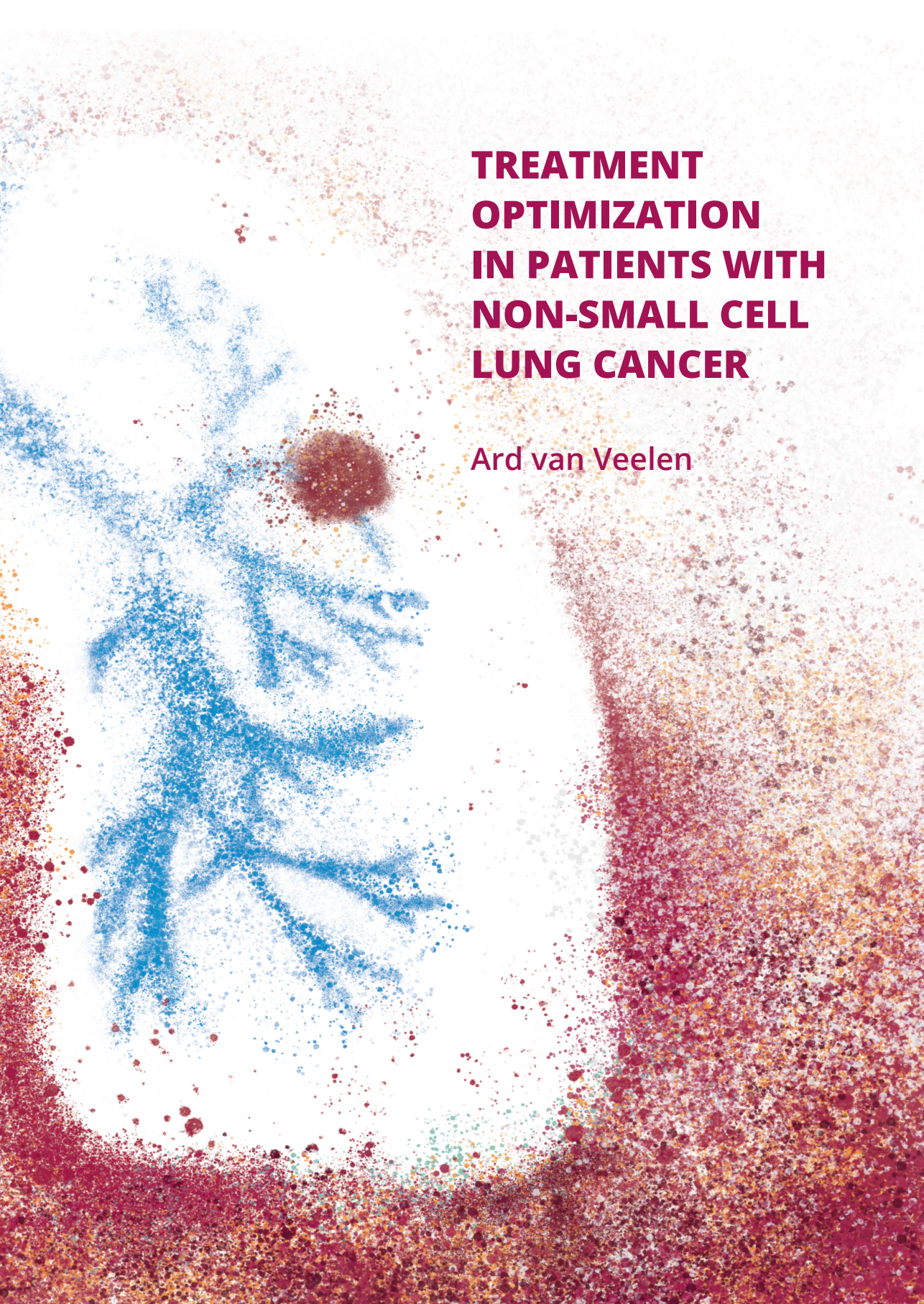
Treatment optimization in patients with non-small cell lung cancer

TREATMENT OPTIMIZATION IN PATIENTS WITH NON-SMALL CELL LUNG CANCER

Ard van Veelen



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Promotor
Prof. dr. V.C.G. Tjan - Heijnen

Copromotores
Dr. S. Croes
Dr. P.C. Souverein (Universiteit Utrecht)
Dr. R.M.J.M. van Geel

BEOORDELINGSCOMMISSIE

Prof. dr. M.A. Joore (voorzitter)
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Prof. dr. H.J.M. Groen (Universitair Medisch Centrum Groningen)
Dr. M.M.H. Hochstenbag

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Chapter 1

INTRODUCTION AND OUTLINE OF THE THESIS

INTRODUCTION

Lung cancer

Cancer is the second major reason for death worldwide, after cardiovascular diseases [1], and may overtake cardiovascular diseases as the number one reason for death if the current trends continue over time [2]. This increase is caused by population growth and aging in general [3]. Lung cancer is one of the most frequently diagnosed subtypes of cancer, both in men and in women. In men, lung cancer is the most commonly diagnosed form of cancer globally. In addition, lung cancer is the number one cause of cancer related death worldwide and in 93 individual countries [3]. In women, lung cancer is the number three most commonly diagnosed form of cancer, and the leading cause for cancer related deaths in 25 countries. Moreover, it ranks second on the list of cancer related deaths worldwide, only behind breast cancer. Overall, in men and women combined, lung cancer is the leading cause for cancer related death, as 18% of all cancer related deaths in 2020 could be attributed to lung cancer [3].

Lung cancer can be subdivided into two major groups, namely small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [4]. Approximately 85% of all lung cancer patients are diagnosed with NSCLC. NSCLC can be further divided into three major histologic subtypes, namely adenocarcinoma, squamous cell carcinoma and giant cell carcinoma [4]. The best-known risk factor for lung cancer is smoking. Smoking increases the risk for all types of lung cancer but is most strongly linked to small cell lung cancer and squamous cell carcinoma. Adenocarcinoma is the most diagnosed subtype in patients who have never smoked [4]. Other factors that can increase the risk for the development of lung cancer include family history of lung cancer, genetic polymorphisms, diet and alcohol, exposure to ionising radiation, and occupational exposures, such as asbestos, and air pollution [5].

Non-small-cell lung cancer (NSCLC)

The understanding of the biology of NSCLC has improved during the past few years. Over time, several driver mutations have been identified that could play a role in the development of NSCLC [6]. Those deviations can occur in different genes, and can be classified as deletion, insertion, rearrangement, or amplification [6 - 8]. A driver mutation leads to continuous proliferative signals, often without trigger from an external factor or ligand. The most frequent driver mutations in patients with NSCLC concern the Kirsten Rat sarcoma viral oncogene homolog gene (*KRAS*, 29%), followed by mutations in the epidermal growth factor receptor (*EGFR*) gene (19%). *EGFR* mutations are more frequently observed in Asian patients (30% – 35%) than in Caucasian patients (10% – 15%) [7]. Other genes that can be affected include anaplastic lymphoma kinase (*ALK*), human epidermal growth factor 2 (*HER2*), c-ROS oncogene 1 (*ROS1*), neurotrophic receptor tyrosine kinase (*NTRK*), rearranged during transfection (*RET*), neuregulin-1 (*NRG-1*), hepatocyte growth factor receptor (*MET*) and proto-oncogene B-Raf (*BRAF*). These mutations are less frequently

observed (all below 5%), compared to *KRAS* and *EGFR*-mutations. All those specific mutated forms can be used as target in the treatment of NSCLC-patients [8].

Another group of targets that can be used in the treatment of NSCLC is the immune checkpoint. Programmed death-1 (*PD-1*) and programmed death 1 ligand (*PD-L1*) are two proteins that play an essential role in the homeostasis of the immune system. Binding of *PD-L1* to *PD-1* decreases the activity of the immune system. The *PD-1* pathway plays a crucial role in down-regulating activated T-cells and is therefore a potential target to improve cancer immunity, as inhibition of this immune checkpoint increases the activity of the immune system and with it the anti-cancer activity of the patient's own body [9, 10].

Treatment options in NSCLC

The discovery of specific driver mutations in patients with NSCLC, among others, has led to the development of multiple new treatment options in the last fifteen years which can be used in patients with locally advanced or metastatic NSCLC. Beforehand, treatment options for patients diagnosed with NSCLC were very limited. Surgery was only performed in patients with an early-stage NSCLC, while chemotherapy was the only option in patients with an advanced or metastatic form of NSCLC. The new drugs that have entered the market, can be divided into two major categories. The first category is the group of targeted therapies, which act on a specific mutated target (driver mutation) [8]. The second category is immunotherapy, which targets *PD-1* or *PD-L1* and boosts the immune system to increase the body's own anti-cancer effect [9]. All new drugs for the treatment of patients with NSCLC that have entered the market since 2005, together with their corresponding pivotal studies, are shown in Table 1. As shown, most tyrosine kinase inhibitors (TKIs) target one specific driver mutation, while some TKIs (e.g., crizotinib) are multi-target drugs and can be used for different driver mutations [10]. All TKIs or immunotherapies that have been approved for the treatment of NSCLC patients is shown in Table 1.

One specific TKI that can be used in *EGFR* mutated NSCLC is osimertinib. It was first approved as second-line treatment option in patients with locally advanced or metastatic NSCLC that had progressed on earlier generation EGFR-TKIs. This was done based on the AURA3 trial, as it has shown better efficacy compared to chemotherapy [11]. Shortly after, osimertinib proved to be a better option in treatment naïve patients as well in the FLAURA trial, where it was compared to erlotinib and gefitinib as first-line treatment option [12]. Recently, osimertinib also showed promise in the adjuvant setting, as the disease-free survival was considerably longer in resected NSCLC patients (stage II – IIIa) compared to placebo, in the ADAURA trial [13].

