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EDITORIAL

Australian consensus statement for best practice *ROS1* testing in advanced non-small cell lung cancer

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Summary

Lung cancer is the most commonly diagnosed malignancy and the leading cause of death from cancer globally. Diagnosis of advanced non-small cell lung cancer (NSCLC) is associated with 5-year relative survival of 3.2%. *ROS proto-oncogene 1* (*ROS1*) is an oncogenic driver of NSCLC occurring in up to 2% of cases and commonly associated with younger age and a history of never or light smoking. Results of an early trial with the tyrosine kinase inhibitor (TKI) crizotinib that inhibits tumours that harbour *ROS1* rearrangements have shown an objective response rate (ORR) of 72% (95% CI 58–83%), median progression free survival (PFS) of 19.3 months (95% CI 15.2–39.1 months) and median overall survival (OS) of 51.4 months (95% CI 29.3 months to not reached). Therefore, with the availability of highly effective ROS1-targeted TKI therapy, upfront molecular testing for *ROS1* status alongside *EGFR* and *ALK* testing is recommended for all patients with NSCLC. We review the tissue requirements for *ROS1* testing by immunohistochemistry (IHC) and fluorescent *in situ* hybridisation (FISH) and we present a testing algorithm for advanced NSCLC and consider how the future of pathology testing for *ROS1* may evolve.

Key words: Non-small cell lung cancer; consensus; ROS1; biomarker testing.

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INTRODUCTION

Lung cancer is the most commonly diagnosed malignancy and the leading cause of death from cancer globally.[1] Non-squamous non-small cell lung cancer (NSCLC) accounts for the majority of lung cancer cases.[1] In Australia, 53% of NSCLC diagnoses occur at stage IV, with 5-year relative survival of 10%.[2] Oncogenic drivers of non-squamous NSCLC are mutually exclusive and include activating mutations in *epidermal growth factor receptor (EGFR)*, *anaplastic lymphoma kinase (ALK)*, *hepatocyte growth factor mesenchymal-epithelial transition factor (MET) receptor*, *Kirsten rat sarcoma (KRAS)*, *human epidermal growth factor receptor 2 (HER2)*, *rearranged during transfection (RET)*, *V-raf murine sarcoma viral oncogene homolog B (BRAF)* and *ROS proto-oncogene 1 (ROS1)*, many of which are targets for therapeutic approaches.[3]

The *ROS1* gene, located on the long arm of chromosome 6 (6q22.1), encodes a receptor tyrosine kinase (RTK) containing a large N-terminal extracellular domain, a hydrophobic single-pass transmembrane region and a C-terminal intracellular tyrosine kinase domain.[4] The tyrosine kinase domain of *ROS1* undergoes oncogenic fusion with multiple partner genes on other chromosomes with at least 27 currently identified.[5] Rearranged *ROS1* was first identified in human glioblastoma cell lines and has since been identified in a range of malignancies including NSCLC.[4] *ROS1* rearrangements have been reported in between 0.9% and 2% of human NSCLC cases and are associated with younger age, and a history of never or light smoking.[6] Although the exact mechanism of *ROS1* kinase on driving neoplasia has not yet been elucidated, transgenic mice expressing an *ezrin* (EZR)-*ROS1* in the lung alveolar epithelium developed multiple adenocarcinoma nodules in both lungs at an early age.[7]

ROS1 is structurally related to the ALK and insulin RTKs. Consequently, some ALK tyrosine kinase inhibitors (TKIs) show activity against both the ALK and ROS1 kinases.[4] Indeed, there are currently approved targeted therapies against *ALK* and *ROS1* gene rearranged NSCLC.[8–12]

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Eligibility for treatment with targeted therapies requires positive confirmation of the gene rearrangement in tumour tissue. Therefore, testing for specific tumour mutations or gene rearrangements is critical to ensure that patients are treated with the most appropriate therapy.

