HHS Public Access

Author manuscript

Histopathology. Author manuscript; available in PMC 2018 February 01.

Published in final edited form as:

Histopathology. 2017 February ; 70(3): 402–411. doi:10.1111/his.13076.

Screening for *ROS1* gene rearrangements in non-small cell lung cancers using immunohistochemistry with FISH confirmation is an effective method to identify this rare target

Christina I Selinger[1], **Bob T Li**[2,3,4], **Nick Pavlakis**[2,4], **Matthew Links**[5], **Anthony J Gill**[4,6], **Adrian Lee**[2], **Stephen Clarke**[2,4], **Thang N Tran**[1], **Trina Lum**[1], **Po Yee Yip**[8,10,12,13], **Lisa Horvath**[4,7,8], **Bing Yu**[4,9], **Maija RJ Kohonen-Corish**[8,10,11], **Sandra A O'Toole**[1,4,8,*], and **Wendy A Cooper**[1,4,10,*]

[1]Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia

[2]Department of Medical Oncology, Royal North Shore Hospital, St Leonards, Australia

[3]Thoracic Oncology Service, Memorial Sloan Kettering Cancer Center, New York, USA

[4]Sydney Medical School, University of Sydney, Camperdown, Australia

[5]Department of Medical Oncology, St George Hospital, Kogarah, Australia

[6]Cancer Diagnosis and Pathology Group, Kolling Institute of Medical Research, Royal North Shore Hospital, St Leonards Australia

[7]Medical Oncology, Royal Prince Alfred Hospital, Camperdown, Australia

[8]The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Darlinghurst, Australia

[9]Department of Medical Genomics, Royal Prince Alfred Hospital, Camperdown, NSW, Australia

[10]School of Medicine, University of Western Sydney, Penrith, Australia

[11]St Vincent's Clinical School, University of New South Wales, Darlinghurst, Australia

[12]Chris O'Brien Lifehouse, Camperdown, Australia

[13]Macarthur Cancer Therapy Centre, Campbelltown Hospital, Campbelltown, Australia

Abstract

Aims—To assess the prevalence of *ROS1* rearrangements in a retrospective and prospective diagnostic Australian cohort and evaluate the effectiveness of immunohistochemical screening.

Corresponding author: Wendy Cooper, Tissue Pathology and Diagnostic Oncology, Building 94, Royal Prince Alfred Hospital, Camperdown, NSW, 2050, Australia, Fax Number: +61 295158405, Telephone Number: +61 295157804, Wendy.Cooper@sswahs.nsw.gov.au.

*Equal last authors

Conflicts of Interest: W. Cooper has participated in Lung Cancer Advisory Boards and has received honoraria from Pfizer Oncology. S. O'Toole has received honoraria from Roche, Pfizer, and Lilly Oncology. A. Gill has received honoraria from Pfizer Oncology and Astra Zeneca. N. Pavlakis has participated in Lung Cancer Advisory Boards for Pfizer, and received honoraria. The other authors state that they have no conflicts of interest.

Methods—A retrospective cohort of 278 early stage lung adenocarcinomas and an additional 104 prospective NSCLC cases referred for routine molecular testing were evaluated. ROS1 immunohistochemistry (IHC) was performed (D4D6 clone, Cell Signaling Technology) on all cases as well as fluorescence in situ hybridisation (FISH) using the ZytoVision and Abbott Molecular *ROS1* FISH probes, with ≥15% of cells with split signals considered positive for rearrangement.

Results—Eighty eight cases (32%) from the retrospective cohort showed staining by ROS1 IHC, and one case (0.4%) showed *ROS1* rearrangement by FISH. Nineteen of the prospective diagnostic cases showed ROS1 IHC staining of which 12 (12%) cases were confirmed as *ROS1* rearranged by FISH. There were no *ROS1* rearranged cases that showed no expression of ROS1 with IHC. The *ROS1* rearranged cases in the prospective cohort were all *EGFR* wildtype and *ALK* rearrangement negative. The sensitivity of ROS1 IHC in the retrospective cohort was 100% and specificity was 76%.

Conclusions—*ROS1* rearrangements are rare events in lung adenocarcinomas. Selection of cases for *ROS1* FISH testing, by excluding *EGFR/ALK* positive cases and use of IHC to screen for potentially positive cases can be used to enrich for the likelihood of a identifying a *ROS1* rearranged lung cancer and prevent the need to undertake expensive and time consuming FISH testing in all cases.

Keywords

c-ros oncogene 1 (ROS1); non-small cell lung cancer (NSCLC); immunohistochemistry (IHC); fluorescence in situ hybridisation (FISH)

Introduction

A new paradigm of targeted therapies has emerged for non-small cell lung cancer following the discovery of a number of targetable, generally mutually exclusive driver mutations such as those involving *epidermal growth factor receptor* (*EGFR*), *anaplastic lymphoma kinase* (*ALK*), and *c-ros oncogene 1 (ROS1).* Like *ALK*, rearrangements involving *ROS1* are strongly predictive of response to the inhibitor crizotinib[1].

ROS1 is the oncogene product of the avian sarcoma RNA tumour virus[2–4] that encodes a transmembrane tyrosine kinase receptor from the insulin receptor subfamily, and has high homology with the intracellular kinase domain and ATP binding site of *ALK.* Activation of *ROS1* leads to signalling through downstream oncogenic pathways including *PI3Kinase/Akt*, *MTOR* and *RAS-MAPK/ERK* pathways [5–7]. Rearranged or activated *ROS1* has been shown to have transforming potential in nude mice with NSCLC[8]. In addition, *ROS1* gene rearrangements have also been identified in other malignancies such as glioblastomas[9], cholangiocarcinoma[10–12], ovarian cancer[13], gastric cancer[14] and colorectal cancer[15].

