abbott Laboratories, Abbott Park, IL, USA), as described previously [21]. Two commercially available *ROS1* break-apart FISH probe sets were used for this study: the Aquarius Pathology *ROS1* Breakapart Probe (Cytocell, Cambridge, GB) and/or the ZytoLight® SPEC *ROS1* Dual Color Break Apart Probe, (ZytoVision, Bremerhaven, Germany). The Aquarius Pathology probe set consists of a green 406Kb 5' (telomeric) probe and two red (299Kb and 171Kb) 3' (centromeric) probes, positioned on each side of the *ROS1*

gene, whereas the ZytoLight probe mix contains an orange 450Kb 5' probe and a green 750Kb 3' probe. Both probes were tested on the *ROS1*-rearranged HCC78 cell line and on *ROS1*-positive patient samples and yielded similar results. Nuclei were counterstained with DAPI/Vectashield (Vektor Laboratories, Burlingame, CA, USA) and were analyzed with a Metafer slide scanning system (Metasystems, Altlussheim, Germany) under a 63 \times oil immersion objective with a fluorescence microscope (Imager Z2, Zeiss) equipped with appropriate filters, a charge-coupled device camera, and the FISH imaging and capturing software Metafer 4 (Metasystems). Signals were enumerated with the ISIS imaging system (Metasystems). Tumor tissues were considered *ROS1* FISH positive (*ROS1*-rearranged) if >15% tumor cells showed split red and green signals (signals separated by ≥ 1 signal diameter) and/or single 3' signals. Otherwise the samples were considered as FISH negative.

2.2. *ROS1* immunohistochemistry

Sections were deparaffinized and incubated with the rabbit primary monoclonal *ROS1* antibody [4] (Clone D4D6, #3287, Cell Signaling Technology, Danvers, MA, USA) at a dilution of 1:50 2 h at 20 °C. A standard three-stage indirect immunoperoxidase technique was performed on a Benchmark XT Ventana staining module using an XT UltraView DAB kit; antigen retrieving was performed with the Cell Conditioning buffer (CC1, Tris/Boric acid/EDTA pH8.0) for 1 h, according to the manufacturer's instructions (Ventana, Tucson, AZ, USA). No signal amplification was required. The specificity of the *ROS1* antibody was assessed by using paraffin-embedded cell blocks of the *ROS1*-rearranged cell line HCC78, which was subsequently used as positive external controls for all tests. Normal lung tissues were used as negative controls. The percentage of positive cells was evaluated, and staining scores were assessed as follows: 0; no staining, 1+; faint cytoplasmic staining, 2+; moderate cytoplasmic staining and 3+; intense granular cytoplasmic staining, similarly to previously described [21]. Membrane staining was recorded when observed.

3. Results

3.1. *ROS1* fluorescence in situ hybridization (Fig. 1)

Among the 121 cases analyzed by both immunohistochemistry and FISH, 9 cases were *ROS1*-rearranged, 98 cases were negative and 14 were not interpretable.

Out of the nine *ROS1*-rearranged specimens, only one showed a classical split pattern (Fig. 1B), 6 had a *ROS1* rearrangement which resulted in the deletion of the 5' region (nuclei with isolated 3' signals, Fig. 1C and D), and 2 had a mix pattern (presence of various isolated 3' and split signals, Fig. 1E). The mean percentage of positive nuclei was 67% (range: 51–87%); the total number of tumor nuclei analyzed ranged from 83 to 244, with a mean of 137.

For the 98 negative cases, as with *ALK* FISH [21,23], nuclei showed various patterns within a given tumor; nuclei with 1 to more than 6 fusions, and/or isolated green and/or red signals, and/or split signals (Fig. 1A). 34 cases (34.7%) showed two fusion signals or very close green and red signals in the majority of nuclei (predominant pattern). In 9 cases, the presence of 3–5 fused *ROS1* signals was the predominant pattern in tumor nuclei and in 32 cases loss of one *ROS1* fusion signal (only 1 gene copy) was the predominant pattern. In addition, 1 case showed 15% or more nuclei with at least 6 *ROS1* fusion signals.

For all *ROS1*-non rearranged cases, mean percent of positive nuclei (percent of nuclei showing a rearrangement pattern in *ROS1*-negative cases) was 3.2%, ranging from 0 to 11.8%.