Guidelines for biomarker testing including *ROS1* have been developed by the National Comprehensive Cancer Network (NCCN),[13] the European Society for Medical Oncology (ESMO),[14] the American Society of Clinical Oncology (ASCO),[15] and the College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP).[16] All guidelines recommend *ROS1* testing for all patients with advanced non-squamous NSCLC irrespective of clinical characteristics. The guidelines also agree that initial screening should be performed using immunohistochemistry (IHC) with confirmation using fluorescent *in situ* hybridisation (FISH) or cytogenetic methods. The guidelines also recommend that *ROS1* testing should be conducted upfront along with *EGFR* and *ALK* testing. Patients who have a positive *ROS1* test are recommended for treatment with an oral TKI targeting *ROS1*.

Crizotinib was the first TKI to demonstrate activity in patients with *ROS1* rearranged NSCLC and recently was reimbursed for this indication by the Pharmaceutical Benefits Scheme (PBS).[8,17] Other agents have since then shown promising activity, including ceritinib and more recently entrectinib, lorlatinib and repotrectinib.

The aim of this guideline is to describe a best practice approach to lung pathology testing in Australia focusing on *ROS1* testing, in order to enable patients to get prompt access to targeted therapy in a timely fashion.

TRIAL EVIDENCE SUPPORTING THE USE OF SMALL MOLECULE TYROSINE KINASE INHIBITORS IN *ROS1*-POSITIVE ADVANCED NON-SMALL CELL LUNG CANCER

The treatment paradigm in support of TKI therapy as best practice for patients with *ROS1*-positive advanced NSCLC has been established in the absence of any randomised phase III trial in view of compelling single arm trial results. A randomised study is unlikely to be conducted due to the rare nature of the disease. A summary of the clinical trial data for TKI therapy in *ROS1* rearranged NSCLC is provide in Table 1.

TISSUE REQUIREMENTS FOR *ROS1* TESTING

The diagnosis of NSCLC may be made on a variety of specimens including resections, endobronchial biopsies, core biopsies and fine needle aspirates from primary or metastatic disease. Any sample containing adequate numbers of tumour cells may be utilised for *ROS1* testing.[16] Cell blocks or other cytological preparations may be used.[16]

Tissue biopsies

As with other biomarker testing, pre-analytic factors should be standardised as much as possible in order to ensure consistent quality of *ROS1* testing. The time between specimen removal from the body and preservation, total fixation time, types of fixative, specimen size, and decalcification can all affect DNA analysis. Delay to preservation can result in reduced IHC and FISH signals, although may not affect polymerase chain reaction (PCR) results; less than 1 hour is the recommended cold ischaemia time[18] although this is frequently beyond the control of the pathology laboratory.

Fixation of tissue samples in 10% neutral buffered formalin is recommended for histological specimens and is routine in the majority of Australian laboratories.[18] The period of fixation should be greater than 6 hours but should not exceed 24 hours, so that antigenicity and DNA integrity are maintained.[3]

Acid decalcification on bone samples frequently results in failure of IHC, FISH and other mutation testing so should be avoided if possible. Manual dissection of soft tissue components of the biopsy specimens and submission in a separate block, thus obviating the need for decalcification, should be performed if appropriate.[19] In contrast, decalcification using ethylenediaminetetraacetic acid (EDTA) reduces background staining, allows for retention of DNA integrity, and results in stronger FISH signals, as compared with acid based methods.[18]

Cytology specimens

A large number of pre-analytical factors can affect the DNA yield and quality of cytological samples. These include fixative, collection media, staining, mounting media, tissue retrieval method, and type of slides as well as sample preparation methods.[20]

Table 1 Summary of clinical trials of tyrosine kinase inhibitor therapy in ROS1-positive advanced non-small cell lung cancer

First author (study)	Publication year	Phase	N	Experimental agent	ORR % (95% CI)	Median PFS months (95% CI)	Median OS months (95% CI)
Shaw (PROFILE1001)[48]	2019	I	53	Crizotinib	72 (58–83)	19.3 (15.2–39.1)	51.4 (29.3–NR)
Wu[49]	2018	II	127	Crizotinib	71.7 (63.0–79.3)	15.9 (12.9–24.0)	32.5 (32.5–NR)
Lim[50]	2017	II	32	Ceritinib	62 (45–77)	9.3 (0–22)	24 (5–43)
Shaw[51]	2017	I	12	Lorlatinib	50 (21–79)	Not reported	Not reported
Doebele (STARTRK-1, STARTRK-2 and ALKA-372-001)[52]	2018	I/II	53	Entrectinib	77.4 (63.8–87.7)	19.0 (12.2–36.6)	NE (NE–NE)
Ou (TRIDENT-1)[53]	2018	I	30	Repotrectinib	80[a] (not reported) 18[b] (not reported)	Not reported	Not reported

