



**Universiteit  
Leiden**  
The Netherlands

## **Trastuzumab and pertuzumab combination therapy for advanced pre-treated HER2 exon 20-mutated non-small cell lung cancer**

Henegouwen, J.M.V.; Jebbink, M.; Hoes, L.R.; Wijngaart, H. van der; Zeverijn, L.J.; Velden, D.L. van der; ... ; Gelderblom, H.

### **Citation**

Henegouwen, J. M. V., Jebbink, M., Hoes, L. R., Wijngaart, H. van der, Zeverijn, L. J., Velden, D. L. van der, ... Gelderblom, H. (2023). Trastuzumab and pertuzumab combination therapy for advanced pre-treated HER2 exon 20-mutated non-small cell lung cancer. *European Journal Of Cancer*, 171, 114-123. doi:10.1016/j.ejca.2022.05.009

Version: Publisher's Version

License: [Creative Commons CC BY 4.0 license](https://creativecommons.org/licenses/by/4.0/)

Downloaded from: <https://hdl.handle.net/1887/3731171>

**Note:** To cite this publication please use the final published version (if applicable).



## Original Research

# Trastuzumab and pertuzumab combination therapy for advanced pre-treated HER2 exon 20-mutated non-small cell lung cancer



J.M. van Berge Henegouwen <sup>a,b</sup>, M. Jebbink <sup>c</sup>, L.R. Hoes <sup>b,d</sup>,  
 H. van der Wijngaart <sup>b,e</sup>, L.J. Zeverijn <sup>b,d</sup>, D.L. van der Velden <sup>f</sup>,  
 P. Roepman <sup>g</sup>, W.W.J. de Leng <sup>h</sup>, A.M.L. Jansen <sup>h</sup>, E. van Werkhoven <sup>i</sup>,  
 V. van der Noort <sup>i</sup>, A.J. van der Wekken <sup>j</sup>, A.J. de Langen <sup>c</sup>, E.E. Voest <sup>b,d</sup>,  
 H.M.W. Verheul <sup>k</sup>, E.F. Smit <sup>l</sup>, H. Gelderblom <sup>a,\*</sup>

<sup>a</sup> Department of Medical Oncology, Leiden University Medical Center, Leiden, the Netherlands

<sup>b</sup> Oncode Institute, the Netherlands

<sup>c</sup> Department of Thoracic Oncology, Netherlands Cancer Institute, Amsterdam, the Netherlands

<sup>d</sup> Department of Molecular Oncology & Immunology, Netherlands Cancer Institute, Amsterdam, the Netherlands

<sup>e</sup> Department of Medical Oncology, Amsterdam University Medical Center, Vrije Universiteit Amsterdam, Cancer Center Amsterdam, Amsterdam, the Netherlands

<sup>f</sup> Department of Radiology and Nuclear Medicine, Amsterdam University Medical Center, Amsterdam, the Netherlands

<sup>g</sup> Hartwig Medical Foundation, Amsterdam, the Netherlands

<sup>h</sup> Department of Pathology, University Medical Center Utrecht, Utrecht, the Netherlands

<sup>i</sup> Biometrics Department, Netherlands Cancer Institute, Amsterdam, the Netherlands

<sup>j</sup> Department of Pulmonary Diseases, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

<sup>k</sup> Department of Medical Oncology, Radboud University Medical Center, Nijmegen, the Netherlands

<sup>l</sup> Department of Pulmonology, Leiden University Medical Center, Leiden, the Netherlands

Received 14 February 2022; received in revised form 23 April 2022; accepted 16 May 2022

Available online 15 June 2022

## KEYWORDS

Non-small lung cancer;  
 HER2 mutation;  
 Targeted therapies;  
 Precision medicine;

**Abstract Introduction:** In 1–3% of non-small cell lung cancer (NSCLC) human epidermal growth factor 2 (*HER2*) mutations are identified as a genomic driver. Nevertheless, no *HER2*-targeted treatment is approved for NSCLC. In the Drug Rediscovery Protocol (DRUP), patients are treated with off-label drugs based on their molecular profile. Here, we present the results of the cohort ‘trastuzumab/pertuzumab for *HER2* exon20 mutation positive (*HER2m+*) NSCLC’.

\* Corresponding author: Department of medical oncology, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, the Netherlands.  
 E-mail address: [a.j.gelderblom@lumc.nl](mailto:a.j.gelderblom@lumc.nl) (H. Gelderblom).

## Whole genome sequencing

**Methods:** Patients with treatment refractory, advanced HER2m+ NSCLC with measurable disease (RECISTv1.1) were eligible. Treatment with intravenous trastuzumab combined with pertuzumab every 3 weeks was administered. The primary end-point was clinical benefit (CB: either objective response or stable disease  $\geq$  16 weeks). Patients were enrolled using a Simon-like 2-stage design, with 8 patients in stage 1 and up to 24 patients in stage 2 if at least 1 patient had CB in stage 1. At baseline, a biopsy for biomarker analysis, including whole genome sequencing, was obtained.

**Results:** Twenty-four evaluable patients were enrolled and treated between May 2017 and August 2020. CB was observed in 9 patients (38%); including an objective response rate of 8.3% (2 patients had a partial response) and 7 patients with stable disease  $\geq$  16 weeks. The most frequently observed *HER2* mutation was p.Y772\_A775dup (71%, n = 20). Median follow-up was 13 months, median progression-free survival and overall survival 4 (95% CI 3–6) and 10 months (95% CI 4 – not reached), respectively. Whole genome sequencing data (available for 67% of patients) confirmed the inclusion mutation in all cases. No unexpected toxicity was observed.

**Conclusion:** Despite the fact that the study did meet its primary end-point, trastuzumab/pertuzumab was only marginally active in a subset of patients with heavily pre-treated *HER2m+* NSCLC.

© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Primary lung cancer is one of the most commonly diagnosed cancers and a leading cause of cancer-related death worldwide [1]. Over 80% of the primary lung cancer cases are non-small cell lung cancers (NSCLCs). At the time of diagnosis, the majority of these patients have regional spread or metastatic disease. Fortunately, major progress has been made in the systemic targeted treatment of metastatic NSCLC in the past decade [2].

The discovery of oncogenic drivers in NSCLC led to the approval of a number of targeted agents, mostly tyrosine kinase inhibitors (TKIs), which are widely used in clinic [2]. Currently, no targeted treatment for the oncogenic driver human epidermal growth factor 2 (*HER2*) is approved, despite its prevalence of 1–3% in NSCLC [2–6]. *HER2* mutation positive (*HER2m+*) NSCLC is more frequently identified in female patients, patients with adenocarcinoma subtype and non-smokers. The genetic diversity of *HER2* mutations in NSCLC is low, as more than 90% are in-frame insertions located in exon 20 and tend to be mutually exclusive of other oncogenic drivers [3,6].