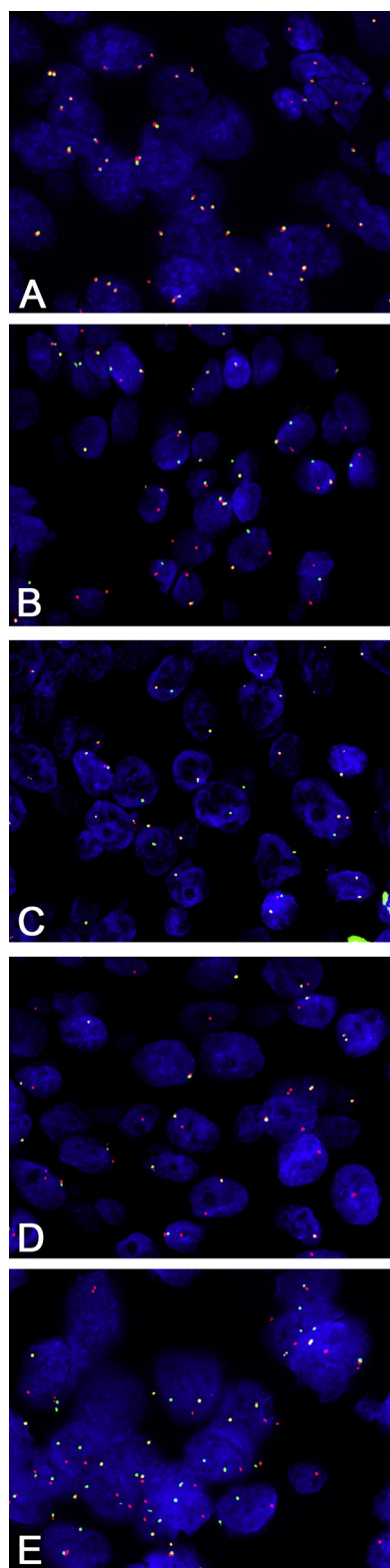


Fig. 1. Detection of *ROS1* gene rearrangements in triple negative lung adenocarcinoma cases by FISH. Non-rearranged *ROS1* (A) shows fusion (orange signals) or very close apposition of the probes close to the 3' and the 5' ends of the gene. A, B, C and E: tumor nuclei hybridized with the ZytoLight® SPEC *ROS1* dual color break-apart probe (ZytoVision). Rearranged *ROS1* is indicated by split 3' (green) and 5' (red) signals (B) or isolated 3' (green) signals (C) or various single 3' and split signals (E). D: tumor nuclei hybridized with the Aquarius® *ROS1* dual color break-apart probe (Cytocell). Rearranged *ROS1* appears here as isolated red (3') signals. Original magnification 630×. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

ROS1 fusion protein detection by IHC in a panel of lung adenocarcinoma FFPE samples with mutated or rearranged protein kinase genes.

Protein kinase gene alteration N = 80, all <i>ROS1</i> FISH negative	<i>ROS1</i> IHC positive	<i>ROS1</i> IHC doubtful	<i>ROS1</i> IHC negative
ALK (n = 17)	1	0	16
HER2 (n = 6)	3	0	3
BRAF (n = 7)	0	0	7
PI3KCA (n = 6)	0	0	6
KRAS (n = 20)	0	0	20
EGFR (n = 24)	0	2	22

Fourteen cases were not interpretable. Thirteen cases showed no or very weak hybridization signals and a high background staining, most probably due to inappropriate preanalytical steps (poor quality of the material, decalcification, delays in fixation, etc.). One case was not interpretable due to an insufficient number of tumor cells analyzable (31 cells, all showing no *ROS1* gene rearrangement).

3.2. *ROS1* immunohistochemistry (Fig. 2)

3.2.1. *ROS1* protein expression in *ROS1*-positive HCC78 cell line and normal human lung tissue

As described initially by Rimkunas et al. [4], HCC78 cells showed a strong cytoplasmic and punctate staining. In a few specimens of normal lung tissue, rare pneumocytes were slightly stained and nonspecific positivity of macrophages and in particular giant cells was noted (Fig. 2A and B).

