original reports abstract

Cost-Effectiveness of Parallel Versus Sequential Testing of Genetic Aberrations for Stage IV Non–Small-Cell Lung Cancer in the Netherlands

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PURPOSE A large number of targeted treatment options for stage IV nonsquamous non–small-cell lung cancer with specific genetic aberrations in tumor DNA is available. It is therefore important to optimize diagnostic testing strategies, such that patients receive adequate personalized treatment that improves survival and quality of life. The aim of this study is to assess the efficacy (including diagnostic costs, turnaround time (TAT), unsuccessful tests, percentages of correct findings, therapeutic costs, and therapeutic effectiveness) of parallel next generation sequencing (NGS)–based versus sequential single-gene–based testing strategies routinely used in patients with metastasized non–small-cell lung cancer in the Netherlands.

METHODS A diagnostic microsimulation model was developed to simulate 100,000 patients with prevalence of genetic aberrations, extracted from real-world data from the Dutch Pathology Registry. These simulated patients were modeled to undergo different testing strategies composed of multiple tests with different test characteristics including single-gene and panel tests, test accuracy, the probability of an unsuccessful test, and TAT. Diagnostic outcomes were linked to a previously developed treatment model, to predict average long-term survival, quality-adjusted life-years (QALYs), costs, and cost-effectiveness of parallel versus sequential testing.

RESULTS NGS-based parallel testing for all actionable genetic aberrations is on average €266 cheaper than single-gene–based sequential testing, and detects additional relevant targetable genetic aberrations in 20.5% of the cases, given a TAT of maximally 2 weeks. Therapeutic costs increased by €8,358, and 0.12 QALYs were gained, leading to an incremental cost-effectiveness ratio of €69,614/QALY for parallel versus sequential testing.

CONCLUSION NGS-based parallel testing is diagnostically superior over single-gene–based sequential testing, as it is cheaper and more effective than sequential testing. Parallel testing remains cost-effective with an incremental cost-effectiveness ratio of 69,614 €/QALY upon inclusion of therapeutic costs and long-term outcomes.

JCO Precis Oncol 6:e2200201. © 2022 by American Society of Clinical Oncology

INTRODUCTION

Non–small-cell lung cancer (NSCLC) has the highest mortality of all types of cancers in the Netherlands. In 49% of patients, NSCLC is detected when it has already metastasized (stage IV), which has a 1 year survival rate of 31% in 2018 in the Netherlands.[1] However, this outcome may be improved as new genetic-aberration–tailored therapy options become available, mostly for nonsquamous NSCLC.

Only specific subgroups of patients who exhibit specific genetic aberrations benefit from these personalized therapies. The Dutch guidelines recommend testing for 12 predictive markers to select patients who are eligible for personalized therapies.[2] Genetic aberrations can be detected with different types of tests, which may come with different sensitivity, specificity, turnaround time (TAT), costs, as well as the number of targets assessed in a single assay.

These different tests can be performed either in parallel to reduce TAT, or sequentially to reduce diagnostic costs. However, the effect of parallel and sequential test strategies on outcomes including costs, TAT, unsuccessful tests, and treatment recommendations is unclear, as it depends on many factors. With

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on June 3, 2022 and published at ascopubs.org/journal/po on July 14, 2022: DOI https://doi.org/10.1200/PO.22.00201

CONTEXT

Key Objective

There is substantial variation in the order and type of molecular tests used for the detection of genetic aberrations for personalized treatment in patients with metastasized nonsquamous non–small-cell lung cancer. This study compares diagnostic testing strategies in the Dutch context, to optimize the detection of druggable targets and the cost-effectiveness of molecular predictive testing.

Knowledge Generated

Model-based analyses showed that parallel testing with next-generation sequencing–based DNA and RNA panels can improve the detection of potential druggable mutations and fusion genes, respectively, without increasing diagnostic costs.

Relevance

Optimal use of diagnostic tests contributes to correct and timely detection of genetic aberrations. This increases the number of patients who receive adequate personalized treatment and improves their survival and quality of life.

respect to TAT, guidelines recommend that test results should be available within 2 weeks.[3]

In the Netherlands, a variety of predictive molecular testing strategies for patients with nonsquamous NSCLC are being used in daily clinical practice.[4] To optimize diagnostics testing, several cost-effectiveness studies have compared next-generation sequencing (NGS) DNA panels to a combination of single-gene tests, or to standard of care.[5-8] These studies found NGS strategies to detect a higher number of genetic aberrations, but differed in conclusions regarding diagnostic costs. None of these studies specified the timing and order of single-gene tests performed, although this may vary in clinical practice and affect both accuracy and TAT of a testing strategy. Therefore, effect of the timing and order of strategies requires investigation aiming to improve testing guidelines.