Driver mutations involving rearrangement of the *ROS1* gene have recently been described in NSCLC and act as a target for tyrosine kinase inhibitors. *ROS1* rearrangements have been identified in 1–3% of NSCLC[8,16,17], but at higher rates in young, never-smoker, lung adenocarcinoma patients[17]. Due to the high homology between the kinase domains of *ROS1* and *ALK*, ALK inhibitors were tested on *ROS1* positive cell lines and tumours and were

found to be inhibitory[18]. *ROS1* rearranged tumours determined by break apart fluorescence in situ hybridisation (FISH) testing were added to eligibility criteria for the PROFILE 1001 study, a phase I study evaluating the ALK, MET/ROS1 tyrosine kinase inhibitor crizotinib, which reported an overall objective response rate of 72% in 50 *ROS1* positive patients[19]. Preliminary studies suggest *ROS1* rearranged patients treated with crizotinib may have longer median response duration compared with *ALK* rearranged patients, perhaps due to crizotinib having a higher binding efficiency and potency to inhibit ROS1[19].

Although some *ROS1* fusion partners are intrachromosomal involving the long (q) arm of chromosome 6, most partners occur on other chromosomes[20]. A total of 12 *ROS1* fusion variants have been identified, with fusion partners that include: *SLC34-A2, CD74, TMP3, SDC4, EZR, LRIG3, GOPC (FIG), KDELR2* and *CCDC6*[8,21,22]. Importantly, all fusions include the receptor tyrosine kinase domain of *ROS1*[8].

Although *ROS1* rearranged lung cancers show promise as targetable tumours, there is a significant challenge in determining the best way to identify this rare alteration, often in small biopsy samples with limited tissue available for analysis. A number of studies show that ROS1 immunohistochemistry can be utilised in conjunction with FISH to reveal *ROS1* rearrangements in NSCLC[23–31]. The finding that some cases with *ROS1* rearrangement show weak ROS1 immunoreactivity has led a number of authors to conclude that although diffuse-positive ROS1 IHC staining with moderate-strong intensity is more commonly associated with *ROS1* rearrangement, this is not always the case[23,24,26]. Furthermore, strongly positive ROS1 IHC cases can sometimes be *ROS1* FISH negative[23–25,27,28].

IHC screening for *ALK* rearrangements in NSCLC has been established as an effective technique to identify ALK positive tumours[32–37]. An ALK IHC assay has also received FDA approval as a diagnostic companion test for screening for *ALK* rearrangements in the USA. We aimed to assess if a similar process of IHC screening could be useful to identify *ROS1* rearrangements. In this study, we assessed the prevalence of *ROS1* rearrangements in a retrospective cohort of Australian NSCLC comparing 2 techniques (IHC and FISH) and then applied the testing process in a prospective cohort of lung cancers referred for mutation assessment.

Materials & Methods

Patient Cohorts

A retrospective cohort of 278 resected stage I–III lung adenocarcinomas from Royal Prince Alfred Hospital (RPAH) and Concord Repatriation General Hospital between January 1990 and May 2002 were included in the study as previously described[38–40]. Formalin fixed paraffin embedded tissue was used to construct tissue microarrays to test for ROS1 expression and *ROS1* gene rearrangement. *EGFR, KRAS* and *ALK* status had previously been assessed in this cohort as previously described [16,19]. 10% (29/278) harboured an activating *EGFR* mutation, 28% (77/278) harboured a *KRAS* mutation, 1% (3/278) had an *ALK* rearrangement and 26% were not mutation tested (72/278) (Table 1).

An additional cohort of 104 NSCLC cases referred for diagnostic molecular testing (*EGFR*, *ALK* or *ROS1*) at RPAH between November 2012 and May 2016 were also included in the study. Ethics approval was granted by the Sydney Local Health District Ethics Review Committee (X12-0313 and HREC/12/RPAH/479). All cases referred for *EGFR* mutation testing underwent ROS1 IHC if sufficient tissue was available. The 104 cases consisted of tumours found to be ROS1 IHC positive on screening, or were separately referred for *ROS1* FISH testing at the request of the clinical team which included cases with enriched likelihood for *ROS1* rearrangement (*EGFR/KRAS−, ALK−,* female never smoker). The cohort consisted of predominantly *EGFR* wildtype cases (66%), however 15 cases (14%) possessed an *EGFR* mutation, 5 cases (5%) had a *KRAS* mutation, 1 case (1%) had a *KIT* mutation and 1 case (1%) had a *BRAF* mutation (Table 1). Eleven cases (11%) had unknown mutation status. These cases were referred for *ROS1* rearrangement testing as requested by the clinical team or selected due to clinical features or ROS1 IHC positivity. Sixty two cases were mutation tested at RPAH in parallel with diagnostic ROS1 testing (ROS1 IHC & FISH). Mutation testing was performed using the Oncocarta v1.0 and OncoFOCUS v3 on the Sequenom MassARRAY platform (Sequenom/Agena Bioscience, San Diego, CA). The other 42 cases had previously undergone *EGFR* testing elsewhere. *ALK* rearrangement status was negative for all cases except for two positive cases which were *ALK* FISH tested in parallel with *ROS1* at the time due to clinician request. All cases in the diagnostic cohort were adenocarcinomas, except for three adenosquamous and three large cell carcinomas.

