CieBOD advies - RET-fusie detectie bij niet-kleincellig longcarcinoom

Leeswijzer:
Het streven wordt om onderstaande tekst te publiceren in het NVVP bulletin en op te nemen in de Richtlijnendatabase (www.richtlijnendatabase.nl), bij richtlijnmodules over middelen waarvoor de onderzochte test wordt ingezet in het kader van doelgerichte therapie.
De methodiek van de cieBOD is gebaseerd op de methodiek voor diagnostische testaccuratesse van GRADE, maar met een aantal aanpassingen. Omdat er geen/weinig studies te verwachten zijn waarin verschillende 'test-treatment' strategieën worden vergeleken (maar alleen studies over concordantie tussen testen, zoals hieronder beschreven) is het niveau van het bewijs hoe dan ook zeer laag. Daarom is de GRADE-waardering achterwege gelaten, omdat het geen onderscheidend vermogen heeft.

Uitgangsvraag
Wat is het optimale testbeleid omtrent RET-fusie detectie bij patiënten met stadium IV niet-kleincellig longcarcinoom?

Inleiding
Bij patiënten met stadium IV niet-kleincellig longcarcinoom (NSCLC) biedt de aanwezigheid van een RET-fusie een potentieel aangrijpingspunt voor doelgerichte therapie. De incidentie van RET-fusies bedraagt ongeveer 1% bij NSCLC en is afhankelijk van leeftijd, rookstatus, histologisch subtype en etniciteit. Meerdere testen worden op het moment gebruikt om RET-fusies te detecteren, zoals Fluorescence In-Situ Hybridization (FISH), Reverse-Transcription-PCR (RT-PCR), DNA-Next Generation Sequencing (DNA-NGS), RNA-NGS en ImmunoHistoChemie (IHC). Uit recente onderzoeken blijkt dat de betrouwbaarheid van sommige testen twijfelachtig is, met fout-positieve en fout-negatieve resultaten en als gevolg daarvan mogelijk over- en onderbehandeling. In deze module brengen we de betrouwbaarheid van de verschillende testen voor detectie van RET-fusies bij NSCLC in kaart aan de hand van de sensitiviteit, specificiteit en concordantie tussen verschillende testen op basis van klinische samples.
For the international exchange of this literature review, the next part is written in English.

Search and select
A systematic review of the literature was performed to answer the following question: Which tests are most accurate for detection of RET fusion for targeted therapy in patients with stage IV non-small cell lung cancer?

Patients: patients with stage IV non-small cell lung cancer
Interventions: immunohistochemistry, FISH, RT-PCR, DNA NGS (broad panel/WGS), RNA NGS
Comparisons: other molecular diagnostic tests
Outcomes: clinical sensitivity, clinical specificity, concordance between different tests
Timing: when systemic therapy is indicated
Setting: molecular diagnostics using tissue samples

Relevant outcome measures
The cieBOD considered sensitivity, specificity and concordance between test results using real-world clinical samples (as opposed to using technical validation samples), including all clinical relevant genetic aberrations of the targets, as critical outcome measures for decision making. In molecular diagnostics the sensitivity and specificity are influenced by a multitude of factors and there is rarely a golden standard. Therefore, the cieBOD did not predefine a

clinically relevant difference, but interpreted the sensitivity, specificity and concordance between test results in the context of the included studies.

Search and select (Methods)

The databases Medline (via OVID) and Embase (via Embase.com) were searched using relevant search terms between January 2017 and April 2022. The detailed search strategy is depicted under the tab Methods. The systematic literature search resulted in 335 hits. Studies were selected based on the following criteria: validation studies of immunohistochemistry, FISH, RT-PCR, DNA NGS (broad panel/WGS), RNA NGS for stage IV NSCLC. Studies on other tumor types or unknown tumor types, non-comparative studies, studies using plasma samples instead of tissue samples and studies with fewer than five RET fusions were excluded. This resulted in a selection of 44 potentially relevant studies based on title and abstract screening. These 44 hits contained 17 conference abstracts, 9 guidelines or reviews, and 1 duplicate, which were excluded. The 17 remaining full text papers were presented to the cieBOD members and screened by at least two members. After screening, 6 studies were included.

Results

Six studies were included in the analysis of the literature. Important study characteristics and results are summarized in the evidence tables. The assessment of the risk of bias is summarized in the risk of bias tables.

Summary of literature

Description of studies

In Baker 2022 (Baker, et al., 2022), the results of Abbott RET break-apart FISH were compared to NGS Oncomine Focus in a training set of 13 RET fusion positive-specimens and 12 RET fusion-negative specimens, and a validation set of 14 RET fusion-positive specimens and 82 RET fusion-negative specimens. The study had a prospective observational design. The study was performed in the US with samples from Cleveland Clinic (8), Loxo Oncology at Lilly (18), Indivumed, iSpecimen and BioIVT (95). The majority of these specimens were from patients with NSCLC. No information was provided on the stage of the tumors. In the training set, the results were bootstrapped using the 19% positive cell cut-off, resulting in a sensitivity of 89% and a specificity of 99% (no confidence intervals provided). In the validation set, the same 19% positive cell cut-off resulted in 12/14 (86%) NGS positive samples correctly identified using FISH, and 81/82 (99%) NGS negative samples correctly identified using FISH (no confidence intervals provided).

In Chu 2022 (Chu, et al., 2022), the results of the Idylla GeneFusion assay in 133 solid tumor samples with and 10 tumor samples without known kinase gene rearrangements from a cohort evaluated by the MSK-IMPACT (DNA NGS panel) or MSK-Fusion (RNA NGS panel) assay are described. The study had a retrospective observational design. The study was performed at the Memorial Sloan Kettering Cancer Center, US. Of these 143 samples, 108 (76%) were retrieved from patients with NSCLC. No information was provided on the stage of the tumors. In total, 31 (23%) RET fusions were detected. One sample was excluded due to low amplification and insufficient material for retesting, resulting in 142 tumor samples tested using both modalities.

The Idylla GeneFusion assay was able to detect 31/31 (100%) of the RET fusions. Also, 111/111 (100%) tumor samples without RET fusion were identified correctly. These tumor samples did have other kinase gene rearrangements such as ALK, ROS1 or NTRK1/2/3.

In Feng 2022 (Feng, et al., 2022), the results of targeted RNA NGS (N=10), FISH (N=30) and IHC (N=57) were compared to the results of DNA NGS in 9,431 tumor samples from patients with NSCLC from 2017 to 2020. The study had a retrospective observational design. The study was performed in two large cancer centers in China. In total, 167 (1.8%) RET fusions were detected. Of these patients, 62 (48.1%) had stage IV cancer. FISH and IHC were only performed on samples from patients with sufficient tissue.
Of the 30 FISH tests performed on positive DNA NGS results, 25 (83%) tested positive for RET-rearrangement.
In the study, IHC 3+ was considered positive. Of the 57 IHC tests performed on positive DNA NGS results, 16 (28.1%) tested positive for RET rearrangement.

In Radonic 2021 (Radonic, et al., 2021), the results of RET break-apart FISH assays were compared to RNA NGS in 4,873 samples from patients with driver negative NSCLC from 2015 to 2019. The study had a retrospective observational design. The study was performed using samples from six university hospitals in the Netherlands and Ireland. Of these samples, 2,858 were initially tested with FISH, of which 48 had a RET rearrangement. The other 2,015 samples were initially tested with RNA NGS, of which 14 samples had a RET fusion. From the 48 and 14 samples with RET rearrangements or fusions, 30 and 9 cases were tested using the other test modality respectively.
Of the 30 samples first tested with FISH, 9 (30%) RET fusions were detected using RNA NGS. Of the 9 samples first tested with RNA NGS, 9 (100%) RET rearrangements were detected using FISH.