Treatment optimization

Although new treatment options provide better outcomes on average, there is still a large proportion of patients who do not benefit equally well or who experience (more) severe adverse events. Together with the high cost of these newer therapies, this calls for further

treatment optimization. In the development of new drugs, the maximum tolerated dosage (MTD) is assessed in phase I trials, and this dosage is then further evaluated in phase II and phase III trials, mainly for efficacy outcomes. However, it is unknown whether a dosage below the MTD would achieve similar efficacy outcomes and whether the use of MTD as standard dose would mean potential under- or over-dosing in individual patients, with potential effect on treatment outcomes [14].

Treatment optimization can be achieved via multiple ways, and can be based on efficacy, safety, costs, or a combination. One approach that can be used is therapeutic drug monitoring (TDM), which mainly tries to improve the effectiveness or safety of a specific treatment. Furthermore, observational studies can be conducted to evaluate which specific patient subgroups might profit from a specific drug, or in which subgroup decreased treatment outcomes can be expected. Approaches that can potentially be used to improve treatment optimization in patients with NSCLC are described in more detail below. Furthermore, options to improve costs associated with the treatment of patients with NSCLC are also discussed.

Therapeutic drug monitoring

TDM can be used to improve the efficacy or the safety of a treatment. The aim of TDM is to optimize pharmacotherapy by maximizing therapeutic efficacy, while minimizing adverse events, in those instances where the blood concentration of the drug is a better predictor of the desired effect(s) than the dose [15]. In clinical practice, drug concentrations can be quantified in different biological fluids (blood, urine, saliva) and the outcome of the analysis can be linked to specific outcomes. Additionally, TDM can be used to personalize the treatment of every individual patient by maintaining or achieving a pre-specified drug concentration [16]. Furthermore, TDM can be used for different purposes: monitoring compliance, monitor and detect drug interactions or guide the treatment steps during the withdrawal of a specific drug [16]. If TDM is used to individualize the treatment of patients, evidence of a relation between the plasma concentration (trough, maximum or other), or other pharmacokinetic parameter and the treatment outcomes (effectiveness or safety) is necessary. TDM has been implemented for several drugs in clinical practice, for example immunosuppressive drugs [17] and antibiotics [18]. However, the use of TDM in oncology patients has not been as widely studied and implemented. TKIs with an unpredictable dose-exposure relationship, a small therapeutic window, or with a defined target concentration are good options for which TDM could be implemented in clinical practice. The absence of an exposure-response (effectiveness or safety) and high inter-individual variation in drug characteristics are potential reasons why TDM should not be implemented in the clinical practice. Irrespective of all mentioned reasons, a bio-analytical validated method to quantify the drug concentration, in the selected biological fluid, is crucial to evaluate the potential role of TDM and, if appropriate, implement TDM in the clinical practice.

Real-world data

Observational research is often seen as the counterpart of randomised clinical trials (RCTs), which are widely considered the gold standard for establishing the efficacy and safety of a new treatment. The main difference between observational research and an RCT is the use of randomisation in clinical trials, as the treatment selection in clinical practice is often based on specific characteristics of the patient [19]. Furthermore, RCTs often use very stringent in- and exclusion criteria, which often limits inclusion to a small, selected group of patients [20]. This leads to very strong internal validity but compromises the external validity of results found in RCTs. Contrary to RCTs, randomisation is not used in observational studies, and data is most often collected in clinical practice, without the strict in- and exclusion criteria. However, due to the absence of randomisation, a major risk for observational research is selection bias, as there may be large (observed and unobserved) differences between treatment groups. If observed differences occur, methodological approaches are available to adjust for those differences. However, this is not possible for unobserved differences. Unobserved differences occur in data that is not consistently available for all patients, or not available at all, and correction for those unobserved differences is, therefore, impossible [19].

Observational research can be used to complement results found in RCTs or as guide for future RCTs. The external validity of results found in RCTs can be tested in observational studies, by including a more representative reflection of real-world patients. Patients that can be included in clinical trials are most often relatively healthy, due to the in- and exclusion criteria that are used. This could lead to treatment outcomes in clinical trials which cannot be achieved in clinical practice. The difference between treatment outcomes in clinical trials compared to clinical practice is often referred to as the efficacy – effectiveness gap [21]. This has been previously shown for different chemotherapy options, and early generation TKIs in NSCLC patients [22]. Observational data and studies can help to inform physicians about potential discrepancies in expected treatment outcomes for specific subgroups, and thereby help to inform patients more precisely.

Furthermore, observational research can be used to formulate hypotheses that can be tested in an RCT and help to establish the appropriate sample size for an RCT. Lastly, real-world data can be used to examine subsets of patients that would benefit more (or less) from a specific treatment option, to guide treatment selection in clinical practice and thereby optimizing use of this specific treatment in the whole population [19].

Characterizing patients that are more probable to benefit from a treatment, or are more prone for treatment failure or toxicity, can be done using electronic health records (EHRs) from hospitals. EHRs are a good source to identify patients that are treated with a (relative) new drug in clinical practice, and subsequently the treatment outcomes related to this (new) treatment. A disadvantage from using EHRs is that normally not all parameters of interest are collected during clinical practice, which could lead to unobserved detection

bias if two treatment options are compared. Furthermore, data collection is often time consuming, as it needs to be extracted from physician's comments.

Other sources that can be used for observational research are large databases, which systematically collect data from (a specific subgroup of) patients in a real-world setting. One example is the Clinical Practice Research Datalink (CPRD), which is a large primary healthcare database and contains data from patients in the United Kingdom. The CPRD has two different databases, named CPRD GOLD and CPRD Aurum. CPRD GOLD exists since 1987, while CPRD Aurum is a relatively new database, launched in 2017. CPRD consists of information on demographics, diagnoses, symptoms, prescriptions, referrals, immunizations, lifestyle factors, tests, and results (from lab tests, but also treatment outcomes) [23, 24]. For both databases, linkage possibilities to other secondary care databases are available. This enables the option to complement the primary care data from CPRD with more detailed data from secondary care centres. CPRD data can be linked to information about the date, place, and cause of death (ONS Death Registration Data), hospital data (Hospital Episode Statistics – HES [25]), oncological data (National Cancer Registration and Analysis Service – NCRAS [26, 27], mental health data and more detailed demographic data (small area-level data) [23, 24]. The NCRAS consists of four different datasets, namely the cancer registration [26], the systemic anti-cancer treatment (SACT) dataset [27], national radiotherapy dataset and the cancer patients experience survey. The large number of patients and the extensive amount of information are two strengths of CPRD (and large databases in general). Furthermore, the large number of patients in the database provides the opportunity to evaluate (more) rare adverse effects, which are normally not seen in phase III trials due to the relatively lower number of patients included in such studies compared to the number of patients in the database. However, missing data on diagnoses and prescriptions (secondary care or over the counter) and variations in coding between practices and over time can be seen as disadvantages [23, 24].
Identifying patient (sub)groups that are more likely to benefit from a specific treatment using observational studies or indicating patients that have a higher chance of developing specific adverse events, could help to improve treatment optimization.

Costs

The costs of healthcare in total are constantly increasing and reached 8.3 trillion dollars worldwide in 2018 [28]. The increase in total healthcare costs is caused by aging, increased prevalence of chronic diseases, and the improvement in technology, drugs, and standards [29]. Oncology drugs take up the biggest part of drug costs, and the costs of new anti-cancer therapies have been increasing over time and exploded from 1995 onwards [30]. The prices of cancer treatments in general are relatively high and are sometimes more based on what pharmaceutical companies think the market can and will bear, and not directly related to the added therapeutic value of a new drug. This often leads to extreme prices for anti-cancer drugs that surpass the cost-effectiveness threshold used in economic analyses, as is shown by a study from Ireland, which saw that the prices of new anti-cancer

drugs surpass the threshold by a considerable margin [31]. Acceptance of high prices for anti-cancer drugs is caused by a greater demand, and the fact that willingness in society to pay extreme prices for anti-cancer drugs is higher, mainly caused by the characteristics of the disease (lethality, morbidity) and the public fear of the disease [32]. Furthermore, the limited options of (generic) anti-cancer treatments due to the rapid development of new (patented) anti-cancer treatments, with added therapeutic value, causes a high budget-impact for cancer drugs [32, 33]. To illustrate this, the ten anti-cancer drugs which had the biggest budget-impact in the USA in 2020, all cost more than 400 million dollars, with five drugs costing more than $100,000 for one year per patient [33].

The budget-impact of osimertinib was almost $800 million in the USA alone, and this will only further increase when osimertinib is approved for use in additional patients, for example in the adjuvant treatment in patients with an earlier stage NSCLC [13]. In addition, the costs of drugs will continue to rise, as new drugs, with added value over the current treatment options, are evaluated against comparators which are already highly priced. This enables the manufacturer to set an even higher price for the new product [33]. An efficient prescribing behaviour, where the right drug is selected in the right patient, based on patient characteristics, could help in slowing down the increase of anti-cancer drug costs [34]. However, more initiatives are needed to slow down the expenditure to anti-cancer drugs, and a collaborative approach of different initiatives would probably yield the most effect.

Outline of this thesis

Treatment optimization is crucial to achieve the best possible effectiveness of a drug, to minimize the toxicity of the same drug and to control the costs associated with the drug. As several new drug options have become available in the treatment of patients with NSCLC, the aim of this thesis was to evaluate options for treatment optimization of patients with NSCLC, with special attention to NSCLC patients treated with osimertinib.