3.2.2. *ROS1* fusion protein detection in EGFR, KRAS and ALK-non mutated/rearranged lung adenocarcinomas

All nine *ROS1* FISH-positive cases showed *ROS1* protein immunostaining. In these tumors, the cytoplasmic staining was at least moderate (2+), in more than 40% of cells, with an additional intense signal (3+) in 2 cases (Fig. 2C). Positive stainings were heterogeneous, with tumor cells faintly to intensely stained and some cells showing no staining. *ROS1* immunostaining was cytoplasmic for all cases, with a membrane reinforcement noted in two cases, and a perivacuolar cytoplasmic positivity in signet ring cells. A striking granular cytoplasmic staining with perinuclear dots was observed in one case (Fig. 2D).

Out of the 98 FISH-negative specimens, 95 did not express *ROS1* protein. Two of the *ROS1* FISH-negative specimens had a positive IHC result with tumor cells stained with 2+ to 3+ intensities and, interestingly, both were *HER2*-mutated. The last specimen showed a doubtful *ROS1* immunostaining result with 80 cells stained with a 1+ intensity.

One case, containing only 20 tumor cells, was characterized by a moderate staining of all cells; unfortunately *ROS1* FISH analysis was not interpretable due to the absence of hybridization signals and the patient did not agree to repeat the trans-thoracic biopsy, having previously suffered from a pneumothorax.

3.2.3. *ROS1* fusion protein detection in ALK-rearranged, *HER2*-, *BRAF*-, *PI3KCA*-, *KRAS*-, or *EGFR*-mutated lung adenocarcinomas (Table 1)

Out of the 17 *ALK*-rearranged cases analyzed, one was *ROS1* IHC-positive, showing a moderate staining in 30% of cells, and a faint staining in 40% of cells. Out of the 6 *HER2*-mutated adenocarcinomas, 3 were *ROS1* IHC-positive with 30–40% of cells stained with a 2+ intensity. There was no *ROS1* expression in tumors showing a *BRAF* mutation (7 cases), a *PI3KCA* mutation (6 cases), or a *KRAS* mutation (20 cases). Out of the 24 cases harboring an *EGFR* mutation (mutation in exons 18/20/21 or deletion in exon 19), a doubtful faint staining was observed in 15% of cells in 2 cases, both of which had a mutation in exon 21. The *HER2*-mutated (n = 3) and