In the current study, a diagnostic microsimulation model was developed to compare the cost-effectiveness of a parallel (ie, DNA- and RNA-based NGS) versus a sequential (ie, single-gene tests followed by NGS-based approaches) predictive testing strategy assuming that all patients with advanced NSCLC are tested until they are either positive for one marker, or negative for all 12 predictive markers. The testing strategies combine a number of molecular diagnostic tests, where each test has its own specific test characteristics. We first investigated the average diagnostic costs and the percentage of patients with a correct test result received within 2 weeks after initiation of a test strategy. Second, the diagnostic model was linked to a Dutch treatment model,[9] to provide output on total medical costs and quality-adjusted life-years (QALYs) lived when patients in the cohort are treated according to the recommended treatments for each diagnostic strategy.

METHODS

General Approach

To study the cost-effectiveness of diagnostic testing strategies in stage IV nonsquamous NSCLC, a diagnostic model was developed and coupled to the output of a Dutch treatment model[9] that predicts life-years lived (LY), QALYs, and costs for each currently used NSCLC drug treatment in the appropriate patient subgroups. The frequencies of detected genetic aberrations in the simulated cohort were determined in the diagnostic model. When combined, the models can estimate the impact of a diagnostic strategy on long-term patient outcomes by calculating the average treatment costs, overall survival, and QALYs for a mixture of patients with a diversity of molecular diagnoses.

Diagnostic Model

The diagnostic model is a microsimulation model, which simulates a cohort of 100,000 individual patients as they undergo a series of tests that are performed in parallel or sequentially, with each test specified according to its sensitivity, specificity, probability of unsuccessful testing, costs, and TAT. In the model, patients are defined by the true mutational status of the tumor and programmed death ligand-1 (PD-L1) status. For each simulated patient, the model subsequently determines the total testing costs, the molecular test status (eg, *EGFR* mutation-positive), and the *testing status* (which can take one of the values *true positive*, *false positive*, *true negative*, *false negative*, and *unsuccessful tests*; including sample depletion, insufficient quality of the sample, and test failure), and *maximum TAT* (TAT_{max}) *exceeded* (patients in whom the diagnostic test strategy failed to deliver a test result within the acceptable time frame according to medical guidelines, ie, 2 weeks).[3,10-12]

Simulated patients only received one test status. *False-negative* results could be overwritten by all other statuses, because it does not terminate sequential testing strategies. TAT_{max} is determined after all tests are completed and overwrites other test statuses.

Individual patient outcomes were pooled per diagnostic strategy to determine the average diagnostic costs, the frequency distribution of molecular diagnoses, and the frequencies of each *testing status* occurring. The *%correct treatment* was subsequently calculated as the combination

of the proportion of true positives and the proportion of patients without genetic aberration who also receive correct treatment on the basis of PD-L1 expression.

Broad Overview of the Treatment Model

The NSCLC treatment model was developed by Mfumbilwa et al[9] (Data Supplement), which allowed investigating the cost-effectiveness of different test and treatment combinations in patients with NSCLC in a real-world setting. The allocated therapy for each molecular subgroup was based on current clinical practice, as described in the Dutch guidelines[2] and discussed with clinical experts. Predicted average LYs, QALYs, and total therapeutic costs for each molecular subgroup were obtained (Data Supplement) and linked to the outcomes of the diagnostic model, to calculate average LYs, QALYs, and total costs of the total simulated NSCLC cohort.

For individuals in the diagnostic model with *false-positive* mutation tests, it is assumed that the incorrect treatment recommendation led to LYs and QALYs equal to what would be achieved under the best supportive care. Patients with *unsuccessful tests* and patients for whom TAT_{max} was exceeded were assumed to be treated according to their PD-L1 status, or with chemotherapy/immunotherapy (ie, pembrolizumab and cisplatin) if their PD-L1 status remained unknown.