To perform TDM and to further evaluate potential approaches to improve treatment optimization, validated bioanalytical methods are needed. In part I of this thesis, three different bioanalytical methods for the quantification of several (new) TKIs are presented. In **Chapter 2.1** an analytical method for osimertinib in EDTA-plasma is described, with a special focus on the stability of osimertinib in serum, plasma, and whole blood. In **Chapter 2.2** an analytical method for four TKIs (alectinib, crizotinib, erlotinib and gefitinib) in EDTA-plasma is presented. The method in this chapter can be combined with the method in **Chapter 2.1**, which gives the opportunity to quantify those five TKIs with a single assay set-up. **Chapter 2.3** describes an analytical method of seven tyrosine kinase inhibitors in EDTA-plasma, which are recently approved (brigatinib, lorlatinib, selpercatinib, pralsetinib).

Part II of this thesis consists of four observational studies. In two studies EHRs of multiple hospitals in the Netherlands were used, while two other studies used data from CPRD. These studies can be used to inform specific patient subgroups more detailed on treatment

outcomes. In **Chapter 3.1**, we evaluated the use of osimertinib and its outcomes in clinical practice, with a special focus on the effect of age, body mass index and the plasma trough concentration of osimertinib on the effectiveness of osimertinib. In **Chapter 3.2**, the efficacy of osimertinib on the prevention of bone metastases and skeletal related events was studied. In the two database studies, we evaluated the similarities and differences between NSCLC patients in clinical studies, and lung cancer patients in clinical practice, with the aim to show potential reasons for the efficacy-effectiveness gap observed in the treatment of NSCLC patients. In **Chapter 4.1**, the potential eligibility of lung cancer patients to be included in twelve RCTs in CPRD GOLD was determined, and the main reasons for exclusion of patients in clinical practice was evaluated. In **Chapter 4.2,** the quality of the data in CPRD Aurum, the new database, was evaluated. This was done by comparing the characteristics of lung cancer patients between both CPRD databases (GOLD and Aurum). In addition, the hypothetical eligibility for inclusion in eleven large, phase III trials was determined for all patients in CPRD Aurum, a similar analysis as was done in Chapter 3.1 for CPRD GOLD.

Part III contains a single chapter, **Chapter 5.1**, which presents results of a clinical study in which we evaluated the boosting capacity of cobicistat on osimertinib exposure in patients with NSCLC. This was a proof-of-concept study, in which cobicistat, a strong cytochrome P450 3A4 (CYP3A4) inhibitor, was added to a treatment regimen with osimertinib for at least three weeks. Cobicistat is a drug that is specifically developed to inhibit CYP3A4. In this study, we evaluated whether osimertinib exposure could be boosted with cobicistat, which could potentially add an option to increase osimertinib treatment exposure, potentially treatment effectiveness outcomes, as well as a reduction of the costs associated with osimertinib treatment.

Table 1: overview of current treatment options for patients with non-small cell lung cancer and corresponding clinical trial.

Targeted therapies (TKIs)			
Target	**Drug**	**RCT + publication**	**Registration**
KRAS			
	Sotorasib	CodeBreaK100 [35]	06-01-2022
	Adagrasib	KRYSTAL-1 [36]	-
EGFR			
First generation	Erlotinib	BR.21 [37], EURTAC [38]	19-09-2005
	Gefitinib	IPASS [39]	24-06-2009
Second generation	Afatinib	LUX-Lung 3 [40]	25-09-2013
Third generation	Dacomitinib	ARCHER 1050 [41]	02-04-2019
	Osimertinib	AURA3 [11], FLAURA [12], ADAURA [13]	02-02-2016
ALK			
First generation	Crizotinib	PROFILE-1014 [42]	23-10-2012
Second generation	Ceritinib	ASCEND-4 [43]	06-05-2015
	Alectinib	ALEX [44], ALUR [45]	16-02-2017
	Brigatinib	ALTA-1L [46]	22-11-2018
Third generation	Lorlatinib	CROWN [47]	06-05-2019
MET			
	Capmatinib	GEOMETRY [48]	-
	Tepotinib	VISION [49]	16-02-2022
	Tivantinib	MARQUEE [50]	-
ROS1			
	Crizotinib	AcSé [51]	23-10-2012
	Lorlatinib	[52]	06-05-2019
	Entrectinib	STARTRK-1, STARTRK-2 and ALKA-372-001 [53]	31-07-2020
BRAF			
	Vemurafenib	AcSé [54]	17-02-2012
	Dabrafenib + trametinib	[55][56]	27-08-2013 30-06-2014
HER2			
	Trastuzumab + deruxtecan	DESTINY [59]	18-01-2021
RET			
	Selpercatinib	LIBRETTO-001 [60]	11-02-2021
	Pralsetinib	ARROW [61]	18-11-2021
Immunotherapy			
PD-1 blockers			
	Nivolumab	CheckMate [62, 63]	19-06-2015
	Pembrolizumab	KEYNOTE [64 - 67]	17-07-2015
PD-L1 blockers			
	Durvalumab	PACIFIC [68]	21-09-2018
	Atezolizumab	OAK [69]	26-08-2019

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Chapter 2

VALIDATION OF ANALYTICAL METHODS FOR TYROSINE KINASE INHIBITORS

Chapter 2.1

Validation of an analytical method using HPLC-MS/MS to quantify osimertinib in human plasma and supplementary stability results.

A. van Veelen, R.M.J.M. van Geel, Y.M. de Beer, A.C. Dingemans, L.M.L. Stolk, R. ter Heine, F. de Vries, S. Croes.

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ABSTRACT

A new method for quantification of osimertinib (OSIM) in human plasma using a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was developed and validated. Methanol was used for protein precipitation and pazopanib (PAZO) as internal standard. Separation was performed on a HyPURITY® C18 analytical column (50 × 2.1 mm; 3 µm) using a gradient elution of ammonium acetate in water and ammonium acetate in methanol, both acidified with formic acid 0.1%. Detection and quantification of OSIM and PAZO was performed by a triple quadruple mass spectrometer with after electrospray ionization. This method led to robust results, as the selectivity, carry-over, precision, and accuracy all met pre-specified requirements. OSIM was stable in human serum when stored at -80°C. Reduced stability was found when stored at 2-4°C or room temperature (RT). Degradation of OSIM slowed down in EDTA-plasma and acidified human serum. The limited stability of OSIM at RT should be considered in transport and sample preparation. Plasma samples should be frozen as soon as possible, and sample preparation should be performed on dry ice. In the future, EDTA-plasma and sample acidification may be used to improve OSIM stability at RT. However, more research and validation of such an approach is required.

INTRODUCTION

Non-small cell lung cancer (NSCLC) is the most prevalent form of lung cancer [1]. In roughly 10% of all NSCLC-patients a mutation in the epidermal growth factor receptor (*EGFR*) can be found [2]. Osimertinib (OSIM) is a third-generation, irreversible, *EGFR* directed tyrosine kinase inhibitor (TKI), which is registered as first-line and second-line treatment in patients with *EGFR* mutated NSCLC [3, 4].

The pharmacokinetics of OSIM have been studied earlier [5 – 7]. Steady state conditions are achieved after 15 days. OSIM has a half-life between 40 – 50 hours, resulting in a relatively flat plasma concentration-time curve during steady-state. OSIM, and its key metabolites AZ5104 and AZ7550, are mainly metabolized by CYP3A and substantial inter-patient variability in exposure is seen after multiple administrations of OSIM [6, 7].

Seven liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods to quantify OSIM in human plasma have previously been reported [8 – 14]. Four methods were single-drug methods for OSIM (and its metabolites) [8, 10, 12, 13], while three other methods focused on the simultaneous determination of multiple TKIs [9, 11, 14]. Short-term stability of OSIM has been evaluated in different types of plasma and in whole blood, but not one study evaluated OSIM stability in EDTA-plasma, heparinized plasma, human serum, and whole blood which makes it difficult to compare the stability results. Therefore, and for research purposes, we developed a simple high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method for the quantitative analysis of OSIM in human plasma. Herein, we describe the validation of our assay together with OSIM stability data in EDTA-plasma, sodium heparin plasma, whole blood, and serum.

METHODS

2.1 Chemicals and reagents

OSIM (free base purity 95%) and pazopanib (PAZO) (free base purity 98%) were purchased from Toronto Research Chemicals (Toronto ON, Canada). Methanol (ULC/MS-CC/SFC grade) was purchased from Biosolve (Valkenswaard, the Netherlands). Acetonitrile (≥99.9%), 2-propanol (≥99.8%) and dimethylsulfoxide (DMSO, ≥99.0%) were obtained from Merck (Darmstadt, Germany). Drug-free serum (frozen, no additives) was purchased from Sanquin (Amsterdam, the Netherlands).

2.2 Preparation of calibration standards and quality controls

For the calibration standards and quality controls of OSIM, two separately prepared stock solutions (dissolved in DMSO) with a concentration of 1 mg/mL were produced. These were diluted with methanol to a concentration of 10 µg/mL (working solutions). The PAZO stock solution was prepared reconstituting approximately 1 mg PAZO with 100 mL

methanol, resulting in a concentration of approximately 10 µg/mL. Both OSIM and PAZO stock solutions were stored at -80 °C until analysis. The calibration standards consisted of six different concentrations plus a zero and a blank sample. These were prepared by spiking human serum with the working solution. The zero sample only consisted of PAZO, while the blank sample did not contain either OSIM or PAZO. Quality control (QC) samples were prepared from the second working-solution for the validation runs at five different concentrations: lowest limit of quantification (LLOQ) = 25.0 ng/mL; QC_{LOW} = 75.0 ng/mL; QC_{MED} = 250.0 ng/mL; QC_{HIGH} = 375.0 ng/mL and upper limit of quantification (ULOQ) = 500 ng/mL. For study sample runs QC_{LOW}, QC_{MED} and QC_{HIGH} were used.