CI, confidence interval; NE, not evaluated; NR, not reached; ORR, objective response rate; OS, overall survival; PFS, progression free survival.
[a] TKI-naïve patients.
[b] TKI pretreated patients.

Traditionally cell block preparations from cytopathology specimens have been used for ancillary testing, using the same IHC protocols as those for formalin-fixed, paraffin-embedded (FFPE) tissue specimens.[3] Rapid on-site evaluation (ROSE) is useful to ensure adequate material is collected and triaged from fine needle aspiration (FNA) procedures.[19,21] However, direct smear cellularity may not be indicative of cellularity of the cell block, and ROSE is not available in all settings.[22]

Cell blocks are often inadequate due to paucity of tumour cells, with up to 57% reported as acellular or of borderline cellularity,[23] resulting in a repeat procedure in order to obtain more material. In addition, standard 4–5 μm sections of the cell block do not represent the entirety of the nuclear diameter and are likely to have lower nucleic acid yields than other cytology preparations.[20]

Other approaches to use of cytology specimens for molecular testing include use of smeared slides, touch imprints, cytospin preparations and liquid based cytology (LBC).[20,24] Although DNA quality obtained from direct smears, cytospin preparations and LBC is of high quality, as there is no formalin fixation, validation of each individual assay for each preparation is required and diagnostic material is sacrificed, making use of these specimens less appealing to laboratories.[20] The latter issue may become less significant in the future with increasing availability and use of digital slide scanning technology that can provide a permanent diagnostic archive of cytological material. To date, studies evaluating *ROS1* in cytology smears are few. Vlajnic *et al.* prospectively analysed 295 cytology specimens, where the authors reported that ROS1 IHC and FISH was reliable in alcohol fixed smears;[25] Bozzetti *et al.* reported on *ROS1* FISH evaluation of direct smears of 12 cases of primary or metastatic lung adenocarcinoma.[26]

Tumour requirements

Most publications use 4 μm thick FFPE tissue sections for both IHC and FISH.[27] Minimum tumour requirements have not been well established for ROS1 IHC with some authors suggesting a minimum of 20 tumour cells.[27] Other authors suggest only a few tumour cells may be sufficient if they show positive staining;[28] however, in specimens of minimal cellularity, absent staining should be interpreted with caution due to the possibility of patchy staining producing a false negative result.[29] At least 50 non-overlapping, intact tumour cells are required for FISH,[5] and other DNA/RNA based molecular techniques have minimum tumour DNA/RNA requirements that will be defined by the method.

Maximising tissue for ancillary testing in NSCLC

The overriding emphasis in NSCLC is establishing diagnosis and preserving adequate material for ancillary testing to identify potential therapeutic targets. Laboratory processes should ensure that material is appropriately handled to provide the treating clinician with this information. This is often challenging in the era of minimally invasive tissue sampling with acquisition of small volume samples.

Optimal communication between the treating clinician, procedural clinician (e.g., radiologist, bronchoscopist) and pathologist is paramount, particularly in cases where tissue is obtained predominantly for molecular testing. In these cases, only a single haematoxylin and eosin (H&E) stained slide is necessary to confirm the presence of tumour and would avoid unnecessary repetition of diagnostic IHC.