*HER2* is a member of the EGFR tyrosine kinase family and is involved in several signalling pathways, including MEK-ERK and PI3K-AKT. The oncogenic activation of *HER2*, at least by activating mutations and possibly by overexpression or gene amplifications, leads to constitutive dimerisation and activation of these pathways and promotes uncontrolled cell growth [7]. Activating *HER2* alterations have been reported in various other tumours, most notably in breast and gastric/gastroesophageal junction carcinomas and are

well-known to be associated with sensitivity to *HER2*-targeting agents in *HER2*-positive breast cancer, prolonging the overall survival (OS) in these patients. Currently, this is considered to be standard of care [8]. In patients with *HER2m+* NSCLC, shorter progression-free survival (PFS) has been observed in comparison with the general population of stage IV NSCLC, presumably due to intrinsic resistance to chemotherapy [9].

Trastuzumab, a recombinant humanised monoclonal antibody, suppresses oncogenic signalling by blocking either *HER2* homodimerization or ligand-independent heterodimerization with *HER3*. However, trastuzumab does not prevent ligand-dependent heterodimerization with other *HER* molecules, potentially creating an escape for tumour cells [10]. The humanised monoclonal antibody pertuzumab, a *HER* heterodimer inhibitor, inhibits heterodimerization of *HER2* with other *HER* molecules and ligand-induced dimerisation with *HER3* [11]. Therefore, combining trastuzumab and pertuzumab provides a more complete dual blockage of *HER2* downstream signalling [12].

In the present article, we describe the treatment outcome of one of the Drug Rediscovery Protocol (DRUP) cohorts, in which patients with metastatic and/or advanced NSCLC harbouring a *HER2* exon 20 mutation have been treated with the combination of trastuzumab and pertuzumab. In DRUP, patients are treated based on their tumour molecular profile with registered targeted treatments outside their labelled indications [13]. Efficacy and safety data are systematically recorded. Moreover, whole genome sequencing (WGS) for extensive biomarker analysis is performed on baseline tumour biopsies.

## 2. Methods

### 2.1. Study design

The DRUP is a national, investigator-initiated, prospective, multicentre, non-randomised clinical basket trial, designed and conducted on behalf of the Center For Personalized Cancer Treatment (CPCT; Amsterdam, The Netherlands). In DRUP, patients with metastatic or advanced solid tumours, multiple myeloma or non-Hodgkin lymphoma are treated with commercially available targeted- and immunotherapies based on their tumour molecular profile, outside the registered indication. Submitted patients are reviewed and matched to the available study drugs based on their tumour molecular profile by the central study team. If deemed necessary, the DRUP central molecular tumour board can be consulted. Patients are then enrolled in multiple parallel cohorts, each defined by a tumour type, tumour profile and study drug [13]. The study protocol has been approved by the independent ethics committee. The study includes only adults aged  $\geq 18$  years and written informed consent is obtained from all the subjects participating in the study.

DRUP is registered with [ClinicalTrials.gov](https://clinicaltrials.gov), number NCT02925234.

### 2.2. Study population

Eligible patients had treatment refractory, metastatic NSCLC with molecular testing (panel-based next-generation sequencing, polymerase chain reaction or WGS) demonstrating a pathogenic *HER2* exon 20 mutation in either the primary tumour or a metastatic deposit. Patients had measurable disease according to the Response Evaluation Criteria in Solid Tumours version 1.1 (RECIST v1.1) and an Eastern Cooperative Oncology Group performance status of 0–2 [14]. Furthermore, patients were required to have adequate bone marrow and organ function, left ventricular ejection fraction  $>50\%$  (assessed by ultrasound, multigated acquisition scan or magnetic resonance imaging) and were required to use adequate contraception for the duration of study treatment and 7 months thereafter.

Patients with known pathogenic mutations in *KRAS*, *NRAS* or *BRAF* were excluded. Additional exclusion criteria included severe dyspnoea at rest due to the complications of advanced malignancy, requiring oxygen therapy; prior treatment with anthracyclines  $<6$  months prior to enrolment; ongoing toxicity of grade 2 or higher (other than alopecia) according to ‘Common Terminology Criteria for Adverse Events (CTCAE 4.03)’, caused by previous treatments; concomitant treatment with any other anti-cancer therapy; known active progressive brain metastases (patients with previously treated brain metastases were eligible, provided that the patient had not experienced a seizure or change

in neurological status  $<3$  months prior to enrolment and had been stable for  $>1$  month without steroid treatment); the presence of any other clinically significant medical condition which made it undesirable to participate in the study. Patients were considered evaluable for the primary end-point if at least 2 treatment administrations of intravenous medication were completed. Non-evaluable patients were replaced.

### 2.3. Treatment and tumour assessment

Patients were treated with intravenous trastuzumab (initial loading dose of 8 mg/kg body weight, followed by a maintenance dose of 6 mg/kg body weight) in combination with intravenous pertuzumab (initial loading dose of 840 mg, followed by a maintenance dose of 420 mg) in 21-day cycles until disease progression or unmanageable toxicity. Thoracic and abdominal CT scans for tumour response assessment were performed at baseline and every 9 weeks (3 cycles) after treatment initiation. If study treatment was continued after 3 response evaluations (i.e. 27 weeks), response evaluations were performed every 12 weeks.

Safety was measured by the frequency of grade  $\geq 3$  adverse events (AEs) and serious AEs (SAEs) occurring up to 30 days after the last dose of study drug. All AEs were graded according to the CTCAE v4.03.

The primary end-points included clinical benefit (CB) rate, defined by confirmed objective tumour response (RECIST v1.1) and absence of disease progression for  $\geq 16$  weeks (stable disease (SD)) after treatment initiation, and treatment-related grade  $\geq 3$  AEs and SAEs. The secondary end-points included PFS and OS, time on treatment (TOT) and TOT ratio (the ratio of TOT of the current study treatment to TOT of prior line of treatment). Sequencing-success rate of pre-treatment biopsies and biomarker analysis using WGS on pre-treatment biopsies formed an exploratory end-point.

### 2.4. WGS on pre-treatment biopsies and biomarker analysis

A fresh frozen tumour biopsy was mandatory before treatment initiation, obtained  $\leq 2$  months before enrolment, after the last line of therapy and without any type of anti-cancer therapy in between. Biopsies during and after the study treatment were optional. All biopsies were sent to the Hartwig Medical Foundation (Amsterdam, The Netherlands) for WGS [15], together with a 10-ml blood sample to determine the background variation of the germline DNA of the patient. If the tumour cell percentage was  $\geq 30\%$  and the DNA yield was  $\geq 300$  ng, WGS and biomarker analyses were performed. In case WGS analysis failed, WGS data obtained either before or after study inclusion were used for the biomarker analysis, if available.