2.3 Instrumentation

For the analysis a Surveyor® Autosampler Plus with a quaternary MS-pump plus and degasser (ThermoFischer, Breda, The Netherlands) as a chromatographic system was used. A TSQ Quantum-Access® triple quad mass-spectrometer (ThermoFischer, Breda, the Netherlands) with an electrospray ionization interface (ESI) combined with Excalibur® software (version 2.2SP1) was used for detection and quantification. Chromatographic separation was performed on a HyPURITY® C18 analytical column (50 × 2.1 mm, 3 µm, ThermoFischer Scientific) combined with a drop-in guard (HyPURITY® C18, 10 × 2.1 mm, 3 µm).

2.4 Sample preparation

The solution for deproteinization was made by adding 2.5 µL PAZO (approximately 10 µg/mL) to 10 mL of methanol. A 20 µL serum sample was pipetted in an Eppendorf cup placed in a container filled with dry ice. Thereafter, 150 µL of deproteinization solution was added and vortexed for 2 minutes. After vortexing, the Eppendorf cups were centrifuged at 11,300 g for 5 minutes. Subsequently, 100 µL supernatant was pipetted in a glass vial and 400 µL water was added to ensure compatibility with the gradient used during chromatography.

2.5 Chromatographic condition and LC-MS/MS settings

Two mobile phases were used for achieving chromatographic separation. Mobile phase A consisted of 2 mM ammonium acetate in water (+ 0.1% formic acid). Mobile phase B consisted of 2 mM ammonium acetate in methanol (+ 0.1% formic acid). The starting gradient was set at 70% A and 30% B for 0.5 minutes. After 0.5 minutes the gradient linearly increased to 100% B in 2.0 minutes. 100% B was maintained for 0.3 minutes after which the gradient was reset to initial conditions and kept steady for 1 minute before a new sample was injected. The flow during the run was maintained at 500 µL/min. Column temperature was set at 60 °C. Auto-sampler temperature was set at 10°C. The divert-valve was set in the waste-position for the first 1.5 minutes.

MS detection was in MRM-mode with the following settings: spray voltage 5000 V, Sheath gas pressure 60, Aux gas pressure 15, Capillary temperature 360 °C, Collision gas pressure 1.5 mTorr. Transition of OSIM and PAZO was set at m/z 500.3 → 72.3 and 438.2 → 357.1,

respectively. Collision energies were 24 eV (OSIM) and 27 eV (PAZO). Tube lens values were 96 (OSIM) and 120 (PAZO).

2.6 Method validation

The validation was based on the most recent guideline 'bio-analytical method validation' by the European Medicines Agency (EMA) [15].

2.6.1 Selectivity and carry-over

Selectivity was evaluated by analysing six different EDTA-plasma samples from patients who were not treated with OSIM. The largest peak close to the retention times of OSIM and PAZO were manually integrated. These values were compared to the response of the lowest response in one of the 5 LLOQ's in the same validation run. The response for OSIM should be lower than 20% of the LLOQ. For PAZO, the response should not exceed 5% of the peak area of the internal standard. Carry-over was tested by injecting a blank plasma sample after an ULOQ sample. Analysis was carried out in five-fold. The limits for carry-over are similar to the limits for selectivity (<15% for OSIM, <20% for LLOQ, and <5% for PAZO).

2.6.2 Lower limit of quantification and linearity

The mean plasma trough concentration in the population has been reported (166 ng/mL) [16] and no unexpected results were encountered while analysing patient samples. The expected concentrations of patient samples in clinical practice will be ≥40 ng/mL, therefore the LLOQ was set at 25 ng/mL. The upper limit of quantification was set at 500 ng/mL, resulting in a calibration range from 25 – 500 ng/mL. In other studies, wider concentration ranges were used (between 0.5 – 4000 ng/mL) [8 - 10, 14], but such a wide concentration range was considered unnecessary for this analytical method because the results in the planned follow-up study are expected to be within the range of 25 – 500 ng/mL. Three calibration curves were constructed (y=ax+b, weighting 1/x) and back-calculated concentrations were not allowed to exceed 15% of the nominal value, except for the LLOQ, which was allowed to remain within 20% of the nominal value [15].

2.6.3 Precision and Accuracy

Precision and accuracy of the developed method were determined by analysing five different QCs (LLOQ, QC_{LOW}, QC_{MED}, QC_{HIGH} and ULOQ) on three different days using freshly prepared calibration standards to construct the calibration curve. On each day, five replicates of each QC were analysed. Precision and accuracy were not allowed to exceed 15% for all QCs except for the LLOQ which was allowed to remain within 20% [15].

2.6.4 Matrix effect

Matrix effects were determined by calculating the ratio of the peak area after spiking the blank matrix with OSIM and PAZO and compare this with OSIM and PAZO in the mobile phase (70%A:30%B). This was done for the QC_{LOW} and QC_{HIGH}.

2.6.5 Dilution integrity

Usually, dilution integrity is tested by diluting plasma (c= 1.5 × ULOQ) 4-fold and 2-fold [15]. However, during the development of this method, we found that the stability of OSIM at RT is shorter than four hours, as mentioned by Rood *et al.* [8]. The precise quantification of OSIM was therefore limited to the whole calibration range, which was sufficient for our planned study.

2.6.6 Stability

Short-term stability and long-term stability of OSIM were determined in human serum (additives-free) for QC_{LOW}, QC_{MED} and QC_{HIGH} at 3 different temperatures (RT, 4 °C and -80 °C). For the stock solution of OSIM as well as for the working solution the long-term stability was determined at -80 °C. Accuracy was not allowed to exceed 15% of the nominal value. 24-hour stability was tested by re-injecting all QC's and calibration standard in the auto sampler (maintained at 10 °C).

Short-term stability of OSIM at RT was evaluated in more detail. Human serum was spiked with OSIM (QC_{LOW} and QC_{HIGH}) and immediately frozen afterwards. Samples were thawed on another day and stored at RT for 0, 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours before sample preparation and analysis.

Short-term stability of OSIM at RT was also investigated in EDTA-plasma, heparinised plasma, and whole blood, which was anti-coagulated with EDTA. Additionally, stability of OSIM was also evaluated with two patient samples. Stability was evaluated after 0, 2, 4, 8 and 24 hours of storage at RT. EDTA-plasma samples, heparinised plasma samples and whole blood samples were spiked with OSIM (QC_{LOW} and QC_{HIGH}).

A study by Kallepalli *et al*. indicated that OSIM was more stable in an acidic environment [17]. The effect of pH on the stability of OSIM in human serum was assessed by acidifying or alkalising human serum with 0.1 mL hydrochloric acid (0.1M) or 0.1 mL sodium hydroxide (0.1M), respectively. Barring the buffering effect of serum, the pH of the samples would be approximately 2 and 12, respectively. Subsequently, the serum samples were spiked with OSIM (QC_{LOW} and QC_{HIGH}). OSIM concentrations were determined after 0, 2 and 4 hours of storage at RT.

RESULTS

3.1 Method development

Initially, the method described by Rood *et al*. was adopted, using a salting-out liquid-liquid extraction (SALLE) [8]. After extraction and centrifugation, the clear and colourless extract was transferred and tested for residual dissolved protein. The extract turned milky after adding methanol indicating residual dissolved protein to be present. As continuous

injection of dissolved protein on the chromatographic column would lead to amorphous peaks and a reduced lifespan of the column, simply adopting this method was not possible. Therefore, a new method for sample preparation was developed in which methanol was used for protein precipitation. This is largely comparable with the sample preparation described in other studies, which used acetonitrile for protein precipitation [9 - 11].

Preferably, labelled OSIM should have been used as internal standard. However, this was not commercially available at the time, and therefore PAZO has been used as internal standard. For OSIM and PAZO the most abundant fragments were chosen (OSIM – 500.3 → 72.3 and PAZO 438.2 → 357.1), which were similar to the transitions used in the study by Rood *et al*. [8].

Several gradients were investigated but no gradient was found competent to co-elute OSIM and PAZO exactly simultaneously, thereby minimizing the risk of ion-suppression or -enhancement. OSIM and PAZO were slightly separated at every evaluated gradient, as is shown in Figure 1. Additionally, calibration curves were created using three different plasma batches (data not shown). This was done prior to the start of the validation. The slope of the three calibration curves were compared and no differences were encountered regarding possible ion suppression or ion enhancement.

During method development carry-over was observed with OSIM. A flush/needle-wash solution of water and methanol (50%/50%) was not sufficient to reduce the carry-over to required levels. Carry-over was significantly reduced by using a flush/needle-wash solution containing water, methanol, acetonitrile, and isopropanol (25% each).