Genetic Aberrations and PD-L1 Expression

Therapeutically relevant genetic aberrations defined in the Dutch Oncology guideline[2] were included in the diagnostic model. These genetic aberrations include common pathogenic mutations in *EGFR classic* (exon 19 deletions, p.L858R), other *EGFR* mutations (eg, p.L861Q, exon 20 insertions), *KRAS* p.G12C, other *KRAS* mutations, BRAF p.V600X, *ERBB2* mutations (in exon 8, 19, 20), *MET* aberrations (ie, amplification and exon 14 skipping), and gene fusions including *ALK*, *ROS1*, *RET*, or *NTRK1/2/3*. Additionally, PD-L1 expression was included. Table 1 shows the frequencies of genetic aberrations in the model as obtained from patients with nonsquamous NSCLC in a Dutch real-world data study that underwent NGS panel testing (N = 3,616).[4] Briefly, mutation frequencies and presence of *ALK/ROS1/RET* fusions were extracted from molecular pathology reports obtained from the nationwide network and registry of histology and cytopathology (Dutch Pathology Registry [PALGA]). These pathology reports were collected in selected time intervals between October 2017 and April 2019.[4,16] *NTRK1,2,3* frequencies were deduced from pathology reports collected from PALGA between 2017 and 2020.[13] In the model, all genetic aberrations are assumed to be mutually exclusive.[17,18] PD-L1 expression was deduced from pathology reports obtained from PALGA between July 2017 and December 2018.[14]

TABLE 1. Prevalence of Genetic Aberrations

Genetic Aberrations	Prevalence (%)	95% CI	Source
KRAS other mutations	25.8	24.5 to 27.0	4
KRAS p.G12C	18.7	17.6 to 19.9	4
EGFR classic (exon 19, p.L858R)	12.8	11.8 to 13.7	4
BRAF (p.V600E)	6.6	5.9 to 7.4	4
MET (amplification/exon 14 skipping)	3.8	3.3 to 4.4	4
EGFR other mutations (p.L861, exon 20 insertions)	3.0	2.6 to 3.5	4
ALK fusion	2.9	2.5 to 3.4	4
ERBB2 mutations	2.0	1.6 to 2.4	4
RET fusion	1.4	1.1 to 1.8	4
ROS1 fusion	1.2	0.9 to 1.5	4
NTRK(1,2,3) fusion	0.1	0.1 to 0.3	13
PD-L1 > 50%[a]	30.9	28.9 to 32.9	14,15
PD-L1 1%-49%[a]	24.1	22.3 to 26.0	14,15

NOTE. The prevalence of genetic aberrations is the prevalence estimated for stage IV nonsquamous non–small-cell lung cancer in the Netherlands.

Abbreviation: PD-L1, programmed death ligand-1.

[a]PD-L1 expression frequencies were adjusted for false-positive tests and false-negative tests using a cross-table, and the sensitivity and specificity of immunohistochemistry.[15]

Diagnostic Testing Strategies

The sequential and parallel testing strategies are defined on the basis of testing patterns commonly observed in Dutch clinical practice (Fig 1). The sequential testing strategy starts with testing for the most common mutation types in *EGFR* and *KRAS*, using two Idylla assays, which is a relatively cheap single-gene test with a short TAT (Table 2). Second, protein expression of ALK, ROS1, and NTRK1,2,3 is analyzed using immunohistochemistry (IHC). IHC positivity requires confirmation with fluorescence in situ hybridization (FISH) because of the high false-positive rates of these IHC tests. In the absence of gene fusions, the presence of remaining mutations is examined using a targeted DNA NGS panel. Finally, *RET* fusions are studied with FISH, and a positive test result is confirmed with a RNA sequencing panel. At each step, testing is only continued in the absence of molecular aberrations in one of the target genes, or when a confirmation test is required. PD-L1 expression is separately tested.

In the parallel testing strategy, the most common genetic aberrations are tested simultaneously with targeted DNA ([hotspot regions of] about 50 genes as described in Pasmans et al[19]) and RNA NGS panels (Archer FusionPlex Lung Panel), and IHC for PD-L1 expression.