WGS data were analysed using an optimised, high-quality bioinformatic pipeline. A summarising report of all relevant findings was created for each patient, including information on tumour purity, ploidy, somatic variants, copy number variations, mutational load and more complex genomic features such as gene fusions, COSMIC mutational signatures, microsatellite (in) stability and homologous repair deficiency [15–17].

### 2.5. Statistical analysis

This cohort was evaluated in a 2-stage design, using a Simon-like 2-stage monitoring plan [18]. If there would be 0 patients with CB in the first 8 included patients, the cohort would be closed early. Otherwise, an additional 16 patients would be included. If 5 or more patients would meet the definition of CB, further investigation would be warranted. This monitoring rule has 85% power and an alpha error rate of 7.8% to reject the null hypothesis that the probability of CB for 16 weeks is 10% if it is actually 30%.

All statistical analyses were performed using R version 4.0.3 (<http://www.R-project.org/>). Patient characteristics, AEs and tumour responses were summarised using descriptive statistics. A waterfall plot was used to illustrate maximum tumour shrinkage compared to baseline. Kaplan–Meier methods were used to estimate TOT, PFS (from the start of treatment to progression or death from any cause, whichever came first and censoring patients alive without progression) and OS (calculated from the first day of treatment administration to the date of death from any cause, censoring patients who were alive at last follow-up). Associations between CB and

COSMIC mutational signatures were explored using the Pearson Chi-square test. For associations between CB and number of potential drivers, the Wilcoxon's rank sum test was used.

## 3. Results

### 3.1. Patients

For the current cohort, patients were included in 10 out of the 35 hospitals in The Netherlands that participated in DRUP between May 2017 and August 2020. A total of 28 patients with advanced *HER2* exon 20-mutated NSCLC were enrolled and treated. All 28 patients were included for baseline characteristics and safety analyses. However, among the 28 included patients, 3 patients were not evaluable for the primary end-point. In addition, during the analysis it appeared that 1 patient harboured a *HER2* exon 19 mutation (p.L755P). Therefore, this patient was excluded from the efficacy analysis, leading to a total of 24 patients with evaluable *HER2* exon 20-mutated NSCLC (Fig. 1). For baseline characteristics and safety analysis, all 28 patients who started study treatment were included.

At data cut-off in May 2021, the median follow-up duration was 13 months (IQR 6.4–20.1). The main reason for the treatment discontinuation of the evaluable patients was progressive disease ( $n = 21$ ; 92%). The other reason for treatment discontinuation was symptomatic deterioration ( $n = 3$ ; 8%).

Baseline characteristics of patients with NSCLC that have been included in the current cohort are presented in

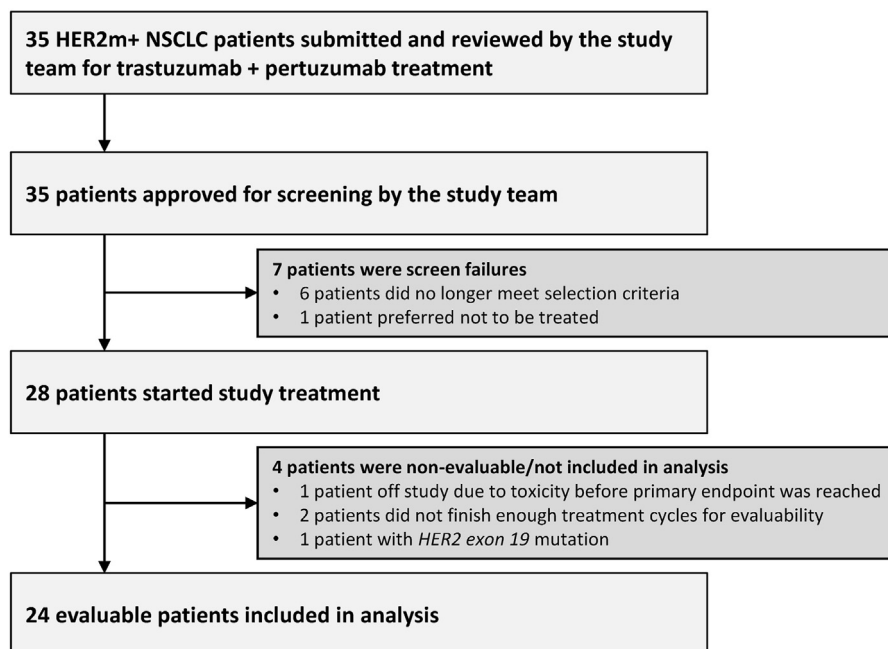


Fig. 1. Case submissions and reasons for non-ac accrual or non-evaluability. Flow chart of patients with *HER2* exon 20-mutated NSCLC submitted to the study team between May 2017 and August 2020, and reasons for screen failure and non-evaluability. *HER2m+* NSCLC: *HER2* exon 20 mutated non-small lung cancer. *HER*, human epidermal growth factor; NSCLC, non-small cell lung cancer.

Table 1  
Baseline characteristics.

No. of patients (%)	Clinical benefit (n = 9)	Objective response (n = 2)	No clinical benefit (n = 15)	Total (n = 28)
<b>Median age, years (range)</b>	70 (47–79)	72 (71–73)	59 (37–75)	59 (37–82)
<b>Gender</b>				
Male	5 (56%)	1 (50%)	7 (47%)	14 (50%)
Female	4 (44%)	1 (50%)	8 (53%)	14 (50%)
<b>WHO performance status</b>				
WHO 0	3 (33%)	–	4 (27%)	7 (25%)
WHO 1	5 (56%)	1 (50%)	10 (67%)	17 (61%)
WHO 2	–	–	–	1 (4%)
Unknown	1 (11%)	1 (50%)	1 (7%)	3 (11%)
<b>Histology,</b>				
Adenocarcinoma	9 (100%)	2 (100%)	15 (100%)	28 (100%)
<b>Median No. of previous systemic therapy lines</b>				
1	2 (22%)	1 (50%)	3 (20%)	7 (25%)
2	7 (78%)	1 (50%)	5 (33%)	12 (43%)
3	–	–	5 (33%)	6 (21%)
4	–	–	1 (7%)	2 (7%)
6	–	–	1 (7%)	1 (4%)
<b>Previous targeted therapy lines</b>				
0	8 (89%)	2 (100%)	10 (67%)	21 (75%)
1	1 (11%)	–	3 (20%)	5 (15%)
2	–	–	2 (13%)	2 (7%)
<b>Prior HER2-targeted therapy</b>				
Afatinib	–	–	4 (27%)	4 (14%)
<b>Smoking status (n = 26)</b>				
Current smoker	1 (11%)	–	1 (7%)	2 (7%)
Former smoker	3 (33%)	–	6 (40%)	11 (39%)
Never smoker	5 (56%)	2 (100%)	8 (53%)	13 (46%)
Unknown	–	–	–	2 (7%)
<b>HER2 mutation type</b>				
T772_A775dup	8 (89%)	1 (50%)	10 (67%)	20 (71%)
G776delins	1 (11%)	1 (50%)	4 (26%)	5 (17%)
V777L	–	–	1 (7%)	1 (4%)
Exon 20 ins (unspecified)	–	–	–	1 (4%)
L755P (exon 19)	–	–	–	1 (4%)