Standard laboratory processes may result in tissue wastage, in particular when tissue blocks are faced in order to obtain a complete cross section, resulting in discarded tissue at the microtome. This may be amplified when multiple tissue fragments are placed in the same block and embedded at different levels. Requests for IHC can result in further tissue wastage if refacing of the block is required.[19]

Implementation of laboratory protocols for handling specimens obtained for workup of NSCLC, such as routinely dividing the specimen between two or more blocks with one for diagnostic workup including IHC and one for molecular testing, and/or standardised H&E and levels on coated slides cut upfront, are processes that can maximise tissue in small biopsy specimens and reduce turnaround time, as multiple tests can thus be run in parallel. Individual laboratories should develop their own procedures for optimal approach based on their local workflow and testing requirements. Any unused cut sections should be stored under cool, dark and dry conditions. Air oxidisation will cause sample degradation and sections must be used within six months.[3]

THE ROLE OF IMMUNOHISTOCHEMISTRY (IHC) IN *ROS1* TESTING

Because of the cost of molecular techniques and the rarity of *ROS1* rearrangements, IHC has been suggested as a screening test to identify tumours with increased ROS1 protein levels in which FISH testing is more likely to identify a gene rearrangement. Review of recent studies[27,29–33] reveals that ROS1 IHC is an effective method to screen for *ROS1*-rearranged NSCLC due to a reported sensitivity of 100% in most studies and specificity ranging from 92–100%, with variability secondary to the threshold used for positivity. ROS1 protein expression in *ROS1*-rearranged NSCLC shows intercellular variability in the presence of uniform *ROS1* gene rearrangement, and it has been suggested that FISH testing should ideally be performed in all cases with any positive labelling.[33] Therefore, FISH remains the gold-standard for confirmation of *ROS1* rearrangements and currently there are no data suggesting that IHC positivity alone predicts response to ROS1 inhibitors.

To date, the D4D6 rabbit monoclonal antibody (Cell Signaling Technology, USA) is the only ROS1 antibody universally commercially available and has been used in most of the studies that have assessed the performance of IHC as a screening test for *ROS1* FISH.[27,29–33] Of note, this antibody has achieved comparable sensitivities and specificities with a wide range of antigen retrieval and detection methods and with dilutions ranging from 1:50 to 1:1000).[27,33,34] This includes both hand-staining and automated staining on various platforms including the Bond Max Ventana Benchmark (Ventana, USA), and various Dako platforms (Dako, Denmark), which suggests that the D4D6 clone is an antibody with robust performance. However, there is now a recent comparison between two anti-ROS1 IHC clones, the SP384 (Ventana) and D4D6 (Cell Signaling Technology) which utilised a training cohort of 51 FISH-positive cases of advanced lung adenocarcinoma, followed by a validation cohort of 714 consecutive cases of advanced lung adenocarcinoma.[35] Various pathology platforms were used.

Importantly, the SP384 and D4D6 clones demonstrated variable sensitivity and specificity with two different cut-off points ≥1+ (all % tumour cells) and ≥2+ (>30% stained tumour cells). The D4D6 yielded better accuracy in predicting *ROS1* rearrangement by FISH in the validation study. However, a follow-up study demonstrated high sensitivity of the SP384 clone without compromising specificity and suggest this antibody as a useful alternative to the D4D6 clone. The frequent labelling of type 2 pneumocytes with this antibody was found to be useful as an *in situ* positive control.[36]

As different antibodies with variable performance profiles become available, laboratories must be aware of the differences prior to incorporating testing into routine clinical practice. This is especially relevant to Australian pathologists, where the Ventana platform is widely used.

Currently there is no consensus as to the optimisation of the ROS1 antibody, but as for any laboratory-developed test, it is essential to include positive control material. Unfortunately, there is no known benign external tissue control for ROS1. Additionally, because of the rarity of *ROS1* rearrangements in NSCLC, spare tumour tissue with confirmed *ROS1* rearrangements may not be readily available in most laboratories. To overcome the difficulty of obtaining positive control tissue, it has been suggested to use a paraffin-embedded cell block of the *ROS1*-rearranged cell line HCC-78 which expresses ROS1 protein at a high level. However, to confirm assay sensitivity, use of the U-118 MG glioblastoma cell line which expresses ROS1 protein at low levels has also been suggested.[31] The Royal College of Pathologists of Australasia Quality Assurance Program (RCPAQAP) has recently established a pilot program for ROS1 IHC in collaboration with UK NEQAS.