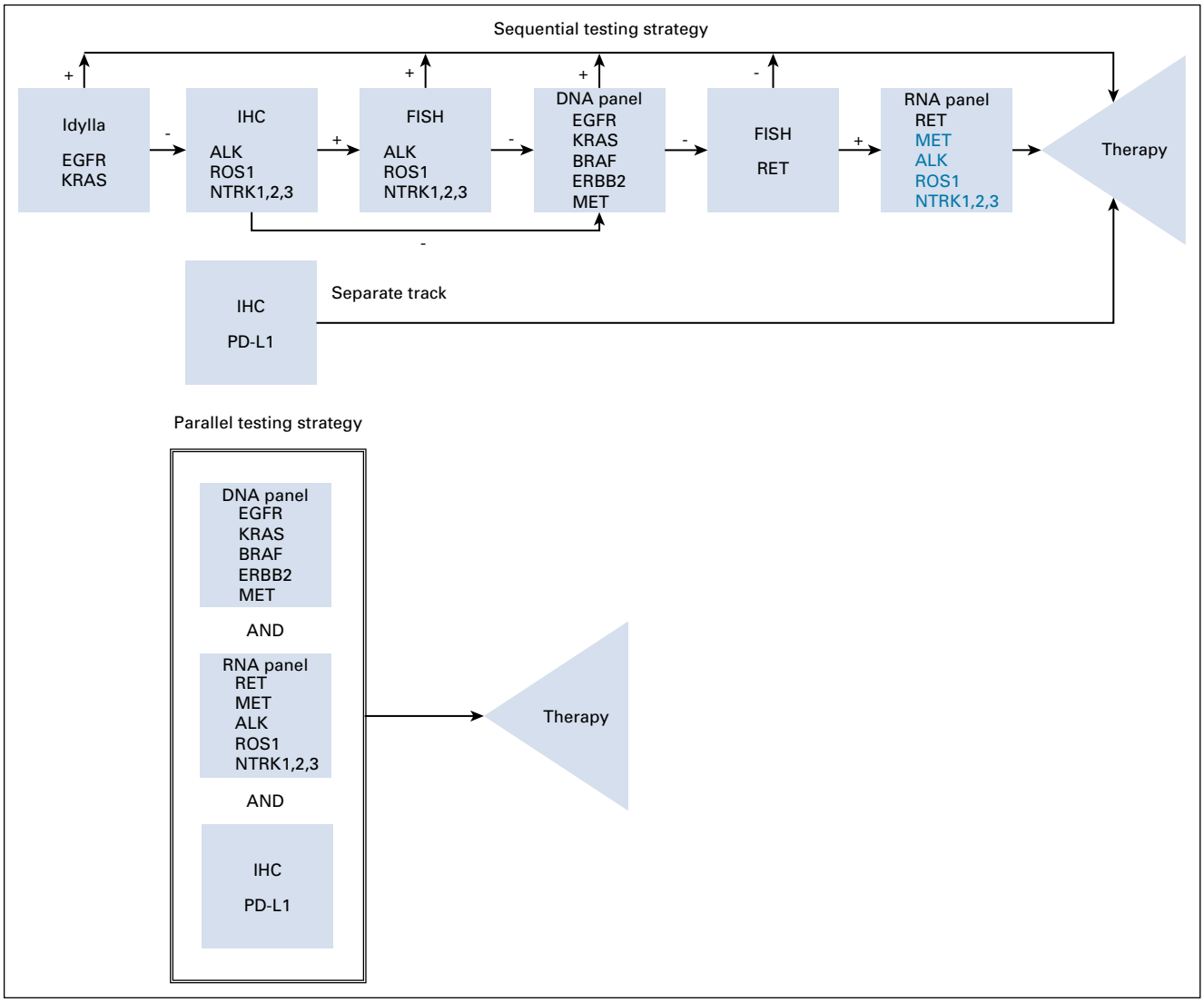

FIG 1. Parallel and sequential testing strategies. Sequential testing starts with tests with short turnaround times for the most common genetic aberrations, and continues testing for less frequently occurring genetic aberrations and fusion genes depending on positive (+) or negative (–) test results. A positive IHC of ALK, ROS1, or NTRK1,2,3 needs to be confirmed with FISH. A positive RET FISH is confirmed with RNA sequencing. PD-L1 expression is tested in a separate independent track. Parallel testing consists of targeted DNA and RNA next-generation sequencing, and IHC testing for PD-L1 simultaneously. FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; PD-L1, programmed death ligand-1.

Model Parameters

All model parameters are shown in Tables 1 and 2 and the Data Supplement. A detailed description of the sources, estimation methods, and assumptions that were used to determine these parameters can be found in the Data Supplement.

Additional Analyses

Three additional analyses investigated how the conclusions of the model were affected by modeling choices made. First, simulations were performed to observe the impact of using alternative, mixed, diagnostic testing strategies (Data Supplement) in combination with the effect of a different TAT_{max}. Second, the impact of including treatment for *KRAS* p.G12C and *RET* fusions was investigated. Finally, a one-way sensitivity analysis was performed, to assess the impact of parameter uncertainty on the difference in *% correct treatment* between sequential and parallel testing. A more extensive description of the additional analyses can be found in the Data Supplement.