In Tan 2020 (Tan, et al., 2020), the results of Abbott RET break-apart FISH were compared to NGS TruSight Oncology 500 panel (DNA + RNA) in a subset of 13 RET fusion positive-specimens from patients with NSCLC. This subset was part of a larger set of 64 patients with RET-rearranged NSCLC treated at the National Cancer Centre Singapore between April 2014 and March 2020. The study had a retrospective nonblinded cohort design. The median age of the 64 patients was 62 (range: 25-85) and 50 (78%) had stage IV cancer. In 6/13 (46%) patients, both FISH and NGS were positive. In 3/13 (23%) patients, only FISH results were positive. In 2/13 (15.5%), only NGS results were positive. In 2/13 (15.5%), FISH results were equivocal and NGS results were positive.

In Yang 2021 (Yang, et al., 2021), the results of RNA NGS (MSK-Fusion), break-apart FISH and IHC were compared with DNA NGS panel (MSK-IMPACT) in 46,897 samples from 41,869 patients between 2014 to 2020. The study had a retrospective observational design. The patients had different types of cancer, including lung cancer. In total, 171 samples harbored RET structural variants as detected by the DNA NGS panel. Of these samples, 105 with a known fusion partner were not tested using RNA NGS. In 34/34 RET positive samples with a known fusion partner and in 12/32 cases with a non-canonical RET fusion the fusion was detected by RNA NGS, implicating that 151/171 samples that were tested positive by DNA NGS had a functional RET fusion. 4,459 RET fusion-negative DNA-NGS samples without a mitogenic driver mutation or clinical samples that were suspected for alterations inducing resistance to a targeted therapy were also tested using RNA NGS, and no RET fusions were detected. 44/48 samples with a RET fusion were also aberrant using break-apart FISH. Especially the sensitivity for detecting NCOA4-RET fusions was low. 0/17 samples without a RET fusion were aberrant using break-apart FISH. 70 RET fusion positive samples and 89 negative samples were tested using IHC. Using a cut-off of >1% tumor cells with cytoplasmic staining 61/70 RET fusion positive cases and 16 RET fusion negative cases were considered fusion positive by IHC.

DNA NGS showed 100% (46/46; 95% CI: 92.3-100%) sensitivity and 99.6% (4,459/4,479; 95% CI: 99.3-99.7%) specificity, as compared to RNA NGS. No results per tumor type were described.

FISH showed 91.7% (44/48; 95% CI: 80-97.7%) sensitivity, varying between the fusion partners, as compared to DNA NGS and RNA NGS. Sensitivity of FISH was higher for lung cancer (100%, 95% CI: 85.8-100%) than for thyroid cancer (87.5%, 95% CI: 61.7-98.4%) or other tumors (75%, 95% CI: 34.9-96.8%).

IHC was first calibrated using a training (19 fusion-positive and 18 fusion-negative samples) and a validation set (51 fusion-positive and 71 fusion-negative samples) to determine and evaluate the optimal staining criteria. After calibration, IHC showed 87.1% (61/70; 95% CI: 77-93.9%) sensitivity, varying between the fusion partners, and 82% (73/89; 95% CI: 72.5-89.4%) specificity, although the comparator test is not described. Sensitivity of IHC was higher for lung cancer (97.6%; 95% CI: 87.1-99.9%) than for thyroid cancer (78.9%, 95% CI: 54.4%-93.9%) or other tumors (60%, 95% CI: 26.2-87.8%). Specificity was consistently ≥80% for all three tumor types

Table 1 Overview of the tests used in the studies

Study	Test 1	Test 2	Test 3	Test 4
Baker 2022	RET break-apart FISH	NGS using different tests		
Chu 2022	Idylla GeneFusion	MSK-IMPACT (DNA NGS)	MSK-Fusion (RNA NGS)	
Feng 2022	RNA NGS	RET break-apart FISH	IHC	DNA NGS
Radonic 2021	RET break-apart FISH	RNA NGS using different tests		
Tan 2020	RET break-apart FISH	NGS TruSight Oncology 500 panel (DNA + RNA)		
Yang 2021	MSK-Fusion (RNA NGS)	MSK-IMPACT (DNA NGS)	RET break-apart FISH	IHC

Risk of bias

The risk of bias was assessed using the QUADAS2 criteria for observational studies.

Baker 2022

The patient selection was not described. In addition, it is unclear what the samples from other institutions than hospitals were, and also patients with thyroid cancer were included. No patient characteristics were reported. It is unclear how the differentiation between the training set and the validation set was made. It is unclear whether the results of the tests were interpreted blinded to the results of the other tests. The interval between the testing using RET break-apart fusion and Oncomine NGS was not described. Also, for the validation set, different techniques of NGS were used. Given these shortcomings in study design and reporting, it can be concluded that there is a high risk of bias.

Chu 2022

The patient selection was only described briefly. In addition, only 76% of the samples came from patients with lung cancer and their tumor stage nor other patient characteristics were described. It is unclear whether the results of the tests were interpreted blinded to the results of the other tests. The interval between the testing using Idylla GeneFusion and DNA NGS or RNA NGS was not described. Given these shortcomings in study design and reporting, it can be concluded that there is a high risk of bias.

Feng 2022
The patient selection was only described briefly. In total, 80/167 (47%) patients were excluded due to insufficient tissue. Patient characteristics were only described for 129/167 (77%) patients, with no clear reason. The described patient population matched the population of our review question in 48.1% of the cases (stage IV NSCLC). Results were not described separately per tumor stage.
The results of the tests were interpreted blinded to the results of the other tests. The interval between DNA NGS and RNA NGS, FISH or IHC was not described. Given these shortcomings in study design and reporting, it can be concluded that there is a high risk of bias.

Radonic 2021
The patient selection was only described briefly. No patient characteristics were described, although all patients had NSCLC of an unknown stage. In total, 23/62 (37.1%) of the patients were excluded due to insufficient tissue. It is unclear whether the results of the tests were interpreted blinded to the results of the other tests. The interval between FISH and RNA NGS was not described. Four different tests for break-apart FISH and three different tests for RNA NGS were used in the participating hospitals. Given these shortcomings in study design and reporting, it can be concluded that there is a high risk of bias.

Tan 2020
The patient selection was only described briefly. Patient characteristics were described of the larger set of 64 patients, but not specifically of the patients who were tested using both FISH and NGS TruSight Oncology. In total, 51/64 (79.7%) patients were excluded due to insufficient tissue. The interpretation of the tests was not blinded. The interval between FISH and NGS TruSight Oncology was not described. Given these shortcomings in study design and reporting, it can be concluded that there is a high risk of bias.

Yang 2021
The patient selection was not described. No patient characteristics were provided, although results are reported separately for different tumor types. No description of exclusions were given. The results of the tests were interpreted blinded to the results of the other tests. The interval between DNA NGS, RNA NGS, FISH or IHC was not described. Given these shortcomings in study design and reporting, it can be concluded that there is a high risk of bias.

