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HEALTH TECHNOLOGY ASSESSMENT:

Tests for detection of ROS1 gene alterations in people with non-small cell lung cancer (NSCLC)

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Authors	Flodgren GM, *Project leader, Senior researcher, Norwegian Institute of Public Health* Hamidi V, *Health economist, Norwegian Instirute of Pulic Health*
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Key messages

The Norwegian Institute of Public Health has been commissioned to evaluate molecular tests for the identification of somatic ROS1 gene alterations in people with locally advanced or metastatic non-small cell lung cancer (NSCLC). People with tumours harbouring ROS1 gene alterations probably make up 1-2% of NSCLC cases. Accurate and reliable detection of ROS1 gene alterations is important for identification of people who may benefit from treatment, as well as ROS1 negative patients, to avoid provision of unnecessary and costly treatment.

We included one systematic review, six narrative reviews, a survey of Norwegian Hospital trusts, and two reviews on the preferences of patients related to molecular testing. Experts were contacted for cost information. The results of this HTA show that:

- There is scarce, incomplete and low-quality evidence on the sensitivity and specificity of tests for the detection of ROS1 gene alterations in people with advanced or metastasised NSCLC
- Positive IHC ROS1 results needs confirmation with FISH or other methods, due to a tendency for false positive staining.
- While the different tests had different pros and cons, single gene testing may be unfeasible, since people with NSCLC typically are tested for more than one type of actionable gene alteration.
- NGS due to its capacity to analyse multiple genes simultaneously, may have the potential to reduce the risk of repeat biopsies.
- The cost for ROS1 using IHC as pre-test with FISH confirmation, is possibly less than for the other methods.
- The cost associated with NGS testing will significantly decrease when parallel tests are to be performed for several biomarkers (i.e. gene panels) from multiple patients. However, at present, the capital and infrastructure as well as maintenance costs are higher for NGS than the other diagnostic methods.
- Future research should focus on conducting larger cohort studies with well-defined patient populations, that follows the patients from testing (or no testing), through treatment and final outcomes.

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Tests for detection of ROS1 gene alterations in people with non-small cell lung cancer (NSCLC)

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Team:
Gerd.M.Flodgren, researcher, and team leader
Vida Hamadi, health economist
Elisabet Hafstad, research librarian
Ingrid Harboe, research librarian

Clinical experts:
Tormod K Guren, OUH, Emilius AM Janssen, UiS, Hege EG Russnes, OUH, Lars Helgeland, UiB

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Ingrid Harboe, research librarian

Kliniske eksperter:
Tormod K Guren, OUH, Emilius AM Janssen, UiS, Hege EG Russnes, OUS; Lars Helgeland, UIB

Folkehelseinstituttet har på oppdrag fra Bestillerforum for nye metoder evaluert molekylære tester for identifisering av somatiske ROS1-genforandringer hos pasienter med lokalavansert eller metastasert ikke-småcellet lungekreft (NSCLC). Pasienter med svulster som har ROS1-genendringer utgjør ca 1-2% av NSCLC-tilfellene, noe som tilsvarer rundt 10 pasienter per år i Norge. Tester som identifiserer hvilke pasienter som kan ha nytte av målrettet medikamentell behandling er viktige for adekvate behandlingsbeslutninger.

Vi inkluderte én systematisk oversikt, seks narrative oversikter, én spørreundersøkelse ved norske sykehus og to oversikter om pasienters preferanser til molekylær testing. Vi kontaktet eksperter for prisinformasjon. Resultatene av den forenklede metodevurderingen viser:

- Det er begrenset dokumentasjon av lav kvalitet for sensitivitet og spesifisitet av tester for påvisning av ROS1-genendringer hos personer med avansert eller metastasert NSCLC.
- Positive IHC ROS1-resultater må bekreftes med FISH eller andre metoder på grunn av tendens til falsk positiv farging.
- Forskjellige tester har ulike fordeler og ulemper, men enkeltgentesting vil ofte være lite hensiktsmessig da personer med NSCLC vanligvis testes for mer enn én type handlingsbar genendring.
- Grunnet muligheten til å analysere flere gener samtidig kan NGS redusere behovet for gjentatte biopsier.
- Testing av ROS1 med IHC som forhåndstest og FISH-bekreftelse, er muligens mindre kostbart enn andre metoder.
- Kostnadene knyttet til NGS-testing vil betydelig reduseres når parallelle tester skal utføres på flere biomarkører fra flere pasienter. Kapital og infrastruktur samt vedlikeholdskostnader er imidlertid per i dag høyere for NGS enn de andre diagnostiske metodene.
- Fremtidig forskning bør fokusere på å gjennomføre store kohortstudier med veldefinerte pasientpopulasjoner, som følger pasientene fra test (eller ingen test), gjennom behandling til endelige resultater.

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Preface

This health technology assessment (HTA) was commissioned by The National System for Managed Introduction of New Health Technologies within the Specialist Health Service in Norway (Nye Metoder). The following commission was given 16.12.2019: "A simplified methods evaluation with a summary of efficacy, safety and costs of entrectinib for the treatment of locally advanced or metastatic ROS1-positive non-small cell lung cancer (NSCLC) is carried out by the Norwegian Medicines Agency (NoMA). The Norwegian Institute of Public Health is responsible for carrying out the assessment of the relevant diagnostic test." (ID2019_115). NIPH initiated the work in September 2020. This HTA includes a summary of reviews reporting on the sensitivity, specificity, concordance, feasibility and cost analysis of four different test methodologies (IHC, FISH, NGS and RT-PCR) for the identification of ROS1 gene alterations in NSCLC. The organisation of genomic tests services, and patient preferences related to molecular testing were also briefly touched upon. The aim of this report is to support well-informed decisions in health care that can lead to improved quality of services.

In addition to the authors, the following people contributed to the protocol and/or present report: Clinical experts: Tormod K Guren, OUH, Åslaug Helland, OUH, Emilius AM Janssen, UiS, Hege EG Russnes, OUH; Information specialists: Elisabet Hafstad, NIPH; Ingrid Harboe, NIPH

Contributions of authors: GMF: screening, data extraction, mapping of data, production of figures, write-up of the review, and project lead; VH: screening, data extraction, cost analysis, and write-up of the health economy results and discussion

The authors and involved experts declare no conflicts of interest.

We emphasise that although the clinical experts have contributed with valuable input and comments, NIPH is solely responsible for the content of this report

Kåre Birger Hagen
Director of Reviews and Health Technology Assessments

Kjetil Gundro Brurberg
Department director of Reviews and Health Technology Assessments

Gerd M Flodgren
Senior Researcher, and Project coordinator

Abbreviations

ALK	Anaplastic Lymphoma Kinase fusion oncogene
AIHTA	Austrian Institute for Health Technology
CI	Confidence Interval
DRG	Diagnosis-related group
EGAPP	Evaluation of genomic applications in practice and prevention
EGFR	Epidermal growth factor receptor proteins
ELSI	Ethical, legal, and social implications
EMA	European Medicine Agency
ESMO	European Society for Medical Oncology
EUnetHTA	European Network for Health Technology Assessment
FDA	American Food and Drug Administration
FFPD	Formalin-fixed Paraffin Embedded samples
FISH	Fluorescence in Situ Hybridisation
HTA	Health Technology Assessment
IHC	Immuno-Histo-Chemistry
INAHTA	International Network of Agencies for HTA
IQWIG	Institute for Quality and Efficiency in Health Care
KCE	Belgian Health Care Knowledge Centre
NGS	Next Generation Sequencing
NIHR	National Institute for Health Research
NIPH	Norwegian Institute of Public Health
NordiQc	Nordic Immunohistochemical Quality Control
NoMA	Norwegian Medicine's Agency
NSCLC	Non-small-cell lung carcinoma
EMQN	European Molecular Genetics Quality Network
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses.
RET	REarrangement during Transfection (RET) oncogene
RHF	Regionale Helse Foretak (Norwegian)
ROS1 gene	Proto-oncogene tyrosine-protein kinase fusion protein

RTK	Receptor tyrosine kinase
RT-PCR	Reverse transcription polymerase chain reaction
PD-LI	Programmed Death Ligand 1
PICO	Population, Intervention, Comparison, Outcomes
SR	Systematic review
TAT	Turn-around time
TKI	Tyrosine kinase inhibitor

Objectives

The main objective of this evaluation was to summarise available evidence on the analytical validity, the clinical validity, and the clinical utility of diagnostic single tests, both single and multigene biomarker analyses (IHC, FISH, RT-PCR, and NGS) for the detection of ROS 1 alterations in patients with locally advanced or metastatic non-small cell lung cancer (NSCLC).

More precisely we aimed to answer the following research questions:

- How accurately and reliably do each of these tests detect the biomarker in the laboratory (technical performance)?
- How accurately and reliably do each of these tests detect the biomarker in samples from patients with locally advanced or metastasised NSCLC (e.g. tumour tissue, circulating cells, or cytology samples)?
- How well do each of these tests predict the effectiveness of treatment (e.g. shrinking of the tumour, or slowing down the disease process)?
- How well do each of these tests predict outcomes of importance to the patient (e.g. overall survival, and quality of life)?
- What are the potential adverse effects of using these tests to guide treatment decisions affecting patients?
- What are the pros and cons of the different tests (i.e. the feasibility of tests in terms of biological tissue requirements, turnarond time, invasiveness, training/expertise needed for running the analyses or interpreting the test results)

Secondary aims included assessing (i) Service delivery/organisational aspects related to test services in Norway, (ii) the ethical, legal, and social implications (ELSI) of molecular testing, (iii) patient preferences related to testing, and (iv) costs.

Background

Condition/disease

Epidemiology

Lung cancer constitute approximately 10% of all new cancer cases in Norway. It is the second most common cancer among men and the third most common in women (1). Non-small-cell lung cancer (NSCLC) dominates, and among its sub-types, adenocarcinoma is most prevalent. The 5-year survival for NSCLC is less than 10% (1).

ROS1 (proto-oncogene tyrosine-protein kinase fusion protein) is a receptor tyrosine kinase (RTK)(2). ROS1 gene alterations occur almost exclusively in adenocarcinomas. In NSCLC a number of fusion partners have been identified, of which the most common are CD74-ROS1, SLC34A2-ROS1, TPM3-ROS1, and SDC4-ROS1 (2). Patients with ROS1 alterations probably make up 1-2% of NSCLC adenocarcinoma cases, which corresponds to around 10 patients per year in Norway (4). Most of these patients are young, female, never-smokers (3).

Expression of the ROS1 fusion protein results in hyperactivation of downstream signaling pathways, which in turn leads to uncontrolled cell division and increased tumor tissue survival (2). Treatment with the tyrosine kinase inhibitor (TKI) Entrectinib has in one small one-armed study, mostly including previously treated patients with NSCLC, been shown to shrink tumours and slow down the disease progression (4, 5). Entrectinib is approved for treatment of ROS1 fusion positive NSCLC by the European Medicines Agency (EMA) (6), and the US Food and Drug Administration (FDA) (7), and in Norway.

Accurate and reliable detection of ROS1 gene alterations is important to ensure that people who may benefit from treatment are correctly identified. Similarly, accurate and reliable detection of ROS1 negative patients can avoid provision of unnecessary and costly treatment.

Progress, treatment, and care pathway for locally advanced or metastatic NSCLC

Patients with NSCLC are typically diagnosed at a late stage in the disease process where curative treatment is not feasible, and when survival is very low (8). Treatment for these patients therefore focus on interventions to prolong life and prevent or alleviate symptoms. Radiation therapy and/or drug treatment is given to most of

these patients (8). It is recommended that all patients with NSCLC are tested for PD-L1 expression, and patients with non-squamous cell carcinoma are tested for EGFR, ALK, and ROS1 alterations. In adenocarcinoma with a detected mutation, targeted treatment is offered in the first line (e.g. TKI treatment with Crizotinib for ROS1), and in some cases second-line treatment is also offered(8). See Figure 1

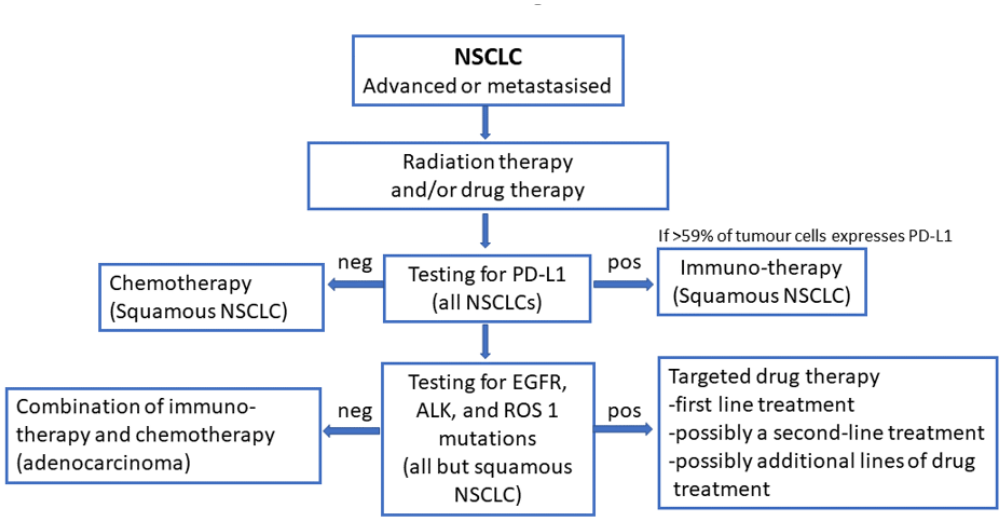


Figure 1. *The recommended testing and treatment algorithm for patients with advanced NSCLC by Norwegian guidelines (8).*

Molecular tests for detection of ROS1 gene alterations

There are four main methods that may be used to detect ROS1 alterations in NSCLC: immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), real time reverse transcription polymerase chain reaction (RT-PCR), and next generation sequencing (NGS) that can either be DNA- or RNA-based (2). IHC is routinely used at Norwegian hospitals to screen for increased ROS1 gene alteration protein expression in patients with NSCLC. In the case of IHC ROS1 positivity, FISH, which is currently considered the gold standard, is used for confirmation (9). While IHC, FISH, and PCR are single protein/gene tests, NGS includes multi-gene panels, by which alterations in hundreds of genes can be detected at the same time. There is at present no companion diagnostic test for Entrectinib (Rozlytrek) approved by the FDA.