RESULTS

Diagnostic Efficacy of Parallel Versus Sequential Testing

In the sequential strategy, 32.5% of the patients exceed the TAT_{max} and consequently did not receive a test result in time (Fig 2). Within this group, most patients harbored genomic aberrations in *BRAF*, *ERBB2*, *RET*, or *MET*, meaning that the TAT_{max} is often reached during the third step of the sequential test strategy (Fig 1). Parallel testing significantly reduces the variance in TAT (Data Supplement), and only 1.5% of the patients exceed the TAT_{max}.

TABLE 2. Test Characteristics

Test	Sensitivity	Specificity	TAT (days)[a]	Costs (€)	Source
Idylla *KRAS*	0.81 (0.65 to 0.93)	0.95 (0.87 to 0.99)	3.85 (1.75 to 8.47)	258	4,19,20
Idylla *EGFR*	0.98 (0.96 to 0.99)	0.96 (0.93 to 0.98)	3.85 (1.75 to 8.47)	318	4,19,21
IHC *ALK*	0.94 (0.92 to 0.96)	0.95 (0.94 to 0.95)	1.37 (1.29 to 1.46)	102	4,11,19
IHC *ROS1*	0.93 (0.86 to 0.98)	0.92 (0.90 to 0.94)	1.37 (1.29 to 1.46)	102	4,11,19
IHC *NTRK*1,2,3	0.88 (0.79 to 0.95)	0.81 (0.77 to 0.85)	1.37 (1.29 to 1.46)	102	4,19,22
IHC PD-L1 0%[b]	0.99 (0.94 to 0.97)	0.79 (0.74 to 0.84)	1.37 (1.29 to 1.46)	102	4,15,19
IHC PD-L1 50%[b]	0.96 (0.93 to 0.98)	0.60 (0.53 to 0.67)	1.37 (1.29 to 1.46)	102	4,15,19
FISH *RET*	0.94 (0.85 to 0.99)	0.71 (0.57 to 0.84)	1.18 (1.09 to 1.28)	134	4,19,23-26
FISH *ALK*, *ROS1*, *NTRK1,2,3*	0.94 (0.85 to 0.99)	0.92 (0.84 to 0.98)	1.18 (1.09 to 1.28)	134	4,19,23-25
DNA sequencing panel	0.95 (0.90 to 0.99)	0.95 (0.90 to 0.99)	8.68 (7.31 to 10.29)	284	4,19,27
RNA sequencing panel[c]	0.95 (0.90 to 0.99)	0.95 (0.90 to 0.99)	8.68 (7.31 to 10.29)	407	4,19,28

NOTE. Diagnostic costs are fixed in the model and include capital costs, maintenance costs, software costs, and operational costs. The sensitivity, specificity, and TAT are reported as mean (95% CI).

Abbreviations: FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; PD-L1, programmed death ligand-1; TAT, turnaround time.

[a]The TATs of tests are drawn from a log-normal distribution.

[b]PD-L1 expression frequencies were adjusted for false-positive tests and false-negative tests using a cross-table, and the sensitivity and specificity of IHC.[15]

[c]Newly estimated using a bottom-up microcosting calculation for Archer FusionPlex Lung Panel, similar to that described by Pasmans et al.[28]

Sequential testing has more *false-positive* test results (3.3%) than parallel testing (1.4%), since each additional tests increases the chance of a *false-positive* result. Likewise, the number of *unsuccessful tests* increases if more tests are required (sequential 6.9% *v* parallel 2.7%). The proportion of *unsuccessful tests* in the sequential strategy in our simulations is slightly higher than observed in our reference cohort[4] (5.7%). However, the reference cohort contains a broad mixture of sequential strategies unlike the simulated cohort and sample depletion was difficult to assess in these data, which might explain this difference.

Differences in PD-L1 test outcomes (data not shown) are insignificant between both strategies because of the same strategy to determine the PD-L1 status (ie, separate track).

Costs of Parallel Versus Sequential Testing

The difference between the sequential and parallel testing strategies in average diagnostic costs is €158, making parallel testing 17% cheaper (Table 3). Sequential testing is cheaper for 45.5% of patients who have mutations in *EGFR* and *KRAS* and hence only require Idylla testing. However, the additional costs for IHC and FISH of *ALK*, *ROS1*, and *NTRK1,2,3* in the next steps already exceed the average parallel testing costs.

Cost-Effectiveness of Parallel Versus Sequential Testing

The diagnostic model predicts parallel testing to be able to correctly identify a substantially higher proportion (20.5%) of therapeutic targets within the TAT_{max} of 2 weeks (Table 3). The treatment models shows that parallel testing obtains an additional 0.17 LY and 0.12 additional QALYs compared with sequential testing. The treatment costs are €8,357 higher for parallel testing, resulting in an incremental cost-effectiveness ratio (ICER) of €69,614/QALY.