ROS1 Immunohistochemistry

Immunohistochemistry was performed on sections, cut at 4um, using the Cell Signaling Technology rabbit monoclonal ROS1 (D4D6) antibody (Danvers, MA, USA) at 1:50 dilution for 2 hours. Staining was performed using the UltraView DAB universal detection kit (Roche, Basel, Switzerland) including an Amplification Kit (Roche), and was performed on a Benchmark ULTRA autostainer (Roche). Positive controls included lung tumor confirmed by FISH to be positive for *ROS1* rearrangement. Any cytoplasmic staining for ROS1 IHC in tumour cells was considered positive. Non-specific staining of macrophages and type II pneumocytes were disregarded. Percentage of cells expressing ROS1 and intensity of expression was also evaluated, in addition to H-scores (% positive cells x intensity [1 mild, 2 moderate, 3 strong staining]).

ROS1 Fluorescence In Situ Hybridisation

Interphase fluorescence in situ hybridisation (FISH) was performed in a NATA (National Association of Testing Authorities) accredited diagnostic laboratory for *ROS1* rearrangement using the ZytoLight SPEC ROS1 Dual Colour Break Apart Probe (ZytoVision) and the LSI ROS1 (Tel) SpectrumOrange Probe and LSI ROS1 (Cen) SpectrumGreen Probe (Abbott Molecular). The ZytoVision FISH probe was used before the Abbott Molecular FISH probe was commercially available in March 2013. The retrospective cohort was analysed using the ZytoVision *ROS1* FISH probe, and the prospective cohort utilised both FISH probes. In our experience both probes show equivalent performance, and both have been utilised and published in international cohorts[23,26,27,43–45]. FISH was performed following the manufacturers guidelines except that Invitrogen Pretreatment solution was used at 98–100°C for 20 minutes. Interphase signals were counted in at least 50

tumour nuclei per cases using an epifluorescence microscope (Zeiss). Cases were classified as *ROS1* FISH positive if they showed ≥15% cells with split signals at least 2 signal distances apart or an isolated centromeric 3′ (green signal) pattern (as indicated by manufacturer and Mazieres et al[45]). The specific tissue region undergoing evaluation was verified by a pathologist and all FISH results were evaluated by a scientist and at least one expert pathologist with considerable experience reviewing lung cancer FISH (WC or SOT).

Results

All cases within the retrospective cohort were evaluated using ROS1 IHC and FISH. Two of the 104 cases in the prospective diagnostic cohort were unable to have ROS1 IHC testing due to limited material available. Both cases were *ROS1* FISH negative.

Within the retrospective cohort a single case (0.4%) showed strong diffuse staining by ROS1 IHC, and this case was confirmed rearrangement positive by *ROS1* FISH. This case was a poorly differentiated adenocarcinoma in a 66 year old female, which showed a growth pattern containing intracytoplasmic mucin. All other cases were *ROS1* rearrangement negative by FISH. ROS1 IHC staining was present in 32% of cases (88/278), however most of these cases showed weak immunoreactivity (1+).

Nineteen cases (Table 1) in the prospective cohort showed ROS1 IHC staining. Twelve cases (12%) were confirmed as *ROS1* rearranged (Table 1). These cases all showed 3+ immunoreactivity with H scores 270–300. The remaining seven IHC positive cases were *ROS1* FISH negative. All of these IHC positive-FISH negative cases showed 3+ immunoreactivity except for one which was 2+. H scores ranged from 40–300.

Four *ROS1* rearranged cases showed a predominantly isolated 3′ green locus (5′ locus deletion) signal pattern (Table 2; One example is presented in Figure 1-A). A ROS1 IHC positive case (adenosquamous carcinoma in a 56yr old female, unknown smoking status, *EGFR* wildtype) that was confirmed rearranged with *ROS1* FISH, showed a signal pattern consisting predominantly of a single set of split signals per cell, with loss of the accompanying set of fusion signals (Table 2; Figure 1-B). The other *ROS1* rearranged case showed a predominantly classical split signal pattern (Table 2).

One of the ROS1 IHC positive - FISH negative cases (adenocarcinoma from a 62yr old female, non-smoker, *EGFR* wildtype) showed an atypical *ROS1* FISH signal pattern of 26% isolated 5′ (red) signals (Table 2; Figure 1-C). Additionally, another strongly ROS1 IHC positive case (adenocarcinoma from a 62yr old male, non-smoker, *EGFR* wildtype) was *ROS1* FISH negative (Table 2; Figure 1-D).

All *ROS1* positive cases were adenocarcinomas except for one adenosquamous carcinoma.

The sensitivity of ROS1 IHC in the retrospective cohort was 100% and specificity was 76%; positive predictive value (PPV) was 1% and negative predictive value (NPV) was 100%. If only 3+ IHC staining was classified as positive, the sensitivity and specificity of ROS1 IHC in the retrospective cohort was 100% with positive predictive value (PPV) and negative predictive value (NPV) also at 100%.

The combined thirteen *ROS1* rearranged cases were all *EGFR* wildtype (except for one case with unknown *EGFR* mutation status in the retrospective cohort) and were all *ALK* rearrangement negative. Two cases were non-smokers and the remaining ten cases had unknown smoking history.

Discussion

Compared with rearrangements involving *ALK* which are reported to occur in around 3% of patients with NSCLC[46], *ROS1* rearrangements have been reported to occur at slightly lower percentages (1%)[46,47]. This was somewhat validated in our retrospective cohort, which showed 0.4% of 278 cases with *ROS1* rearrangement. As the prospective cohort of 104 cases was enriched with patients that were previously confirmed to be *EGFR* and *ALK* wildtype, the rate of *ROS1* rearrangements was higher (12%). Despite the lower rate of *ROS1* rearrangement, compared with that of *ALK*, the rate of response to targeted therapy appears slightly higher with a longer median duration of response and progression-free survival[19], adding a valuable incentive to screen for *ROS1* rearrangements.