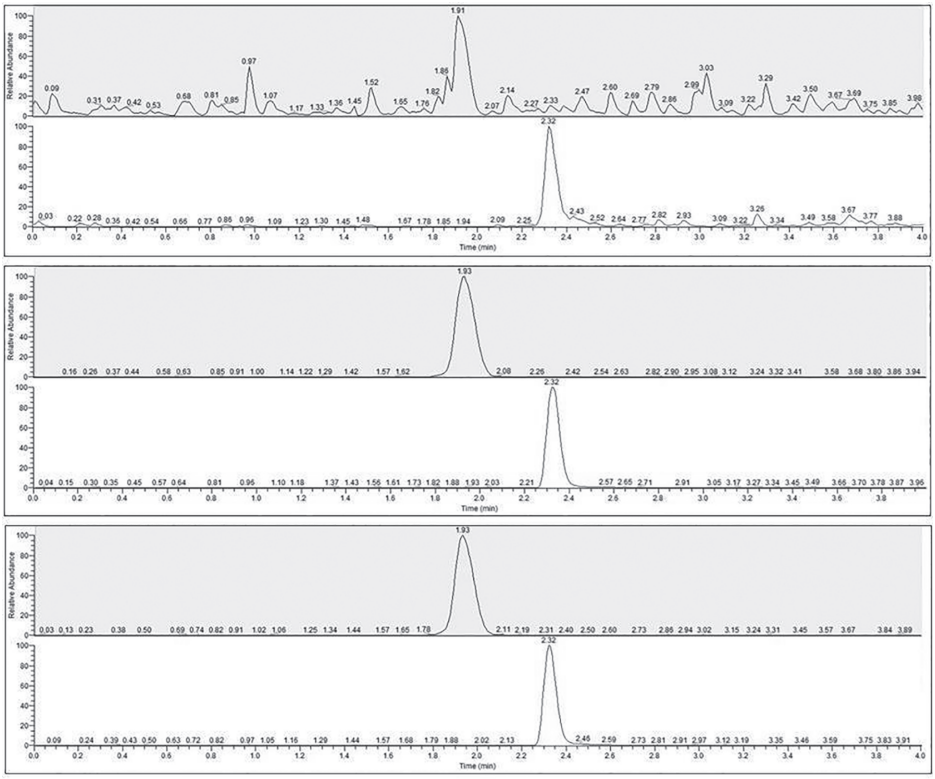

Figure 1: chromatograms of OSIM (grey) and PAZO (white) for a blank sample (upper), the LLOQ (middle) and a patient sample (lower). Maximum signal for osimertinib in blank sample was 4.91E2, 1.04E5 in LLOQ, and 1.16E5 in the patient sample.

The optimization of the sample preparation was carried out by varying the volume of methanol. Dilution varied from approximately 1:1 to 1:7 (sample/methanol). At every extract, additional methanol was added to visually check for residual protein (milky extract). Dilution 1:1 to 1:5 resulted in milky extracts (visually expected). Dilution 1:6 gave a clear extract. To assure sufficient deproteinization a dilution of 1:7.5 was used at the start of the method validation of the method (20 µL sample + 150 µL methanol).

As stability of OSIM at RT was considerably worse than described by Rood *et al*. [8], sample preparation was performed on dry ice to reduce degradation during sample preparation. It has been shown that OSIM is stable when stored on ice [11].

3.2 Method validation

3.2.1 Selectivity and carry-over

Six blank human plasma samples showed no interfering peaks. Blank OSIM responses were all below 10% (range 0.8% - 6.7%). Blank IS responses were all below 0.1% (range 0.0% – 0.1%). The carry-over effect for OSIM did not exceed 20% of LLOQ (range: 7.3% - 11.1%), while the carry-over effect for PAZO was lower than 0.2% of IS (range = 0.1% - 0.2%).

3.2.2 Calibration and linearity

The calibration curves were linear over the examined range (25 ng/mL – 500 ng/mL). The coefficient of determination varied between 0.9964 – 0.9989.

3.2.3 Precision and accuracy

The results of the precision and accuracy of the analysis are shown in Table 1. The mean intra-day precision and inter-day precision of OSIM were 5.0% and 3.9%. The mean intra-day accuracy and mean inter-day accuracy were 91.2% and 94.7%. All precisions and accuracies met the pre-specified requirements (<15% or <20%).

3.2.4 Matrix effect

The matrix effect was evaluated using QC_{LOW} and QC_{HIGH}. The coefficient of variation was 12.8% and 12.2%, respectively.

Table 1: intra- and inter-day accuracy and precision of osimertinib in spiked human serum samples.

Nominal concentration (ng/L)	Mean concentration (n=15) (ng/mL)	Intra-day precision (n=5) (%)	Inter-day precision (n=15) (%)	Intra-day accuracy (n=5) (%)	Inter-day accuracy (n=15) (%)
LLOQ (25.0)	24.9	8.0	4.7	98.1	99.5
QC_{LOW} (75.0)	65.9	3.9	2.0	85.9	87.9
QC_{MED} (250.0)	233.8	3.3	3.2	90.5	93.5
QC_{HIGH} (375.0)	357.6	6.9	3.9	90.4	95.4
HLOQ (500.0)	485.4	2.8	5.6	90.9	97.1
Mean	-	5.0	3.9	91.2	94.7

Abbreviations: LLOQ, lower limit of quantification; QC_{LOW}, quality control low-level; QC_{MED}, quality control mid-level; QC_{HIGH}, quality control high-level; ULOQ, upper limit of quantification; n = number of samples; ng/mL = nanogram per millilitre;

Table 2: stability of osimertinib in human serum at various storage conditions.

Temperature (°C)	Time (days)	Accuracy QC_{LOW} (%)	Accuracy QC_{MED} (%)	Accuracy QC_{HIGH} (%)
-80	30	105.3	102.2	104.6
-80	90	109.2	109.3	105.0
-80	180	93.5	95.2	95.9
2 to 8	1	55.3	61.5	63.3
15 to 25 (RT)	1	0.8	1.9	7.5

Abbreviations: RT = room temperature; QC_{LOW}, quality control low-level; QC_{MED}, quality control mid-level; QC_{HIGH}, quality control high-level. Results are mean concentration compared with nominal value. All concentrations were analysed in duplicate.

3.2.5. Stability

3.2.5.1 Short- and long-term stability

The results of the short-term and the long-term stability of OSIM in human serum (additives-free) are shown in Table 2. Stability was tested using three different concentrations (QC_{LOW}, QC_{MED}, and QC_{HIGH}). The concentration of OSIM in human serum declined rapidly when stored at RT. After 24 hours, the concentration of OSIM had decreased to 0.8% (QC_{LOW}), 1.9% (QC_{MED}) and 7.5% (QC_{HIGH}). When stored at 4°C, the decline in concentration was less considerable than at RT, however, after 24 hours the concentrations of OSIM had fallen to 55.3% (QC_{LOW}), 61.5% (QC_{MED}) and 63.3% (QC_{HIGH}). Because the concentrations after 24 hours were far below the required norm, stability at those temperatures was not further evaluated. Nonetheless, OSIM proved to be stable when stored at -80 °C, which was tested after 1, 3 and 6 months (QC_{LOW}: 105.3%, 109.2% and 93.5%; QC_{MED}: 102.2%, 109.3% and 95.2%; QC_{HIGH}: 104.6%, 105.0% and 95.9%).

Short-term stability of OSIM in human serum at RT was investigated in more detail (Table 3 and Table S1). The calculated concentrations were compared with the starting concentration at T = 0. Similar to the earlier results, the concentration of OSIM decreased drastically over time. After four hours of storage at RT the concentration of OSIM had dropped to 18.6% (QC_{LOW}) and 54.1% (QC_{HIGH}), but the concentrations of OSIM in human serum declined below 85% of the original concentration after 0.5 hours for QC_{LOW} and after 1 hour for QC_{HIGH}.

The results of OSIM stability in two patient samples (heparinized plasma) are shown in Table 3 and Figure S1. After four hours of storage at RT the concentration of OSIM had fallen to (84.4% and 84.5%, respectively). The degradation of OSIM seen in patient plasma was considerably lower than the degradation observed in human serum (QC_{LOW} - 18.6% and QC_{HIGH} - 54.1%), while the concentrations of the two patient samples (102.58 ng/mL and 303.60 ng/mL) were in the same range as the concentration of the quality controls.

The type of anticoagulation could possibly influence OSIM stability, as shown in Table 3 and Figure S1. After four hours of storage at RT the concentration of OSIM in EDTA-plasma had decreased to 91.9% (QC_{LOW}) and 86.4% (QC_{HIGH}). In heparinised plasma the decrease was noticeably larger, as the concentration declined to 67.1% (QC_{LOW}) and 72.1% (QC_{HIGH}) after four hours. After 24 hours at RT the concentration fell to 60.1% (QC_{LOW}) and 64.4% (QC_{HIGH}) in EDTA-plasma. In heparinised plasma the concentration fell to 2.0% (QC_{LOW}) and 2.9% (QC_{HIGH}).

The stability of OSIM in whole blood at room temperature is shown in Table 3. After four hours of storage at room temperature the concentration of OSIM had decreased to 88.4% (QC_{LOW}) and 96.3% (QC_{HIGH}). After 24 hours at RT the concentration fell to 54.7% (QC_{LOW}) and 93.6% (QC_{HIGH}). Upon storage at 4°C the concentration of OSIM had decreased to 79.2% (QC_{LOW}) and 96.6% (QC_{HIGH}) after 4 hours and to 53.6% (QC_{LOW}) and 91.0% (QC_{HIGH}) after 24 hours (results not shown).

The effect of pH on OSIM stability is shown in Table 3. OSIM was more stable in acidified serum compared to alkalised serum. After four hours of storage at RT the concentration of OSIM decreased to 79.5% (QC_{LOW}) and 91.1% (QC_{HIGH}) in an acidic environment, while the concentration of OSIM fell to 3.2% (QC_{LOW}) and 14.9% (QC_{HIGH}) when human serum was alkalised. These results match the results presented by Kallepalli *et al.* [17], which showed that OSIM was relatively stable in an acidic environment but degraded rapidly in an alkaline environment.