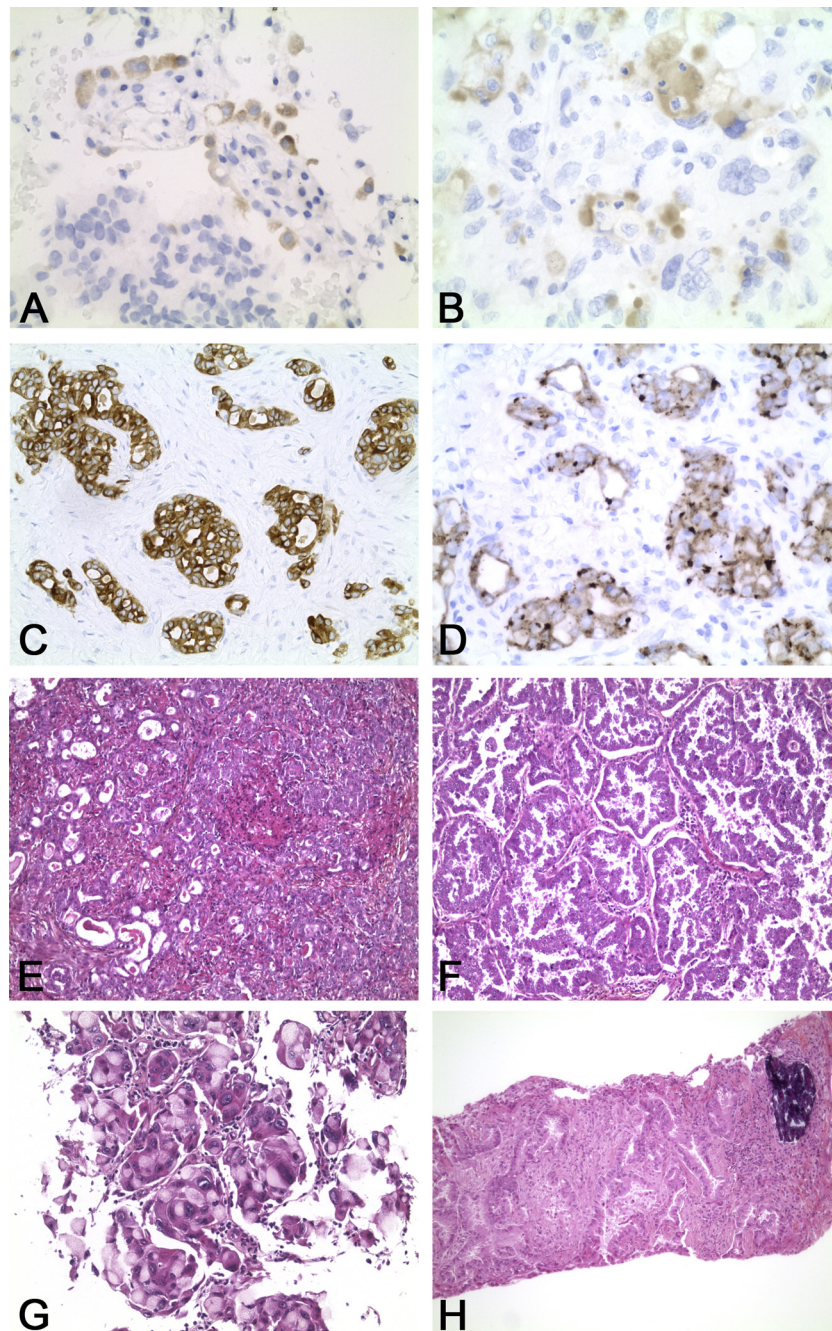


Fig. 2. Representative histologic features and expression of the ROS1 protein in ROS1-rearranged lung adenocarcinomas. Non-specific ROS1 positivity was sometimes observed in type II pneumocytes (A, ROS1 IHC 400 \times) and in multinucleated giant cells (B, ROS1 IHC 400 \times). ROS1 IHC was positive in all these tumors, with a moderate to strong cytoplasmic staining observed in more than 40% tumor cells, sometimes with a membrane reinforcement (C, ROS1 IHC 200 \times). One case showed vesicular paranuclear dots (D, ROS1 IHC 400 \times). ROS1-positive specimens were mainly characterized by a solid cribriform and acinar pattern (E, HES 100 \times), and two of them by a micropapillary pattern (F, HES 100 \times). Signet ring cells were noted in 5 cases (G, HES 200 \times), and calcifications in 3 cases (H, HES 100 \times).

ALK-rearranged ($n = 1$) tumors showing a ROS1 positive staining by IHC, as well as the *EGFR*-mutated tumors ($n = 2$) showing a doubtful ROS1 immunostaining, were all ROS1 FISH-negative.

In summary, considering a positivity threshold of 2+ staining intensity, the sensitivity of the ROS1 D4D6 antibody compared to FISH in this study is 100% and the specificity is 96.9% (Table 2).

3.3. Correlation between ROS1 rearrangement and clinical and histological data (Tables 3 and 4)

ROS1 positive patients' mean age was 60.9 years (41–78 years), 5 patients were female and 4 male. ROS1 negative patients' mean

Table 2
Correlation between ROS1 FISH and IHC results.

N = 121	FISH positive, $n = 9$	FISH negative, $n = 98$	FISH not interpretable, $n = 14$
IHC positive (2+/3+) $n = 11$	9/9	2/98 (<i>HER2</i> mutated)	0
IHC doubtful (1+) $n = 2$	0	1/98	1/14
IHC negative $n = 108$	0	95/98	13/14

Table 3Correlation between *ROS1*-rearrangement and clinical data.

Patient	Sex	Age at diagnosis	TNM	Stage	Smoking habit	Specimen
1	M	41	T4N2M0	IIIa	Non smoker	Transthoracic needle biopsy
2	F	49	T2aN3M1	IV	Non smoker	Transthoracic needle biopsy
3	M	50	T3N2M1	IV	Non smoker	Pleural biopsy
4	F	71	T3N2M1b	IV	Non smoker	Mediastinal lymph node
5	M	61	T4N0M0	IIlb	Non smoker	Lung resection
6	F	78	T1aN2M0	IIIa	Non smoker	Lung resection
7	F	58	T2bN3M0	IIlb	Non smoker	Bronchial biopsy
8	M	64	T2 N2 M1	IV	Ex-smoker 2,5 pack-year	Cervical lymph node
9	F	76	T3N2M1	IV	Non smoker	Pleural biopsy

Table 4Correlation between *ROS1*-rearrangement and histological data.