Presumably due to the high sensitivity of ROS1 IHC, our retrospective and prospective cohorts showed a notable level of ROS1 immunoreactivity in cases that were negative for a *ROS1* rearrangement by FISH. Although theoretically break apart FISH can detect all rearrangements involving the common breakpoint region, it has been suggested that it may be difficult to identify *ROS1* fusions involving intrachromosomal rearrangements on the same chromosome, such as *ROS1-EZR* by breakapart FISH [16] due to close location of the split signals. *ROS1* rearrangements involving *GOPC* (formally known as *FIG*) would not be detected by some break apart FISH assays as the 5′ probe overlaps or includes *GOPC*, which is only 134kb upstream [13][17]. The ZytoVision *ROS1* FISH probe is able to detect *GOPC-ROS1* fusions, however as the Abbott Molecular *ROS1* FISH probe is designed with the 5′ telomeric probe covering both *ROS1* and *GOPC* genes, it is thought that the FISH probe cannot detect *GOPC-ROS1* fusions. Fusions with *GOPC* however, are thought to make up only 3% of *ROS1* fusion partners in NSCLC[47], although further data is required to characterise the frequency of this fusion partner with certainty.

High ROS1 IHC expression without *ROS1* FISH positivity may be caused by a number of factors. A fusion gene not revealed by FISH, such as a *GOPC-ROS1* fusion, could be responsible for a subset of these cases – however the ZytoVision FISH probe which can detect this fusion, was used to confirm FISH results in all but 3 of these cases, where further tissue was not available for assessment. Alternative cryptic *ROS1* rearrangements could also explain why some cases show high protein ROS1 expression with no detected *ROS1* rearrangement by FISH. Alternative methods such as RT-PCR and sequencing[20,26,31,43] have revealed *ROS1* rearrangements in similar cases, and would be of great benefit when feasible diagnostically. In addition, activation of the *ROS1* oncogene could take place independent of structural DNA aberrations at the *ROS1* locus, and be epigenetically driven, such as by alternative transcript initiation - which has been documented for *ALK*[48]. Other mechanisms of RNA and protein conformational activation of ROS1 could also theoretically contribute, but have not yet manifested in studies so far.

During the early stage of this study, limited commercial options for *ROS1* FISH probes were available, therefore we utilised the ZytoVision *ROS1* break apart FISH probe before the Abbott Molecular *ROS1* FISH probe was also available. Many studies have used either the Abbott Molecular *ROS1* FISH probe set, or the ZytoVision probe set [23,26,27,43,44], including the EUROS1 cohort conducted in six European countries[45].

Interestingly, Warth et al[23] described in 1478 cases, ROS1 IHC positivity but not *ROS1* rearrangement, was associated with prolonged overall survival. This suggests that ROS1 immunoreactivity may have more clinical significance than previously understood, however, its association with response to ROS1 inhibitors has not been investigated.

No false negative ROS1 IHC cases were observed in the retrospective cohort. The only *ROS1* rearranged case in the retrospective cohort expressed ROS1 with 3+ intensity IHC, and similarly in the prospective cohort, all *ROS1* rearranged cases expressed ROS1 with 3+ IHC intensity.

In our study ROS1 IHC identified all *ROS1* rearranged tumours, as all *ROS1* FISH-positive cases in our cohorts were immunoreactive. Our data suggests that IHC for ROS1 is a highly sensitive method to screen for *ROS1* rearrangements with a very high negative predictive value but not as specific. As such, we recommend IHC screening, followed by FISH confirmation. While our results suggest FISH could be undertaken only on those cases with diffuse strong intensity IHC staining (3+ staining in at least 90% of tumour cells, H score ≥ 270), assessment of intensity is subjective and other studies have shown occasional FISH+ cases may be missed with such an approach[24,26] so in our centre we have adopted an approach of FISH testing cases with any ROS1 IHC positivity. A similar approach has been shown to be effective in identifying *ALK* rearranged lung cancer[32].

Compared with performing FISH as a routine screening method for *ROS1* rearrangements in all *EGFR/ALK*-negative NSCLC cases, ROS1 IHC is preferable, due to the reduced cost and reduced labour involved in interpretation. Confirmatory FISH could be reserved for cases showing diffuse and moderately strong ROS1 IHC staining in centres where FISH testing is not readily available.

In conclusion, potentially targetable *ROS1* gene rearrangements occur in a very small percentage of lung adenocarcinomas and are largely mutually exclusive with *EGFR* mutations and *ALK* rearrangements. Screening with IHC is highly sensitive, but not as specific, and may be a suitable method of reducing the number of cases requiring FISH to identify the *ROS1* genetic abnormality. Selection of cases for *ROS1* FISH testing such as exclusion of *EGFR/ALK* positive cases and use of ROS1 IHC to screen for potentially positive cases can be used to enrich for the likelihood of a identifying a *ROS1* rearranged lung cancer and prevent the need to undertake expensive and time consuming FISH testing in all cases.

Acknowledgments

Clinical Professor Sandra O'Toole received funding from the CINSW, the Sydney Breast Cancer Foundation and the NHMRC. This research was also supported by generous donations from the Tag family foundation, Mr David

Paradice, ICAP and the O'Sullivan family. Bob Li received funding from the NIH/NCI Cancer Center Support Grant P30 CA008748.