Baseline characteristics of the 28 patients enrolled in the cohort. WHO, World Health Organization.

**Table 1.** The median age was 59 years (range 37–82) and 50% of patients were men. All patients had adenocarcinomas and almost half of patients (46%) were never smokers. All patients received prior systemic treatment and 75% of patients (n = 21) received at least 2 prior treatment lines. Seven patients (25%) received at least 1 prior line of targeted therapy and among them were 4 patients who received prior HER2-targeted therapy, the second-generation pan-HER TKI afatinib. No significant differences were found in baseline characteristics between patients with CB and without CB, although patients without CB numerically received more prior systemic therapies compared to patients with CB (1.8 versus 2.5; p = 0.09). Based on the molecular pathology reports used for inclusion, most patients (n = 20; 71%) in this cohort were found to have the *HER2* 12 base pair exon 20 insertion/duplication p.Y772\_A775dup.

### 3.2. CB and survival

At data cut-off, 38% (n = 9) of the evaluable patients had CB. The objective response rate (ORR) was 8.3%. Two patients achieved a partial response (PR) and 7

patients had SD at 16 weeks (actual SD duration median 27 weeks; range 25–51 weeks). Fig. 2 is a waterfall plot depicting the greatest changes in the sum of target lesions for each patient. The median time on treatment for the patients with CB was 5.6 months (IQR 5.55–11.73). Of these patients, 8 were found to have the *Y772\_A775dup* mutation, whereas the other patient harboured the *G776delins* mutation. None of the patients that received prior HER2-targeted therapy (afatinib) before inclusion experienced CB.

The median PFS and OS were 4 months (95% CI 3–6 months) and 10 months (95% CI 6 - NA), respectively (Fig. 3A and B). The median TOT ratio for all patients was 1.1 (95% CI 0.9–2.4). The median TOT for patients with CB was 1.6 (95% CI 0.7–5) and 1.0 (95% CI 0.7–1.4) for patients without CB.

### 3.3. Baseline biopsies and WGS results

Pre-treatment study biopsies were performed in 21 out of 24 evaluable patients. Fourteen out of 21 biopsies were sequenced successfully (67%), confirming the inclusion target in all cases. Eight biopsies were not suitable for

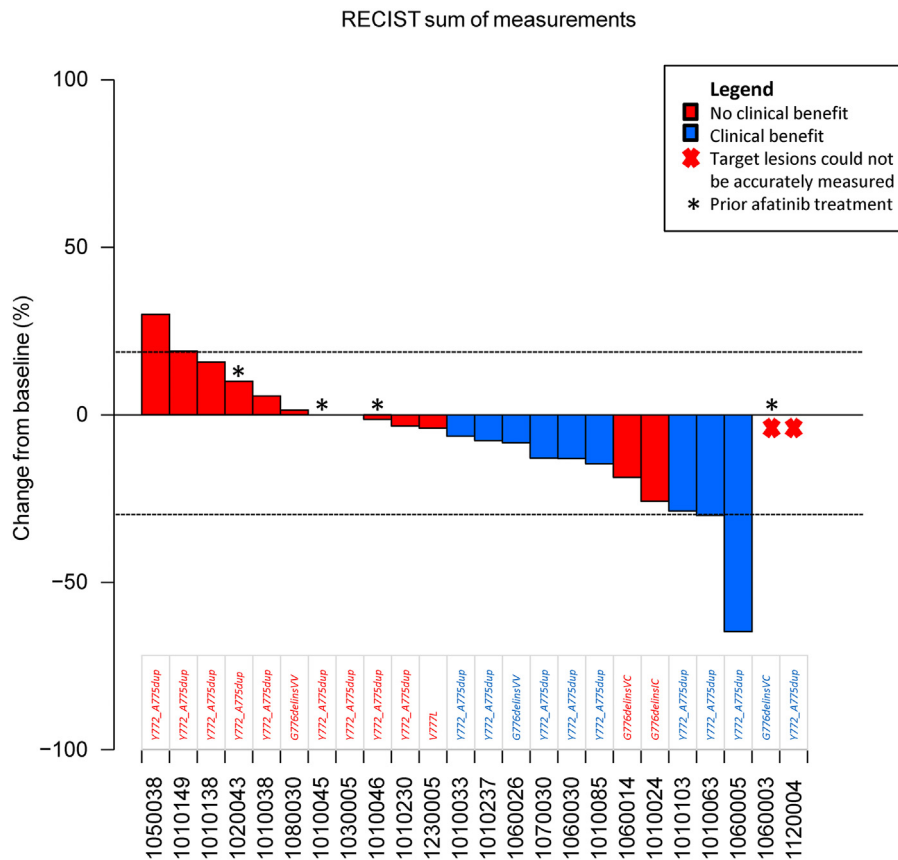


Fig. 2. Waterfall plot of best response. Best percentage of change in target lesion size from baseline. The upper dashed line at 20% represents the threshold for progressive disease, and the lower dashed line at -30% represents the threshold for partial response. Clinical benefit is defined as confirmed objective response (RECIST v1.1) and absence of disease progression for  $\geq 16$  weeks after treatment initiation. HER2 mutations and patient ID's are displayed at the bottom of the figure. HER, human epidermal growth factor.

sequencing due to low tumour cell percentage (n = 6) or insufficient DNA yields (n = 2). Failed biopsies were obtained from lymph nodes (n = 3), lung tissue (n = 3) and bone tissue (n = 2). For 2 patients with failed WGS results, available WGS data, obtained either before or after study inclusion, were used for biomarker analysis. All detected potential drivers by WGS are shown in Fig. 4. Based on the available genomic data, patients with CB had less potential drivers compared to patients without CB (average of 2.8 versus 4.5; p = 0.04). The most commonly found concomitant potential driver was TP53 mutation, which was found in 60% of patients with CB and in 73% of patients without CB (p = 0.61). Additionally, several alterations in the cyclin-dependent kinase (CDK) pathway were found (e.g. CCNE1 amplification, CDKN2A loss/mutation or homozygous RBI loss). Similarly, no significant difference was found between patients with and without CB (40% versus 46%; p = 0.84).