Overwegingen – van bewijs naar aanbeveling

<u>Conclusie per test en de kwaliteit van het bewijs</u>

<u>Conclusion IHC</u>
We selected two studies that evaluated RET IHC compared to NGS-based techniques. (Yang, et al., 2021) (Feng, et al., 2022) Using a RET antibody (Abcam, ab134100) and a custom scoring algorithm, Yang et al. reported a sensitivity and specificity of 86.3% and 80.3%, respectively. Using the same antibody, Feng, at al. 2022 came to similar conclusions. The latter study used a more standard scoring algorithm and reported a concordance rate of 28.1% between NGS and IHC. Both studies reported substantial variation in staining patterns and intensity between different types of RET fusions
Both studies conclude that RET IHC is not recommended as IHC is likely to result in both false positive and false negative results. Although a limited number of papers have been published

regarding IHC for RET gene fusion detection, the data that have been published are consistent. We therefore do not consider IHC as a suitable screening tool for RET gene fusions.

Conclusion FISH

We selected 3 studies in which FISH (using break apart probes) were compared to RNA NGS. (Baker, et al., 2022) (Radonic, et al., 2021) (Tan, et al., 2020) In general, few samples were used for direct comparison and/or the test group was not representative for study population due to possible selection bias. Baker et al compared RET FISH to RNA NGS using a test set and subsequent validation set; using a cut-off of 19% in FISH analysis, 86% of NGS positive samples were also positively identified in FISH, whereas 99% of NGS negative samples were also negatively tested in FISH. Other studies confirmed these findings that not all NGS RET positive samples were indeed positive in FISH. (Feng, et al., 2022) (Yang, et al., 2021) Tan et al performed a non-blinded re-analyses of previously found RET positive cases in routine diagnostics: only 13 cases were directly compared using FISH and RNA NGS, in which 5/13 were discrepant. Radonic et al reported a large, multicenter study in which FISH was compared to RNA NGS. However, only a small, selected group was tested with both techniques: of 30 FISH positive samples only 9 were correctly identified having an RNA fusion using RNA NGS, whereas 9 NGS positive samples were also tested positive in FISH. It was concluded that FISH was a sensitive technology, but unspecific: FISH often generated false positive results and always needed confirmation using RNA NGS.
In conclusion: When using FISH for RET fusion detection, it is important to use a well validated cut-off level. However, taking this into account, FISH and NGS did not correlate well. But even with an optimal cut-off level the false-positive rate for FISH is very high.

Conclusion DNA NGS for RET fusion detection

In total three studies were selected. (Chu, et al., 2022) (Tan, et al., 2020) (Yang, et al., 2021) Two of these studies used the DNA-NGS as comparator for other techniques such as Idylla fusion or IHC/RNA-NGS/FISH. (Chu, et al., 2022) (Tan, et al., 2020) The set-up of these studies does not allow an evaluation of the performance of the DNA NGS with respect to RET fusion detection. Yang et al studied the performance of RET fusion detection (MSK-IMPACT) compared to RNA sequencing (MSK-Fusion). This retrospective analysis showed a 100% sensitivity and 99,6% specificity for the MSK-IMPACT when compared to the MSK-Fusion. This is based on a selected 4,479 samples (based on cases with DNA-level structural variant of unknown significance (SVUS) involving kinase genes, de novo solid tumors lacking MAPK driver alterations; and cases with clinical evidence of progression to a targeted or hormone therapy but lacking a resistance mechanism) for which both test were performed and 151 RET fusions detected on DNA which were selected for the MSK-Fusion panel. False positive results for DNA NGS were seen in non-lung and non-thyroid tumors and the RNA-based approach showed no false negative results. RNA-based approaches were still valuable in evaluation of RET variants of unknown significance. The sensitivity and specificity of a DNA NGS approach is likely to depend on the used assay. Since the conclusions are based on only one large study, and no other studies have confirmed this performance or have evaluated other NGS assays, we can only state that DNA NGS for RET fusion using the MSK-IMPACT detection is a reliable method to detect RET fusions in lung cancer. This does not automatically mean that other DNA-based NGS assays are as reliable as this is dependent on the coverage of intronic RET rearrangements spread over very large distances.

Conclusion RNA NGS for RET fusion detection

Three studies were selected in which RNA-based analyses were one of the comparators. (Chu, et al., 2022) (Radonic, et al., 2021) (Yang, et al., 2021)

Chu et al used a targeted DNA-based NGS panel targeting 505 genes (MSK-IMPACT) as gold standard to evaluate the Idylla GeneFusion assay. Idylla GeneFusion detected 31/31 cases with a RET fusion identified by the MSK-IMPACT assay. 28/31 were based on a fusion-specific result and 3/31 were identified by exploiting the 3'to 5'expression imbalance, with which the fusion partner remains unclear. The specificity of the test was assessed by excluding RET fusion detection in samples with other fusions or MET exon 14 skipping and thus is not representative for all tumors. Of note is that the Idylla GeneFusion is unable to detect NRG1 fusions.

Radonic et al used three different RNA-based NGS tests (Archer, oncomine, assuragen quantidex) to evaluate the results of FISH analyses on NSCLC cases. In only 9/30 cases with a RET rearrangement based on FISH a fusion gene was detected using RNA-based NGS analyses, whereas in 9/9 cases with RET fusion as determined with an RNA-based NGS analysis a fusion gene was detected with FISH. False positive FISH results were attributed to genomic events not leading to a functional fusion product by using whole genome sequencing.

Yang et al used a targeted RNA-based NGS panel utilizing anchored multiplex PCR technology targeting selected regions of 123 genes (MSK-Fusion) as gold standard to evaluate the DNA-based test evaluating 505 genes (MSK-IMPACT (see also Chu et al). DNA NGS showed 100% (46/46; 95% CI: 92.3-100%) sensitivity and 99.6% (4,459/4,479; 95% CI: 99.3-99.7%) specificity as compared with the MSK-Fusion test.

Overall RNA-based analyses seem to be superior over FISH analyses. As all functional fusions that are detected by a DNA-based analyses can be detected on RNA level and as for most FFPE-tumors a result can be obtained, RNA-based fusion detection is a suitable approach to efficiently detect RET fusions. The papers also demonstrate a similar result for other fusion genes that are relevant in NSCLC like ALK, ROS1, NTRK1, NTRK2 and NTRK3, and MET exon 14 skipping. There are limited data on the performance of different assays and thus for the choice of an RNA-based assay critical review of the test specifics and validation for RET fusion detection are required.

Because of the relevance of the local context for the considerations, the next part is written in Dutch.

<u>Andere relevante literatuur</u>

In de European Society of Medical Oncology (ESMO) aanbevelingen voor RET-fusie detectie (Belli, et al., 2021) staan verschillende schema's voor het detecteren van RET-fusie bij onder andere NSCLC, op basis van een review van de literatuur. In deze aanbevelingen zijn studies samengevat die gepubliceerd zijn tussen 2012 en 2019. Het is op basis van de gepubliceerde aanbevelingen niet te achterhalen hoe men tot de keuze van deze studies is gekomen: het betreft geen systematische review. Er is geen risk of bias beoordeling gedaan. Hierdoor is het proces van de centrale vraag naar de aanbevelingen (in de vorm van stroomschema's) niet helder en transparant. Desondanks worden er in deze ESMO-aanbevelingen op basis van een aantal wetenschappelijke studies en consensus expert opinion adviezen gegeven over welke RET-fusie detectie assays de voorkeur hebben. De ESMO adviseert FISH als één van de methode van voorkeur voor de detectie van RET-fusies omdat het - tot recent - wereldwijd de meest gebruikte methode is met een relatief hoge specificiteit en sensitiviteit. De ESMO-aanbevelingen geven echter ook aan dat FISH niet optimaal is omdat de fusiepartner niet bepaald wordt wat steeds meer impact krijgt in de klinische praktijk, en er met betrekking tot de moleculaire diagnostiek van longkanker vele FISH-testen per patiënt nodig zijn. Daarbij laten de studies gevonden in dit cieBOD advies zien dat RET FISH kan leiden tot hoge aantallen vals-positieve bevindingen. De ESMO ontmoedigt zeer het gebruik van IHC vanwege beperkte sensitiviteit en specificiteit met vals-positieve bevindingen tot

wel 40%, dat ook bevestigd wordt in ons cieBOD advies. In lijn met onze bevindingen adviseert de ESMO ook targeted RNA-NGS als de voorkeurstest voor de detectie van RET-fusies.