CIS performed the research and wrote the paper. BTL, NP, ML, AG, AL, SC and LH provided data and assisted with the manuscript. TNT assisted with cohort construction and assisted with the manuscript. TL performed the IHC and assisted with the manuscript. PYY assisted with cohort construction, provided data and assisted with the manuscript. BY assisted with mutation testing analysis and assisted with the manuscript. MRJKC assisted with cohort construction and assisted with the manuscript. SOT & WC analysed and interpreted the results, designed the study and assisted with the manuscript.

References

1. Ou S-HI, Tan J, Yen Y, Soo RA. ROS1 as a "druggable" receptor tyrosine kinase: lessons learned from inhibiting the ALK pathway. Expert Rev Anticancer Ther. 2012 Apr; 12(4):447–56. [PubMed: 22500682]
2. Shibuya M, Hanafusa H, Balduzzi PC. Cellular sequences related to three new onc genes of avian sarcoma virus (fps, yes, and ros) and their expression in normal and transformed cells. J Virol. 1982 Apr; 42(1):143–52. [PubMed: 6177868]
3. Wang LH, Hanafusa H, Notter MF, Balduzzi PC. Genetic structure and transforming sequence of avian sarcoma virus UR2. J Virol. 1982 Mar; 41(3):833–41. [PubMed: 6284974]
4. Balduzzi PC, Notter MF, Morgan HR, Shibuya M. Some biological properties of two new avian sarcoma viruses. J Virol. 1981 Oct; 40(1):268–75. [PubMed: 6270379]
5. Uttamsingh S, Zong CS, Wang L-H. Matrix-independent activation of phosphatidylinositol 3-kinase, Stat3, and cyclin A-associated Cdk2 Is essential for anchorage-independent growth of v-Ros-transformed chicken embryo fibroblasts. J Biol Chem. 2003 May 23; 278(21):18798–810. [PubMed: 12646574]
6. Charest A, Wilker EW, McLaughlin ME, et al. ROS fusion tyrosine kinase activates a SH2 domain-containing phosphatase-2/phosphatidylinositol 3-kinase/mammalian target of rapamycin signaling axis to form glioblastoma in mice. Cancer Res. 2006 Aug 1; 66(15):7473–81. [PubMed: 16885344]
7. Nguyen KT, Zong CS, Uttamsingh S, et al. The role of phosphatidylinositol 3-kinase, rho family GTPases, and STAT3 in Ros-induced cell transformation. J Biol Chem. 2002 Mar 29; 277(13): 11107–15. [PubMed: 11799110]
8. Takeuchi K, Soda M, Togashi Y, et al. RET, ROS1 and ALK fusions in lung cancer. Nat Med. 2012 Mar; 18(3):378–81. [PubMed: 22327623]
9. Birchmeier C, Sharma S, Wigler M. Expression and rearrangement of the ROS1 gene in human glioblastoma cells. Proc Natl Acad Sci U S A. 1987 Dec; 84(24):9270–4. [PubMed: 2827175]
10. Liu P, Wu Y, Sun L, Zuo Q, Shi M. ROS kinase fusions are not common in Chinese patients with cholangiocarcinoma. Nan Fang Yi Ke Da Xue Xue Bao. 2013 Apr; 33(4):474–8. [PubMed: 23644102]
11. Gu T-L, Deng X, Huang F, et al. Survey of tyrosine kinase signaling reveals ROS kinase fusions in human cholangiocarcinoma. PloS One. 2011; 6(1):e15640. [PubMed: 21253578]
12. Peraldo Neia C, Cavalloni G, Balsamo A, et al. Screening for the FIG-ROS1 fusion in biliary tract carcinomas by nested PCR. Genes Chromosomes Cancer. 2014 Dec; 53(12):1033–40. [PubMed: 25231053]
13. Birch AH, Arcand SL, Oros KK, et al. Chromosome 3 anomalies investigated by genome wide SNP analysis of benign, low malignant potential and low grade ovarian serous tumours. PloS One. 2011; 6(12):e28250. [PubMed: 22163003]
14. Lee J, Lee SE, Kang SY, et al. Identification of ROS1 rearrangement in gastric adenocarcinoma. Cancer. 2013 May 1; 119(9):1627–35. [PubMed: 23400546]
15. Aisner DL, Nguyen TT, Paskulin DD, et al. ROS1 and ALK fusions in colorectal cancer, with evidence of intratumoral heterogeneity for molecular drivers. Mol Cancer Res MCR. 2014 Jan; 12(1):111–8. [PubMed: 24296758]
16. Davies KD, Le AT, Theodoro MF, et al. Identifying and targeting ROS1 gene fusions in non-small cell lung cancer. Clin Cancer Res Off J Am Assoc Cancer Res. 2012 Sep 1; 18(17):4570–9.