Whilst paraffin-embedded tissue has been used for the studies assessing the utility of IHC as a screening test for FISH, use of cytology cell block material for this purpose is widely accepted in practice.[3] In addition, the use of conventional ethanol-fixed cytology preparations has also been successful, but this approach has not been widely validated.[3] IHC positive cases typically show granular cytoplasmic labelling, but globular and membranous staining patterns are also recognised, and may also be associated with *ROS1* gene rearrangements (Fig. 1).[29,33] Analogous to ALK IHC, assessment of mucinous adenocarcinomas with signet cells is problematic because the cytoplasm of tumour cells in such tumours is largely replaced by mucin, which can lead to false-positive staining but may also make it difficult to detect genuine labelling.[33] Occasionally, weak positive labelling may be observed in hyperplastic type II pneumocytes, alveolar macrophages and, in the case of bone metastases, osteoclast-like giant cells, which should not be regarded as positive.

A variety of scoring systems has been employed, including H scores and intensity of staining (0–3+).[33–35] H scores of at least 100 to 150 had reported sensitivities of 100%,[34] but FISH positivity has been reported in a single case with an H score of 5, although multiplex real time (RT)-PCR on fresh frozen tissue failed to identify a *ROS1* fusion transcript in this case.[33] Therefore, the sensitivity of IHC as a screening test is clearly dependent on the cut-offs used. It is well recognised that diffuse moderate to strong staining is more commonly associated with *ROS1* rearrangement by FISH or PCR, and test specificity can certainly be improved by increasing cut-offs (e.g., using strictly 3+ cut-offs) and H scores of 150 or more, but this reduces the sensitivity of the test. Since scoring of staining intensity is already somewhat subjective and observer-dependent, we suggest that consideration should be given to performing FISH testing on all cases with any degree of ROS1 IHC positivity that lack *EGFR*, *ALK* and *KRAS* (if available) alterations (as *ROS1* alterations are mutually exclusive with these driver mutations). This approach is in line with international recommendations by expert panels.[3,30] However, laboratories should be aware that current Australian reimbursement only covers FISH testing for cases exhibiting 2+ or 3+ positivity by IHC (Fig. 2).

FLUORESCENT *IN SITU* HYBRIDISATION (FISH) TESTING

Interphase FISH assays using break-apart probes designed to flank the common breakpoints in *ROS1* can be used to identify *ROS1* translocations in FFPE tissue samples of lung cancer. The most recent IASLC/CAP/AMP guidelines recommend FISH or molecular methods [RT-PCR or next generation sequencing (NGS)] to confirm a *ROS1* rearrangement in ROS1 IHC positive lung adenocarcinomas prior to considering TKI therapy.[16] Currently, in Australia *ROS1* FISH assays are reimbursed by Medicare for IHC 2+ or 3+ positive non-squamous NSCLC (that lack an activating *EGFR* mutation and are ALK IHC negative), and confirmation of a *ROS1* gene rearrangement by FISH is required to access crizotinib (Fig. 2).

Several commercially available break-apart probes are available and can identify *ROS1* rearrangements regardless of the fusion partner. While FISH probe sets may vary, many of the commercially available probe sets label the 3′ end (containing the *ROS1* tyrosine kinase domain) green and the smaller 5′ end red or orange. A split pattern (with separation of a red and green signal by at least one signal diameter) or isolated 3′ signal pattern, is considered positive for *ROS1* gene rearrangement if found in at least 15% of tumour cells with a minimum of 50 tumour cells recommended for analysis[5] (Fig. 1).

While a threshold of 15% is commonly cited in the literature as the cut-off point to define a positive tumour,[37] laboratories should undertake their own internal validation against an alternative testing method or another laboratory's results to confirm this value. Fused signal patterns or isolated 5′ signal patterns are considered negative. While FISH assays are considered highly sensitive and specific for identifying *ROS1* rearrangements involving the common breakpoint region, false negatives can be seen with some intra-chromosomal rearrangements involving nearby partner genes such as TPD52L1, CEP85L and GOPC, located very close to *ROS1* on chromosome 6q.[5] Some probe set designs are more vulnerable to such false negatives than others and each laboratory should be aware of any shortcomings in the chosen probe design. Alternatively, false positive *ROS1* FISH may rarely arise from non-activating fusions involving *ROS1*. Unusual patterns such as isolated 5′ signals, while technically considered negative, should ideally undergo alternative testing such as NGS to exclude a cryptic or complex rearrangement not detectable by FISH.