Additional Analyses

Additional analyses were performed to study the effect of variations in testing strategies, model parameters, and inclusions of additional targeted therapies. Details can be found in the Data Supplement. In brief, we examined whether alternative testing strategies affected the outcomes of this study (Data Supplement).

Parallel testing remains the best strategy to detect targetable genetic aberrations, unless the TAT_{max} is 1 week (Data Supplement). However, all strategies perform poorly if TAT_{max} is set at 1 week; it is questionable whether such a TAT_{max} is necessary. TAT_{max} depends on guidelines, which in turn are determined by both the clinical urgency for patients and the feasibility for laboratories.

Sequential DNA-based followed by RNA-based NGS analyses is the best alternative for the parallel testing strategy. With DNA-based NGS analyses, often, a driver aberration is detected and therefore the sequential strategy leads to an average cost reduction of 36% (€280) compared with the parallel testing strategy. However, this alternative strategy leads to an increase in TAT, and 5.1% of the test results exceeded the TAT_{max}, unless the TAT_{max} is increased to 4 weeks.

In addition, the effect of new targeted therapies on the outcomes was evaluated. Parallel NGS-based testing remained the dominant strategy upon inclusion of targeted therapies for *KRAS* p.G12C mutations and *RET* fusions in the model (Data Supplement). Including these therapies resulted in increased survival of patients and an ICER of