Why is it important to conduct this assessment?

In this HTA we have summarised the evidence of the sensitivity, specificity, concordance, pros and cons of different tests for the detection of ROS1 gene alterations, as well as information on organization and delivery of services, and patient preferences related to molecular testing. In addition, we have conducted an economic evaluation of the relevant diagnostic methods for detection ROS1. This assessment was conducted to assist decision makers in making informed decisions regarding the delivery and organisation of genomic tests services in Norway.

Method

We have, due to time constraints, chosen to conduct an overview of reviews to respond to this commission. The purpose of the molecular tests under study was prediction of treatment response or adverse events. We used a combination of the EGAPP framework (10, 11), and the extended framework described by Pitini et al. to guide our assessment (12). A glossary is found in Appendix 1.

Literature search

Research librarian Elisabet Hafstad (EH) developed the main search strategy with input from the authors, and ran the electronic searches for reviews comparing diagnostic tests for the detection of ROS1 alterations in patients with locally advanced or metastasised NSCLC, in terms of analytical validity, clinical validity, clinical utility and/or feasibility. Another librarian (Ingrid Harboe) peer reviewed the search strategy. The search did not have any language restrictions. We however found no eligible reviews in other languages than English. The main search strategy is provided in Appendix 2.

We systematically searched for literature from January 2015 and up to October 2020, in the following electronic databases:

- Epistemonikos
- MEDLINE (Ovid)
- Embase (Ovid)
- INAHTA database
- EUnetHTA Relative Effectiveness Assessments
- Guidelines International Network (GIN)
- HTAi vortal + IQWIG
- AIHTA
- KCE
- NIHR Journal Library

In addition, Ingrid Harboe, developed a search strategy for ELSI and patient preferences and ran the searches for related reviews of pharmacogenomic testing in oncology, in Epistemonikos, and in PubMed (the search strategy can be provided after contact with authors). We also searched the reference lists of included studies and contacted experts in the field.

Inclusion criteria

We used the PICO (population, intervention, comparison and outcomes) framework to describe the inclusion criteria (13). Only summarised evidence were eligible for inclusion.

Population:	People with locally advanced or metastatic non-small cell lung cancer (NSCLC), not previously treated with ROS 1 inhibitors
Intervention (index test (s)):	Immunohistochemistry (IHC), Fluorescence in situ hybridization (FISH), Real-time reverse transcription polymerase chain reaction (RT-PCR), and Next generation sequencing (NGS) used for the detection of ROS1 gene alterations
Comparison (reference test(s)):	Head-to-head comparisons of the tests listed above
Outcomes:	Analytical validity, clinical validity, clinical utility, feasibility, ELSI, and patient preferences
Language:	English, Norwegian, Swedish, Danish, Icelandic and Persian
Study design:	Systematic reviews, and non-systematic reviews

Exclusion criteria

Study design:	Original studies, Conference abstracts, etc.
Population:	Patients with other types of cancer than NSCLC
Intervention:	Other tests than those listed above
Outcome:	Outcomes not related to the accuracy, or feasibility of tests

Other exclusion criteria were reviews that did not report a comparison between tests, or with cell-lines with known mutation status, or reviews written in other languages than those listed above.

Selection of reviews

We downloaded all titles and abstracts retrieved by the electronic searches into the reference management program EndNote (14) and removed duplicates. Two review authors (GMF and VH) independently assessed the remaining titles and abstracts against the inclusion criteria using Rayyan (15). We obtained full text copies of potentially relevant reviews, and assessed them in duplicate. We resolved disagreements by discussion. Reasons for exclusion of publications read in full text but subsequently excluded are reported in Appendix 3.

Data extraction and management

Two reviewers (GMF and VH) independently extracted data from each included review into a standardised piloted data extraction form, which was adapted for use in this HTA. Any disagreements were resolved through discussion among review authors. We extracted the following data: citation, year of publication, setting, country, funding, conflicts of interest, study designs, language, and the PICOs:

- *Participants*: number, age, gender, race/ethnicity, socioeconomic status, time since diagnosis, previous treatment received, concomitant therapy/medication, etc.
- *Molecular tests*: technical details of tests, regulatory status, in-house or commercial test, previous tests conducted, sequence of tests if more than one test, test turnaround time, type and amount of biological tissue needed, etc.
- *Comparisons*: head-to-head-comparisons, index test(s) versus reference tests if applicable, or cell-lines with known mutation status
- *Outcomes*: analytical validity (sensitivity, specificity, assay robustness, quality control), clinical validity (i.e. sensitivity and specificity, positive and negative predictive values), clinical utility (e.g. response rate to treatment, overall survival, quality of life), pros and cons of the different tests etc.

Quality assessment

We used the AMSTAR tool (16) to assess the quality of included systematic reviews.

Compilation of results

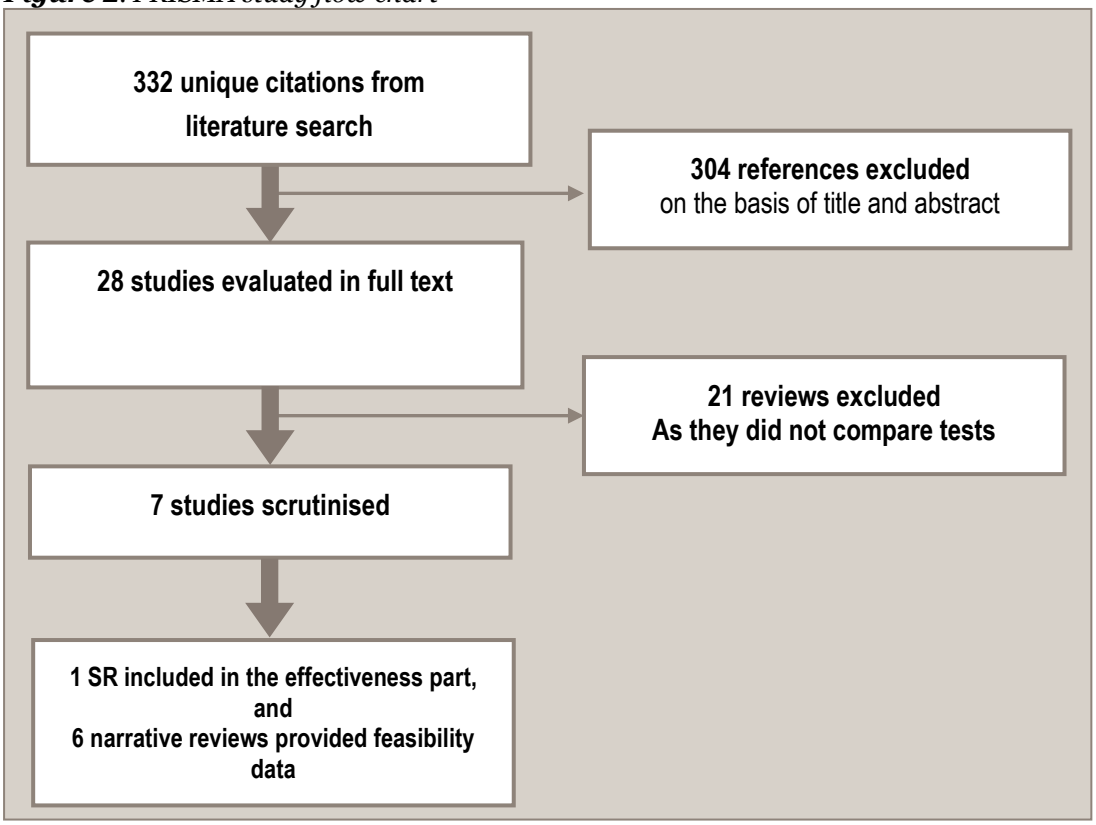
We have provided a narrative summary of the available evidence for the analytical validity, the clinical validity, and the clinical utility of different tests used for the detection of ROS1 gene alterations from included reviews in text and tables. We have, when available, incorporated data received from experts of from the test suppliers. Also results related to service delivery/organizational aspects, resource use, ELSI, and patient preferences are reported narratively.

Review Results

Search results

See Figure 2. PRISMA study flow chart below.
The main search of the electronic data bases yielded 332 citations. Three-hundred and four of these were irrelevant and directly excluded at title and abstract screening stage, leaving 28 citations to be retrieved in full text for further scrutiny. Seven publications, that provided data on comparisons between tests for the detection of ROS 1 alterations in NSCLC, were considered relevant for this review. Only one of these qualified as a systematic review (SR) (17). Six were narrative reviews or consensus documents with no methods section (9, 18-23). Studies read in full text but subsequently excluded are listed in Appendix 3, along with the reasons for exclusion.

***Figure 2**. PRISMA study flow chart*



The search for reviews related to ELSI, and patients' preferences yielded 64 citations, of which none was direct relevant for pharmacogenomic testing in NSCLC. One review read in full text and subsequently excluded was concerned with ELSI in gene expression profiling tests for breast cancer prognosis (24). Two eligible publications were identified when searching PubMed: one review summarizing studies of patient attitudes/preferences related to genomic testing in oncology in general (25), and one review explored patients (and providers') needs and preferences in understanding pharmacogenomics and genomic testing in cancer precision medicine(26).

Characteristics of included systematic reviews

One systematic review by Yang et al. (27), which included 21 studies from various countries, compared IHC with molecular tests for the detection of ROS1 alterations in patients with NSCLC (N=10,898 participants). Nine of these studies were included in the concordance analysis, and five studies provided data for the pooled sensitivity and specificity. *Study designs*: One of the included studies was a prospective cohort and one study a retrospective cohort, while the study design for the remaining studies was unclear. *Description of tests and sample populations*: The antibody clone D4D6 (Cell Signaling Technology) was used in all IHC analyses. The molecular comparison tests varied across studies (FISH: N=16, DNA-sequencing: N=2, and NanoString technique: N=2). No information was provided on the characteristics of the participants, the samples, the reference standards or cut-offs, biological material requirements, or test turnaround time. No information on how concordance had been calculated was provided in the review. *Conflict of interest*: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of their article.

Quality assessment: The review was of critically low quality according to AMSTAR. See Appendix 4.

Sensitivity, specificity, and concordance

IHC versus molecular tests

Yang et al. (27) reported an overall concordance rate between ROS1 IHC and molecular tests of 93.4% (95% CI 78.3, 98.2), which varied between ROS1 positive and ROS1 negative cases (Table 1). The pooled sensitivity was 0.90 (95% CI 0.70, 0.99) and the pooled specificity 0.82 (95% CI 0.79, 0.84). The sensitivity of IHC ranged from 0.33 to 1.00 across studies, and the specificity from 0.50 to 1.00. No sub-

group analysis by type of molecular test could be performed, due to the few studies included.

Table 1. *Yang et al. (27) IHC vs molecular tests for detection of ROS1 alterations*

No of included studies (patients)	IHC and molecular tests	Rate	95% confidence interval
9 studies (N=unclear)	Concordance rate (all)	93.4%	78.3 to 98.2
	Concordance (positive cases)	79.0%	43.3 to 94.9
	Concordance (negative cases)	97.0%	83.3 to 99.5
5 studies (N=unclear)	Pooled Sensitivity	0.90	0.70 to 0.99
	Pooled Specificity	0.82	0.79 to 0.84
	Diagnostic Odds Ratio (OR)	118.01	11.81 to 1179.67
	AUC-ROC curve*	0.9417	

* Area under the curve of the summary receiver operating characteristic curve. An overall summary of diagnostic accuracy, with 1.0 corresponding to perfect accuracy.

The results of the narrative reviews (9, 18, 20-22, 28) are briefly summarized in Appendix 5.

Feasibility

See Table 2

Below we have summarised information on the feasibility of the different test retrieved from the narrative reviews (9, 18, 20-22, 28), a survey of Norwegian Health Trusts (29), ESMO fact sheet on ROS1 (30), and information from experts. Summarised information on different NGS systems/panels received from five NGS suppliers (Archer, Cari's Life Sciences, Illumina, Roche, Thermo Fisher) is found in Appendix 6.