Efficiënt gebruik van weefsel

Voor stadium IV NSCLC moeten meerdere targets worden gedetecteerd (o.a. EGFR, HER2, KRAS, BRAF, ROS, ALK, NRG1 en NTRK1/2/3, en MET exon 14-skipping, zie de richtlijn niet kleincellig longcarcinoom).

Voor de moleculaire diagnostiek van gemetastaseerd longkanker wordt tumormateriaal over het algemeen verkregen via transthoracale naaldbiopten of bronchusbiopten waarbij de hoeveelheid tumorweefsel vaak beperkt is. De grootte van het aangeleverde materiaal (aantal en type monsters) voor predictieve testen is gerelateerd aan de kans op een betrouwbare uitslag (zie de richtlijn niet kleincellig longcarcinoom).

Het is mogelijk dat getrapte analyse efficiënter uitpakt qua weefselgebruik, bijvoorbeeld wanneer er direct een KRAS-mutatie wordt gevonden. Dit leidt dan tevens tot kosten- en tijdsbesparing, echter voor een deel van de gevonden targets. Een nadeel van getrapte analyses kan juist ook zijn dat deze voor een deel van de patiënten tot inefficiënt gebruik van het beschikbare tumorweefsel kan leiden omdat voor alle verschillende testen steeds tumorweefsel nodig is. Het gevaar is dat er voor de laatste testen in de sequentie geen tumorweefsel meer beschikbaar is en nieuw tumormateriaal verkregen zal moeten worden om de predictieve analyse compleet te maken. Omdat RET-fusie één van de laatste targets is waarnaar wordt gekeken, zal het voor RET-fusie detectie vrijwel altijd ongunstiger uitpakken. Een recente internationale studie (Dall'Olio, et al., 2020) laat zien dat NGS-gebaseerd parallelle analyse gemiddeld een efficiënter weefselmanagement heeft dan trapsgewijze analyse van single-gene methoden.

Efficiënt gebruik van het beschikbare tumorweefsel wordt daarom als belangrijk gezien om een volledige analyse te garanderen.

Waarden en voorkeuren van patiënten (in overleg met NFK)

De patiëntenverenigingen zijn algemeen van mening dat er getest moeten worden voor alle targets waarvoor doelgerichte therapie beschikbaar is inclusief voor klinische studies. Met betrekking tot doorlooptijden geven patiëntenverenigingen aan dat doorlooptijden erg belangrijk zijn en dat het wachten op de volledige uitslag van moleculaire NGS-testen bijna altijd mogelijk en daarmee wenselijk is.
Een completer beeld geeft een grotere kans op de best passende behandeling. Wanneer de arts het doel van het testen uitlegt, zal de patiënt begrijpen waarom de behandeling niet meteen kan starten als het beeld niet compleet is. Daarom hebben patiëntenverenigingen voorkeur voor brede en complete analyse in een keer, boven single gene testen/ getrapte analyse.

Kosten (middelenbeslag)

De frequentie van RET-fusies in patiënten met longkanker (0.6-0.9%) en adenocarcinoom (1.2-2%) is laag. (Steeghs, et al., 2022) Gezien deze lage frequentie is de analyse met een single-gene test (die alleen RET-fusies detecteert) kosten-inefficiënt. De aanbeveling van de huidige richtlijn niet-kleincellig longcarcinoom voor de moleculaire diagnostiek bij longkanker is om, naast RET-fusies, ook andere weinig-frequent voorkomende fusies (ROS, ALK, NTRK1/2/3, NRG1, en MET exon 14-skipping) te analyseren. Hierdoor is een RNA-

gebaseerde NGS test efficiënter waarin tegelijkertijd ook voor fusies in ROS, ALK, NRG1 en NTRK1/2/3, en MET exon 14-skipping getest wordt.

Een recente Nederlandse kosten-effectiviteitsstudie (Wolff, et al., 2022) laat zien dat NGS-gebaseerde parallelle analyse voor de huidige 13 predictieve markers gemiddeld €158 goedkoper is dan trapsgewijze analyse met single-gene methoden. NGS-gebaseerde parallel analyse detecteert aanvullende genetische afwijkingen relevant voor doelgerichte therapie in 20,5% van de gevallen met als gevolg dat therapeutische kosten stegen met €8.358 en 0,12 QALY's waren gewonnen, wat leidt tot een incrementele kosteneffectiviteitsratio van €69.614/QALY voor parallelle versus sequentiële testen inclusief therapeutische consequenties. In deze studie werd DNA-NGS parallel met RNA-NGS getest. Als kan worden voldaan aan een acceptabele totale doorlooptijd, zouden kosten verder te besparen zijn door sequentieel eerst een DNA-NGS gevolgd door een RNA-NGS uit te voeren. Of er kosten kunnen worden bespaard is afhankelijk van meerdere factoren en zal per situatie moeten worden beoordeeld. Dat NGS-gebaseerde aanpak over alle patiënten gemiddeld goedkoper is, wordt ook door andere studies ondersteund. (Dall'Olio, et al., 2020) (Pennell, et al., 2019)

Verder is vanwege de inzet van NGS-gebaseerde testen (als ook de uitbreiding van nieuwe targets) ook een besparing van de algemene zorgkosten mogelijk, omdat relatief meer longkankerpatiënten met mutatie of fusie gezien worden die in aanmerking komen voor doelgerichte therapie, waarbij de follow-up alleen op de polikliniek plaats vindt. Patiënten zonder deze mutaties/fusies krijgen (chemo-)immunotherapie waarvoor ze iedere drie weken naar het ziekenhuis komen voor een infuus via het dagcentrum voorafgegaan door een polikliniek bezoek en infusiebereiding via de apotheek.

<u>Doorlooptijd</u>

Internationaal wordt een doorlooptijd van 10 werkdagen gehanteerd vanaf ontvangst materiaal in het moleculair laboratorium tot rapportage van alle resultaten. (Lindeman, et al., 2018) In Nederland is in de SONCOS normen gedefinieerd dat het totale diagnostische traject maximaal drie weken mag duren. (Stichting Oncologische Samenwerking (SONCOS), 2022)

Een recente Nederlandse kosten-effectiviteitsanalyse (Wolff, et al., 2022) laat zien dat NGS-gebaseerd parallel analyse voor de huidige 13 predictieve markers gemiddeld een kortere doorlooptijd hebben dan trapsgewijze analyse van single-gene methoden. De kans dat de maximale doorlooptijd voor alle patiënten gehaald wordt met de NGS analyse is groter dan wanneer er een getrapte analyse wordt ingezet. Omdat RET-fusie één van de laatste targets is waarnaar wordt gekeken, zal het voor RET-fusie detectie ongunstig uitpakken bij een getrapte analyse met single gene testen. Dat NGS-gebaseerde aanpak over alle patiënten sneller is wordt ook door andere studies ondersteund. (Dall'Olio, et al., 2020) (Pennell, et al., 2019) (Matsuda, et al., 2022). Voor de parallelle NGS-analyse kan worden overwogen voor een sequentiële analyse met eerst de DNA-NGS gevolgd door de RNA-NGS als kan worden voldaan aan een acceptabele totale doorlooptijd, met name om daarmee kosten mogelijk verder te besparen. Dit is afhankelijk van meerdere factoren en zal per situatie moeten worden beoordeeld.