ROS1 break apart FISH assays provide a reliable method for identifying *ROS1* rearrangements in lung cancer specimens that is particularly useful following IHC screening.[37]

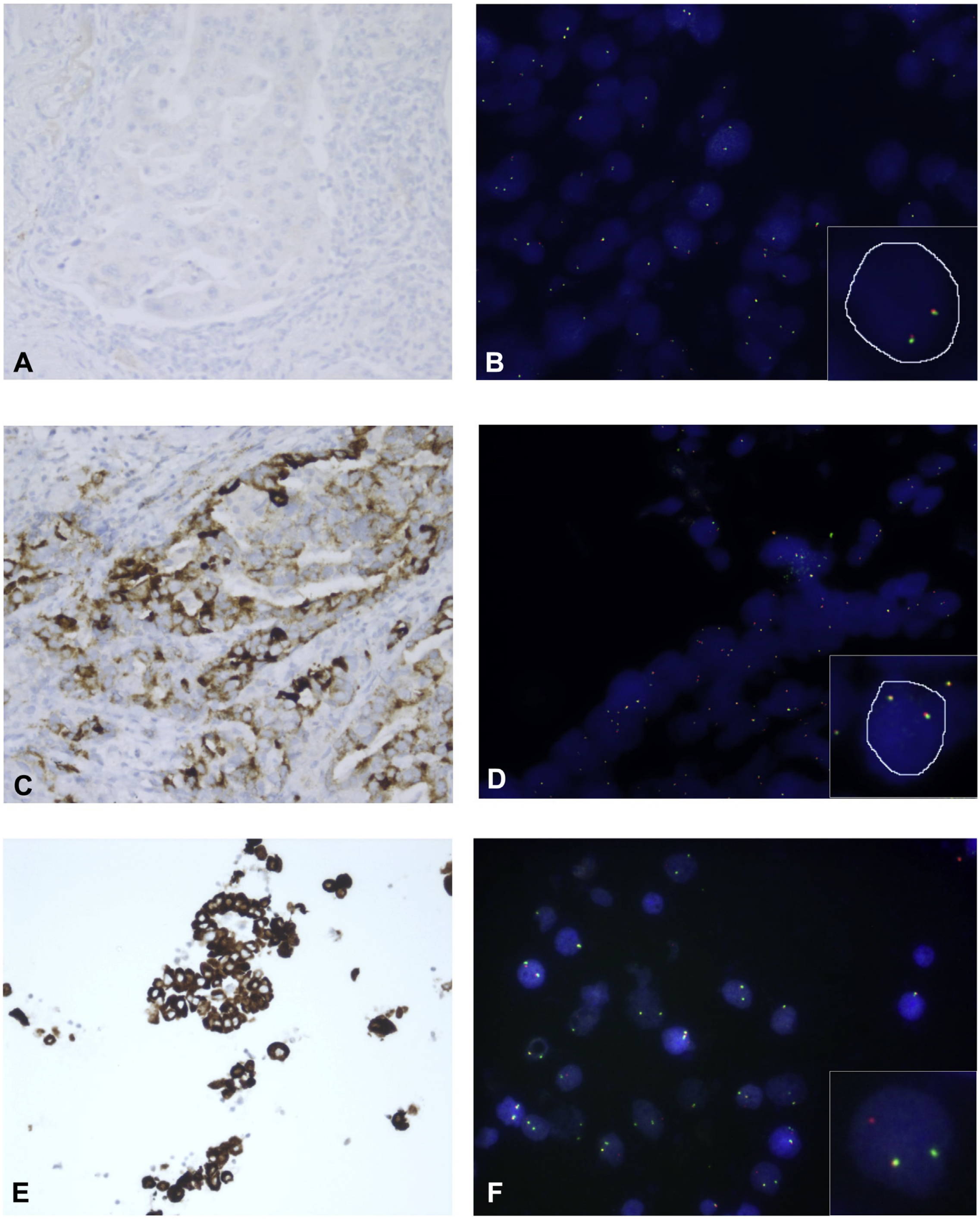

Fig. 1 ROS1 IHC and FISH images of non-small cell lung cancer. (A) A ROS1-negative case that was IHC-negative and (B) FISH-negative with fused signals; (FISH was not required in this case but was performed upon the clinician's request). (C) A ROS1-negative case that was IHC positive (patchy 2+ and 3+ staining) and (D) FISH negative. (E) A ROS1-positive case (cytology sample) that was IHC-positive (diffuse 3+ staining) with (F) *ROS1* rearrangement confirmed by FISH showing a split signal pattern.

However, dependable results require careful laboratory validation and interpretation by suitably qualified pathologists with appropriate knowledge of the assay strengths and limitations.

FUTURE TESTING

Currently in the Australian context *ROS1* testing is downstream and dependent for reimbursement purposes on prior negative testing for *EGFR* and ALK (triaged by negative IHC before mandated FISH for *ALK* IHC positive cases). This is a sub-optimal process; frequently there are delays due to cell block/tissue retrieval for upfront *EGFR* testing which is often compounded by subsequent further delays from referral to another laboratory after *EGFR* testing, as not all laboratories perform all techniques. As in the above section on FISH, it can also be challenging to acquire adequate tumour material,

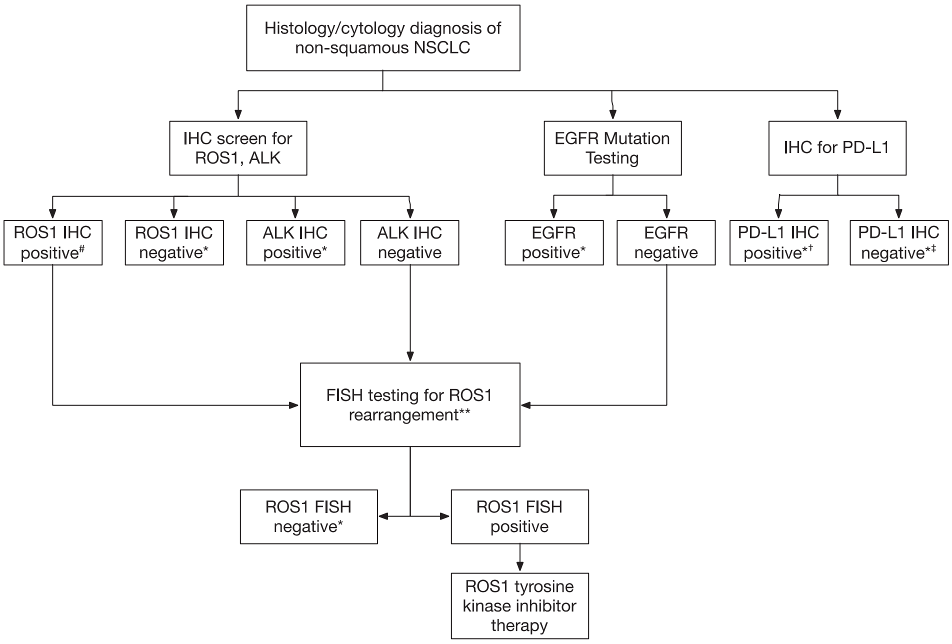

Fig. 2 Testing algorithm for advanced stage non-squamous non-small cell lung cancer. FISH, fluorescent *in situ* hybridisation; IHC, immunohistochemistry; NSCLC, non-small cell lung cancer. #ROS1 IHC positive: any degree of ROS1 IHC positivity should be considered for FISH although diffuse moderate to strong staining is more commonly associated with ROS1 rearrangement. MBS reimbursement for FISH testing in Australia is only available for cases exhibiting 2+ or 3+ positivity by IHC. †PD-L1 IHC positive: tumour proportion score ≥50%. ‡PD-L1 IHC negative: tumour proportion score <50%. *Refer to Guidelines from the National Comprehensive Cancer Network (NCCN),[13] the European Society for Medical Oncology (ESMO),[14] the American Society of Clinical Oncology (ASCO),[15] and the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology.[16] **FISH testing for *ROS1* rearrangement (if ROS1 IHC positive and EGFR negative and ALK IHC negative).