Freeze-thaw stability of OSIM was performed in other studies and all reported sufficient accuracy after three freeze-thaw cycles [8 – 11]. Due to the rapid decline of OSIM at RT encountered in this study, it was decided not to evaluate the freeze-thaw stability of OSIM because storage at RT would inevitably lead to lower OSIM-concentrations.

A limitation of this validation was that during stability-testing the concentration of OSIM repeatedly declined below 25 ng/mL. As the calibration range was set from 25 – 500 ng/mL some concentrations could not be calculated completely accurate. As this was solely seen during stability tests, this was not considered serious. However, this uncertainty should be kept in mind while evaluating the stability results of OSIM as the concentrations below 25 ng/mL.

Table 3: short term stability of osimertinib at room temperature in serum, plasma, and whole blood.

	Human serum		Patient samples		EDTA plasma		Heparinised plasma		Whole blood		Acidified serum		Alkalised serum	
Time (hour)	**$[C]/[C_0]$ QC_{LOW} (%)**	**$[C]/[C_0]$ QC_{HIGH} (%)**	**$[C]/[C_0]$ patient I (%)**	**$[C]/[C_0]$ patient II (%)**	**$[C]/[C_0]$ QC_{LOW} (%)**	**$[C]/[C_0]$ QC_{HIGH} (%)**	**$[C]/[C_0]$ QC_{LOW} (%)**	**$[C]/[C_0]$ QC_{LOW} (%)**	**$[C]/[C_0]$ QC_{LOW} (%)**	**$[C]/[C_0]$ QC_{HIGH} (%)**	**$[C]/[C_0]$ QC_{LOW} (%)**	**$[C]/[C_0]$ QC_{HIGH} (%)**	**$[C]/[C_0]$ QC_{LOW} (%)**	**$[C]/[C_0]$ QC_{HIGH} (%)**
0.0	100	100	100.0	100	100.0	100.0	100.0	100.0	100	100	100	100	100	100
2.0	37.6	73.6	89.6	90.6	99.1	96.1	86.4	83.4	88.7	104.0	89.4	95.6	14.1	37.8
4.0	18.6	54.1	84.5	84.4	91.9	86.4	67.1	72.1	88.4	96.3	79.5	91.1	3.2	14.9
8.0	-	-	57.7	60.4	83.9	86.3	37.1	46.3	74.5	94.8	-	-	-	-
24.0	-	-	5.3	5.8	60.1	64.4	2.0	2.9	54.7	93.6	-	-	-	-

Abbreviations: [C] = concentration; $[C_0]$ = concentration at start (t = 0.0); QC_{LOW} = quality control low level; QC_{HIGH} = quality control high level;

3.2.5.2 Stock stability
OSIM proved stable in DMSO when stored at -80°C. After three weeks, three months, and six months all accuracies were between 95% and 105%. OSIM proved instable when dissolved in methanol. After three weeks the concentration had dropped to 68.6% and was therefore not further evaluated. Results are shown in Table S2 in the supplementary material.

The long-term stability of PAZO was examined previously, and PAZO proved to be stable in plasma at different temperatures [18 – 20]. PAZO was stable up to nine months at 20°C and up to three months at RT and at 2 - 8°C. Therefore, PAZO was considered to be stable, and no additional analyses were performed.

3.2.6 Clinical applicability
This assay was developed to support clinical studies as well as individual patient care in a real-world clinical setting. We analysed multiple patient samples of patients treated with OSIM. All measured concentrations were between 51.8 ng/mL and 303.6 ng/mL and therefore within the pre-specified range of 25 – 500 ng/mL used for this analytical method. Until now, incurred sample reanalysis (ISR) was performed for one sample, which was reanalysed in a different run after storage at -80 °C for 6 weeks. The OSIM concentrations were similar in both measurements (102.6 ng/mL and 105.0 ng/mL), indicating good reproducibility of the method. ISR assessment will be performed in additional patient samples as part of an on-going clinical trial (NCT03858491).

3.3 Comparison with previous studies

Other analytical methods for OSIM have been published previously. While some have mainly focused on OSIM alone or in combination with its metabolites [8, 10, 12, 13], others developed an analytical method for multiple TKIs [8, 11, 14]. As the active metabolites of OSIM account for approximately 10% of the total OSIM AUC [6, 21], they could contribute to the efficacy and toxicity of osimertinib, with AZ5104 being the most likely option due to the increased potency seen in in-vitro studies [22]. Although AZ5104 was not included in our method, AZ5104 exposure could be estimated based on the osimertinib exposure, as the intra- and inter-patient variability in the osimertinib to AZ5104 ratio is small. Furthermore, since elimination rates for OSIM and AZ5104 are similar, the parent to metabolite ratio remains consistent over time within each dosing interval [21].

In our method, HPLC was used for separation, whereas other studies mainly used ultra-high-performance liquid chromatography (UPLC) for separation [8, 10 - 14]. As UPLC may not be available in all laboratories, the use of HPLC could enable more laboratories to implement the quantification of OSIM. Although HPLC methods usually have longer run times [9], we achieved a run time of 3.8 minutes, which is comparable to the run time of 2-5 minutes reported for the UPLC methods 8, 10 – 12, 14]. Furthermore, our sample preparation was largely comparable to the methods used in UPLC methods but is considerably shorter than the only other reported HPLC method [9]. Therefore, this analytical method could serve as

an easy, quick, and relatively cheap option in other laboratories, in which UPLC systems are not available.

Contrasting results have been reported regarding the short-term stability of OSIM, especially at RT. While multiple studies described good stability of OSIM at RT for at least six hours [8 – 10, 12], others reported more limited stability, namely 3-4 hours [11, 14]. However, differences in OSIM concentrations and type of plasma complicate adequate comparison of the results of these studies. Therefore, we decided to assess the short-term stability of OSIM in serum, plasma (EDTA and heparinized) and whole blood, using invariable concentrations of OSIM and fixed laboratory conditions.

The short-term stability of OSIM in heparinized plasma in our study was approximately 2 hours, which was considerably shorter than the previously described 6 hours [8, 10]. Our results were comparable with the results reported by Veerman *et al.*, who described short-term stability of OSIM in heparinized plasma for at least 3 hours and a recovery after 24 hours of less than 20% [11].

According to previous studies, OSIM was stable for at least 4 to 6 hours in EDTA plasma at RT [12, 14]. Similarly, we found OSIM was stable in EDTA-plasma for at least 4 hours at RT. As indicated by a 60-65% recovery of OSIM in EDTA-plasma after 24 hours at RT, the rate of OSIM degradation was considerably less than in heparinized plasma.

In whole blood, OSIM was reported to be stable for at least 5 hours at RT, and for 1 hour at 37 °C [11]. Mitchell *et al*. reported OSIM to be stable for at least two hours at RT. Prior to storage at RT, the blood samples were heated to 37 °C to mimic the situation in clinical practice [12]. In our study, OSIM was stable for at least 4 hours at RT, which is comparable to the results reported by Veerman *et al*.

To the best of our knowledge the effect of pH on the short-term stability was not previously evaluated. We showed that acidification of serum samples has a positive effect on the short-term stability of OSIM compared to alkalinisation or no pH-modification.

The long-term stability of OSIM was evaluated under freezing conditions (-20 °C, -30 °C, -70 °C, -80 °C) and similar results were found in all studies. OSIM proved to be stable in freezing conditions for at least 1 month, but longer stability was reported (up to 9 months) in multiple studies [8 – 10, 14].

CONCLUSION

An HPLC-MS/MS method to quantify OSIM was successfully developed and validated with a similar run-time as previously published UPLC-MS/MS methods. Accuracy, precision, carry-over, and matrix effect were in accordance with the EMA-guidelines. The short-term stability of OSIM in human serum, heparinized- and EDTA plasma is limited at RT. Although, the degradation rate of OSIM is lower in an acidic environment and EDTA-plasma, storage at room temperature should be minimised in order to maintain reliable analysis. Preferably, blood samples should be transported on ice upon collection and stored in the freezer as quickly as possible. Sample workup should ideally be performed on dry ice. EDTA containing tubes and plasma sample acidification may be used. However, in order to implement such an approach, further research to develop specific instructions and subsequent the validation of such a method would be necessary. The clinical applicability of our method was demonstrated by quantitative analysis of blood samples from lung cancer patients treated with OSIM. This bio-analytical assay will be extensively used as part of an on-going clinical trial (NCT0385491) and may be used as part of routine care in the future.

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APPENDIX A

Table A1: short-term stability of osimertinib at room temperature in human serum.

Time (hour)	[C] QC_{LOW} (ng/mL)	[C] QC_{HIGH} (ng/mL)	[C]/[C_0] QC_{LOW} (%)	[C/C_0] QC_{HIGH} (%)
0.0	83.65	420.82	100	100
0.5	67.66	368.74	80.9	87.6
1.0	51.05	339.48	61.0	80.7
1.5	44.75	307.60	53.5	73.1
2.0	31.45	309.73	37.6	73.6
3.0	24.35	247.34	29.1	58.8
4.0	15.52	227.78	18.6	54.1
5.0	15.44	181.33	18.5	43.1
6.0	10.24	150.89	12.2	35.9

Abbreviations: [C] = concentration; [C_0] = concentration at start (t = 0.0); QC_{LOW} = quality control low-level; QC_{HIGH} = quality control high-level;

Table A2: long-term stability of osimertinib in stock solutions.