<u>Plaatsbepaling, haalbaarheid en implementatie</u>

Binnen NSCLC stadium IV is binnen de richtlijn vastgesteld dat naast de RET-fusie detectie de volgende 11 predictieve genen worden geanalyseerd: EGFR, KRAS, ALK, ROS1, BRAF, MET, HER2, NTRK1/2/3 en NRG1. Verder wordt ook IHC voor PDL1 aanbevolen.

Vanuit het traject moleculaire diagnostiek vanuit ZIN wordt gewerkt aan de lijst klinisch minimaal noodzakelijke targets (LKMNT) voor NSCLC, waarbij de NVMO, NVALT en NVVP betrokken zijn. Deze lijst is door de NVALT recent geaccordeerd (december 2022) en zal regelmatig worden aangepast als er wijzigingen zijn in het behandelen van longkanker in het kader van moleculaire targets. Deze LKMNT lijst zal in de toekomst leidend zijn. Als er relevante wijzigingen zijn in LKMNT lijst voor longkanker, kan dit aanleiding zijn om ons huidige advies aan te passen.

RET-fusie detectie wordt gezien de incidentie en de kosten vaak laat in het diagnostische traject aangevraagd. Zoals hierboven aangegeven kan sequentieel smal testen enerzijds leiden tot een lange doorlooptijd en anderzijds is dit kosten-inefficiënt. Gezien deze overwegingen is het advies om de predictieve analyse met NGS-technieken uit te voeren. In dit kader is het relevant om aan te geven dat er op dit moment een toename is van de toepassing van 'bredere' NGS-testen. Een bredere test heeft de intentie om naast de bekende targets ook relevante co-mutaties in beeld te brengen, potentiële biomarkers te bepalen die nog niet gearriveerd zijn maar waar wel veel evidentie over is (bijvoorbeeld TMB en HRD). Gelijktijdig geeft het de ruimte voor inzet van deze test voor meerdere indicaties (naast de doeltumor ook andere maligniteiten) en geeft deze test een aanzienlijke versimpeling van de workflow. Om het testen duurzaam in te richten kan het daarom efficiënter zijn om breder te testen dan strikt noodzakelijk voor de doeltumor. De inzet van moleculaire diagnostiek nu en in de toekomst wordt landelijk geëvalueerd door het Zorginstituut Nederland in opdracht van het Ministerie van Volksgezondheid, Welzijn en Sport.

Erfelijke aanleg

Momenteel zijn er voor NSCLC geen aanwijzingen dat er RET-fusies in de kiembaan voorkomen. RET-fusies worden op dit moment ook niet vermeld in tabel 3 van de projectgroep tumor en erfelijkheid.

Aanbevelingen

Samenvatting cieBOD advies: RET-fusie detectie bij patiënten met NSCLC stadium IV	
Conclusie wetenschappelijke literatuur (per test) - DNA-NGS - RNA-NGS - FISH - IHC	DNA-NGS: eventueel geschikt na validatie voor fusiedetectie en bij onbekende fusiepartners of ongewone herrangschikkingen bevestiging met RNA-NGS RNA-NGS: geschikt, indien alle targets gedetecteerd kunnen worden FISH: niet geschikt door lage specificiteit IHC: niet geschikt door lage sensitiviteit en lage specificiteit
Betrouwbaarheid bewijs	Vergelijkende, veelal retrospectieve niet-geblindeerde studies met een hoog risico op bias
Efficiënt gebruik van weefsel	Voordeel voor DNA-NGS en/of RNA-NGS
Waarden en voorkeuren van patiënten (op indicatie)	Voordeel voor DNA-NGS en/of RNA-NGS wegens completere uitslag
Kosten	Voordeel voor DNA-NGS en/of RNA-NGS wegens gemiddeld lagere kosten
Doorlooptijd	Voordeel voor DNA-NGS en/of RNA-NGS wegens gemiddeld kortere doorlooptijd

Plaatsbepaling	Voordeel voor DNA-NGS en/of RNA-NGS wegens detecteren meerdere genetische afwijkingen bij NSCLC (zie LKMNT)
Haalbaarheid en implementatie	Voordeel voor DNA-NGS en/of RNA-NGS wegens organisatorische voordelen
Rol van erfelijke aanleg	Niet relevant
Aanbevelingen:	

- Gebruik RNA-NGS voor het testen op RET-fusie bij stadium IV NSCLC, of voor fusiedetectie gevalideerde DNA-NGS. Bevestig onbekende fusiepartners of ongewone herrangschikkingen gedetecteerd met DNA-NGS middels een RNA-NGS.
- Analyseer RET-fusies bij stadium IV NSCLC alleen in dezelfde NGS-test waarin tenminste ook de analyse van fusies in ROS1, ALK, NRG1 en NTRK1/2/3, en MET-skipping tegelijk plaats heeft. Analyseer RET-fusies niet met single gene testen.
- Test RET-fusies altijd in de context van overige relevante targets in NSCLC.
    - Zet zowel IHC (voor PD-L1), DNA/RNA-gebaseerde analyses van alle predictieve markers (volgens de actuele lijst klinisch minimaal noodzakelijke targets*) gelijktijdig in, waarbij gebruik wordt gemaakt van brede NGS-technieken.
- Bespreek patiënten met stadium IV NSCLC en een positieve test voor RET-fusie gezien de zeldzaamheid in een regionale moleculaire tumorboard, zonder dat dit leidt tot vertragingen anders dan een niet-zeldzame afwijking.
- Verwijs patiënten niet voor genetische counseling bij de detectie van RET-fusies.

* De lijst klinisch minimaal noodzakelijke targets is op moment van het schrijven van dit advies nog niet openbaar beschikbaar, maar al wel geaccordeerd door de NVALT.

Kennislacunes

Vanuit methodologisch perspectief worden idealiter volledige test-treatment strategieën met elkaar vergeleken. Dergelijke studies zijn niet gevonden, waardoor de kwaliteit van het bewijs laag is.

De analyse van ctDNA: in dit advies voor de detectie van RET-fusies in NSCLC was het uitgangsmateriaal tumorweefsel (chirurgische resecties, weefselbiopten als vries of in paraffine-blokjes) en is de analyse op celvrij DNA uit plasma niet uitgevoerd (dit was een exclusiecriterium). Gezien in ~30% van patiënten met gemetastaseerd NSCLC geen (geschikt) tumorweefsel verkregen kan worden, is de analyse van celvrij DNA een mogelijkheid die momenteel onderzocht wordt. Belangrijk is dat de testen voor de detectie van RET-fusies en de andere 11 predictieve markers (exclusief PD-L1) waarin mutaties met een VAF van 0,1% tot 1% gedetecteerd dienen te worden, andere typen moleculaire testen benodigen.