For 16 biopsies, COSMIC mutational signature-distribution was analysed. Signature 2 and 8 were the strongest observed signature, followed by signature 13 and 5. None of the signatures was significantly associated with CB, although signature 18 showed some

association with CB, present in 2 patients with CB and in 0 in patients without CB (p = 0.083). Not much is known regarding the biology of signature 18, only that is likely representing reactive oxygen species induced DNA damage and that is it similar to signature 36 [19].

### 3.4. Safety

No AEs > grade 4 were observed in this cohort. One patient discontinued study treatment permanently because of pneumonitis grade 4, possibly related to study treatment. Other AEs (possibly) related to study treatment that were reported included diarrhoea (grade 3) and fever (grade 1). All AEs are listed in Table 2.

## 4. Discussion

HER2 exon 20 mutations are present in 1–3% of patients with NSCLC, and effective HER2-targeted treatment is still an unmet need in this type of cancer [2–6]. In the described cohort, we have treated heavily pre-treated patients with advanced HER2 exon 20 mutated NSCLC with trastuzumab/pertuzumab

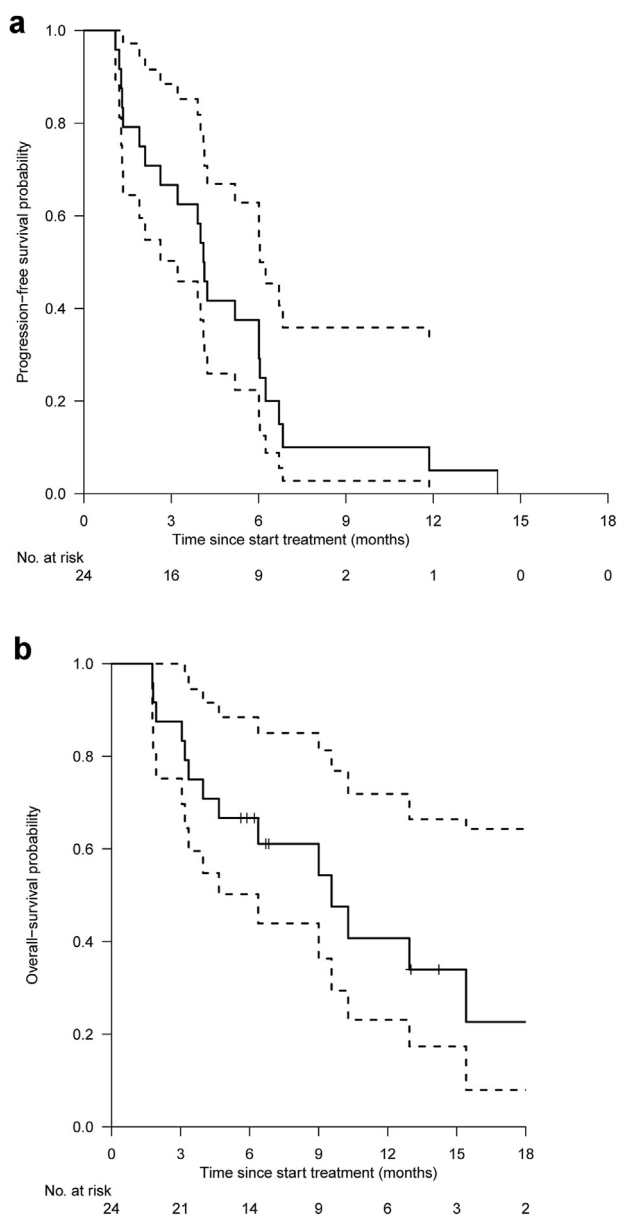


Fig. 3. Progression-free survival and overall survival curves. Kaplan-Meier curve for estimated progression-free survival (3a) and overall survival (3b), with 95% confidence interval (dashed lines).

combination therapy. Overall, trastuzumab/pertuzumab had a modest antitumour activity with CB in 38% of treated patients, including an ORR of 8.3% (2 patients with a confirmed PR), with a median PFS of 4 months.

To the best of our knowledge, this is the largest prospective cohort that investigated the clinical activity of trastuzumab/pertuzumab in patients with *HER2*-mutated NSCLC. Our results are in line with previous reports on the activity of *HER2*-targeted monoclonal antibodies and various pan-*HER*-directed TKIs [20–27]. In the first prospective cohort/basket study MyPathway, an ORR of 21% and a disease control rate of 43% were reported for 14 patients with *HER2*-

mutated NSCLC treated with trastuzumab/pertuzumab combination therapy [23]. Additionally, Mazières *et al.* studied docetaxel treatment in combination with trastuzumab/pertuzumab in *HER2* exon 20 mutated NSCLC that progressed after  $\geq 1$  platinum-based treatment. They observed an OR in 29% patients and SD in 58% patients [27]. Furthermore, several pan-*HER* TKIs have been investigated in *HER2*-mutated NSCLC with ORR ranging from 0 to 19% [21,22,24–26]. Very recently, the results of the DESTINY-Lung01 study, evaluating the efficacy of trastuzumab deruxtecan (a *HER2* antibody-drug conjugate) in patients with *HER2*-mutated NSCLC, have been published. In the study, a confirmed ORR of 55% ( $n = 91$ ) was observed with a median PFS of 8.2 months. Although the response rates are considerably higher than trastuzumab monotherapy or combined with pertuzumab, trastuzumab deruxtecan led to much higher toxicity rates. Ninety-seven percent of patients had at least 1 adverse event related to trastuzumab deruxtecan and 20% had serious drug-related adverse events. In 26% of patients interstitial lung disease was observed, which has resulted in 2 treatment-related deaths [28]. Consequently, this combination treatment will not be suitable for all patients with *HER2*-mutated NSCLC.

Adverse events observed in our cohort, reported as at least possible related to the treatment, were similar to those previously observed in the pivotal trials for patients with *HER2*-positive breast cancer, including diarrhoea, fever and one case of pneumonitis [29–32].