Patient	Predominant pattern	Other histological element	ROS1 IHC	ROS1 FISH pattern	Number of rearranged nuclei
1	Invasive mucinous	Calcification	1+: 10%; 2+: 90%	Split	87% (130/150)
2	Solid (cribriform) and acinar	No	1+: 50%; 2+: 40% ^a	5' deletion	66% (69/104)
3	Acinar and solid (cribriform)	Calcification, signet ring cells <10%	1+: 30%; 2+: 50%	5' deletion	71% (116/164)
4	Solid and acinar	Signet ring cells <10%	1+: 60%; 2+: 40% ^a	5' deletion	83% (96/116)
5	Micropapillary and papillary	No	Not interpretable	5' deletion	55% (58/105)
6	Solid (cribriform) and acinar	Calcification	2+: 80% with perinuclear dot	5' deletion	66% (92/140)
7	Solid	Signet ring cells >10%	1+: 30%; 2+: 40%	5' deletion	54% (45/83)
8	Micropapillary	No	1+: 40%; 2+: 40%; 3+: 20%	Split and 5' del, polysomia	69% (168/244)
9	Solid (cribriform) and acinar	Signet ring cells <10%	3+: 90%	5' deletion	51% (46/90)

^a With membrane reinforcement.

age was 62.3 years (31–88 years), 46 were female and 56 male. Out of the nine positive patients, eight were non-smokers with, for one of them, a passive exposure to tobacco smoke. Only one patient was a former light-smoker (2.5 packs/years). Five patients were at stage IV, two were at stage IIlb and two were at stage IIIa at the time of the *ROS1*-rearrangement diagnosis. Three patients had lung surgery. Five patients had a partial response under pemetrexed alone or associated with a platinum compound, one had a stable disease under pemetrexed alone. After tumor progression under pemetrexed, two patients were included in a crizotinib expanded access program, with a partial response ($\geq 50\%$ at 2 months), but with a dose reduction for one of them, due to toxicity (bilateral pleurisy, severe leg edema, facial erythroderma). One of these two crizotinib-treated patients progressed after 5 months, suggesting a secondary resistance. One patient, treated by carboplatin and vinorelbine, died 2 years after his initial diagnosis of lung cancer.

Specimens were either surgical lung resections (2 cases), or pleural biopsies (2), lymph node biopsies (2), bronchial biopsies (1) or trans-thoracic fine-needle biopsies (TTFNB) (2). The median percentage of malignant cells was 60%. *ROS1*-positive adenocarcinomas were solid and acinar in 7 cases (78%), with an associated cribriform pattern in 4 cases (44%) (Fig. 2E), and a lepidic architecture was also associated in one case. One lung resection showed a micropapillary and papillary pattern. For one case, the lung resection showed a solid, acinar, micropapillary and lepidic adenocarcinoma, the corresponding synchronous lymph node metastasis was only micropapillary (Fig. 2F). Interestingly, one case was a mucinous invasive adenocarcinoma, diagnosed through a TTFNB. Signet ring cells were noted in 4 cases (representing less than 10% of tumor cells in 3 cases, and 20% in 1 case) (Fig. 2G). No extracellular mucus was found, but, interestingly, calcifications were noted in 3 cases (in the lumen of cribriform formations or in the stroma) (Fig. 2H).

All *ROS1*-positive tumors were TTF1 positive, with a heterogeneous staining (from 1+ in 40% of cells to 3+ in 90% of cells). P63 expression was moderate in 2 cases (40% and 60%), negative in all other cases.

4. Discussion

By using both *ROS1* IHC and FISH, we identified 9 patients with *ROS1*-rearranged lung adenocarcinomas, leading to a prevalence

of 7.4% in an enriched cohort of 121 “triple-negative” cases. This study is the third one addressing *ROS1* fusion detection in Caucasian patients, 70 *ROS1*-fused NSCLC cases out of 84 having been reported in Asian patients to date [4,6,9,10,12,14–16].