RET-fusies zijn ook beschreven in chronisch myeloide leukemie (zeldzaam) en papillair schildkliercarcinoom 20-40% (Mulligan, 2014), maar al deze studies zijn geëxcludeerd in dit advies.

Literatuur

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Richtlijn niet-kleincellig longcarcinoom - module NGS versus PCR bij testen op mutaties. (2020). Retrieved from Richtlijnendatabase: https://richtlijnendatabase.nl/richtlijn/niet_kleincellig_longcarcinoom/diagnostiek/ngs_versus_pcr_bij_testen_op_mutaties.html

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Table of excluded studies

Reference	Reason for exclusion
Ambrosini-Spaltro 2022	Unclear how many tissue samples were from lung cancer patients
Kato 2017	No direct comparison between tests, only genetic landscape
Lee 2020	No direct comparison between tests
Li 2021	Only a prediction of therapeutic response
Reguart 2017	Only two RET fusions included
Rogers 2017	Only one RET fusion included
Sakai 2019	Only three RET fusions included
Shi 2022	No direct comparison between tests, only genetic landscape
Vaughn 2018	Unclear source of samples, only two RET fusions
Xiang 2022	Only an unknown minority of the population had stage IV lung cancer
Xu 2018	Only sequencing data, no clinical samples

Zoekverantwoording

Algemene informatie

Diagnostiek RET fusion in patients with nsclc	
Uitgangsvraag:	
Database(s): Ovid/Medline, Embase	Datum: 7-4-2022, 11-5-2022, 7-6-2022
Periode:	Talen: nvt
Literatuurspecialist: Ingeborg van Dusseldorp	
BMI zoekblokken: voor verschillende opdrachten wordt (deels) gebruik gemaakt van de zoekblokken van BMI-Online https://blocks.bmi-online.nl/ Bij gebruikmaking van een volledig zoekblok zal naar de betreffende link op de website worden verwezen.	
Toelichting: **7-6-2022** Op basis van de voorgaande strategie is de terminologie op 25-5 opnieuw vastgesteld en is de zoekstrategie opnieuw uitgevoerd. 11-5-2022 Op 7 april is een oriënterende strategie opgezet. Er wordt een beperkt aantal referenties gevonden maar weinig relevante artikelen. Op 11 mei wordt op basis van een 19 tal mogelijke sleutelartikelen opnieuw een strategie opgezet, met de volgende elementen: RET gene AND gene rearrangement, fusion, high troughput sequencing AND lung cancer AND diagnostics sensitivity, specificity De adviseur heeft uit de 19, 8 sleutelartikelen geselecteerd die aan gestelde criteria voldoen. De referenties zijn in Rayyan gezet en de 8 sleutelartikelen, zie bestand inclusie sleutelartikelen Joppe, als geïncludeerd gemarkeerd. Met de AI functie van Rayyan wordt een relevance ranking toegepast, wat de selectie van de artikelen makkelijker maakt.	
Te gebruiken voor richtlijnen tekst: In de databases Embase en Ovid/Medline is op 11 mei 2022 met relevante zoektermen gezocht naar diagnostische studies. De literatuurzoekactie leverde 714 unieke treffers op.	

5 ***Zoekopbrengst***

Diagnostische studies 7-6-2022	**EMBASE**	**OVID/MEDLINE**	**Ontdubbeld t.ov. set 11-5-2022**
SRs	319	60	
Overige			
Totaal			
Diagnostische studies 11-5-2022	**EMBASE**	**OVID/MEDLINE**	**Ontdubbeld**

SRs	20	6	18
Overige	672	189	696
Totaal			714

Zoekstrategie
Embase
7-6-2022

No.	Query	Results
#23	#20 AND #22	8
#22	#15 AND #21	319
#21	#13 AND [1-1-2017]/sd NOT ('editorial'/it OR 'letter'/it OR 'note'/it) NOT ('animal'/exp NOT 'human'/exp)	743
#20	#9 AND #16	8
#19	#16 NOT #18	558
#18	#16 AND #17	11
#17	'meta analysis'/exp OR 'meta analysis (topic)'/exp OR metaanaly*:ti,ab OR 'meta analy*':ti,ab OR metanaly*:ti,ab OR 'systematic review'/de OR 'cochrane database of systematic reviews'/jt OR prisma:ti,ab OR prospero:ti,ab OR (((systemati* OR scoping OR umbrella OR 'structured literature') NEAR/3 (review* OR overview*)):ti,ab) OR ((systemic* NEAR/1 review*):ti,ab) OR (((systemati* OR literature OR database* OR 'data base*') NEAR/10 search*):ti,ab) OR (((structured OR comprehensive* OR systemic*) NEAR/3 search*):ti,ab) OR (((literature NEAR/3 review*):ti,ab) AND (search*:ti,ab OR database*:ti,ab OR 'data base*':ti,ab)) OR (('data extraction':ti,ab OR 'data source*':ti,ab) AND 'study selection':ti,ab) OR ('search strategy':ti,ab AND 'selection criteria':ti,ab) OR ('data source*':ti,ab AND 'data synthesis':ti,ab) OR medline:ab OR pubmed:ab OR embase:ab OR cochrane:ab OR (((critical OR rapid) NEAR/2 (review* OR overview* OR synthes*)):ti) OR ((((critical* OR rapid*) NEAR/3 (review* OR overview* OR synthes*)):ab) AND (search*:ab OR database*:ab OR 'data base*':ab)) OR metasynthes*:ti,ab OR 'meta synthes*':ti,ab	822864
#16	#14 AND #15	451

No.	Query	Results
#15	'sensitivity and specificity'/de OR sensitiv*:ab,ti OR specific*:ab,ti OR predict*:ab,ti OR 'roc curve':ab,ti OR 'receiver operator':ab,ti OR 'receiver operators':ab,ti OR likelihood:ab,ti OR 'diagnostic error'/exp OR 'diagnostic accuracy'/exp OR 'diagnostic test accuracy study'/exp OR 'inter observer':ab,ti OR 'intra observer':ab,ti OR interobserver:ab,ti OR intraobserver:ab,ti OR validity:ab,ti OR kappa:ab,ti OR reliability:ab,ti OR reproducibility:ab,ti OR ((test NEAR/2 're-test'):ab,ti) OR ((test NEAR/2 'retest'):ab,ti) OR 'reproducibility'/exp OR accuracy:ab,ti OR 'differential diagnosis'/exp OR 'validation study'/de OR 'measurement precision'/exp OR 'diagnostic value'/exp OR 'reliability'/exp OR 'predictive value'/exp OR ppv:ti,ab,kw OR npv:ti,ab,kw OR (('optimal method*' NEAR/4 test*):ti,ab,kw)	9164566
#14	#13 NOT ('editorial'/it OR 'letter'/it OR 'note'/it) NOT ('animal'/exp NOT 'human'/exp)	1008
#13	#10 AND #11 AND #12	1049
#12	'lung cancer'/exp OR (((lung OR pulmonary) NEAR/3 (cancer* OR tumor* OR tumour* OR neoplasm* OR malignan*)):ti,ab,kw)	5142332
#11	'ret gene'/exp OR 'oncogene ret'/exp OR ret:ti,ab,kw	14707
#10	'immunochemistry'/exp OR 'fluorescence in situ hybridization'/exp OR 'reverse transcription polymerase chain reaction'/exp OR 'gene panel'/exp OR 'whole genome sequencing'/exp OR 'high throughput sequencing'/exp OR 'dna next generation sequencing':ti,ab,kw OR 'rna next generation sequencing':ti,ab,kw OR 'nanostring'/exp OR immunochemistr*:ti,ab,kw OR 'fluorenscence in situ hybridi?ation':ti,ab,kw OR 'rt pcr':ti,ab,kw OR 'gene panel':ti,ab,kw OR 'whole genome sequencing':ti,ab,kw OR 'high throughput sequencing':ti,ab,kw OR 'dna sequencing'/exp OR 'rna sequencing'/exp OR 'sequence analysis'/exp OR nanostring:ti,ab,kw	1881675
#9	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8	8
#8	molecular AND characterization AND clinical AND outcomes AND in AND 'ret rearranged' AND nsclc:ti	1
#7	a AND performance AND comparison AND of AND commonly AND used AND assays AND to AND detect AND ret AND fusions	1
#6	fluorescence AND situ AND hybridization AND analysis AND is AND a AND sensitive AND but AND highly AND unspecific AND screening AND method AND for AND ret AND fusions AND in AND lung AND cancer	1
#5	highly AND sensitive AND fusion AND detection AND using AND plasma AND 'cell free' AND rna AND in AND 'non small cell' AND lung AND cancers	1