In the current cohort, the majority of patients harboured the *HER2* variant p.Y772\_A775, which in line with previous reports [33]. Furthermore, based on the available WGS data, all of these *HER2* mutations were mutually exclusive with other well-known drivers in NSCLC, which is also consistent with available literature [3,33]. However, it is important to note that 2 patients did harbour a copy number variation in *EGFR* (gain 13) or *BRAF* (gain 7). Both patients were non-responders, which suggests that these alterations may have influenced treatment outcome. Previous research in patients with *HER2*-positive gastric cancer ( $n = 37$ ) showed 2 patients with *EGFR* amplification in the trastuzumab resistant group ( $n = 20$ ) compared to no *EGFR* alterations in the trastuzumab sensitive group [34]. In addition, in the genomic analysis of the HERACLES trial, in which patients with *HER2*-positive metastatic colorectal cancer were treated with pertuzumab and T-DM1, a *BRAF* amplification was identified in circulating tumour DNA at progression in a patient with a durable PR.

Unfortunately, WGS data were only available for 2 of the 5 best responding patients, both harbouring the p.Y772\_A775dup variant, revealing only 2 and 3 concomitant potential drivers: mutations in *CDKN2A* and *TP53* and mutations in *RBI*, *TP53* and *PABPC1*, respectively. Overall, patients with CB had less potential



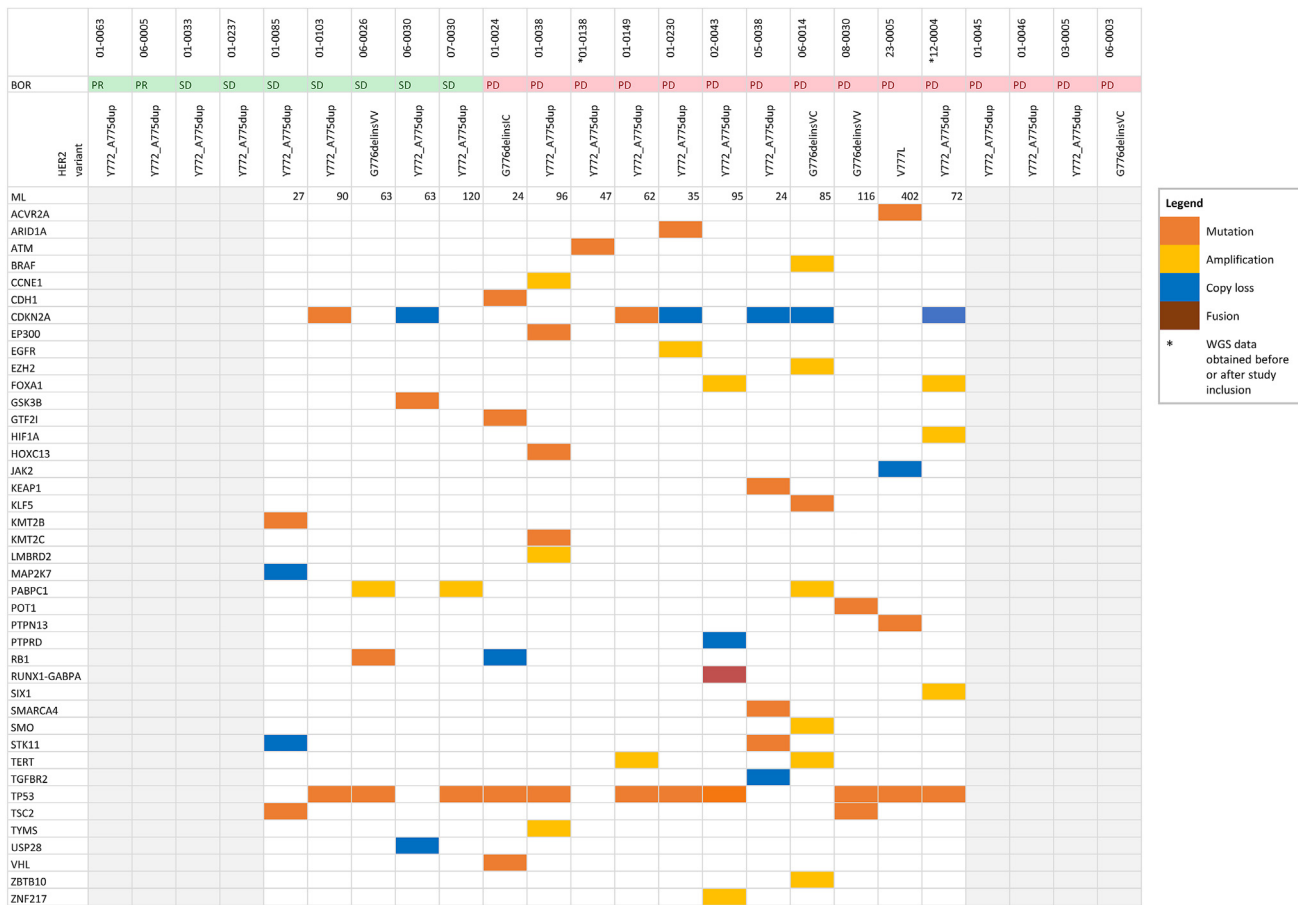


Fig. 4. All detected potential drivers by whole genome sequencing. Co-occurrence of potential drivers as detected by WGS. For all patients the best overall response according to RECIST v.1.1, detected HER2 variant, mutational load and detected potential drivers are shown. BOR, best overall response; ML, mutational load; PR, partial response; SD, stable disease; PD, progressive disease; WGS, whole genome sequencing.

drivers detected by WGS, compared to patients without CB (average of 2.8 versus 4.6;  $p = 0.04$ ). Although it remains unclear to which degree these concomitant

alterations explain the lack of CB, they may have influenced the current observations.

Table 2  
Adverse events.

	Grade 1	Grade 2	Grade 3	Grade 4
Back pain			1	
<b>Diarrhea*</b>			1	
Dyspnoea				1
Fatigue			1	
<b>Fever*</b>	1			
Hypercalcaemia		1		
Malaise		1		
Pain			1	
Pleural effusion			1	
Pneumonia			1	
<b>Pneumonitis*</b>				1
Pneumothorax			1	
Pneumosepsis				1
Thromboembolic event			1	

All reported adverse events are shown in Table 2. \*For the adverse events in bold, the relation to the treatment was scored as either ‘possible’, ‘probable’, or ‘definite’.

Limitations of the current study include the absence of both randomisation and a control group. Another limitation is the missing WGS data in a subset of patients. From the 21 evaluable patients that underwent a pre-treatment biopsy, WGS sequencing data were missing for 7 patients due to the insufficient quality of the biopsy tissue. First, it must be realised that more tumour tissue is required for WGS, compared to sequencing techniques using smaller panels. Additionally, in another major biopsy study (CPCT-02), Bins *et al.* found that the WGS analysis of 469 tumour biopsies was successful in 74% [35]. Possible factors affecting the quality of the biopsy in lung cancer include the size of the nodule, possible movement of the nodule during respiration and depth of the biopsy since some nodules may have central necrosis [36].