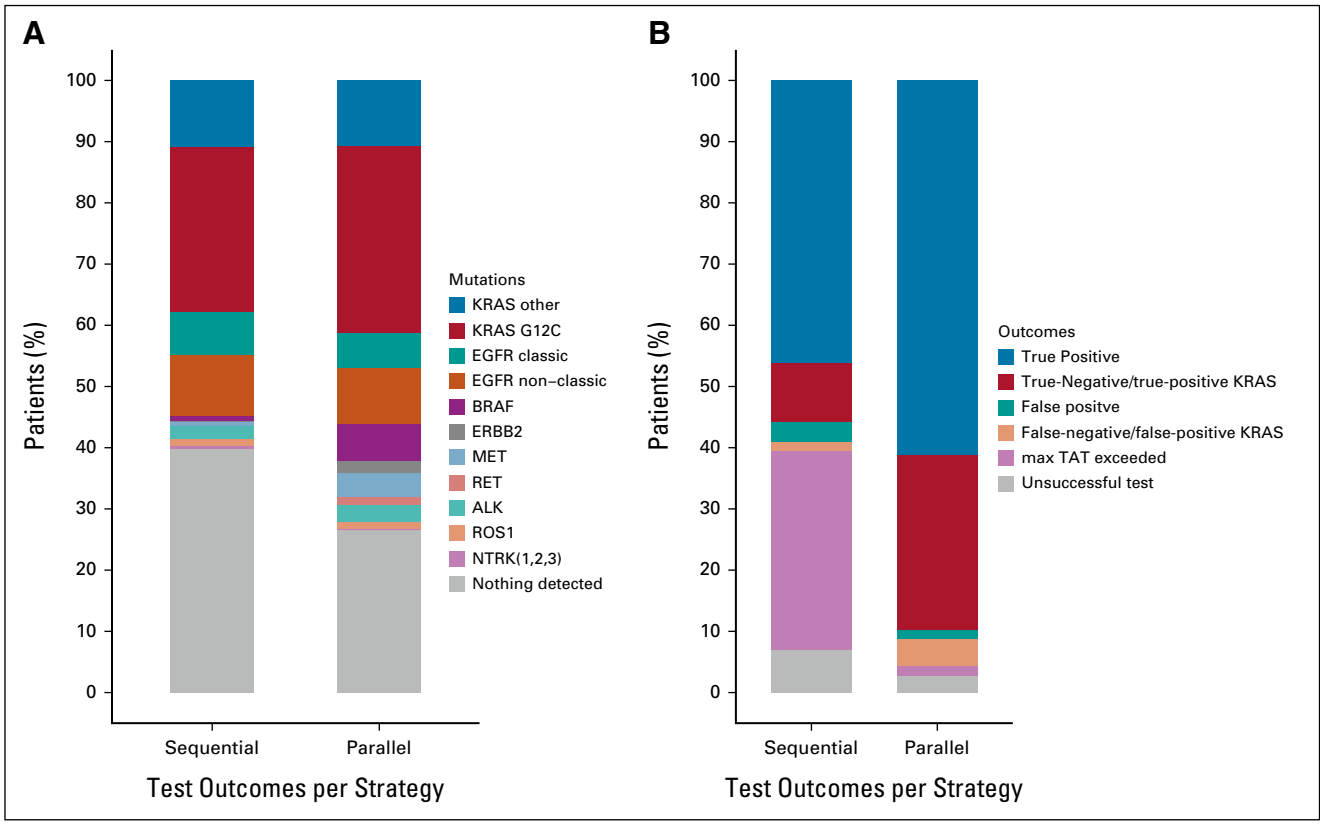

FIG 2. Outcomes of testing strategies. (A) The positive tests show how often mutations are found (true- and false-positive results are pooled). Negative test results, max TAT exceeded, and unsuccessful tests are pooled in the category nothing detected. (B) The test status, that is, the proportion of tests that are true positive with druggable targets, the proportion of true-negative results as well as those positive for KRAS (other), and the proportion of unsuccessful tests (including sample depletion, insufficient quality of the sample, and test failure). Programmed death ligand-1 outcomes are not included in this figure. max TAT, maximum turnaround time.

€54,985/QALY. The expansion of the lists of therapeutic options therefore likely increases the beneficial effect of correctly identifying therapeutic targets, making parallel testing and usage of DNA and RNA NGS panel tests an even more attractive choice.

Finally, we performed a sensitivity analysis with regard to the prevalence of aberrations and sensitivity, specificity, and TAT of the different tests (Data Supplement). Uncertainty concerning parameter estimates only had a relatively small effect on the outcomes of the model. Taken together,

TABLE 3. Cost-Effectiveness Outcomes for the Comparison of the Parallel and the Sequential Testing Strategy and Their Treatments, Including All Therapeutic Targets That Are Currently Recommended by the Dutch Guidelines

	Diagnostic Model		Treatment Model		
Strategy	**Test Costs (€)**	**% Correct Treatment**	**Total Costs (€)**	**LYs**	**QALYs**
Sequential	936	69.4	149,158	2.21	1.56
Parallel	778	89.9	157,515	2.39	1.68
Difference	–158	20.5	8,357	0.17	0.12
Additional QALYs per % additional correct treatment: 0.0058					
ICER: €69,614/QALY					

NOTE. Average test costs per patient and the percentage of patients who received correct treatment with targeted therapy or immunotherapy using a maximum turnaround time of 2 weeks were compared in the diagnostic model. Subsequently, these results were used in the treatment model, to calculate the average total costs including therapeutic and test costs, and the average LYs and QALYs per patient (see the Data Supplement for therapy specific input parameters). The difference between the sequential and the parallel testing strategy and the ICER for parallel versus sequential testing are found in the lower two rows of the table.

Abbreviations: ICER, incremental cost-effectiveness ratio; LY, life-years lived; QALY, quality-adjusted life-year.

outcomes remained robust to variations in testing strategies, model parameters, and inclusion of additional targets.

DISCUSSION

Large variation exists in predictive molecular testing strategies for patients with nonsquamous NSCLC in daily clinical practice. Parallel testing (ie, DNA- and RNA-based NGS panels) assesses all druggable molecular alterations at the same time, thereby obtaining a relatively low TAT for assessing all genetic markers relevant for treatment decisions. Alternatively, molecular testing can also be performed sequentially (ie, single-gene tests followed by NGS-based approaches), which aims to save costs and obtain a low TAT for the most abundant genetic aberrations (ie, *EGFR* and *KRAS*). We performed a microsimulation of a cohort of 100,000 patients with stage IV nonsquamous NSCLC and showed that parallel testing for genetic aberrations is cost-effective compared with sequential testing with a cost-saving of €158 (17%) in diagnostic costs, and 20.5% additional patients receiving appropriate treatment within 2 weeks after test initiation. These results were robust to variations in testing strategies, model parameters, and inclusion of additional targets (*RET* fusions and *KRAS* p.G12C).

The increase in proportion of correct treatments in the parallel strategy resulted in additional treatment costs of €8,357, for an increase of 0.12 QALYs compared with sequential testing, ie, for each percentage point increase in correct treatments, 0.0058 QALYs were gained. Parallel testing showed an ICER of €69,614/QALY upon inclusion of therapeutic costs and survival benefits in the diagnostic strategy comparison. This is below the Dutch recommended willingness-to-pay threshold of €80,000/QALY,[29] and can therefore be considered cost-effective.