Type of samples

Any biological specimen containing tumour cells may be used for the analysis of ROS 1 gene alterations (i.e. needle biopsies, cytology specimens, surgically resected tumour biopsies and liquid biopsies) (30). Forty percent of all NSCLC diagnoses are based on analyses of cytology samples (28). The most used sample preparation in lung cancer is the formalin-fixed paraffin embedded (FFPE) samples, either needle-biopsies or surgically resected tumors (30). We did not find any studies that compared the sensitivity and specificity of different tests for detection of ROS 1 gene alterations when different type of samples was used. One of the NGS suppliers reported that some assays used liquid samples for detection of ROS 1 alterations, and one supplier reported on ongoing work in adapting the technique for plasma samples (personal communication). It was not clear from the information provided in

the included reviews whether one type of sample is more suitable than others for a specific type of test. However, based on the results of a survey of Norwegian hospital trusts, it appears that FFPE samples with poor quality RNA may be challenging for NGS analyses (29).

Material requirements

IHC may require only few cells for an interpretable result , and in addition may function on technically suboptimal material, depending on the antibody and antigen involved (30). FISH requires 50 to 100 cells, and NGS between 600 and 1000 cells (30). Information received from suppliers, suggest that different NGS systems have different tissue requirements (between 10 and 250 ng, or 3-5 sections of FFPE tissue depending on biopsy size/tumor area) per analysis (personal communication). NGS analysis requires high quality RNA, and some sample types (e.g. FFPE) may make the analysis challenging. According to data from one of the suppliers, iterative testing for gene fusions and alterations relevant for patients with NSCLC (e.g. ALK, EGRF, ROS1, NTRK) using reflexive single-gene tests would require as much as 29 slices FFPE (Illumina, data on file, 2019).

Cut-off or reference standard used for positivity

For IHC there is no international scoring standard or cutoff in use (9). For FISH analysis a cut-off between 10% and 15% break-apart is considered an acceptable threshold for positivity (30). According to the information received from NGS suppliers, there is no internationally agreed cut-off or reference for ROS 1 positivity for NGS. Different NGS platforms also use slightly different methods to calculate ROS 1 gene fusions (information from suppliers). See Appendix 5.

Coverage

Even though FISH is often mentioned as being the 'gold standard', there appear to be some variation in the fusion coverage of FISH, i.e. the number of fusion partners identified may differ between different probe sets (20). RT-PCR may also miss identifying some fusions (22). Illumine based NGS however, is capable of detecting both known and unknown fusion partners (22).

Turnaround time

The turnaround time (TAT) is the interval between when a test is requested to the time a treatment decision is made, and includes nine steps: (i) ordering, (ii) collection, (iii)identification, (iv) transportation, (v) preparation, (vi)analysis, (vii) interpretation, (viii) reporting, and (ix) action(31). While evidently some of the steps are the same for the different tests, the time requirement for the analysis, interpretation

of results, and communicating the results to the treating physician in an accessible form, may differ. TAT is in addition dependent on whether the test is 'in-house', or centralised to certain hospitals, or to facilities outside the hospital. Time requirements for the tests assessed in the included reviews were reported in a vague and unprecise manner (See Table 2), and the actual TAT was not provided. Information from the suppliers suggest that NGS analyses, may take between 'a one-day workflow' up to 11 days (personal communication). Laboratories at Norwegian hospital trusts typically run NGS only one day a week (information from experts), although the use of NGS for cancer diagnostics is now rapidly increasing (29).

Interpretation and reporting of results

Time and expertise required for interpreting the results of different tests, and communicating these to the treating physician, was rarely touched upon in the included reviews. The use of electronic scoring systems for IHC, and 'data analysis pipelines' for NGS analyses were mentioned, but not further elaborated on. Results from a survey of Norwegian hospital trusts suggest variations in the reporting of results of NGS analyses across trusts, i.e. some reporting results for actionable gene mutations only, while others also report results for mutations for which no targeted treatment is available. The survey also revealed a lack of reporting guidelines for NGS, as well as guidelines for deciding which gene panels to use, and which genes to test for (29).

Table 2. *Pros and cons of using single markers (IHC, FISH, RT-PCR) and multiple markers (NGS) for detection of ROS 1 gene alterations-*

	IHC -Immunohisto-chemistry[1]	**FISH** -Fluorescence in situ hybridization	**NGS** -Next Generation Sequencing[2]	**RT-PCR** -Reverse transcription polymerase chain reaction[3]
Availability	widely available	widely available	widely availability	not widely available
Multiplexing (Yes/No)	No	No	Yes	No
Sensitivity, specificity, and reliability	Sensitive, but lacks specificity (positive cases need confirmation with FISH or other method)	High sensitivity, and specificity ("gold standard")	Very high sensitivity and specificity (information from some suppliers)	Specific technique, but lacking in sensitivity and reliability (30)
Minimum read (no of tumour cells needed for analysis)	A few cells	50 assessable cells	600-1000 cells (ESMO 2016); 10ng DNA/ RNA or approx. 1000-1500 human cells (18). 3-5 slices FFPE (personal information)	600 -1000 cells
Sample quality requirements	May be used on poor materials	No information	Good mRNA quality and quantity	Good mRNA quality and quantity
Clarity of test	False positive staining, and subjectivity of interpretation of staining may be a problem (20, 32)	Split signals are usually clear	No information	No information
Acceptable threshold (positive test)	No standardized scoring system; different cutoffs for positivity used (20)	10% to >15% abnormal cells	No universally agreed cut-off [4]	No information

Coverage	Not reported	Coverage of fusions may vary across probe sets (9)	Detects both known and unknown fusions, depending on assay type/instrument (22)	May miss some rare fusions (22)
Personnel and expertise required	Interpretation requires an experienced pathologist	Requires two readers/expert pathologist	Requires Data Analyst	Requires special expertise, and is labour intensive (expert opinion)
Turnaround time	"Carried out and read in a couple of hours"- same day or next day service	The analysis takes ca 30 minutes hands-on time for staining. Depending on the type of material the counting of cells takes ca. 20-40 minutes per slide (personal communication).	Between 'a one-day workflow' up to 11 days (information from suppliers)	Takes 30 min hands-on time, plus 2.5 h in PCR machine, and 15 min analysis (information from experts)

1 Plays an important role for the determination of lung-cancer subtypes. Provides information on cellular localization of proteins in the context of tumor structure (Inamura 2018); 2 ROS1 is usually amongst the fusion genes in commercial NGS panels; 3 PCR not recommended for single use for ROS1 fusion detection. 4 Varying cut-offs are used between gene panels but most assays have induvial established cut-off.

ELSI and patient preferences

ELSI

We found no review reporting on ethical, legal or social implications of pharmacogenomic testing in patients with locally advanced or metastasised NSCLC.

Patient preferences

Results from one review, including three small studies; and participants with various cancer types, suggest a number of factors of importance for patient preferences related to pharmacogenomic testing: (i) regulatory (NHS) approval; (ii) test turnaround time; (iii) invasiveness of testing; (iv) physician approval; (v) test sensitivity; (vi) test specificity; (vii) prevalence of variant; (viii) distance to travel; (ix) implications for family and (x) family endorsement for testing (25). Results of one of the studies included in the review, also suggest that cancer type and prognosis may affect patient preferences regarding testing, but this study included very few patients.

Results of a qualitative review (N=36 studies), which used a grounded theory approach to assess the needs and preferences of patients (and providers) in understanding pharmacogenomics and genomic testing, suggest that many patients need more information on how genes can affect response to a medication, and the difference between pharmacogenomic testing and disease risk testing. The results of the review suggest that good quality face-to-face communication with healthcare providers and printed educational materials with accessible information, may be decisive for test uptake (26).

Organisational aspects and service delivery

Detection of ROS 1 rearrangements at Norwegian Hospital Trusts

Currently IHC is routinely used to screen for ROS 1 gene alterations at Norwegian hospitals, with confirmatory FISH for all ROS 1 IHC positive samples. According to information from experts, no national IHC standards, or training in IHC ROS 1 scoring exist in Norway, and all laboratories use a manual "eyeballing" method to assess the ROS1 IHC results. However, most laboratories have a very low threshold for ordering confirmatory FISH, i.e. all samples with more than 1+ stain intensity will be tested by FISH, which reduces the risk of missing ROS 1 positive samples (personal communication). There may however be some variability in ROS 1 gene fusion coverage between different FISH probe sets (9). Different commercially available CE-IVD approved FISH probes are being used at Norwegian hospitals, and it is not known if they differ significantly. Most laboratories will use standard methods for preparing samples for FISH analysis, and the technical TAT may take up to two days. Taking into account laboratory capacity the complete TAT may be up to a week.

Current and planned use of NGS at Norwegian Hospital Trusts

According to a survey of Norwegian hospital trusts a majority of the 16 trusts have already invested in NGS technology, and it is expected that NGS will be available at all trusts during 2021 (29). Reported challenges with implementing NGS from the survey were: low rates, lack of competence, small area, lack of guidelines on which genes to analyse (size of gene panel), and which findings to report. Other challenges related to NGS analysis were poor quality DNA/ RNA, due to the type of samples (FFPE) most often used. The survey also showed that panel size, and reporting of results varied across hospital trusts (29).

Factors affecting the turnaround time of NGS analysis

According to information from the suppliers an NGS analysis takes somewhere between 24 hours (run time) and up to 11 days (personal communication). Probably the latter refer to the total turnaround time (TAT) i.e. time from ordering of a test to the results being handed to the treating physician in an accessible form, but also reflect TAT for the largest gene panels (analyzing more than 500 cancer related genes). For the NGS systems currently in use in Norway the TAT will vary from 7-12 days (personal communication). The TAT depends on the fact that DNA/RNA are isolated only certain days a week, and that the laboratories only run NGS once a week. This is done to optimize and most efficiently use the reagents, kits, chips, and flow-cells, by running samples from different patients in parallel. Newer NGS systems from both

Illumina and Thermo Fisher will be able to deliver a one-day workflow (24 h) i.e. here referring to the time from retrieval of DNA to when the analysis is done for some gene panel assays. For reduced TAT, laboratories may want to isolate DNA/RNA and run NGS every day, but it is unclear whether this will happen. For some NGS systems the reagents used for one sample will be the same as for 10 samples, and the flow-cell may be reused (personal communication).

There are ongoing discussions at Norwegian hospitals whether DNA/RNA should be isolated from all samples from the start, or isolate RNA first after the NGS-analyses turn negative for the usual mutations. Both the quality and quantity of RNA is lower if it is isolated simultaneously with DNA. A separate RNA isolation may be preferable if enough material is available (personal communication). Panels with simultaneous DNA and RNA sequencing may solve this problem in the future (18, 33).

All the Norwegian hospital trusts using NGS, also use bioinformatic tools that are integrated in the platforms, and this use vary locally. UNN also use a sky-based solution. The labs also uses COSMIC https://cancer.sanger.ac.uk/cosmic), CliVar (https://www.ncbi.nlm.nih.gov/clinvar/), My Cancer Genome (https://www.mycancergenome.org), IGV 11 (visualisation tool, http://software.broadinstitute.org/software/igv/), and other free of charge data bases to help with interpretation of NGS results (29). It is not clear how these tools compare, in terms of time used for interpretation of the results.

Detection of ROS 1 alterations using RT-PCR

In the literature, RT-PCR is not recommended as a stand-alone test for detection of ROS 1 gene alteration as it may miss some fusions (22). Based on feedback from individual hospitals, and experts, we have found out that RT-PCR is not used to detect ROS1 or other gene alterations (ALK/RET/NTRK) at pathology labs in Norway. We have therefore not included PCR in our cost-analysis. According to one expert, PCR do not perform as good as NGS and is more demanding in terms of hands-on labour (personal communication).

Economic Evaluation

General

The health care sector, similarly, to society in general, is restricted by limited resources and budget constraints. In Norway, health service interventions are to be evaluated against three prioritization criteria: the benefit criterion (increased longevity and/or improved health-related quality of life), the resource criterion and the severity criterion (absolute shortfall) (34). Norwegian policy documents indicate that the priority-setting criteria are to be evaluated together and weighted against each other. This is to be done by means of a health economic evaluation.

Health economic evaluations are important tools for decision makers facing questions of how to prioritize health technologies and maximize health benefits using limited resources. The basic aim of any economic evaluation is to identify, measure and compare costs and consequences of the alternatives under consideration in an incremental analysis-one in which the differences in costs are compared with differences in consequences.

Identifying the place of a molecular test within care pathways is crucial, not only to guide the selection of a relevant comparator, but also to guide the use of the companion drug and subsequent treatment pathways to be modeled. The exact place along the treatment pathway where testing occurs may change the cost-effectiveness of the intervention because of differences in the type of treatment subsequently received and the costs and outcomes arising from these (35).

In the published health economic evaluations of precision medicine, the cost of genomic testing and the accuracy of a test were reported as important factors, which influence the cost-effectiveness of targeted interventions (36, 37). Ideally, diagnostic interventions should be supported by studies that follow patients from testing via treatment to final clinical outcome, so-called end-to-end studies (38). This combination of data will enable decision-makers to evaluate the overall cost-effectiveness of using a particular test-drug combination versus not using the drug at all or using the drug without the test (39). In other words, this will enable decision-makers to evaluate the predictive value of the test and the relevant biomarker, and also assess the health outcomes and economic consequences of using genomic test and the consecutive treatment in combination.

Key data needed for an economic evaluation of a genomic test include outcome data on the clinical effectiveness and utility of the technology, changes in health status as well as resource use and related costs of the affected patient population and the uptake of the test. Fundamentally, the challenges relating to the data requirements for the economic analysis of a pharmacogenetic intervention revolve around the availability and quality of existing data (35).