In conclusion, despite the fact that the study did not meet its primary end-point, trastuzumab/pertuzumab was only moderately active in a subset of patients with heavily pre-treated *HER2*-mutated NSCLC. The best

responses were observed in patients with less potential other drivers, suggesting that activation of other oncogenic pathways could have contributed to the lack of effectivity in our cohort. We therefore believe that future research should also aim at developing combinatorial-targeted strategies, targeting multiple activated oncogenic pathways, underscoring the importance of broad genomic sequencing.

#### Author contributions

All authors contributed extensively to the work presented in this paper. H.M.W. Verheul, E.E. Voest and H. Gelderblom initiated and led the trial as principal investigators; J.M. van Berge Henegouwen, L.R. Hoes, H. van der Wijngaart, L.J. Zeverijn, D.L. van der Velden coordinated the trial. J.M. van Berge Henegouwen analysed the data and wrote the manuscript. M. Jebbink assisted in the data analysis and substantively revised the manuscript. A.J. van der Wekken, A.J. de Langen, E.F. Smit contributed significantly to patient enrolment and clinical data collection. P. Roepman, W.W.J. de Leng and A.M.L. Jansen helped with interpretation of tumour profiles by performing variant calling and pathogenicity assessments. P. Roepman performed sequencing of tumour biopsies, generated sequencing reports and was involved in biomarker analyses. E. van Werkhoven and V. van der Noort contributed to data extractions and statistical design and analyses.

#### Disclosure of funding

The DRUP trial is supported by the Barcode for Life Foundation (BFL); the Dutch Cancer Society [grant number 10014]; the Hartwig Medical Foundation (HMF) and all participating pharmaceutical companies: Amgen; AstraZeneca; Bayer; Boehringer Ingelheim; Bristol-Myers Squibb; Clovis Oncology; Eisai; Janssen; Lilly; MSD; Novartis; Pfizer; Roche.

#### Credit statement

**J.M. van Berge Henegouwen:** Investigation, Data Curation, Writing - Original Draft, Project administration **M. Jebbink:** Resources, Writing - Review & Editing **L.R. Hoes:** Investigation, Data Curation, Project administration, Writing - Review & Editing **H. van der Wijngaart:** Investigation, Data Curation, Project administration, Writing - Review & Editing **L.J. Zeverijn:** Investigation, Data Curation, Project administration, Writing - Review & Editing **D.L. van der Velden:** Investigation, Data Curation, Project administration, Writing - Review & Editing **P. Roepman:** Formal analysis, Data Curation, Writing - Review & Editing **W.W.J. de Leng:** Formal analysis, Writing - Review & Editing **A.M.L. Jansen:** Formal analysis,

Writing - Review & Editing **E. van Werkhoven:** Formal analysis, Methodology, Writing - Review & Editing **V. van der Noort:** Formal analysis, Writing - Review & Editing **A.J. van der Wekken:** Resources, Writing - Review & Editing **A.J. de Langen:** Resources, Writing - Review & Editing **E.E. Voest:** Conceptualisation, Methodology, Supervision, Writing - Review & Editing **H.M.W. Verheul:** Conceptualisation, Methodology, Supervision, Writing - Review & Editing **E.F. Smit:** Writing - Review & Editing, Resources **H. Gelderblom:** Conceptualisation, Methodology, Writing - Review & Editing, Supervision.

#### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: AW received grants from Boehringer Ingelheim, Pfizer, AstraZeneca, Roche and Takeda and was involved in advisory boards for Lilly, Boehringer Ingelheim, Pfizer, AstraZeneca, Roche, Takeda and Janssen. All outside the submitted work and all money has been received by the UMCG. The other authors declare no competing interests.

#### Acknowledgements

The authors thank the Hartwig Medical Foundation for their in-kind support by performing sequencing and biomarker analyses on baseline biopsies; the Independent Data Monitoring Committee for their advice on cohort decisions and monitoring of preliminary safety data; and the authors would like to thank the patients, the investigators and research staff from all participating hospitals.

#### References

- [1] Fitzmaurice C, Akinyemiju TF, Al Lami FH, Alam T, Alizadeh-Navaei R, Allen C, et al. Global, regional, and national cancer incidence, mortality, years of Life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2016: a systematic analysis for the global burden of disease study. *JAMA Oncol* 2018;4:1553–68.
- [2] Planchard D, Popat S, Kerr K, Novello S, Smit EF, Faivre-Finn C, et al. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2018;29:iv192–237.
- [3] Pillai RN, Behera M, Berry LD, Rossi MR, Kris MG, Johnson BE, et al. HER2 mutations in lung adenocarcinomas: a report from the Lung Cancer Mutation Consortium. *Cancer* 2017; 123:4099–105.
- [4] Shigematsu H, Takahashi T, Nomura M, Majumdar K, Suzuki M, Lee H, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. *Cancer Res* 2005;65:1642–6.
- [5] Barlesi F, Mazieres J, Merlio JP, Debieuvre D, Mosser J, Lena H, et al. Routine molecular profiling of patients with advanced non-small-cell lung cancer: results of a 1-year nationwide programme of the French Cooperative Thoracic Intergroup (IFCT). *Lancet* (London, England) 2016;387:1415–26.