whether it be the clinical condition of the patient or access to the primary and/or metastatic site. Lung biopsies when they are undertaken, either tissue or cytology-based, are usually small and preservation of tissue becomes paramount. The original histological diagnosis by H&E is often supplemented by IHC to determine histological type which might leave little available tissue to serially test for *EGFR* or *ALK* alterations, let alone for *ROS1*. An obvious and easy solution is to perform an all in one assay that detects all mutations and fusions that are actionable. Into the future, the model of serial rather than parallel testing is unsustainable as there is a high likelihood that further gene mutational information will be required from the small amount of material received currently.

For all of these reasons the adoption of techniques that allow parallel rather than serial testing should be encouraged, notwithstanding the lack of reimbursement for the test and eligibility for drug approval in positive patients that currently mandates FISH testing. Technologies are already available in many pathology labs that present an opportunity for such an approach. A further advantage is the testing of the full complement of genes as outlined in the recent 2018 guidelines[15,16] without substantial increase in costs.

One technique that is widely available is based on massively parallel sequencing or NGS. NGS has the ability for high throughput sequencing of multiple genes in multiple patient samples and can detect somatic mutations including single nucleotide variants (SNVs), indels, fusions (including *ROS1* and *ALK* fusions) and copy number changes in biopsy material with a neoplastic cell component as low as 5%. It also has the advantage over FISH in being able to identify the mutations which might provide additional prognostic information, e.g., *ALK* V3 positive lung cancer[38] and *CD74-ROS1* variants that are more likely to present with brain metastases.[39] NGS methodologies also provide additional information such as tumour mutational burden that may help guide immunotherapeutic treatment. Nevertheless, as with all assays, it is likely that inherent limitations mean that a proportion of cases will be missed.[40]

There are many available platforms and though they may be based on different chemistry, they have the same library construction and amplification before sequencing. The template for these tests can be DNA and/or RNA, with some tests using both depending on the design. RNA-based tests identify a higher proportion of fusions than DNA based tests due to design and/or ability to be more robust with low tumour cellularity samples.[41] The platforms and tests vary in time taken to perform the assay, analysis, performance characteristics and coverage and selection of the test will likely vary depending on local laboratory requirements. Not all 'off the shelf' tests provided by many suppliers cover all desired regions and any used clinically require careful evaluation and validation. Issues around research only tests versus clinical grade CE-IVD tests should also be considered. Although the parallel testing cost is higher than serial testing it is likely that a whole of healthcare systems approach that takes into account lab to lab referrals and time spent on re-biopsy, etc., would provide data to show its cost effectiveness. Other technologies could also potentially be used such as NanoString for the detection of somatic mutations relevant to lung cancer.[42]

Although broadly not recommended currently,[43] another likely change will be the upfront use of circulating tumour (ct)DNA[44] as the matrix for testing over tissue or cytology samples.[45] There are obvious advantages to patients, clinicians and laboratories with this approach and it is currently used for detection of *EGFR T790M* mutation in tyrosine kinase resistant lung carcinoma to assess suitability for third line TKI.[46] Although not widespread, with advances in technologies and a reduction in costs of testing it is likely that a broader panel of tests will be available, giving similar advantages to tissue testing.[47]

CONCLUSION

ROS1-positive NSCLC, a biologically distinct and an oncogenic driven rare subgroup of NSCLC, is characterised by high response to TKI therapy. This has led to published guidelines recommending targeted therapy with TKIs as first-line treatment in advanced *ROS1*-positive NSCLC over other potential systemic therapy options, including chemotherapy and checkpoint inhibitors. The ability to offer *ROS1* targeted therapy is dependent on the pathologist determining the *ROS1* alteration status in an individual patient's tumour. As such, ROS1 testing should be incorporated upfront in the testing algorithm for non-squamous NSCLC.

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