Method

Health economic evaluation of using genomic testing for ROS1 to determine eligibility for treatment with entrectinib in patients with metastatic or advanced NSCLC, preferably, should be performed based on an integrated test-treatment model to follow the NSCLC patient from diagnostic test for the detection of ROS1 mutations via treatment to clinical outcomes. Based on the results of our review, none of the included studies evaluated the tests' clinical utility. Moreover, the feedback from the suppliers of the diagnostic methods and the relevant pharmaceutical company supported the results of our review, *i.e.* there are no end-to-end studies available for the detection of ROS1 mutations in patients with NSCLC.

Hence, in the absence of the relevant clinical utilities data, we have in collaboration with the experts from the regional health authorities, estimated the costs associated with each diagnostic method in Norway. The analyses were performed based on the micro-costing method. Micro-costing is a highly detailed health economic costing approach in which all of the underlying resources required for an intervention or activity, such as equipment, consumables, and staff time are identified, and then unit costs are attached to this resource use to generate an overall cost (40).

Implementing precision medicine in healthcare is potentially a costly investment and it requires testing multiple patients to identify a specific group of responders. Currently, more and more multiple tests and multiple precision medicines for particular diseases become available (41). For example, in non-small cell lung cancer, a set of parallel tests are to be performed on a number of molecular biomarkers to decide between a range of precision medicines (41). Therefore, in the estimation of the costs related to the diagnostic methods, we have also considered the multigene testing and testing samples from multiple patients.

In addition, we contacted suppliers of relevant tests to procure information about technical details, the costs related to operation as well as investment requirements for establishing routine testing in the Norwegian hospitals. We received feedback from five supplies Archer, Caris' Life Science, Illumina, Roche and Thermo Fisher. We have presented a summary of the information received from these suppliers regarding the costs associated with different methods in Appendix 6.

Furthermore, we have calculated the costs per investigation associated with the relevant diagnostic methods based on the current Norwegian tariff rates for clinical laboratory services. A comparison between the estimated costs based on the micro-costing method and the costs calculated based on the tariff rates may assist the decision makers to make decision regarding the revision of the current tariff rates relevant for using genomic tests. We expressed relevant costs in 2020 Norwegian kroner (NOK).

Eligible population for detection of ROS1 gene alterations

In 2019, about 3,320 new cases of lung cancer were diagnosed in Norway (42). Approximately 85% of lung cancer cases were classified as non-small cell lung cancer (43). Of these, 30%-50% were adenocarcinomas (43), and around 75% had locally advanced or metastatic disease at the time of diagnosis (44). Approximately 90% of these patients were eligible for first line treatment, ending up with about ~711 patients eligible for ROS1 testing per year in Norway (Table 3).

Table 3. *Estimated number of patients eligible for detection of ROS1 gene alterations*

	Number of patients	Comments
Lung cancer	3,320	New cases, Cancer Registry of Norway 2019 (42)
NSCLC	2,822	About 85% (43)
Adenocarcinomas	1,129	About 40% (43)
Locally advanced or metastatic	790	About 70% (44)
First line treatment	711	About 90%
Candidate for ROS1 testing	**711**	

Results

In this section, the costs associated with different diagnostic methods based on a micro-costing analysis and based on current Norwegian tariff rates for clinical laboratory services are presented. A summary of the information received from the commercial suppliers regarding the costs associated with the NGS panels presented in Appendix 6.

Costs associated with different diagnostic methods based on data of micro-costing analysis received from the Norwegian hospitals

In order to identify and measure the resource use and costs associated with the relevant diagnostic methods for the detection of ROS1, we contacted the four Norwegian regional health authorities. We received information about resource required for performing detection of ROS1 from three University hospitals: Stavanger University Hospital, St. Olav's University Hospital and Oslo University Hospital. The estimated costs varied according to the approach used to estimate costs by different hospitals, however, all these estimates included direct costs such as consumables and supplies costs and the cost associated with staff time. The resource used and unit costs are presented in Table 4. It should be noted that the costs for NGS are estimated for a panel that can identify rearrangement in ROS1, NTRK1,2,3, ALK and RET. For the other methods the costs were estimated for testing one biomarker, i.e. the costs for testing several biomarkers with these tests will be higher.

Due to data consistency, our analyses are based on information received from Stavanger University Hospital. However, the data sent from St. Olav's University Hospital and Oslo University Hospital are presented in Appendix 7.

Table 4. *Estimated cost for ROS1 testing with IHC and FISH and for testing several biomarkers with NGS**

Diagnostic methods	**IHC**		**FISH**		**NGS****	
	1 patient NOK	**10 patients NOK**	**1 patient NOK**	**10 patients NOK**	**1 patient NOK*** **	**10 patients NOK**
Reagent costs	1200	1,200	1,500	1500	14,480	1,730
Personal costs	494 (Bioengineer, pathologist)	457	671 (Bioengineer, Molecular biologist, pathologist)	654	1,821 (Bioengineer, Molecular biologist, pathologist)	594
Sum	**1,694**	**1,657**	**2,171**	**2,154**	**16,301**	**2,325**

** Based on feedback from individual hospitals, RT-PCR is not used to detect ROS1 or other gene alterations (ALK/RET/NTRK) at pathology labs in Norway. We have therefore not included PCR in our cost-analysis.*

*** The panel can analyse 8 samples og 6 biomarkers simultaneously.*

**** The estimated costs are associated with testing one sample.*

The cost estimation associated to detection ROS1 are presented in two different scenarios.

Scenario 1
Based on the Norwegian national guidelines and review of the literature on the available methods for the detection of ROS1 gene fusions. IHC may be used to triage upregulated ROS gene. If the ROS1 IHC is positive, FISH or other testing is required to confirm the diagnosis. As previously mentioned in this report, FISH is by many considered the gold standard test for the identification of ROS1 gene alterations (45).

In this scenario, we have assumed that all patients undergo at least one ROS1 IHC testing. IHC will be used as a pre-test with FISH confirmation. This resulted that approximately 5% of ROS1 IHC tested patients had a positive result and received FISH testing (personal communication by professor Emiel Janssen, Unit Head Molecular and Quantitative Pathology, Stavanger University Hospital). 100% test accuracy for FISH testing (gold standard test) was assumed for estimation of the number of eligible patients for confirmatory FISH testing. This assumption is discussed later in this report.

The utilisation of ROS1 IHC testing as a pre-test and patients eligible for FISH is presented in Table 5.

***Table 5**. Estimated number of IHC positive patients eligible for ROS1 FISH testing*

	Number of patients	Comments
Patients eligible for ROS1 testing with IHC	711*	
IHC positive patients confirmed by FISH testing	35	Expert opinion 5%**
ROS1-positive patients confirmed by FISH testing	**11**	FISH confirmation (100% sensitivity and specificity).

** See Table 4*

*** Source: (46)*

Total costs associated with ROS1 gene rearrangement testing by using IHC as a pre-test with FISH confirmation were estimated to be approximately NOK 1,277,500 for ROS1 testing in about 711 eligible patients per year in Norway.

The costs per IHC testing and per FISH testing are based on the mean costs presented in the Table 6.

Table 6. *Estimated cost related to ROS1 testing by using IHC as pre-test with FISH confirmation*

	Number of eligible patients	Cost per test* (NOK)	Cost of testing ROS1 in eligible patients (NOK)
Cost of ROS1 IHC testing	711	1,690	1,201,590
Cost of ROS1 FISH testing	35	2,170	75,950
Total costs by using IHC as pre-test with FISH confirmation			**1,277,540**

**Source: (46)*

Scenario 2

Running a sequence of single-gene tests can be time consuming and may require a relatively large tissue sample, which is not always available as NSCLC is often detected at an advanced stage and only small biopsy samples are usually available (47). Based on the experts' opinions, testing samples from several patients at once can save the use of resources and consequently the costs associated to the diagnostic methods. Thus, in two different analyses, we have presented a set of parallel tests performed on a number of molecular biomarkers relevant for advanced NSCLC (testing 2 or 3 biomarkers at the same time). The costs were calculated for the multigene testing and testing sample from one patient, and/or 10 patients as an example.

Scenario 2a: we presented the cost associated with using different diagnostic methods for testing ROS1 and NTRK in advanced NSCLC.
Scenario 2b: we presented the cost associated with using different diagnostic methods for testing ROS1, ALK and NTRK in advanced NSCLC.

In both scenarios the costs are presented when we run the test just for one patient or ten patients. All estimated costs are based on data that we received from Stavanger University Hospital (46). The results of scenario 2a and 2b are presented in Table 7.

Table 7. *Estimated costs associated with using different diagnostic methods for testing 2 or 3 biomarkers for one and 10 advanced NSCLC patients*

Diagnostic methods	Testing ROS1 and NTRK		Testing ROS1, NTRK and ALK	
	1 patient NOK	10 patients NOK	1 patient NOK	10 patients NOK
IHC*	2,890	2,850	4,160	4060

FISH	7,020	4,160	10,150	5,860
RT-PCR**	3,450	1,490	3,870	1,310
NGS* **	16,300	2,330	16,300	2,230

Source: (46)

** IHC positive results are required to be confirmed by the other methods.*

*** Based on feedback from individual hospitals, RT-PCR is not used to detect ROS1 or other gene alterations (ALK/RET/NTRK) at pathology labs in Norway. We have therefore not included PCR in our cost-analysis.*

*** The costs are estimated for Oncomine Focus panel. The panel can analyse 6 biomarkers simultaneously including ALK, RET, NTRK 1, 2, 3 and ROS1*

The results of the cost analyses showed that NGS can be one of the most affordable diagnostic methods. The method is considerably more expensive if only one patient (one sample) would be tested. However, the foremost advantage of the NGS technologies is the massively parallel sequencing capability. It means sequencing of multiple targeted genomic regions from the multiple samples in the same run. As the results showed in Table 7, the cost associated with NGS testing will be significantly decreased (approximately NOK 2,000 per patient) when parallel tests are performed on several biomarkers from multiple patients. Assuming about 711 patients are eligible for ROS1 testing annually in Norway, the costs are estimated to be about NOK 1,422,000.

IHC is less costly for testing one biomarker in one NSCLC patient. However, the costs will be increased as more than one biomarker are going to be tested at the same time. Furthermore, IHC is just recommended as pre-test for detection of ROS1 and NTRK. The positive results are required to be confirmed by the other relevant methods. Consequently, total costs can be increased for testing several biomarkers and samples.

The costs associated with using RT-PCR is less than the costs related to other tests. However, RT-PCR is a very specific technique, but it lacks somewhat in sensitivity and reliability. Rare fusion genes may be missed if the primer set for the multiplex PCR reaction does not cover the fusion gene in question, and quality mRNA may not be available from formalin fixed paraffin embedded (FFPE) tissue, the usual source of lung cancer diagnostic material. This technology is not widely available and requires special expertise. By our knowledge, none of Norwegian hospitals uses RT-PCR for the detection of ROS1.

Other relevant costs

Preparing the biopsy

According to our experts, the costs associated with preparing the biopsy is the same for all diagnostic methods. Therefore, the biopsy costs are not included in the comparison between diagnostic methods. Based on data we have received from St. Olav's University Hospital the cost per biopsy per patients is approximately NOK 270 (Table 8).

Table 8. *Costs per biopsy per patient*

	Costs per biopsy NOK	**Comments**
Formaldehyde 20 ml	12.66	50 pieces per package
Biopsy forceps	163.50	20 pieces per package
Biopsy wraps	1.24	Bio-wraps (100 pieces per package)
Personnel costs: nurses	92.00	10 minutes per biopsy
Total cost per biopsy per patient	**269.40**	

Source: personal communication St. Olav's University hospital

The costs related to the infrastructure, quality assurance and maintenance

IHC and FISH

Based on the information from the experts, all hospitals in Norway can perform IHC. For FISH testing there is a need for a fluorescence microscope/ scanner to be able to interpret the results in addition to the equipment for IHC. All university hospitals and some of the regional hospitals can perform FISH in Norway. However, the infrastructure costs for IHC and FISH is estimated to be around NOK 750,000 and NOK 2,750,000, respectively (personal communication, Oslo University Hospital)

Most pathology departments already have a maintenance plan and agreements on their IHC machines, thus there is no extra cost associated with the introduction of ROS1 or NTRK with IHC or FISH. External quality rounds (NordiQc, EMQN) is estimated to cost around NOK 5,000 per year.

NGS

Currently, about nine pathology departments in Norway have equipment (six hospitals have Ion Torrent S5 and three hospitals MiSeq) to run RNA sequencing.

Depending on the equipment capacity, it is estimated that the costs related to equipment and supplies investments are approximately 3 to 4 million NOK. The maintenance costs of NGS instruments are calculated to be about 150,000 per year.

Based on the information from the experts, the validation process takes a lot of time and different types of expertise (incl. bioengineers, pathologists, engineers) are involved in this process. At least one NGS kit is used for the validation process. The price of such a kit varies from NOK 30,000 to 80,000. The validation process is only performed once, when the method is established. After that external quality control system is used to check that all is still functioning adequately.

Costs associated with different diagnostic methods based on current tariff rates for clinical laboratory services

Currently, the reimbursement of the expenses related to using diagnostic methods in pathology laboratories calculated based on the Norwegian tariff rates for clinical laboratory services (48). In Table 9, we present the cost per investigation associated with different diagnostic methods for the detection of ROS1 based on the tariff rates. We have received feedback from the experts that PCR is not usually used for testing ROS1 in Norway, and therefore it is not presented here. The average cost is calculated as the total of the tariff per investigation and the patient's co-payment, multiplied by two (49).