No.	Query	Results
#4	analytical AND accuracy AND of AND ret AND fusion AND detection AND by AND 'break apart' AND fluorescence AND in AND situ AND hybridization	1
#3	identification AND validation AND of AND noncanonical AND ret AND fusions AND in AND 'non–small cell' AND lung AND cancer AND through AND dna AND rna AND sequencing NOT 1148p:ti NOT potential:ti	1
#2	clinical AND utility AND performance AND of AND an AND 'ultra rapid' AND multiplex AND 'rna based' AND assay AND for AND detection AND ret	1
#1	the AND role AND of AND 'next generation' AND sequencing AND in AND detecting AND gene AND fusions AND with AND known AND unknown AND partners AND a AND 'single center'	1

11-5-2022

No.	Query	Results
#87	#76 AND #83 sleutelartikelen gevonden	8
#86	#83 NOT #85 Overige diagnostisch	672
#85	#83 AND #84 SR diagnostisch	20
#84	'meta analysis'/exp OR 'meta analysis (topic)'/exp OR metaanaly*:ti,ab OR 'meta analy*':ti,ab OR metanaly*:ti,ab OR 'systematic review'/de OR 'cochrane database of systematic reviews'/jt OR prisma:ti,ab OR prospero:ti,ab OR (((systemati* OR scoping OR umbrella OR 'structured literature') NEAR/3 (review* OR overview*)):ti,ab) OR ((systemic* NEAR/1 review*):ti,ab) OR (((systemati* OR literature OR database* OR 'data base*') NEAR/10 search*):ti,ab) OR (((structured OR comprehensive* OR systemic*) NEAR/3 search*):ti,ab) OR (((literature NEAR/3 review*):ti,ab) AND (search*:ti,ab OR database*:ti,ab OR 'data base*':ti,ab)) OR (('data extraction':ti,ab OR 'data source*':ti,ab) AND 'study selection':ti,ab) OR ('search strategy':ti,ab AND 'selection criteria':ti,ab) OR ('data source*':ti,ab AND 'data synthesis':ti,ab) OR medline:ab OR pubmed:ab OR embase:ab OR cochrane:ab OR (((critical OR rapid) NEAR/2 (review* OR overview* OR synthes*)):ti) OR ((((critical* OR rapid*) NEAR/3 (review* OR overview* OR synthes*)):ab) AND (search*:ab OR database*:ab OR 'data base*':ab)) OR metasynthes*:ti,ab OR 'meta synthes*':ti,ab	822864

No.	Query	Results
#83	#81 AND #82	692
#82	'sensitivity and specificity'/de OR sensitiv*:ab,ti OR specific*:ab,ti OR predict*:ab,ti OR 'roc curve':ab,ti OR 'receiver operator':ab,ti OR 'receiver operators':ab,ti OR likelihood:ab,ti OR 'diagnostic error'/exp OR 'diagnostic accuracy'/exp OR 'diagnostic test accuracy study'/exp OR 'inter observer':ab,ti OR 'intra observer':ab,ti OR interobserver:ab,ti OR intraobserver:ab,ti OR validity:ab,ti OR kappa:ab,ti OR reliability:ab,ti OR reproducibility:ab,ti OR ((test NEAR/2 're-test'):ab,ti) OR ((test NEAR/2 'retest'):ab,ti) OR 'reproducibility'/exp OR accuracy:ab,ti OR 'differential diagnosis'/exp OR 'validation study'/de OR 'measurement precision'/exp OR 'diagnostic value'/exp OR 'reliability'/exp OR 'predictive value'/exp OR ppv:ti,ab,kw OR npv:ti,ab,kw OR (('optimal method*' NEAR/4 test*):ti,ab,kw)	9164566
#81	#80 NOT ('editorial'/it OR 'letter'/it OR 'note'/it) NOT ('animal'/exp NOT 'human'/exp)	1636
#80	#77 AND #78 AND #79	1715
#79	'lung cancer'/exp OR (((lung OR pulmonary) NEAR/3 (cancer* OR tumor* OR tumour* OR neoplasm* OR malignan*)):ti,ab,kw)	514232
#78	'ret gene'/exp OR 'oncogene ret'/exp OR ret:ti,ab,kw	14646
#77	'high throughput sequencing'/exp OR 'gene rearrangement'/exp OR 'gene fusion'/exp OR (((gene* OR dna OR ret) NEAR/3 (fusion* OR rearrangement* OR alteration*)):ti,ab,kw)	272966
#76	#68 OR #69 OR #70 OR #71 OR #72 OR #73 OR #74 OR #75 sleutelartikelen	8
#75	molecular AND characterization AND clinical AND outcomes AND in AND ' ret rearranged' AND nsclc:ti	1
#74	a AND performance AND comparison AND of AND commonly AND used A ND assays AND to AND detect AND ret AND fusions	1
#73	fluorescence AND situ AND hybridization AND analysis AND is AND a AND sensitive AND but AND highly AND unspecific AND screening AND method AND for AND ret AND fusions AND in AND lung AND cancer	1
#72	highly AND sensitive AND fusion AND detection AND using AND plasma A ND 'cell free' AND rna AND in AND 'non small cell' AND lung AND cancers	1
#71	analytical AND accuracy AND of AND ret AND fusion AND detection AND b y AND 'break apart' AND fluorescence AND in AND situ AND hybridization	1
#70	identification AND validation AND of AND noncanonical AND ret AND fusi ons AND in AND 'non–small	1

No.	Query	Results
	cell' AND lung AND cancer AND through AND dna AND rna AND sequencing NOT 1148p:ti NOT potential:ti	
#69	clinical AND utility AND performance AND of AND an AND 'ultra rapid' AND multiplex AND 'rna based' AND assay AND for AND detection AND ret	1
#68	the AND role AND of AND 'next generation' AND sequencing AND in AND detecting AND gene AND fusions AND with AND known AND unknown AND partners AND a AND 'single center'	1