Eight of our *ROS1*-positive patients were non-smokers, and one was a former light-smoker (2.5 PY), consistent with the previously described higher prevalence of *ROS1*-positive-NSCLCs in non-smokers [9,15]. *ROS1*-positive patients' mean age was around 61 years, not different from *ROS1*-negative patients (mean age 62.3 years), in accordance with the studies of Yoshida et al. [16] and Kim et al. [12], but not with another series in which *ROS1*-positive patients were found to be twelve years younger than negative patients [9].

There was no gender-predominance in our series. Interestingly, considering all the *ROS1*-positive cases published to date, 50 out of 71 documented cases were described in women [6,9,10,12,14–16]. However, as most were from Asia, there is a need for further epidemiological studies on Caucasian populations [24].

Among the *ROS1*-positive patients we detected, 6 had a partial response or a stable disease with pemetrexed, 2 had a partial response with crizotinib, but one of them progressed, developing a probably secondary resistance after 5 months of crizotinib (the mechanism of resistance is currently under investigation). As this is a prospective study, longer clinical outcome and survival data are not yet available. However, our observations are concordant with the promising results of clinical trials evaluating the sensitivity of *ROS1*-rearranged tumors to pemetrexed and crizotinib [19,20,25,26].

Histologically, *ROS1*-rearranged tumors did not exhibit a specific morphological pattern; they were predominantly solid and acinar, and a focal cribriform aspect was associated in 44% of cases, without mucinous features. Signet ring cells were found in five cases, representing more than 10% of tumor cells in one case. Compared to *ALK*-rearranged adenocarcinomas, the presence of a cribriform pattern and of signet ring cells seems lower in *ROS1*+ tumors [21,27,28], but, similarly to *ALK*-rearranged tumors [27], calcifications were noted in nearly one third of our cases.

Considering a positivity threshold for *ROS1* IHC of 2+ staining intensity, all *ROS1* FISH-positive tumors were positive by IHC. The intensity and the distribution of the immunostainings were heterogeneous, with a cytoplasmic granular stain, sometimes associated

with a membrane reinforcement, as seen with ALK fusion-protein staining in solid tumors [21]. A striking staining pattern with positive perinuclear dots was observed in one case, which could correspond to a FIG-ROS1 fusion protein, as a deletion of the 5' probe was observed by FISH in this tumor [4,8].

In our hands, the D4D6 ROS1 antibody was positive in 4 ROS1 FISH-negative cases harboring an ALK (1 case) or HER2 rearrangement/mutation (3 cases), suggesting that the D4D6 clone recognizes an epitope present on one or several common protein(s) involved in ALK, HER2 and ROS1 signaling pathways. Of note, a recent paper by Lee et al. also suggests a possible (over)expression of the ROS1 protein, which could occur through epigenetics mechanisms, such as promotor hypomethylation, without any gene translocation [29]. This phenomenon could perhaps explain the faint positivity of ROS1 immunostaining in some FISH-negative cases. In view of these results, we recommend looking for HER2 mutations for all ROS1 FISH-negative cases showing a positive ROS1 IHC.

Despite its limitations (cost, limited tissue and cell morphology, signal fading), FISH remains the gold standard technique, and its clear advantage over alternative techniques (RT-PCR, sequencing technologies, etc.) is its ability to detect a translocation, whatever the fusion partner, as at least 9 different partners have been described for ROS1. Interestingly, the predominant ROS1+ FISH pattern obtained was not the classical split pattern (observed in only one specimen), but a 5' deletion, sometimes associated with split signals and/or polysomy, suggesting, as previously discussed [16], that ROS1 fusions develop through complex genomic events associating deletion, insertion and copy number gains. In addition, these FISH patterns do not seem to be related to the fusion partner [16], and studies are needed to compare FISH patterns and fusion variants to tyrosine-kinase inhibitor response.

5. Conclusion

In conclusion, given the prevalence of ROS1 fusions in up to 7.4% EGFR, KRAS and ALK non-mutated/rearranged adenocarcinomas, we recommend a prescreening by ROS1 IHC of these “triple-negative” tumors, followed by FISH in positive or doubtful cases or when the clinical and histopathological presentation are suggestive of a ROS1 rearrangement. We also recommend testing for HER2 mutations all cases with a positive ROS1 IHC and a negative ROS1 FISH.

Conflict of interest statement

None declared.

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