Table 9. *Average costs associated with using the relevant diagnostic methods based on the Norwegian tariff rates*

Diagnostic methods	Costs per test NOK	Comments
ROS1 IHC	714 (245+469)	705b:3-7 blocks, 705k: 4 or more analyses
ROS1 FISH	445	701g: 1-3 probes
ROS1 NGS*	14,363 (107+14,256)	701b: Organic extraction of DNA/RNA, 705s: DNA/RNA gene sequencing analysis package

Source: Lovdata poliklinikk-takster 2020 (48). The numbers are rounded off.
RT-PCR is not used for testing ROS1 in Norwegian hospitals; therefore, it is not presented.
**The tariff is used per analysis package, if both RNA and DNA sequencing are performed, the tariff can be used twice per patient. For testing ROS1, we included just the costs associated with RNA sequencing. The tariff is only used for outpatients or samples sent to the laboratory.*

In Norway, outpatient laboratory services are financed on block grants, co-payments from the patients, and tariff rates while laboratory services for inpatients are financed on case-based payment (Diagnosis-related group, DRG).

A comparison between the estimated costs based on the micro-costing method and the costs estimated based on the current tariff rates indicated that the current tariff rates are generally insufficient to cover the costs of running IHC and FISH methods. Regarding NGS testing, the analyses showed that the current tariff rate for NGS-testing can cover the costs of running the small NGS assay (for example, using Oncomine Focus assay gene fusion detection for 23 selected genes). However, based on the information received from the suppliers of the commercial tests and the feedback from the experts, the reimbursement rates of 2020 are probably not sufficient to cover the costs of running the NGS panels to assess a wide range of biomarkers[1].

[1] Our analyses are preformed based on the tariff rates for 2020. However, the larger NGS panels have received a new tariff rate in 2021 (705u).

Discussion

Summary of main results

We have in this HTA summarised available evidence on the analytical validity, the clinical validity, and the clinical utility of single gene tests (IHC, FISH, RT-PCR) and multigene panels (NGS) for the detection of ROS1 gene alterations in people with locally advanced or metastasised NSCLC. We have also summarised information addressing the advantages and disadvantages of these tests, and qualitative evidence on the preferences of patients related to pharmacogenomic testing.

We identified and included one systematic review (27) of critically low quality that provided comparative data on the sensitivity, specificity, concordance of tests for the identification of ROS1 gene alterations; six narrative reviews that provided information on the pros and cons of these tests (9, 18-22, 27). A survey of Norwegian hospital trusts reporting barriers to implementation and challenges with NGS analyses in general (29), and two reviews of patient preferences related to pharmacogenomic testing (25, 26) were also included. We did not identify any end-to-end studies.

Pooled results from the one systematic review (27)suggest that IHC, due to its tendency for false positive staining, can only be used to screen for ROS1 alterations, if positive samples are confirmed by FISH or other methods, which was supported by un-pooled results from three of the narrative reviews (9, 20, 21, 27). This is in line with the current testing routine for detection of ROS1 gene alterations at Norwegian hospitals.

Un-pooled results from one small narrative review (in total 11 samples) comparing IHC/FISH with NGS (18) indicate high sensitivity but lower specificity of NGS, and likewise un-pooled results from another small narrative review (2 studies, 59 samples) suggest good concordance of NGS with gold standard (FISH) in detecting ROS1 gene alterations in NSCLC. However, as these results are based on evidence from non-systematic reviews, including few participants, they are very uncertain and need to be verified in larger, well-defined cohorts of patients See Appendix 4.

None of the included reviews reported on outcomes of importance to patients (e.g. overall survival, quality of life), or how well the tests predict the treatment effects (e.g. shrinking of the tumour or slowing down of the disease progression).

In conclusion, the evidence-base for the diagnostic accuracy of tests for detection of ROS1 gene alterations in people with locally advanced or metastasized NSCLC is scarce and incomplete. End-to-end studies are non-existent, and therefore the clinical utility of the tests remains unknown.

Feasibility of tests

See Table 2 for details
There are pros and cons with all tests used for the identification of ROS1 alterations, i.e. few if any tests have low tissue requirements, can work on poor material, have clear cut-offs/standardised scoring, short turnaround time, good coverage (do not miss some fusions), require little hands-on-time, and do not require extensive expertise to run and interpret the analyses, and can analyse many genes simultaneously.

IHC has the advantage of being widely available at Norwegian laboratories, while PCR is not. NGS is becoming increasingly available. FISH for ROS1 is only established in a few university hospitals, and all other hospitals send their samples to these hospitals. A second advantage with IHC is the low material requirement for the analysis -only a few cells, while FISH require a little more, and both NGS and PCR require up to 1000-1500 cells for the analysis. Another advantage with IHC is that it can be run successfully on technically suboptimal materials, while both NGS and PCR require high quality RNA, and poor RNA from FFPE samples can cause problems with the NGS analysis.

One advantage with NGS is that hundreds of genes can be analysed in the same run, while the other tests can only analyse one gene at the time. Another advantage with NGS is that it is capable of identifying both known and unknown fusions. FISH even though considered the 'gold standard', may differ slightly in coverage between different probe sets, and PCR may miss identifying some fusions. We found no information for the coverage of IHC.

IHC lacks a standardized cut-off and scoring systems, has a tendency for false positive staining, and problems with subjectivity of interpretation, while FISH has a standardised cut-off, and typically a clear break-apart signal. We found no

information for NGS and PCR on the clarity of tests, and for NGS there are no internationally agreed cut-offs or standards.

An advantage with IHC and FISH is that both methods can be performed relatively rapidly, and that interpretation is usually straightforward, without the use of advanced software. NGS on the other hand employs advanced technology and personnel with molecular and bioinformatic expertise, typically requiring 2 weeks.
There are thus some differences in the expertise (and training) needed, as well as in the amount of hands-on-work required for running of the different tests, but this information was not available for all included tests.

Conserving tissue and reducing the risk of adverse events

It should be noted that ROS1 is one of an increasing number of biomarkers with available targeted treatment that are relevant to test for in patients with NSCLC. Due to this and the limited size of lung biopsies (28), it has been suggested that using sequential single-gene tests may result in exhaustion of the available material and patients not being tested for all relevant biomarkers (18, 28). This may potentially result in increased risk of adverse events due to repeat biopsies, pain and discomfort for the patient. Whether exhaustion of the sample tissue, and repeat biopsies, are common problems when single gene tests are used, could not be addressed in this HTA, as none of the included reviews provided any data for such a comparison.

Saving time from test ordering to administration of treatment

Short test turnaround time enabling timely administration of targeted drug treatment without unnecessary delay, potentially play an important role for patients with advanced or metastasised NSCLC. Results from a modeling study, conducted in the US, suggest that NGS analyses will save time compared to sequential single-gene analyses (50). However, as there appear to be relatively large differences in turnaround time between different NGS systems, the time that potentially can be saved by using NGS, as compared to sequential single-gene tests, must depend heavily on which NGS system that are used. Also, the organization of test services plays an important role for the turnaround time and thus for the timeliness of the treatment decisions, e.g. whether the laboratories in question run NGS analyses every day, or once a week, which at present is the case at Norwegian hospital trusts.

ELSI and patient preferences

We did not identify any reviews concerned with the ELSI of pharmacogenomic testing, (e.g. confidentiality, disclosure of genomic test results, ownership of data, panel testing for mutations with no available treatment, and the costs of scaling up of pharmacogenomic testing and targeted therapies) (51). We believe that due to its complexity, ELSI need to be addressed in a separate publication.

Patients' preferences related to pharmaco-genomic testing appear to depend on several factors, of which some are related to the test per se. One factor of importance to patients is the invasiveness of the test (52). As mentioned earlier, NGS may, due to its capacity to analyse numerous genes simultaneously, have the potential to conserve material and reduce the risk of repeat biopsies (18). There is also ongoing research and method development related to the use of liquid biopsies for pharmacogenomic testing, which if moved into routine use, would relieve patients from the pain and unpleasantness of many biopsies (53). Another factor of suggested importance to the preferences of patients is the sensitivity and specificity of the test i.e. the rate of false negative tests resulting in a missed treatment opportunity, or the risk of having to endure unnecessary drug treatment due to a false positive test. The sensitivity and specificity of tests is probably not an issue if IHC is used to screen for ROS1 gene alterations and FISH or other methods (e.g. NGS) are used for confirmation.

Yet, another factor of relevance for patients, is the prevalence of the gene alteration. ROS1 gene alterations are not very common in advanced or metastasized NSCLC (1-2 %), which means that maybe as few as 10 people of around 700 available for testing, may potentially benefit from being tested and receiving the targeted treatment. There are however other targetable gene alterations of relevance for this patient group (e.g. ALK, EGFR, NTRK, RET). If these biomarkers are simultaneously tested for using multi-gene panels, the potential for finding targetable gene mutations increases, which may be a motivator for the patient to take the test.

Challenges with this assessment

This is one of the first assessments of diagnostic tests for the identification of targetable gene mutations in patients with cancer conducted at NIPH, in which we also pilot a new assessment framework for pharmacogenomic tests.

We experienced numerous challenges when conducting this assessment. Some of which were directly related to the commission per se, since the task we were given

was very general and therefore called for an unusually exploratory approach. For example, it was from the beginning unclear (i) what the tests of interest were (IHC, FISH, PRC, NGS, or other); (ii) what test versions, or specific applications to consider; (iii) where in the clinical pathway the tests were to be used (i.e. a screening test used first in a row of tests, a confirmatory test, or a stand-alone test); (iv) what the outcomes of interest were, i.e. whether the report was expected to cover diagnostic test accuracy, feasibility, organizational/service delivery aspects, costs, ELSI, patient preferences, or even healthcare professionals attitudes and experiences of pharmacogenomic tests.

Other challenges were, first, the lack of a real-world scenario, in which more than one gene alteration was tested for, which typically would be the case in a clinical situation. Second, the lack of information on how the results of the report will be amalgamated with the report on the effectiveness and safety of drugs conducted by NoMA. Third, how the report will be used by decision-makers. Due to these challenges it was difficult to ensure the usefulness of this assessment for decision-makers.

It may be helpful for future commissions to consider the guideline on the assessment of companion tests and treatment effects produced by CADTH (CADTH 2019). The guideline states that the decision problem should encompass both the treatment and the companion diagnostic, and that the companion diagnostic(s) to be evaluated should be clearly described. The guideline further recommend that the decision problem should clearly specify where and when in the clinical pathway the companion diagnostic will be used and how its result will inform the subsequent treatment decision, In addition, factors that may impact upon the clinicians' behavioural response to a companion diagnostic result, patient acceptance of test and results, and adherence to treatment based on the test results, should during the scoping of the decision problem be considered to determine whether they are relevant for consideration (CADTH 2019).

We hope this pilot will be of help in detailing future commissions and in deciding what assessments of diagnostic tests should cover, and not cover, as this is crucial to ensure that future assessments are of help to decision makers.

Limitations with this review

We did not, due to time constraints, search for primary studies, and may therefore have missed including important evidence. However, we have done simplified searches without finding many relevant studies.

We identified one eligible systematic review for inclusion in this HTA, which according to AMSTAR was of critically low quality, and a handful of narrative non-systematic reviews. While systematic reviews are considered the highest level of evidence, non-systematic or narrative reviews, are generally considered as low-level evidence. Normally we would not include narrative reviews in an HTA, but as the narrative reviews seem to provide valuable information on the feasibility of tests, we chose to include these reviews to demonstrate the construct of the new framework we are piloting in this HTA.

We did not find any reviews comparing the accuracy, turnaround time, material requirements etc. of different NGS-systems, and have summarised the information provided by five NGS suppliers in text and tables. The aim with this HTA also focused mainly on comparing different type of tests, and not NGS systems from different suppliers.

The reviews included in this HTA, provided very little information on the participating patients, other than that they had locally advanced or metastasised NSCLC. We therefore do not know whether they had previously received TKIs (e.g. criznotinib), which was one of our exclusion criteria.

As mentioned earlier, we did not find any end -to-end studies, and cannot therefore say anything about the outcomes of importance to patients (e.g. overall survival, quality of life), or how well the tests can predict the effectiveness of treatment (e.g. shrinking of the tumour, or slowing down the disease process). Nor can we say anything about whether taking a test, or not taking a test makes a difference for overall survival.

Economic evaluation

Several testing strategies for the detection of ROS1 mutation in NSCLC exist, however there is still lack of the evidence on the comparative economic implications of using these strategies in patients with NSCLC in Norwegian clinical practice. We assessed the costs associated with the relevant diagnostic methods based on the data received from Norwegian hospitals, the laboratories of molecular biology.

Our assessment showed that the costs with NGS approach are likely to be higher than the other diagnostic methods if it is used for one patient only, which is due to the higher reagent costs of the NGS test. However, as NGS technology allows massively parallel sequencing and testing samples from several patients at once, the cost associated with NGS testing will significantly be decreased when parallel tests are to be performed on several biomarkers from multiple patients. In addition, the need for tissue preservation and the burden and comorbidity of repeat biopsies is likely to decrease.