Time (days)	Medium	Temperature	Accuracy (%)
21	DMSO	- 80°C	105.24
60	DMSO	- 80°C	98.20
90	DMSO	- 80°C	103.74
21	methanol	- 80°C	68.60

DMSO = dimethylsulfoxide;

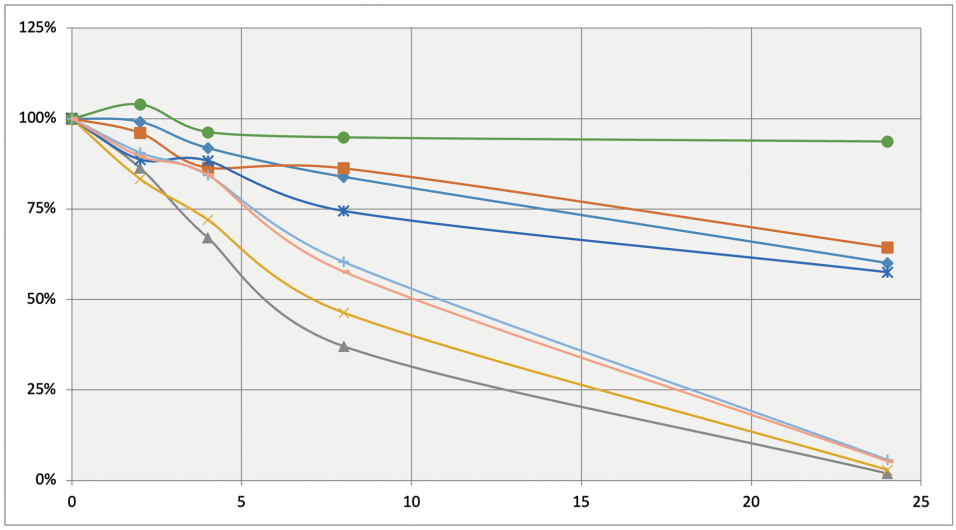

Figure A1: short-term stability of osimertinib in plasma at room temperature
(whole blood – QC_{HIGH} = circle, whole blood – QC_{LOW} = asterisk, EDTA-plasma – QC_{HIGH} = rhombus, EDTA-plasma – QC_{LOW} = square, heparinized plasma – QC_{HIGH} = multiplication-sign, heparinized plasma – QC_{LOW} = triangle, patient sample – high = minus, patient sample – low = plus); x-axis = time (hours), y-axis = accuracy.

Chapter 2.2

Development and validation of an HPLC-MS/MS method to simultaneously quantify alectinib, crizotinib, erlotinib, gefitinib and osimertinib in human plasma samples, using one assay run.

A. van Veelen, R.M.J.M. van Geel, R. Schoufs, Y.M. de Beer,
L.M.L. Stolk, L.E.L. Hendriks, S. Croes.

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ABSTRACT

A liquid chromatography-tandem mass spectrometry method was developed and validated to quantify alectinib, crizotinib, erlotinib and gefitinib. This assay can be combined with our method for osimertinib, allowing quantification of the most used anaplastic lymphoma kinase (*ALK*) and epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitors (TKIs) in non-small cell lung cancer with a single-assay setup. Chromatographic separation was performed on a HyPURITY® C18 analytical column using an elution gradient of ammonium acetate in water and in methanol, both acidified with formic acid 0.1%. Detection and quantification were performed by a triple quad mass spectrometer with an electrospray ionization interface. This method led to robust results, as the selectivity, carry-over, precision, and accuracy all met pre-specified requirements. The assay was validated over a linear range of 100 – 2000 ng/mL for alectinib and erlotinib and 50 – 1000 ng/mL for crizotinib and gefitinib. Alectinib, crizotinib, erlotinib and gefitinib were all stable for at least 4 hours in whole blood (at room temperature (RT) and at 4°C) and for at least one month in EDTA-plasma when stored at -80 °C, while osimertinib proved to be unstable at RT. Although high-performance liquid chromatography was used, the run time was short and comparable with other methods using ultra-high performance liquid chromatography.

INTRODUCTION

The prognosis of patients with metastatic non-small cell lung cancer (NSCLC) has improved the last years, mainly due to the introduction of tyrosine kinase inhibitors (TKIs) for those with an oncogenic driver, and due to the development of immune checkpoint inhibitors for most of the other NSCLC patients [1, 2].

The oncogenic drivers, for which the most TKI options are available, are the activating epidermal growth factor receptor (*EGFR*) mutations and the anaplastic lymphoma kinase (*ALK*) gene fusions. For patients with *EGFR* mutations, erlotinib, gefitinib (first generation TKI), afatinib, dacomitinib (second generation TKI), and osimertinib (third generation TKI) are available in standard of care. For patients with an *ALK* gene fusion, crizotinib (first generation TKI), ceritinib, alectinib, brigatinib and lorlatinib (next generation TKI) are available. Those TKIs have proven to be effective in comparison to either chemotherapy or an earlier generation TKI in clinical trials [3 – 13]. For all these TKIs, a fixed dose is given, and for example body weight-based dosing is not considered. As plasma levels can vary between patients, resulting in either under treatment or toxicity, the interest for therapeutic drug monitoring (TDM) is growing [14].

Single drug assays to quantify alectinib, crizotinib, erlotinib, gefitinib or osimertinib in human plasma have been published abundantly, but multi-drug assays to quantify multiple TKIs simultaneously have been developed and validated less often [15 – 18]. For research purposes and TDM, we developed and validated a simple and fast high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method for the simultaneous quantification of alectinib, crizotinib, erlotinib and gefitinib in human plasma which can be combined with our previous validated method to quantify osimertinib, allowing quantification of the currently most frequently used TKIs in NSCLC with a single assay setup. Using this assay for TDM may further optimize treatment with these TKIs by revealing under or over exposure.

METHODS

2.1 Chemicals and reagents

Alectinib (free base purity (FBP) = 98%), crizotinib (FBP = 98%), erlotinib (FBP = 97%), gefitinib (FBP = 98%), crizotinib-D5 (chemical purity 97%, isotopic purity 99,2%), erlotinib-D6 (FBP = 98%) and gefitinib-D3 (chemical purity = 98%, isotopic purity = 99,7%) were purchased from Bio Connect (Huissen, the Netherlands). Methanol (ULC/MS-CC/SFC grade) was purchased from Biosolve (Valkenswaard, the Netherlands). Acetonitrile (≥ 99.9%), 2-propanol (≥ 99.8%) and dimethylsulfoxide (DMSO, ≥ 99.0%) were obtained from Merck (Darmstadt, Germany). Ammonium acetate (Normapur) was purchased from VWR (Leuven, Belgium). Sodium citrate plasma (frozen, no additives) was purchased from

Sanquin (Amsterdam, the Netherlands). K2-EDTA whole blood, and subsequently, K2-EDTA-plasma was obtained from volunteers.

2.2 Preparation of calibration standards and quality controls

For the calibration standards and quality controls of crizotinib and gefitinib, two separately prepared stock solutions (1 mg/mL) were produced in DMSO. This led to two crizotinib solutions and two gefitinib solutions, one for calibration purposes, and one for quality control purposes. One crizotinib solution and one gefitinib solution were combined and diluted in methanol to 20 µg/mL (solution 1). The same was done for the second crizotinib/gefitinib solutions, which was used for quality control (QC)-batches (solution QC_1). For alectinib and erlotinib, a similar approach was taken, as two separate stock solutions (1 mg/mL) were prepared in DMSO as well for both drugs, resulting in two alectinib solutions and two erlotinib solutions. Those four solutions were used to form solution 2 and solution QC_2, in a similar way as described for crizotinib and gefitinib. Both solutions (2 and QC_2) were subsequently diluted in methanol to 40 µg/mL. A working solution was produced by combining solution 1 and solution 2 1:1 to form solution 3 and by combining solution QC_1 and QC_2 forming solution QC_3.

The stock solutions of the internal standards (crizotinib-D5, erlotinib-D6 and gefitinib-D3) were prepared reconstituting approximately 1 mg of each compound separately in 100 mL methanol (10 µg/mL). All stock solutions (solution 1, 2, QC_1 and QC_2) were stored at -80 °C until analysis. Calibration standards consisted of six different concentrations, a zero sample and a blank sample (Table 1). The calibration standards were prepared by spiking citrate plasma with solution 3. The zero sample only consisted of all internal standards, while the blank sample did not contain any TKI or internal standard. Quality control samples were prepared at five different concentrations from solution QC_3: lowest limit of quantification (LLOQ), QC_{LOW}, QC_{MED}, QC_{HIGH} and upper limit of quantification (ULOQ). QC_{LOW}, QC_{MED} and QC_{HIGH} were used for sample runs. The nominal concentrations of all QCs can be found in Table 1.

2.3 Instrumentation

For the analysis a Surveyor® Autosampler Plus with a quaternary MS-pump plus and degasser (ThermoFischer, Breda, the Netherlands) as a chromatographic system was used. A TSQ Quantum-Access® triplequad mass-spectrometer (ThermoFischer, Breda, the Netherlands) with an electrospray ionization interface (ESI) combined with Excalibur® software (version 2.2SP1) was used for detection and quantification. Chromatographic separation was performed on a HyPURITY® C18 analytical column (50 × 2.1 mm, 3 µm, Thermo Fischer Scientific) combined with a drop-in guard (HyPURITY® C18, 10 × 2.1 mm, 3 µm).

Table 1: concentrations for calibration curve and quality controls.