Parallel testing strategies outperformed sequential testing in the current study for two reasons. First, combining two tests that focus on the same genetic aberration (Idylla and NGS or IHC and FISH) affects the *false-positive* and *false-negative* rate, resulting in fewer *true positives* for the sequential testing strategy. Second, using multiple sequential tests increased the total TAT for all biomarkers, risk of sample exhaustion,[4] and diagnostic costs. Instead, comprehensive DNA and RNA NGS panels enable simultaneous detection of all predictive markers and are therefore cheaper when compared with the average sequential strategy.

Our results are in line with other publications showing that comprehensive NGS strategies outperformed single-gene tests with respect to diagnostic yield.[5-8,17,18] Differences in diagnostic costs varied per study. However, these publications differ from the current study. For example, we combined different molecular techniques and included TAT in determining the usefulness of test outcomes.

Some simplifying modeling assumptions had to be made for this study. First, we assumed that all test results obtained after the TAT_{max} are disregarded and the patient is treated with immunotherapy or chemotherapy depending on their PD-L1 status. However, we also show that parallel testing remained the most effective strategy upon considering higher TAT_{max} values acceptable. Hence, these results support our conclusions.

A second assumption involves the sensitivity and specificity of the molecular tests, which were unknown for targeted DNA and RNA NGS analyses, as there is no gold standard to compare these tests to. Therefore, we assumed a 95% sensitivity and specificity, which is a conservative estimate.[30] The true sensitivity and specificity may be higher and therefore would increase the difference in effectiveness between sequential and parallel testing, favoring parallel testing.

Third, the probability of *unsuccessful tests* was simplified in the model, by assuming that this probability is the same for all tests. This automatically results in a higher cumulative probability in the sequential testing strategy. Real-world data from Dutch clinical practice[4] showed that *unsuccessful tests* are significantly higher for single-gene testing strategies compared with NGS-based approaches. This difference might even be larger as sample depletion is under-reported in this real-world data set. Similarly, Dal-l'Olio et al[6] show that stepwise testing with a higher number of tests also has a significantly higher sample exhaustion rate than a large panel testing strategy. Taken together, the difference in *unsuccessful tests* between the parallel NGS-based and sequential single-gene–based testing strategy is likely larger than assumed in the model, which strengthens the conclusions of this study.

A fourth simplification is that rebiopsies are not taken into account. The samples that are obtained at baseline are assumed not to affect the observed differences in outcomes such as the cost-effectiveness ordering between the different strategies. Rebiopsies are rarely taken, and the obtained data on this topic were of poor quality. Rebiopsies are both relatively expensive and increase the TAT, and would therefore negatively affect the cost-effectiveness of strategies. It is likely that rebiopsies are more common in strategies with higher frequencies of *unsuccessful tests*, which is shown to be higher for single-gene tests. Inclusion of rebiopsies would therefore further strengthen the conclusions of this study.

The results of the diagnostic model can be generalized to other countries, although genetic aberration frequencies might differ. For example, *EGFR* mutation frequencies are much lower in Europe than in Asian countries.[31] Nevertheless, other studies found that NGS-based DNA-panel testing is also cost-effective in Asian populations.[7]

In addition to providing added value for current treatment recommendations in NSCLC, targeted DNA and RNA NGS panels have additional benefits. These comprehensive analyses can potentially detect additional genetic aberrations, enabling inclusion into clinical trials. Implementation of even larger targeted NGS panels or whole genome sequencing would enable even broader analyses

(eg, additional targets and tumor mutational burden). Implementation of such strategies in routine clinical practice requires a similar evaluation of diagnostic accuracy, costs, and test-TAT as presented here. Furthermore, expertise and volume are important factors to cost-effectively implement such broad testing strategies.[32,33] Sample volume per laboratory, for example, has a large effect on diagnostic costs and TAT. Centralization of these sequencing analyses could reduce both costs and TAT.[19]

In conclusion, parallel testing with targeted DNA- and RNA-NGS approaches has a higher true-positive testing rate than sequential testing with multiple single-gene tests in combination with targeted DNA-based NGS, resulting in more adequate treatment and improved survival.

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SUPPORT

Supported by ZonMw (846001001).

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Financial support: Katrien Grünberg, Marjolijn J.L. Ligtenberg
Provision of study materials or patients: Katrien Grünberg, Marjolijn J.L. Ligtenberg
Collection and assembly of data: Elisabeth M.P. Steeghs, Zakile A. Mfumbilwa, Harry J.M. Groen
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Manuscript writing: All authors
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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

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No other potential conflicts of interest were reported.

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