The results showed that the cost associated with using NGS was around NOK 16,000 per sample. However, if several samples and biomarkers, e.g. NTRK, ROS1, RET and ALK, are tested with NGS at the same time, the costs will be reduced to approximately NOK 2,000 per patient. Assuming about 711 patients are eligible for ROS1 testing annually in Norway, the costs are estimated to be about NOK 1,422,000. Alternatively, if we assume that patients are tested for ROS1 in line with the current guidelines, using IHC as pre-test with FISH confirmation, the costs were estimated to be approximately NOK 1,277,500 for ROS1 testing in 711 eligible patients per year. The costs did not include overhead, capital, and other infrastructure costs.

Based on the data from the Norwegian pathology departments, the capital and infrastructure costs (including overhead costs) are higher for NGS than the other diagnostic methods. It has been estimated that the costs related to NGS equipment and supplies investments is approximately NOK 3-4 million. Further, the costs associated to maintenance the NGS instruments is more expensive (around 30,000- 80,000 NOK) than the other methods. The validation process for any of the techniques is challenging as there are so few positive cases reported in Norway. A validation with 5-10 positive cases would be preferable but this is in practice very difficult. As such, the validation process at local hospital is dependent on positive cases/controls from other countries/companies.

A comparison between the estimated costs based on the micro-costing method and the costs estimated based on the current tariff rates indicated that the current tariff rates are generally insufficient to cover the costs of running IHC and FISH methods. The analyses also showed that the current tariff rate for NGS-testing can cover the costs of running the small NGS assay. Based on the tariff rates of 2020, the current reimbursement rates are probably not sufficient to cover the costs of running the larger NGS panels. However, these panels have received a new tariff rate in 2021 (705u) but this is not included in our calculations.

Multiple labs also reported differences in reimbursement for internal versus external testing (inpatient versus outpatient) which may led to apparently unintended consequences, such as clinicians triaging groups of patients to prioritize for external testing when assays were not available in-house, or electing to perform diagnostics in an outpatient rather than inpatient setting if possible (54).

All hospitals in Norway can perform IHC. FISH for ROS1 is only established in a few university hospitals, and all other hospitals send their samples to these hospitals.

About eleven Norwegian hospitals have invested in NGS technology and some of them have already access to the equipment of two commercial suppliers of NGS (Thermo Fisher and Illumina). The price sent from Illumina (TruSight™ Oncology 500, covers

523 cancer-relevant genes)[1] and Thermo Fisher (Oncomine™ Focus Assay, gene fusion detection for 23 selected genes), is approximately [redacted] per test (the maximum batch size of seven samples) and [redacted] (excl. value added tax) per sample, respectively.

In addition, we have received the information from three other NGS suppliers (Roche, Caris' Life Science and Archer). For using the technology of these three suppliers, the patients' tumor samples should be sent abroad to the external laboratories. After preparation and conducting the test, a clinical and biological report will be sent to the local hospitals. If eligible, they will return of remaining parts of the tumor block. One can discuss that this approach may to some cost-saving due to the work performed by local experts at the pathology departments need to conduct the test will be saved. However, it is important to mention that precision medicine is an interdisciplinary field that requires multidisciplinary collaboration among different field of expertise including pathology, oncology, and the laboratory. In addition, the legal and ethical consequences of this approach should be considered and assessed. The list price sent from these three suppliers (Roche, Caris' Life Science and Archer) is approximately between NOK 25,000-37,000. Roche offers a net price of [redacted] excluding value added tax (FoundationOne® CDx, covers 324 genes). The price includes all the relevant procedures from pick up and transport of tumor sample to deliver the clinical report and return of remaining parts of the tumor block if eligible. Archer and Caris are also opened to discuss the price of RNA -sequencing analysis.

Although, we have tried to conduct our analysis based on the best available data, lack of cost data comparing different diagnostic methods was the most important limitation of this economic analysis. We contacted all Norwegian regional health authorities. We received data from three hospitals based on the relative different approaches. The data related to different diagnostic methods for ROS1 testing were presented in different scenarios by Stavanger University Hospital, due to data consistency, our analyses are based on data received from this hospital.

The estimation of the costs associated with ROS1 IHC testing, followed by confirmation ROS1 FISH testing (scenario 1) was estimated based on the 100% test accuracy for Fish testing. However, it is reasonable to assume that 100% sensitivity and specificity was only true for FISH testing of ROS1 gene rearrangements in ideal circumstances. The size of the population eligible for ROS1 FISH testing was also dependent in ROS1 IHC sensitivity and specificity in Norwegian practice.

[1] We did not receive cost information for the smaller gene panels from Illumina (e.g. Illumina AmpliSeq 52 genes).

In scenario 1, it is also reasonable that some patients might not undergo ROS1 FISH. Additionally, some ROS1 FISH testing would be repeated. We have not included these scenarios in our analyses.

We have not considered the additional re-biopsy costs in our analyses. It should be noted that a large group of patients with advanced NSCLC have indeed inadequate biopsy tissue for additional testing.

Challenges

Recently, the number of economic evaluations on precision medicine has noticeably increased. However, an important number of these analyses focus purely on the assessment of the actual therapeutic treatment, failing to include the impact that the actual tests have on the overall economic value of the test–drug combination (55). Even, when both testing and therapeutic decisions are taken into consideration, the weight of the accuracy of the companion tests on the overall results is rarely explored (55).

In principle, the public sector benefit from the availability of companion diagnostics that accurately identify responders, reduce the number needed to treat, and thereby improve the efficient use of resource. Therefore, the consequences of implementation test-and-treat interventions and system integration challenges should be considered by the reimbursement authorities. In addition to that a good biomarker test should measure the biomarker with a high degree of accuracy and demonstrate analytical validity, the biomarker test should optimally demonstrate clinical utility, meaning that it improves patient's outcomes compare to a no-testing approach.

This requires establishing the relationship between the test results and the consecutive treatment, and outcomes. Generating evidence to support the economic case of a precision medicine in practice, however, can be a challenge. Manufacturers, analysts, and funders of research may improve their research and development activities by considering the evidenced required by later-stage decision-makers at an earlier time period in the process of evidence generation.

Principally, the basic principles of cost-effectiveness should be applied to biomarkers. However, cost-effectiveness estimates for recent pharmaceutical-diagnostic combinations have been highly variable among major HTA markets, suggesting that methods for incorporating test information into economic evaluations are inconsistent. Key issues include gaps in the evidence supporting clinical utility and cost effectiveness of diagnostics (56).

Usually, information on treatment patterns and on the costs and outcomes relating to using diagnostic methods, is the most limitation of the published economic evaluation of precision medicine interventions, especially data about false-positive and false-negative test results (56). The higher specificity rates of the diagnostic method will

help to reduce the potential treatment of 'false positives' and consequently engaging in high spending for a proportion of the patient population for which the targeted therapy would not be effective or for which it could even produce some harm. Furthermore, the analysis can even become more complex if different tests are combined or sequentially used. This potential complexity can be handled by explicitly showing how these tests are going to be used in practice and then working with the combined sensitivities and specificities of the tests (39).

Moreover, the tests available and the test sequence employed in the clinical study, may differ in their ability to accurately select patients who will likely benefit from target therapy (57). San Miguel and co-writer (55) has studied the potential impact that changes in diagnostic sensitivity and specificity may have on the economic value of test–intervention combinations. The results of this study have shown that in the field of targeted therapy in oncology, test accuracy becomes even more crucial given the high effectiveness but also high prices of some of these therapies. The importance of the test specificity becomes even more crucial if very small population subsets are to be identified using the biomarker, as in the example of lung cancer that the mutations are often present in no more than 1 to 2% of a specific tumor type (58). The importance of test specificity; for the patient it is crucial to receive the correct targeted treatment; for society the use of (often expensive) targeted treatment in patients that do not benefit from it because the marker was not tested accurately is a waste of money.

In addition to the issues related to properly assessing the cost effectiveness of test-drug mentioned above, there are regulatory barriers to the development and adoption of precision medicine. First, the regulation of marketing approval is insufficiently harmonized. It varies across countries and is different for drugs and diagnostic tests. In the United States, marketing approval for drugs and diagnostics is done by the FDA. The joint approval process performed by a single agency ensures scientific knowledge-sharing and provides an effective way to approve precision medicines. However, in Europe, no single European agency regulates both medicines and tests. The European Medicines Agency (EMA) regulates the marketing approval of drugs, whereas it is each European Union member state's Notified Body that monitors the performance standards of diagnostic tests (59).

Furthermore, the requirements for marketing approval of tests are still relatively lenient. In Europe, the test manufacturer is currently required to demonstrate the clinical validity (predictive capability) but not the clinical utility (effect on clinical outcomes) of the test. However, in 2017 the EU parliament and council agreed on a new set of regulations on in vitro diagnostics (60). Based on these new regulations, companion diagnostics will need to meet stricter performance requirements, including clinical evidence and there will be a link between the assessment of a diagnostics by a notified body and the corresponding medical product by a medicine regulatory authority. It means that if a companion diagnostic is necessary to identify whether a patient is likely to benefit from a corresponding medical product, the evidence

regarding its impact on patient outcome, i.e. clinical utility, will be carefully considered by the medicine authorities, in determining the benefit/risk of the medical product. Consequently, these evidence on clinical utility of companion diagnostics can be used in evaluation of health economic consequences of the test and and the consecutive treatment. The regulations become full effective in 2022 (60).

Another important challenge related to assess the cost-effectiveness of diagnostic methods is that, currently, the manufacturer of a new test does not need to demonstrate its effectiveness if a similar test already exists. Moreover, laboratory developed tests, that is, tests performed within a single laboratory or hospital (not commercialized)– do not require a full regulatory review (59). Therefore, there is a lack of standardized evidence of the performance of biomarker tests in terms of their impact on health outcomes. This results in uncertainty for health authorities who make decisions on pricing and reimbursement based on the value of treatment produced by the biomarker test.

Conclusion

The evidence for the diagnostic accuracy of tests for the detection of ROS1 alterations in people with locally advanced or metastasised NSCLC is scarce and incomplete. This HTA supports that IHC can be used to screen for ROS1 gene alterations only if verified by FISH or other methods, which is in line with the current testing routine at Norwegian hospitals.

Data on concordance between IHC plus FISH versus NGS is scarce, and thus need to be confirmed in larger cohort studies. We did not identify explicit data on adverse events related to the different tests, but NGS may potentially, due to its capacity to analyse multiple genes simultaneously, reduce the risk of depletion of scarce biomaterial and the need for repeated biopsies, and thereby related morbidity, pain, and discomfort for the patient.

Single testing for ROS1 using IHC as pre-test with FISH confirmation is possibly less costly than NGS method, it needs to be considered that patients with advanced NSCLC also need to be tested for other biomarkers. The cost associated with NGS testing will decrease significantly when parallel tests are to be performed on several biomarkers from multiple patients. However, at present, the capital and infrastructure as well as maintenance costs are higher for NGS than the other diagnostic methods.

The current tariff rates are generally insufficient to cover the costs of running IHC and FISH methods. Based on the tariff rates of 2020, the reimbursement rate for NGS-testing can cover the costs of running the small NGS assays.

Biomarker tests should optimally demonstrate clinical utility, meaning that it improves patient's outcomes compared to a no-testing approach. We did not identify end-to-end studies and were therefore unable to explore associations between test strategy and clinical outcomes of importance to patients. The consequences of implementation test-and-treat interventions and system integration challenges should be considered by the reimbursement authorities. Future research should focus on conducting large cohort studies with well-defined patient populations, that follows the patients from testing (or no testing), through treatment and final outcomes.