17. Bergethon K, Shaw AT, Ou S-HI, et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol Off J Am Soc Clin Oncol. 2012 Mar 10; 30(8):863–70.
18. Tanizaki J, Okamoto I, Okamoto K, et al. MET tyrosine kinase inhibitor crizotinib (PF-02341066) shows differential antitumor effects in non-small cell lung cancer according to MET alterations. J Thorac Oncol Off Publ Int Assoc Study Lung Cancer. 2011 Oct; 6(10):1624–31.
19. Shaw AT, Ou S-HI, Bang Y-J, et al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. N Engl J Med. 2014 Nov 20; 371(21):1963–71. [PubMed: 25264305]
20. Suehara Y, Arcila M, Wang L, et al. Identification of KIF5B-RET and GOPC-ROS1 fusions in lung adenocarcinomas through a comprehensive mRNA-based screen for tyrosine kinase fusions. Clin Cancer Res Off J Am Assoc Cancer Res. 2012 Dec 15; 18(24):6599–608.
21. Rimkunas VM, Crosby KE, Li D, et al. Analysis of receptor tyrosine kinase ROS1-positive tumors in non-small cell lung cancer: identification of a FIG-ROS1 fusion. Clin Cancer Res Off J Am Assoc Cancer Res. 2012 Aug 15; 18(16):4449–57.
22. Rikova K, Guo A, Zeng Q, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell. 2007 Dec 14; 131(6):1190–203. [PubMed: 18083107]
23. Warth A, Muley T, Dienemann H, et al. ROS1 expression and translocations in non-small-cell lung cancer: clinicopathological analysis of 1478 cases. Histopathology. 2014 Aug; 65(2):187–94. [PubMed: 24456475]
24. Yoshida A, Tsuta K, Wakai S, et al. Immunohistochemical detection of ROS1 is useful for identifying ROS1 rearrangements in lung cancers. Mod Pathol Off J U S Can Acad Pathol Inc. 2014 May; 27(5):711–20.
25. Sholl LM, Sun H, Butaney M, et al. ROS1 immunohistochemistry for detection of ROS1-rearranged lung adenocarcinomas. Am J Surg Pathol. 2013 Sep; 37(9):1441–9. [PubMed: 23887156]
26. Shan L, Lian F, Guo L, et al. Detection of ROS1 gene rearrangement in lung adenocarcinoma: comparison of IHC, FISH and real-time RT-PCR. PloS One. 2015; 10(3):e0120422. [PubMed: 25742289]
27. Mescam-Mancini L, Lantuéjoul S, Moro-Sibilot D, et al. On the relevance of a testing algorithm for the detection of ROS1-rearranged lung adenocarcinomas. Lung Cancer Amst Neth. 2014 Feb; 83(2):168–73.
28. Cha YJ, Lee JS, Kim HR, et al. Screening of ROS1 rearrangements in lung adenocarcinoma by immunohistochemistry and comparison with ALK rearrangements. PloS One. 2014; 9(7):e103333. [PubMed: 25058391]
29. Cao B, Wei P, Liu Z, et al. Detection of lung adenocarcinoma with ROS1 rearrangement by IHC, FISH, and RT-PCR and analysis of its clinicopathologic features. OncoTargets Ther. 2016; 9:131–8.
30. Boyle TA, Masago K, Ellison KE, Yatabe Y, Hirsch FR. ROS1 Immunohistochemistry Among Major Genotypes of Non-Small-Cell Lung Cancer. Clin Lung Cancer. 2014 Oct 24.
31. Lee SE, Lee B, Hong M, et al. Comprehensive analysis of RET and ROS1 rearrangement in lung adenocarcinoma. Mod Pathol Off J U S Can Acad Pathol Inc. 2015 Apr; 28(4):468–79.
32. Selinger CI, Rogers T-M, Russell PA, et al. Testing for ALK rearrangement in lung adenocarcinoma: a multicenter comparison of immunohistochemistry and fluorescent in situ hybridization. Mod Pathol Off J U S Can Acad Pathol Inc. 2013 Dec; 26(12):1545–53.
33. Boland JM, Erdogan S, Vasmatzis G, et al. Anaplastic lymphoma kinase immunoreactivity correlates with ALK gene rearrangement and transcriptional up-regulation in non-small cell lung carcinomas. Hum Pathol. 2009 Aug; 40(8):1152–8. [PubMed: 19386350]
34. Camidge DR, Hirsch FR, Varella-Garcia M, Franklin WA. Finding ALK-positive lung cancer: what are we really looking for? J Thorac Oncol Off Publ Int Assoc Study Lung Cancer. 2011 Mar; 6(3): 411–3.
35. Houang M, Toon CW, Clarkson A, et al. Reflex ALK immunohistochemistry is feasible and highly specific for ALK gene rearrangements in lung cancer. Pathology (Phila). 2014 Aug; 46(5):383–8.
36. Mino-Kenudson M, Chirieac LR, Law K, et al. A novel, highly sensitive antibody allows for the routine detection of ALK-rearranged lung adenocarcinomas by standard immunohistochemistry. Clin Cancer Res Off J Am Assoc Cancer Res. 2010 Mar 1; 16(5):1561–71.

37. Takeuchi K, Choi YL, Togashi Y, et al. KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. Clin Cancer Res Off J Am Assoc Cancer Res. 2009 May 1; 15(9):3143–9.

38. Yip PY, Yu B, Cooper WA, et al. Patterns of DNA mutations and ALK rearrangement in resected node negative lung adenocarcinoma. J Thorac Oncol Off Publ Int Assoc Study Lung Cancer. 2013 Apr; 8(4):408–14.

39. Selinger CI, Cooper WA, Al-Sohaily S, et al. Loss of special AT-rich binding protein 1 expression is a marker of poor survival in lung cancer. J Thorac Oncol Off Publ Int Assoc Study Lung Cancer. 2011 Jul; 6(7):1179–89.