- [6] Ninomiya K, Hata T, Yoshioka H, Ohashi K, Bessho A, Hosokawa S, et al. A prospective cohort study to define the clinical features and outcome of lung cancers harboring HER2 aberration in Japan (HER2-CS STUDY). *Chest* 2019;156:357–66.
- [7] Moasser MM. The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. *Oncogene* 2007;26:6469–87.
- [8] Chmielecki J, Ross JS, Wang K, Frampton GM, Palmer GA, Ali SM, et al. Oncogenic alterations in ERBB2/HER2 represent potential therapeutic targets across tumors from diverse anatomic sites of origin. *Oncologist* 2015;20:7–12.
- [9] Wang Y, Zhang S, Wu F, Zhao J, Li X, Zhao C, et al. Outcomes of Pemetrexed-based chemotherapies in HER2-mutant lung cancers. *BMC Cancer* 2018;18:326.
- [10] Ghosh R, Narasanna A, Wang SE, Liu S, Chakrabarty A, Balko JM, et al. Trastuzumab has preferential activity against breast cancers driven by HER2 homodimers. *Cancer Res* 2011;71:1871–82.
- [11] Agus DB, Akita RW, Fox WD, Lewis GD, Higgins B, Pisacane PI, et al. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. *Cancer Cell* 2002;2:127–37.
- [12] Cortés J, Fumoleau P, Bianchi GV, Petrella TM, Gelmon K, Pivot X, et al. Pertuzumab monotherapy after trastuzumab-based treatment and subsequent reintroduction of trastuzumab: activity and tolerability in patients with advanced human epidermal growth factor receptor 2-positive breast cancer. *J Clin Oncol* 2012;30:1594–600.
- [13] van der Velden DL, Hoes LR, van der Wijngaart H, van Berge Henegouwen JM, van Werkhoven E, Roepman P, et al. The Drug Rediscovery protocol facilitates the expanded use of existing anticancer drugs. *Nature* 2019;574:127–31.
- [14] Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Euro J Cancer (Oxford, England: 1990)* 2009;45:228–47.
- [15] Roepman P, de Bruijn E, van Lieshout S, Schoenmaker L, Boelens MC, Dubbink HJ, et al. Clinical validation of whole genome sequencing for cancer diagnostics. *J Mol Diagn* 2021;23:816–33.
- [16] Priestley P, Baber J, Lolkema MP, Steeghs N, de Bruijn E, Shale C, et al. Pan-cancer whole-genome analyses of metastatic solid tumours. *Nature* 2019;575:210–6.
- [17] Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, et al. COSMIC: somatic cancer genetics at high-resolution. *Nucleic Acids Res* 2017;45: D777–d83.
- [18] Jung SH, Lee T, Kim K, George SL. Admissible two-stage designs for phase II cancer clinical trials. *Stat Med* 2004;23:561–9.
- [19] SBS18 – GRCh37 – COSMIC v94. COSMIC catalogue of somatic mutations in cancer.
- [20] Mazières J, Barlesi F, Filleron T, Besse B, Monnet I, Beau-Faller M, et al. Lung cancer patients with HER2 mutations treated with chemotherapy and HER2-targeted drugs: results from the European EUHER2 cohort. *Ann Oncol* 2016;27:281–6.
- [21] Besse B, Soria J, Yao BJEC. Neratinib with or without temsirolimus in patients with non-small cell lung cancer carrying HER2 somatic mutations: an international randomized phase II study. 2014.
- [22] De Grève J, Teugels E, Geers C, Decoster L, Galdermans D, De Mey J, et al. Clinical activity of afatinib (BIBW 2992) in patients with lung adenocarcinoma with mutations in the kinase domain of HER2/neu. *Lung Cancer (Amsterdam, Netherlands)* 2012;76:123–7.
- [23] Hainsworth JD, Meric-Bernstam F, Swanton C, Hurwitz H, Spigel DR, Sweeney C, et al. Targeted therapy for advanced solid tumors on the basis of molecular profiles: results from MyPathway, an open-label, phase IIa multiple basket study. *J Clin Oncol* 2018;36:536–42.
- [24] Jebbink M, de Langen AJ, Boelens MC, Monkhorst K, Smit EF. The force of HER2 - a druggable target in NSCLC? *Cancer Treat Rev* 2020;86:101996.
- [25] Kris MG, Camidge DR, Giaccone G, Hida T, Li BT, O'Connell J, et al. Targeting HER2 aberrations as actionable drivers in lung cancers: phase II trial of the pan-HER tyrosine kinase inhibitor dacomitinib in patients with HER2-mutant or amplified tumors. *Ann Oncol* 2015;26:1421–7.
- [26] Li BT, Lee A, O'Toole S, Cooper W, Yu B, Chaft JE, et al. HER2 insertion YVMA mutant lung cancer: long natural history and response to afatinib. *Lung Cancer (Amsterdam, Netherlands)* 2015;90:617–9.
- [27] Mazières J, Lafitte C, Ricordel C, Greillier L, Negre E, Zalcman G, et al. Combination of trastuzumab, pertuzumab, and docetaxel in patients with advanced non-small-cell lung cancer harboring HER2 mutations: results from the IFCT-1703 R2D2 trial. *J Clin Oncol* 2022;40:719–28.
- [28] Li BT, Smit EF, Goto Y, Nakagawa K, Udagawa H, Mazières J, et al. Trastuzumab deruxtecan in HER2-mutant non-small-cell lung cancer. *N Engl J Med* 2021;386(3):241–51.
- [29] Gianni L, Pienkowski T, Im YH, Tseng LM, Liu MC, Lluch A, et al. 5-year analysis of neoadjuvant pertuzumab and trastuzumab in patients with locally advanced, inflammatory, or early-stage HER2-positive breast cancer (NeoSphere): a multicentre, open-label, phase 2 randomised trial. *Lancet Oncol* 2016;17:791–800.
- [30] Schneeweiss A, Chia S, Hickish T, Harvey V, Eniu A, Waldron-Lynch M, et al. Long-term efficacy analysis of the randomised, phase II TRYPHAENA cardiac safety study: evaluating pertuzumab and trastuzumab plus standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer. *Euro J Cancer (Oxford, England: 1990)* 2018;89:27–35.
- [31] Swain SM, Miles D, Kim SB, Im YH, Im SA, Semiglazov V, et al. Pertuzumab, trastuzumab, and docetaxel for HER2-positive metastatic breast cancer (CLEOPATRA): end-of-study results from a double-blind, randomised, placebo-controlled, phase 3 study. *Lancet Oncol* 2020;21:519–30.
- [32] von Minckwitz G, Procter M, de Azambuja E, Zardavas D, Benyunes M, Viale G, et al. Adjuvant pertuzumab and trastuzumab in early HER2-positive breast cancer. *N Engl J Med* 2017;377:122–31.
- [33] Arcila ME, Chaft JE, Nafa K, Roy-Chowdhuri S, Lau C, Zaidinski M, et al. Prevalence, clinicopathologic associations, and molecular spectrum of ERBB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. *Clin Cancer Res* 2012;18:4910–8.
- [34] Pietrantonio F, Fucà G, Morano F, Ghoghini A, Corso S, Aprile G, et al. Biomarkers of primary resistance to trastuzumab in HER2-positive metastatic gastric cancer patients: the AMNESIA case-control study. *Clin Cancer Res* 2018;24:1082–9.
- [35] Bins S, Cirkel GA, Gadellaa-Van Hooijdonk CG, Weeber F, Numan IJ, Bruggink AH, et al. Implementation of a multicenter biobanking collaboration for next-generation sequencing-based biomarker discovery based on fresh frozen pretreatment tumor tissue biopsies. *Oncologist* 2017;22:33–40.
- [36] Priola AM, Priola SM, Cataldi A, Errico L, Di Franco M, Campisi P, et al. Accuracy of CT-guided transthoracic needle biopsy of lung lesions: factors affecting diagnostic yield. *La Radiologia Medica* 2007;112:1142–59.