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Appendices

Appendix 1 Glossary

Adenocarcinoma	a cancer type that begins in glandular cells that are found in tissue that lines certain internal organs like for example the lungs
Advanced cancer	locally advanced cancer is cancer has grown outside the body part it started in but has not yet spread to other body parts
Analytical validity	or technical performance, is a test's ability to accurately and reliably *measure* a biomarker of interest (sensitivity, specificity, assay robustness, and quality control). According to EGAPP definitions https://www.cdc.gov/genomics/gtesting/egapp/recommend/method.htm
Gene alteration	a somatic gene alteration
Antibody clone	selected antibodies with the characteristics that work well for immunohistochemistry (IHC)
Chemotherapy	a drug treatment aimed at killing cancer-cells
Clinical utility	impact on patient outcomes, refers to how likely it is that using the test to guide clinical decisions will significantly improve outcomes related to patients health and well-being (benefits vs. harms, whether using the tests gives added value to not using it, effectiveness, and efficacy). According to EGAPP definitions https://www.cdc.gov/genomics/gtesting/egapp/recommend/method.htm.
Clinical validity	or the strength of clinical correlation, is a test's ability to accurately and reliably identify or *predict* the disorder of interest (sensitivity, specificity, positive predictive value, negative predictive value). According to EGAPP definitions https://www.cdc.gov/genomics/gtesting/egapp/recommend/method.htm
Concordance rate	the number of subjects that are concordant (in agreement) over the total number of subjects assessed
Confidence interval	a type of estimate computed from the statistics of the observed data that proposes a range of plausible values for an unknown parameter (e.g. the mean)

End-to-end study	a study that follows patients from testing, through treatment, to final outcomes
Entrectinib	an anti-cancer medication, and a selective tyrosine kinase inhibitor that may be used to treat ROS1-positive non-small cell lung cancer and NTRK fusion-positive solid tumors
Fluorescence in situ hybridisation (FISH)	a laboratory method for detecting and locating a specific DNA sequence on a chromosome that relies on exposing chromosomes to a small DNA sequence called a probe that has a fluorescent molecule attached to it; the probe sequence binds to its corresponding sequence on the chromosome.
Fusion gene	a gene made by joining parts of two different genes
Genomics	a term that refers to the molecular composition of a tumor
Immuno-Histo-Chemistry (IHC)	a laboratory method that uses antibodies to check for certain antigens in tissue samples. The antibodies are usually linked to an enzyme or a fluorescent dye, which when activated allows the antigen to be seen under a microscope
Immunotherapy	a type of cancer treatment that helps your immune system fight cancer
Metastatic cancer	a cancer that spreads from its site of origin to other parts of the body
Molecular test	a laboratory test that checks for certain genes, proteins, or other molecules in a sample of tissue, blood, or other body fluid, or that check for certain changes in a gene or chromosome
Multi-gene panel	a genetic test that use next-generation sequencing to test multiple genes simultaneously
Multiplexing	A method for detecting multiple genetic alteration simultaneously
Mutation (somatic)	a genetic alteration acquired by a cell that can be passed to the progeny of the mutated cell in the course of cell division.
NanoString nCounter	a variation on the DNA microarray technique that uses molecular "barcodes" and microscopic imaging to detect and count up to several hundred unique transcripts in one hybridization reaction
Next Generation Sequencing (NGS)	also called massively parallel, deep sequencing or multigene panel, is a DNA sequencing technology by which entire human genome can be sequenced within a single day
Non-squamous lung cancer	a type of NSCLC that typically originates in peripheral lung tissue
Non-small-cell lung carcinoma (NSCLC)	any type of epithelial lung cancer other than small-cell lung carcinoma (SCLC), which accounts for about 85% of all lung cancers

Radiation therapy	a type of cancer treatment that uses high energy beams most often X-rays, but also protons or other types of energy, to kill cancer cells
Gene rearrangement	a programmed DNA recombination event that occurs during cellular differentiation to reconstitute a functional gene from gene segments separated in the genome
ROS1 gene	a gene that makes a protein that is involved in sending signals in cells and in cell growth: the protein is a type of receptor tyrosine kinase
Reverse transcription polymerase chain reaction	or RT-PCR, is a laboratory technique combining reverse transcription of RNA into DNA and amplification of specific DNA targets using polymerase chain reaction
Sensitivity	the ability of a test to correctly identify those with the disease (true positive rate)
Single-gene test	a test that look for changes in only one gene.
Specificity	the ability of the test to correctly identify those without the disease (true negative rate)
Somatic gene mutation	a type of alteration in DNA that occurs after conception.
Squamous cell carcinoma	a type of NSCLC that typically originates in one of the main bronchi of the lungs
Systematic review	a review of the evidence on a clearly formulated question that uses systematic and explicit methods to identify, select and critically appraise relevant primary research, and to extract and analyze data from the studies that are included in the reviewy
Turnaround time (TAT)	the total test cycle which include ordering of test, collection, identification, transportation, preparation, analysis, reporting, interpretation and action
Therapeutic TAT	the interval between when a test is requested to the time a treatment decision is made

Appendix 2 Full Search strategy

Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily 1946 to September 30, 2020; Embase 1974 to 2020 September 30

	Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily 1946 to September 30, 2020 **Embase** 1974 to 2020 September 30	
1	(Sequence Analysis, RNA/ OR Sequence Analysis, DNA/ OR High-Throughput Screening Assays/) use ppezv OR (exp Sequence Analysis/ OR High Throughput Screening/) use oemezd OR (NGS OR sequencing OR minisequenc* OR pyrosequenc* OR profiling OR molecular diagnostic* OR ((multigene OR multi-gene OR multiplex OR multi-plex) ADJ6 (assay* OR panel*)) OR ((fusion* OR mutation* OR rearrang* OR re-arrang* OR mRNA OR sequenc*) ADJ6 analys*) OR ((high-throughput OR messenger RNA OR mRNA OR phosphoproteomic*) ADJ3 screen*)).tw,kw,kf.	1698336
2	In Situ Hybridization, Fluorescence/ use ppezv OR Fluorescence In Situ Hybridization/ use oemezd OR ((fluorescen* in-situ ADJ (hybridisation OR hybridization)) OR FISH).tw,kw,kf.	454528
3	Reverse Transcriptase Polymerase Chain Reaction/ use ppezv OR Reverse Transcriptase Polymerase Chain Reaction/ use oemezd OR (polymerase chain reaction OR PCR OR ddPCR OR rtPCR).tw,kw,kf.	1693961
4	exp Immunohistochemistry/ use ppezv OR exp Immunohistochemistry/ use oemezd OR (antigen retrieval OR immunohistochemistry OR immunohistocytochemistry OR immunocytochemistry OR immunolabel* OR ((goodpasture OR immunogold* OR immunoproxidase OR immunophosphatase OR peroxidase) ADJ (stain* OR techni*)) OR immunostaining OR IHC).tw,kw,kf.	1514554
5	(Oncomine* OR FoundationOne* OR TruSight* OR TSO500* OR Caris Molecular* OR OncoDEEP* OR FusionPlex* OR MSK Impact* OR MSKImpact* OR "Archer DX" OR Illumina* OR ThermoFisher* OR Thermo Fisher* OR Qiagen* OR Therascreen* OR MassARRAY* OR Sequenom* OR SNaPshot* Multiplex* OR Rabbit* mAb* OR VENTANA*).tw,dm,dv.	216518
6	("proto-oncogene tyrosine-protein kinase" OR ROS1 OR "ROS-1" OR "ROS 1" OR "ROS proto-oncogene 1" OR MCF3).tw,kw,kf	5096
7	(("proto-oncogene tyrosine-protein kinase" OR ROS1 OR "ROS-1" OR "ROS 1" OR "ROS proto-oncogene 1" OR MCF3) ADJ6 (detect* OR screen* OR test*)).tw,kw,kf	950
8	(("proto-oncogene tyrosine-protein kinase" OR ROS1 OR "ROS-1" OR "ROS 1" OR "ROS proto-oncogene 1" OR MCF3) AND (detect* OR screen* OR test*)).ti	191

9	Carcinoma, Non-Small-Cell Lung/ use ppezv OR Non small cell lung cancer/ use oemezd OR (NSCLC OR ((lung OR pulmon*) ADJ3 (adenocarcinoma* OR cancer* OR carcinoma*))).tw,kw,kf	535611
10	(Meta-Analysis.pt. OR Systematic Review.pt OR Review.pt. OR "Review Literature as Topic"/ OR "Meta-Analysis as Topic"/ OR "Technology Assessment, Biomedical"/ OR exp Guideline/ OR Cochrane Database of Systematic Reviews.jn.) use ppezv OR (Systematic Review/ OR Review/ OR Meta Analysis/ OR Biomedical Technology Assessment/ OR Practice Guideline/ OR Cochrane Database of Systematic Reviews.jn.) use oemezd OR (((systematic* OR evidence OR research OR literature) ADJ3 (overview* OR synthes*)) OR review OR reviews OR guideline* OR meta-anal* OR metaanal* OR metanal* OR technology assessment* OR HTA OR pubmed OR medline OR embase OR cinahl OR cinhal OR cochrane OR handsearch* OR ((comprehensiv* OR systematic* OR manual OR hand OR database) ADJ3 search*)).tw,kw,kf	8263382
11	(2015 OR 2016 OR 2017 OR 2018 OR 2019 OR 2020).yr.	16055811
12	((OR/1-5) AND 6) OR 7 OR 8	3043
13	9 AND 10 AND 11 AND 12 [MEDLINE: 133; Embase 292	425

Epistemonikos

	Søkegrensesnitt: Advanced search – Title/Abstract Filters: Publication Year 2015-2020	
	ROS1 OR "ROS-1" OR "ROS 1" OR MCF3 OR "ROS proto-oncogene 1"	Broad Synthesis: 3 Structured Summary: 1 Systematic Review: 16

INAHTA database

	ROS1 OR "ROS-1" OR "ROS 1" OR MCF3 OR "ROS proto-oncogene 1" OR "c-ros-1"	1

Appendix 3 List of excluded studies

Study First author (reference no.)	Cause for exclusion of study
Aguado 2016(53)	Not a review. Descriptive. No real comparison of FISH and NGS.
Coffman 2018(61)	Abstract only.
Conde 2018 (62)	Abstract only. Belongs with the FT by Conde below.
Conde 2019 (63)	Not a review. Novel ROS 1 IHC clone , and D4D 6 antibody studies using FISH positive and negative samples. Original paper.
Dagago-Jack 2014(64)	Abstract only. Detection of ROS 1 in plasma using NGS.
Dooms 2019(65)	Abstract only. Describes predictive molecular testing on small tissue samples.
Godre 2017(66)	Abstract only. MA of concordance between IHC and FISH. No FT available.
Hofman 2018(67)	Not a review. Compares FISH with molecular approaches.
Hung 2018(68)	Not an SR or NSR. Discusses the role of diagnostic and predictive IHC for NSCLC.
Inamura 2018 (32)	Not a SR or NSR. Describes pros, and cons, and pitfalls with IHC.
Laggner 2018(69)	Not a review. Abstract only.
Lambros 2017 (70)	Correspondance only. Costs of testing.
McDonough 2018 (71)	Not a review. Abstract only. No FT available. NGS ROS 1 testing, in house expertise and clinical utility.
Marchetti 2017 (72)	Not a review. Original study.
Pal 2017 (2)	Not a review. Descriptive.
Park 2020(45)	Not a review. Original paper comparing single gene assays (FISH as golden standard) with targeted NGS for ROS 1 detection in any type of lung cancer.
Pinsolle 2019 (73)	Not a review. More general about testing with IHC, FISH, PCR, and NGS.
Roy-Chowdhuri 2018 (74)	Not a review. Discusses various preanalytical and analytical factors that impact immunocytochemistry (ICC) in cytologic specimens.
Schluckebier 2017(75)	Abstract only.Belongs with the FT by Schluckebier below.

Schluckebier 2020(76)	Not a review. Compares cost-effectiveness of testing with NGS and FISH.
Viola 2016 (77)	Not a review. Original paper. Validation study.

Appendix 4 AMSTAR Yang 2018

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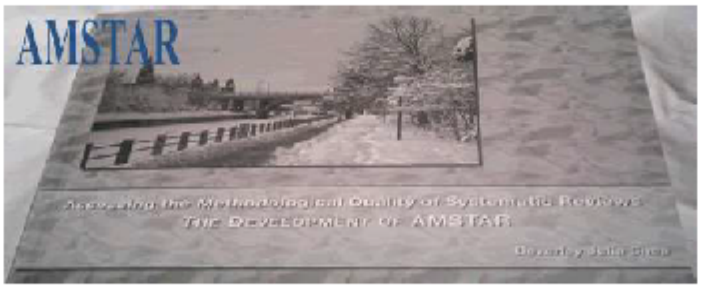

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AMSTAR 2 Results

Printer Friendly Version

Article Name: Yang 2018

You are currently logged on as Guest. You need to be logged on as a member to submit your score.
Log On

Yang 2018 is a Critially Low quality review

1. Did the research questions and inclusion criteria for the review include the components of PICO?	Yes Yes Yes Yes Yes Yes
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	NoYesYesYes
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Yes
4. Did the review authors use a comprehensive literature search strategy?	Partial Yes Yes Yes Yes
5. Did the review authors perform study selection in duplicate?	No
6. Did the review authors perform data extraction in duplicate?	Yes
7. Did the review authors provide a list of excluded studies and justify the exclusions?	No Yes
8. Did the review authors describe the included studies in adequate detail?	No Yes

Appendix 5 Results from the narrative reviews (N=5)

IHC and/or FISH versus NGS

Brisudova et al. reported 100% concordance between IHC/FISH and NGS for detection of ROS1 gene alterations in patients with NSCLC. Two small studies (7 and 52 participants) concerned with the detection of ROS1 gene alterations were included in this review (18).

NGS versus FISH

Pisapia and colleagues reported 100% sensitivity and 96% specificity for NGS compared with FISH for detection of ROS1 gene alterations in cytology samples (22). Only one study of ROS 1 in NSCLC (11 samples), was included in this review.

IHC versus FISH

Three reviews compared IHC with FISH for the detection of ROS 1 gene alterations in NSCLC (9, 20, 21). All studies used the antibody clone (D4D6), but applied at different dilutions, using different scoring methods and a variety of amplification kits. The sensitivity and specificity of ROS 1 IHC varied across the studies included in the reviews.

Comparisons of tests: sensitivity, specificity, and concordance

Author Year	No of studies (no patients)	Sensitivity (%)	Specificity (%)	Concordance (%)
IHC and/or FISH vs NGS				
Brisudova 2020(18)	2 studies (59 pts)	-	-	100%
NGS vs FISH				
Pisapia 2017(22)	1 study (11 samples)[1]	100%	96%	-
IHC vs FISH				
Luk 2018(20)	6 studies (N=unclear)[2]	Range: 94% to 100%	Range: 76% to 100%	-
Ross 2017 a (9)	7 studies (3,236 pts)	Range: 33% to 100%	Range: 15% to 100%	-
Ross 2017 b (21)	1 study (556 pts)[3]	100%	100%	-

[1] Three types of cytology samples, and two types of preparations were included [2]Some overlap with the studies included in Ross 2017a. [3] This study was also included in Ross 2017a.