40. Cooper WA, Kohonen-Corish MRJ, Chan C, et al. Prognostic significance of DNA repair proteins MLH1, MSH2 and MGMT expression in non-small-cell lung cancer and precursor lesions. Histopathology. 2008 Apr; 52(5):613–22. [PubMed: 18370958]

41. Tran TN, Selinger CI, Yu B, et al. Alterations of insulin-like growth factor-1 receptor gene copy number and protein expression are common in non-small cell lung cancer. J Clin Pathol. 2014 Nov; 67(11):985–91. [PubMed: 25118293]

42. Tran TN, Selinger CI, Kohonen-Corish MRJ, et al. Alterations of MET Gene Copy Number and Protein Expression in Primary Non-Small-Cell Lung Cancer and Corresponding Nodal Metastases. Clin Lung Cancer. 2015 Aug 18.

43. Lira ME, Choi Y-L, Lim SM, et al. A single-tube multiplexed assay for detecting ALK, ROS1, and RET fusions in lung cancer. J Mol Diagn JMD. 2014 Mar; 16(2):229–43. [PubMed: 24418728]

44. Scheffler M, Schultheis A, Teixido C, et al. ROS1 rearrangements in lung adenocarcinoma: prognostic impact, therapeutic options and genetic variability. Oncotarget. 2015 Apr 30; 6(12): 10577–85. [PubMed: 25868855]

45. Mazières J, Zalcman G, Crinò L, et al. Crizotinib therapy for advanced lung adenocarcinoma and a ROS1 rearrangement: results from the EUROS1 cohort. J Clin Oncol Off J Am Soc Clin Oncol. 2015 Mar 20; 33(9):992–9.

46. Rosell R, Karachaliou N, Wolf J, Ou S-HI. ALK and ROS1 non-small-cell lung cancer: two molecular subgroups sensitive to targeted therapy. Lancet Respir Med. 2014 Dec; 2(12):966–8. [PubMed: 25466349]

47. Gainor JF, Shaw AT. Novel targets in non-small cell lung cancer: ROS1 and RET fusions. The Oncologist. 2013; 18(7):865–75. [PubMed: 23814043]

48. Wiesner T, Lee W, Obenauf AC, et al. Alternative transcription initiation leads to expression of a novel ALK isoform in cancer. Nature. 2015 Oct 15; 526(7573):453–7. [PubMed: 26444240]

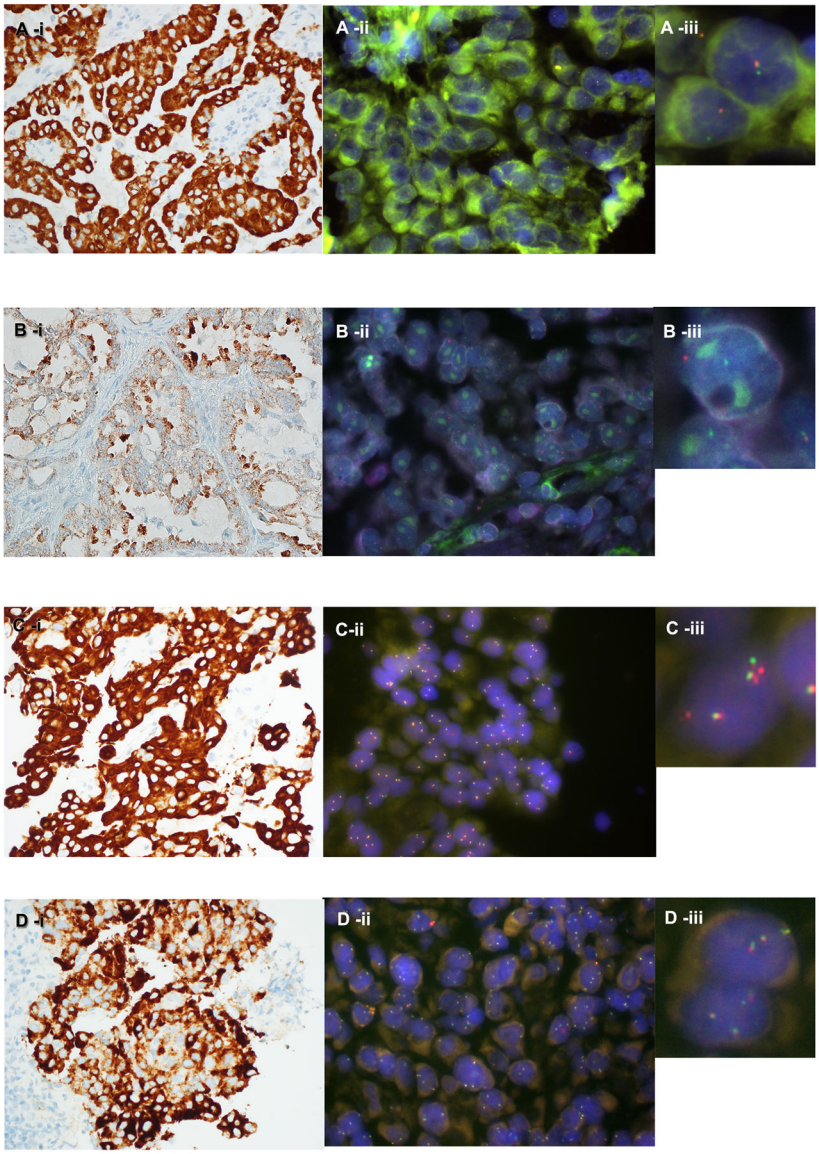

Figure 1.