7 -4- 2022

No.	Query	Results
#41	#39 NOT #40	215
#40	#36 AND #38 Prognostisch	57
#39	#36 AND #37 Diagnostisch	257
#38	Prognostisch 'area under the curve'/exp OR 'brier score'/exp OR 'computer prediction'/exp OR 'c statistic'/exp OR 'c statistics'/exp OR 'integrated discrimination improvement'/exp OR 'net reclassification improvement'/exp OR 'net reclassification index'/exp OR 'prediction'/exp OR 'predictive model'/exp OR 'predictive modeling'/exp OR 'predictive validity'/exp OR 'predictive value'/exp OR 'regression analysis'/exp OR 'statistical model'/exp OR 'area under the curve':ti,ab,kw OR 'brier score*':ti,ab,kw OR 'c statistic*' OR 'computer prediction':ti,ab,kw OR 'decision curve anal*':ti,ab,kw OR (('net reclassification' NEAR/2 (improvement OR index)):ti,ab,kw) OR (((predict* OR statistical*) NEAR/3 (model* OR validity OR value)):ti,ab,kw) OR 'proportional hazards model*':ti,ab,kw OR 'r square*':ti,ab,kw OR regression:ti,ab,kw OR predict*:ti OR multivariate:ti,ab,kw	2854325
#37	Diagnostisch 'sensitivity and specificity'/de OR sensitiv*:ab,ti OR specific*:ab,ti OR predict*:ab,ti OR 'roc curve':ab,ti OR 'receiver operator':ab,ti OR 'receiver operators':ab,ti OR likelihood:ab,ti OR 'diagnostic error'/exp OR 'diagnostic accuracy'/exp OR 'diagnostic test accuracy study'/exp OR 'inter observer':ab,ti OR 'intra observer':ab,ti OR interobserver:ab,ti OR intraobserver:ab,ti OR validity:ab,ti OR kappa:ab,ti OR reliability:ab,ti OR reproducibility:ab,ti OR ((test NEAR/2 're-test'):ab,ti) OR ((test NEAR/2 'retest'):ab,ti) OR 'reproducibility'/exp OR accuracy:ab,ti OR 'differential diagnosis'/exp OR 'validation study'/de OR 'measurement	9116871

No.	Query	Results
	precision'/exp OR 'diagnostic value'/exp OR 'reliability'/exp OR 'predictive value'/exp OR ppv:ti,ab,kw OR npv:ti,ab,kw	
#36	#33 AND #34 AND #35	557
#35	'genetic screening'/exp OR genetic*:ti,ab,kw	1489274
#34	'lung cancer'/exp OR 'lung cancer':ti,ab,kw	476997
#33	'ret gene'/exp OR ret:ti,ab,kw	14355
#32	fluorescence AND situ AND hybridization AND analysis AND is AND a AND sensitive AND but AND highly AND unspecific AND screening AND method AND for AND ret AND fusions AND in AND lung AND cancer Sleutelartikel	1

Ovid/Medline
7-6-2022

#	Searches	Results
8	7 not ((exp animals/ or exp models, animal/) not humans/) not (letter/ or comment/ or editorial/)	60
7	limit 6 to yr="2017 -Current"	60
6	4 and 5	100
5	exp "Sensitivity and Specificity"/ or (Sensitiv* or Specific*).ti,ab. or (predict* or ROC-curve or receiver-operator*).ti,ab. or (likelihood or LR*).ti,ab. or exp Diagnostic Errors/ or (inter-observer or intra-observer or interobserver or intraobserver or validity or kappa or reliability).ti,ab. or reproducibility.ti,ab. or (test adj2 (re-test or retest)).ti,ab. or "Reproducibility of Results"/ or accuracy.ti,ab. or Diagnosis, Differential/ or Validation Study/	7400403
4	1 and 2 and 3	219
3	exp Immunochemistry/ or exp In Situ Hybridization, Fluorescence/ or exp Reverse Transcriptase Polymerase Chain Reaction/ or exp Whole Genome Sequencing/ or exp High-Throughput Nucleotide Sequencing/ or exp Sequence Analysis, DNA/ or exp Sequence Analysis, RNA/ or exp Sequence Analysis/ or dna next generation sequencing.ti,ab,kf. or rna next generation sequencing.ti,ab,kf. or immunochemistr*.ti,ab,kf. or fluorenscence in situ hybridi?ation.ti,ab,kf. or rt pcr.ti,ab,kf. or gene panel.ti,ab,kf. or whole genome sequencing.ti,ab,kf. or high troughput sequencing.ti,ab,kf. or nanostring.ti,ab,kf.	1006374

2	Lung Neoplasms/ or ((lung or pulmonary) adj3 (cancer or neoplasm* or tumor* or tumour* or malignan*)).ti,ab,kf.	321681
1	Proto-Oncogene Proteins c-ret/ or ret.ti,ab,kf.	9643

Search Strategy:

#	Searches	Results
11	6 not 10 Overige diagnostisch	189
10	8 and 9 SR diagnostisch	6
9	(meta-analysis/ or meta-analysis as topic/ or (metaanaly* or meta-analy* or metanaly*).ti,ab,kf. or systematic review/ or cochrane.jw. or (prisma or prospero).ti,ab,kf. or ((systemati* or scoping or umbrella or "structured literature") adj3 (review* or overview*)).ti,ab,kf. or (systemic* adj1 review*).ti,ab,kf. or ((systemati* or literature or database* or data-base*) adj10 search*).ti,ab,kf. or ((structured or comprehensive* or systemic*) adj3 search*).ti,ab,kf. or ((literature adj3 review*) and (search* or database* or data-base*)).ti,ab,kf. or (("data extraction" or "data source*") and "study selection").ti,ab,kf. or ("search strategy" and "selection criteria").ti,ab,kf. or ("data source*" and "data synthesis").ti,ab,kf. or (medline or pubmed or embase or cochrane).ab. or ((critical or rapid) adj2 (review* or overview* or synthes*)).ti. or (((critical* or rapid*) adj3 (review* or overview* or synthes*)) and (search* or database* or data-base*)).ab. or (metasynthes* or meta-synthes*).ti,ab,kf.) not (comment/ or editorial/ or letter/ or ((exp animals/ or exp models, animal/) not humans/))	565651
8	7 not ((exp animals/ or exp models, animal/) not humans/) not (letter/ or comment/ or editorial/)	128
7	6 not ((exp animals/ or exp models, animal/) not humans/) not (letter/ or comment/ or editorial/)	128
6	4 and 5	130
5	exp "Sensitivity and Specificity"/ or (Sensitiv* or Specific*).ti,ab. or (predict* or ROC-curve or receiver-operator*).ti,ab. or (likelihood or LR*).ti,ab. or exp Diagnostic Errors/ or (inter-observer or intra-observer or interobserver or intraobserver or validity or kappa or reliability).ti,ab. or reproducibility.ti,ab. or (test adj2 (re-test or retest)).ti,ab. or "Reproducibility of Results"/ or accuracy.ti,ab. or Diagnosis, Differential/ or Validation Study/	7371144
4	1 and 2 and 3	330

3	Gene Rearrangement/ or Gene Fusion/ or exp High-Throughput Nucleotide Sequencing/ or ((gene* or dna or ret) adj3 (rearrangement* or fusion* or alteration*)).ti,ab,kf. or hight throughput sequenc*.ti,ab,kf.	95370
2	Lung Neoplasms/ or ((lung or pulmonary) adj3 (cancer or neoplasm* or tumor* or tumour* or malignan*)).ti,ab,kf.	320388
1	Proto-Oncogene Proteins c-ret/ or ret.ti,ab,kf.	9604