Appendix 6. Information received from suppliers of NGS systems

Characteristics of NGS panels for which documentation was received from suppliers

Parameter	**ArcherDx Fusion Plex Library preparation kits** (a complement to NGS)	**MI Transcriptome panel**	**TruSight™ Oncology 500** (and TSO comprehensive- under development) [1]	**FoundationOne® CDx**	***Oncomine™ Focus Assay*[2]**
Supplier	**Archer**	**Caris' Life Science**	**Illumina**	**Roche**	**Thermo Fisher**
No of genes in panel	NR	NR (*Note: ROS1 is currently not part of the MI Transcriptome panel*)	523	324	52
Analytical sensitivity and specificity, and precision within run	NR	NR	TSO500 has an analytical sensitivity of 95% down to 5% VAF for all variant types and an analytical specificity of 99.9998% for small variants in normal FFPE tissue samples	Test sensitivity of 95–99% across all alteration types with high specificity (positive predictive value of >99%). High reproducibility (97%)	The accuracy and specificity for RNA fusion (above LOD) 98%; 100% at ≥255 fusion copies[iv]
LOD and LOQ, and precision within run	NR	The assay met mass input acceptance criteria for 50-250 ng RNA. The assay met LOD acceptance criteria down to 10% predicted	NR	The precision (within run) for RNA fusion was determined with 100% at ≥255 fusion copies. The accuracy and specificity for RNA fusion	The precision (within run) for RNA fusion was determined with 100% at ≥255 fusion copies.

		tumor content (dilution study).		(above LOD) 98%; 100% at ≥255 fusion copies[iv]	
Cut-off/reference standard	Laboratories do their own assessment on cut of points and values	NR	NR	NA	NA
Type of samples	NR	FFPE	FFPE	FFPE	FFPE (other assays can use liquid samples)
Material requirements (amount, ng)	NR	50-250 ng	5 slides/40ng	50 ng of dsDNA (from 40 µm of tissue (5 to 25 mm² of tumor surface with 20% of minimal tumor cellularity)	2-3 slides/10 ng
Turnaround time (days)	NR	9-11 d (from specimen receipt through result reporting)	3-4 days (from nucleic acid to variant report)	NR	3-4 days (from sample to answer)
Cost	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
FDA approved	NR	no	no	yes	no
CE-marked	NR	no	no	yes	no

Available for in-house use: yes/no	yes	no	yes	no	yes

1 We did not receive information for the smaller gene panels from Illumina (e.g. Illumina Am-pliSeq 52 genes). 2 Other assays that can detect ROS1 gene fusions from FFPE tissue are: Oncomine™ Solid Tumour Fusion Transcript Kit (CE-IVD) and the Oncomine™ Dx , the Oncomine™ Comprehensive Assay Plus, a large DNA+RNA panel that enables testing for SNV, CNV, Microsatellite instability status (MSI), Tumor mutational burden (TMB) and gene fusion for over 500 cancer related genes; and the Oncomine™ Comprehensive Assay-v3, a DNA+RNA panel covering 161 genes. Additional assays that can detect ROS1 fusions from liquid biopsy samples (i.e. cell free nucleic acid), are the Oncomine™ Lung cfTNA Assay or the Oncomine™ Pan-Cancer Assay. 3 Based on the information from the experts, the price for TSO500 is probably higher due to more hands-on time require. 4 Based on the information from the experts, Ion Torrent needs less hands-on time and may thus be relatively cheaper in the long run.

LOD= the lowest concentration tested that has a peak height that is greater than or equal to the average of a blank sample (no analyte) plus three standard deviations (SD) of the blank. The acceptance criterion is that the LOD has to be less than 20% of the LOQ.

LOQ= the lowest concentration that quantitatively measured suitably with accuracy and precision

Precision within run= is the result of running the same sample several times in the same run.

Descriptions of the technology used by different NGS systems

Suppliers	***Description of technologi****
Archer	FusionPlex RNA kits, utilizes a proprietary technology that only need to use 1 Gene specific primer (GSP) for detection of the fusions in cancer. The benefit of this technology is that there is no need to designing the opposite primer to detect a fusion, instead a universal primer that the GSP reads towards is utilised. This approach not only allows finding the sought after Fusion, but all the present fusions, know or unknown, on the examined gene fragment.
Caris Life sciences	The MI Transcriptome design-controlled assay uses RNA extracted from FFPE tissue to detect fusions and transcript variants using an unbiased hybrid-capture NGS based method. MI Transcriptome interrogates the entire human transcriptome and reports out fusions that are in-frame and likely to be expressed. Note: ROS 1 is at present not covered.
Illumina	TSO Comprehensive is a hybrid capture–based CGP assay with a deoxyribonucleic acid (DNA) + ribonucleic acid (RNA) workflow, intended for the genomic analysis of solid tumor samples. The assay, will be available as an in-vitro diagnostic.
Roche	FoundationOne CDx™ is based on a validated high-throughput hybrid capture-based NGS platform that interrogates the entire coding sequence of 324 cancer-related genes plus select introns from 28 genes often rearranged or altered in solid tumors, one of them *ROS1.*
Thermo Fisher	Ion Torrent™ technology directly translates chemically encoded information (A, C, G, T) into digital information (0, 1) on a semiconductor chip using simple chemistry and proprietary technology. Ion AmpliSeq is a targeted sequencing technology enabling researchers to rapidly and simply amplify thousands of targets, and the Ion Torrent™ Oncomine™ Focus Assay uses these technologies to provide tumour profiling by annotation of cancer driver fusions as ROS1.

**As described by the suppliers.*

Costs associated with the different commercial diagnostic methods

We contacted suppliers of relevant tests to procure information about technical details, unit costs as well as investment requirements for establishing routine testing at Norwegian hospitals. We received feedback from five suppliers on the following tests: Archer (FusionPlex RNA kits), Caris Life science (MI Tumor Seek), Illumina (TruSight™ Oncology 500, TSO500), Roche (FoundationOne CDx™) and Thermo Fisher (Oncomine™ Focus Assay). A summary of the information sent from the suppliers is presented in the table above. Here, we report more information regarding the costs associated with the different commercial diagnostic methods.

Archer

Archer's Library preparation kits are a complement to today's already established NGS technology. According to Archer, there are no costs related to the product more than the cost of the requested Kits. In every kit quality control is provided for control on a RT-PCR instrument. Laboratory technicians, molecular biologist and potentially a bioinformatician is needed to preform and interpret the results.

FusionPlex Oncology Research has a list price of €3,120 (NOK 33,449) and FusionPlex CTL Kit of €2,320 (NOK 24,872). These list prices are given for eight reactions. Any discounts are calculated on the estimated yearly purchase (personal communication by Business development manager Nordics, ArcherDX). The supplier also offers 1.5–2 days training for all new customers. The cost for 1.5-2 days training is €2,500 (NOK 26,801).

Caris' Life Science

Based on information we received from Caris, ROS1 is currently a single site Laboratory Developed Test (LDT), but it will be part of the Molecular Intelligence (MI) Transcriptome panel for both LDT and the companion diagnostic (CDx) in the future, which is currently in development. MI Transcriptome™ uses RNA extracted from FFPE tissue.

Since the technology is a single site test, there would be no investment for Norway (all tests are conducted abroad). The instrumentation used will all be at Caris Life Science. The current list price of MI Tumour Seek is USD3,200 (NOK 30,081). This price includes all the relevant procedures: pick-up and transport of tumour sample from the hospital to the laboratory, control and preparation of the tumour sample by a pathologist, testing for genomic alterations, delivery of a clinical and biological report, return of remaining parts of the tumour block from the laboratory to the hospital and training cost. The training needed would be physician's offices and hospitals being able to fix and ship the FFPE block to Caris Life Sciences. Logistics are handled via a contract with Fed-Ex (24h-service), cost for this is included in the price mentioned above.

Caris is open to discuss the price of RNA-sequencing analysis for identification of NTRK and ROS1 fusions.

Illumina

TruSight Oncology 500 (TSO500) covers 523 genes that are designed to be included in the TSO Comprehensive, which is under development.

With TSO500 running at the maximum batch size of seven samples and 100% efficiency, the cost per result is [redacted], including [redacted] for capital equipment, [redacted] for personnel, and [redacted] for consumables. The cost per result varies by the batch size and efficiency. The lower the batch size, the higher the cost per result. We did not receive information for the smaller gene panels from Illumina (e.g. Illumina AmpliSeq 52 genes).

Roche

Foundation One CDx is an end-to-end integrated CGP service which covers 324 genes. The service covers extraction of patients' samples, NGS sequencers identifying genomic alterations, and the delivery of a clinical and biological report. All tests for the Northern European market are conducted in Penzberg, Germany. DNA extraction is automated, performed on a Promega Maxwell type instrument, and the extracted DNA is then quantified using PicoGreen.

The interpretation of the bioinformatic analysis is reviewed by a team of multidisciplinary experts. The generation of a report is the final step, consisting of cross-checking the alterations identified with therapies available on the market, as well as those under development.

The only work required for the treating clinic in Norway is to retrieve the tumor sample. The list price of Foundation One CDX is NOK 37, 097 excluding value added tax. If public reimbursement is granted, Roche offers a net price of [redacted] excluding value added tax. The cost of tests in clinical practice adds up to approximately NOK 5,500. The price includes the following procedures: Pick-up and transport of tumor sample from the hospital to the lab in Penzberg, control and preparation of the tumor sample by a pathologist, testing for genomic alterations in 324 cancer-related genes using cutting-edge technology ensuring fast and high-quality process, delivery of a clinical and biological report exhaustively referenced by continually updated scientific publications, and if eligible, return of remaining parts of the tumor block from the lab in Penzberg, Germany to the hospital.

Thermo Fisher Scientific

Some Norwegian hospitals have already different types of Thermo Fisher instruments (29), depending on the total workload. However, a trend is to have the S5 Plus and

Prime. On Ion Torrent all the reagents and assays for ROS1 tests are the same for 10 samples for any S5 instrument.

The cost for running ROS1 with the Oncomine Focus assay (both DNA &RNA) is around [redacted] excluding value added tax, when running on 520 Chip with any S5 instrument (personal communication by senior clinical Account manager, Nordic and Baltic, Thermo Fisher Scientific).

To run the Oncomine™ Focus Assay (for FFPE sample) on Ion Torrent S5 NGS platform, one needs purchase panel and other consumables, which are all produced by Thermo Fisher Scientific and available in Norway.

One-year warranty is included in the new instrument. Thermo Fisher also offers different options for maintenance of different types of instruments for the second year. For the Ion S5 sequencer the cost will be around NOK 80,000. The vendor will also offer training on the technology and the specific assay implementation.

Appendix 7 Estimated cost for ROS1 testing with different methods, St. Olav's University Hospital and Oslo University Hospital

IHC

Estimated cost for ROS1 testing with IHC, St Olav's University Hospital and Oslo University Hospital

	St. Olav's University Hospital		Oslo University Hospital
	1 patient NOK	10 patients NOK	1 patient NOK
Reagent cost	408	408	250
Personal cost	564 (bioengineer: 384.62, pathologist: 179.49)	269 (bioengineer: 89.74, pathologist: 179.49)	300 (bioengineering: 210, Pathologist: 90)
Sum	**972**	**677**	**550**

FISH

Estimated cost for ROS1 testing with FISH, St Olav's University Hospital and Oslo University Hospital

	St. Olav's University Hospital		Oslo University Hospital
	1 patient NOK	10 patients NOK	1 patient NOK
Reagent cost	1,500	1,500	1,094
Personal cost	930 (bioengineer: 810, pathologist: 120)	364 (bioengineer: 244, pathologist: 120)	1,967 (bioengineering: 1,280, Pathologist: 687)
Sum	**2,430**	**1,864**	**3,061**

RT-PCR

*Estimated cost for ROS1 testing with RT-PCR, St Olav's University Hospital**

	St. Olav's University Hospital	
	1 patient NOK	**10 patients NOK**
Reagent cost	1,200	1,200
Personal cost	930 (bioengineer: 810, pathologist: 120)	364 (bioengineer: 244, pathologist: 120)
Sum	**3,113**	**1,419**

** RT-PCR is not used for ROS1 testing in Oslo University Hospital*

NGS

Estimated cost for ROS1 testing with NGS, St Olav's University Hospital and Oslo University Hospital

	St. Olav's University Hospital*		Oslo University Hospital**
	1 patient NOK	**10 patients NOK**	
Reagent cost	17,580	2,580	7,168
Personal cost	1,462 (bioengineer: 948.70, molecular biologist: 333.33 pathologist: 179.49)	705 (bioengineer: 192.30, molecular biologist: 333.33 pathologist: 179.49)	3,800 (bioengineering:3,000, Pathologist: 800)
Sum	**19,042**	**3,285**	**10,968**

**Ion Torrent S5 (personal communication by Liv Solvår Nymark, St. Olav's University Hospital)*

***Ion Torrent S5, Oncomine Childhood Cancer Research Assay (personal communication by Martin Andreas Furu, Oslo University Hospital)*

Appendix 8. Progress log

Suggestion submitted	24.11.2019
HTA commissioned	16.12.2019
External clinical experts pointed out by RHA	20.08.2020
Protocol published	23.09.2021
Report sent for expert review	04.06.2021
Internal approval at NIPH	08.07.2021
Report submitted	09.07.2021

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