Examples of ROS1 expression and *ROS1* rearrangement patterns observed: A-i) Strong ROS1 IHC staining, with *ROS1* FISH A-ii) and A-iii) showing a predominantly positive isolated green signal pattern. B-i) Moderate-strong ROS1 IHC staining with *ROS1* FISH B-ii) & B-iii) showing predominantly a single set of split signals. C-i) Strong ROS1 IHC staining, C-ii) & *ROS1* FISH C-iii) showing a negative signal pattern of subtle isolated red signals. D-i) Strong ROS1 IHC staining, however *ROS1* FISH D-ii) & D-iii) showed less than 15% of tumour nuclei with *ROS1* rearrangement.

Table 1

Clinicopathological characteristics of all cohorts

	Retrospective cohort (n=278)			Prospective cohort (n=104)		
***ROS1* FISH**	**All cases (278)**	***ROS1* − (277)**	***ROS1* + (1)**	**All cases (104)**	***ROS1* − (92)**	***ROS1* + (12)**
Age (years)						
Median (range)	69 (40–87)	69 (40–87)	66	62 (34–85)	64 (34–85)	59 (34–81)
Sex						
Male	167 (60%)	167 (60%)	0 (0%)	42 (40%)	39 (42%)	3 (25%)
Female	111 (40%)	110 (40%)	1 (100%)	62 (60%)	53 (58%)	9 (75%)
Histology						
Adenocarcinoma	277 (99.6%)	276 (99.7%)	1 (100%)	98 (94%)	87 (95%)	11 (92%)
Squamous cell carcinoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Large cell carcinoma	0 (0%)	0 (0%)	0 (0%)	3 (3%)	3 (3%)	0 (0%)
Adenosquamous	1 (0.4%)	1 (0.3%)	0 (0%)	3 (3%)	2 (2%)	1 (8%)
Smoking history						
Never	11 (4%)	10 (3.6%)	1 (100%)	29 (28%)	27 (29.4%)	2 (17%)
Ever	74 (26.6%)	74 (26.7%)	0 (0%)	14 (13%)	14 (15.2%)	0 (0%)
Unknown	193 (69.4%)	193 (69.7%)	0 (0%)	61 (59%)	51 (55.4%)	10 (83%)
Mutation status						
EGFR wildtype	99 (36%)	98 (35.4%)	0 (0%)	69 (66%)	57 (62%)	12 (100%)
EGFR mutant	29 (10%)	29 (10.5%)	0 (0%)	15 (14%)	15 (16%)	0 (0%)
Other mutation[^]	78 (28%)	78 (28.1%)	0 (0%)	9 (9%)	9 (10%)	0 (0%)
Unknown	72 (26%)	72 (26%)	1 (100%)	11 (11%)	11 (12%)	0 (0%)
ROS1 IHC						
Positive	89 (32%)	88 (32%)	1 (100%)	19 (18%)	7 (8%)	12 (100%)
Negative	189 (68%)	189 (68%)	0 (0%)	83 (80%)	83 (90%)	0 (0%)
Unable to be tested	0 (0%)	0 (0%)	0 (0%)	2 (2%)	2 (2%)	0 (0%)

[^] *KRAS, PIK3CA, KIT, MET, FGFR1, HRAS, BRAF* mutation or *ALK* rearrangement.

Table 2

ROS1 IHC positive cases

Case #	*KRAS*/*EGFR*/*ALK* status	ROS1 IHC - % cells positive	ROS1 IHC - Intensity	ROS1 IHC - H score (% x intensity)	*ROS1* FISH
Retrospective Cohort					
1	*KRAS & EGFR* unknown/*ALK*−	90%	3+	244	+ (single set of split signals)
2–81	23 *KRAS+*, 13 *EGFR+*, 4 *PIK3CA+*, 2 *MET+*, 1 *ALK +*, 1 *BRAF+*, 1 *FGFR1+*, 1 *HRAS+*, 6 unknown	5–80% (mean =24)	1+	5–80 (mean=24)	-
82–90	*3 KRAS+*, 5 *EGFR+*, 0 unknown	20–78 (mean 40)	2+	40–120 (mean 79)	-
Prospective cohort					
1	All Negative	100%	3+	300	+ (classic split signal pattern)
2	All Negative	100%	3+	300	+ (classic split signal pattern)
3	All Negative	100%	3+	300	+ (single set of split signals)
4	*KRAS* unknown *EGFR*−/*ALK*−	100%	3+	300	+ (classic split signal pattern)
5	*KRAS* unknown *EGFR*−/*ALK*−	100%	3+	300	+ (isolated 3′ green signal pattern)
6	All Negative	100%	3+	300	+ (classic split signal pattern)
7	All Negative	90%	3+	270	+ (isolated 3′ green signal pattern)
8	All Negative	100%	3+	300	+ (classic split signal pattern)
9	All Negative	100%	3+	300	+ (classic split signal pattern)
10	All Negative	90%	3+	270	+(isolated 3′ green signal pattern)
11	All Negative	100%	3+	300	+ (classic split signal pattern)
12	All Negative	100%	3+	300	+ (isolated 3′ green signal pattern)
13	*KRAS* unknown *EGFR*−/*ALK*−	100%	3+	300	− (isolated 5′ red signal pattern)
14	All Negative	25%	3+	75	-
15	All Negative	80%	3+	280	-
16	*EGFR+/KRAS−/ALK−*	33%	3+	99	-
17	*EGFR+/KRAS−/ALK−*	20%	2+	40	-
18	*KRAS* unknown *EGFR*−/*ALK*−	30%	3+	90	-

Case #	*KRAS*/*EGFR*/*ALK* status	ROS1 IHC - % cells positive	ROS1 IHC - Intensity	ROS1 IHC - H score (% x intensity)	*ROS1* FISH
19	*KRAS* unknown *EGFR*−/*ALK*−	20%	3+	60	-