Calibration curve	Level 1 (ng/mL)	Level 2 (ng/mL)	Level 3 (ng/mL)	Level 4 (ng/mL)	Level 5 (ng/mL)	Level 6 (ng/mL)
Quality controls	LLOQ	QC_{LOW}		QC_{MED}	QC_{HIGH}	ULOQ
Alectinib	100	200	500	1000	1500	2000
Crizotinib	50	100	250	500	750	1000
Erlotinib	100	200	500	1000	1500	2000
Gefitinib	50	100	250	500	750	1000

Abbreviations: LLOQ, lower limit of quantification; QC_{LOW}, low quality control; QC_{MED}, middle quality control; QC_{HIGH}, high quality control; ULOQ, upper limit of quantification; ; ng/mL = nanogram per millilitre.

2.4 Sample preparation

The solution for deproteinization was made by adding 40 µL of the stock solution of crizotinib-D5, 90 µL of the stock solution of erlotinib-D6 and 20 µL of the stock solution of gefitinib-D3 to 10 mL methanol. 150 µL of deproteinization solution was added to a 20 µL plasma sample. The mixture was vortexed for two minutes and centrifuged at 11,300 g for five minutes. Subsequently, 100 µL supernatant was mixed with 400 µL mobile phase A to ensure compatibility with the gradient used during chromatography.

2.5 Chromatographic condition and LC-MS/MS settings

Two mobile phases were used for achieving chromatographic separation. Mobile phase A consisted of 2 mM ammonium acetate in water (+ 0.1% formic acid). Mobile phase B consisted of 2 mM ammonium acetate in methanol (+ 0.1% formic acid). The starting gradient was set at 80% A and 20% B for 0.5 minutes. After 0.5 minutes the gradient linearly increased to 100% B in 2.0 minutes and was maintained for an additional 0.3 minutes after which the gradient was reset to initial conditions and kept steady for 1 minute before a new sample was injected. The flow during the run was maintained at 500 µL/min. Column temperature was set at 60 °C. Auto-sampler temperature was set at 10°C. The divert-valve was set in the waste-position for the first 1.5 minutes. A flush/needle-wash solution of water (25%), methanol (25%), acetonitrile (25%) and isopropanol (25%) was used to reduce carry-over.

MS detection was in MRM-mode with the following settings: spray voltage 4500 V, Sheath gas pressure 60, Aux gas pressure 15, Capillary temperature 360 °C, Collision gas pressure 1.5 mTorr. Transitions used for the different compounds were as follows: 483.3 – 396.2 (alectinib), 450.2 – 260.1 (crizotinib), 394.2 – 278.0 (erlotinib), 447.2 – 128.1 (gefitinib), 455.2 – 265.1 (crizotinib-D5), 400.3 – 278.0 (erlotinib-D6) and 450.2 – 128.1 (gefitinib-D3). The collision energy and tube lens value were 23 eV and 85 for alectinib, 22 eV and 87 for crizotinib, 30 eV and 91 for erlotinib, 25 eV and 71 for gefitinib, 24 eV and 88 for crizotinib-D5, 29 eV and 91 for erlotinib-D6, and 23 eV and 88 for gefitinib-D3.

2.6 Method validation

The validation was based on the most recent European Medicines Agency (EMA) guideline 'bio-analytical method validation' [19].

2.6.1 Selectivity and carry-over

Selectivity was evaluated by analysing six different EDTA plasma samples from patients who were not treated with any of the measured TKIs. The largest peak close to the retention times of the TKIs or internal standards were manually integrated. These values were compared to the response of the lowest response in one of the 5 LLOQs in the same validation run. The response should be lower than 20% of the LLOQ. For all internal standards, the response should not exceed 5% of the peak area of the internal standard. Carry-over was tested by injecting a blank plasma sample after an ULOQ sample. Analysis was carried out in five-fold. The limits for carry-over are similar to the limits for selectivity (<15% for QCs of TKIs, <20% for LLOQ of TKIs, and <5% for internal standards).

2.6.2 Lower limit of quantification and linearity

For all TKIs a mean or median plasma trough concentration at steady state ($C_{min,SS}$) in the population has been reported: alectinib – 572 ng/mL, crizotinib – 274 ng/mL, erlotinib – 1,010 ng/mL, and gefitinib – 291 ng/mL [14]. In addition, threshold plasma trough concentrations in steady state have been proposed for alectinib (> 435 ng/mL) and erlotinib (> 500 ng/mL). In our analytical method we used a calibration range of 100 – 2000 ng/mL for alectinib and erlotinib, and 50 – 1000 ng/mL for crizotinib and gefitinib. Three calibration curves were constructed (y = a * x + b, weighing 1/x). Calculated concentrations were not allowed to exceed 15% of the nominal value of all QCs, with exception of the LLOQ, which had to be within 20% of the nominal value.

2.6.3 Precision and Accuracy

Precision and accuracy of the developed method were determined by analysing five different QCs (LLOQ, QC_{LOW}, QC_{MED}, QC_{HIGH} and ULOQ) on three different days using freshly prepared calibration standards to construct the calibration curve. On each day, five replicates of each QC were analysed. Precision and accuracy were not allowed to exceed 15% for all QCs except for the LLOQ which should remain within 20% of the nominal value [19].

2.6.4 Matrix effect

The matrix effect was evaluated using QC_{LOW} and QC_{HIGH}. Matrix effect was the ratio between the peak area of spiked blank matrix and the peak area in spiked mobile phase (80% A : 20% B).

2.6.5 Dilution integrity

Dilution integrity is tested by diluting human plasma (c= 1.5 × ULOQ) 4-fold and 2-fold [19]. All back calculated concentrations should be within 15% of the nominal value.

2.6.6 Stability

Short-term stability and long-term stability of all TKIs were determined in human EDTA and sodium citrate plasma for QC_{LOW}, QC_{MED} and QC_{HIGH} at four different temperatures (room temperature (RT), 4 °C, -20°C and -80 °C). In addition, short term stability was evaluated for all TKIs in EDTA whole blood, at RT and at 4°C. For all stock solutions the long-term stability was determined at -80 °C. Accuracy was not allowed to exceed 15% of the nominal value. Post-preparation stability in the auto sampler was assessed by re-injecting processed QCs and calibration standards (maintained at 10 °C in the auto-sampler for 24 hours).

RESULTS

3.1 Method development

In view of the previously developed assay for the analysis of osimertinib, the new method for the other four TKIs should preferably be similar due to simultaneous use in a routine setting in the future. Detailed validation and stability results for osimertinib are published elsewhere, and therefore will only be highlighted when substantially different from the other TKIs [20].

To minimize differences in sample preparation, we used methanol for protein precipitation. The starting gradient, compared to osimertinib, was altered to ensure an elution time above 1.5 minutes for alectinib, crizotinib, erlotinib and gefitinib. Using 2 mM ammonium-acetate in the dilution step prior to injection resulted in higher sensitivity for all TKIs, which made it possible to lower the injection volume.

At the start of the method development, deuterated alectinib and M4, the active metabolite of alectinib, were not commercially available and could not be included in this method. Therefore, erlotinib-D6 was used as internal standard for the quantification of alectinib.

The chromatograms for the four TKIs (alectinib, crizotinib, erlotinib and gefitinib) and the three internal standards used (crizotinib-D5, erlotinib-D6 and gefitinib-D3) are shown in Figure 1, where plasma samples were spiked with the LLOQ concentration. Furthermore, Figure 2 shows the chromatograms of the TKIs and the internal standards in blank samples. Because the chromatograms report relative abundance, the absolute abundances are described separately. The absolute abundances were considerably higher for the LLOQ chromatograms, and the ratio between the absolute abundances in the spiked sample compared to the blank sample was 17.4 for alectinib, 55.2 for crizotinib, 44.2 for erlotinib, 45.0 for gefitinib, 423.3 for crizotinib-D5, 718.1 for erlotinib-D6, and 796.7 for gefitinib-D3.

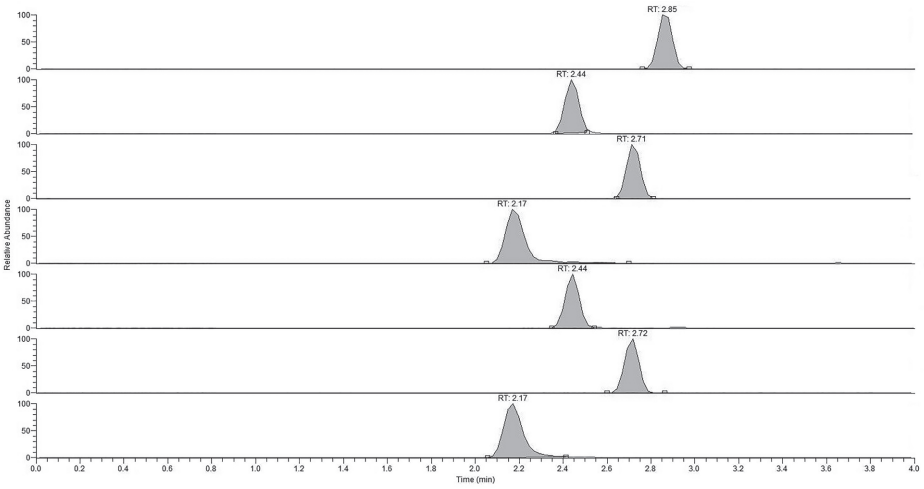

Figure 1: chromatograms of LLOQs of alectinib, crizotinib, erlotinib, gefitinib, crizotinib-D5, erlotinib-D6 and gefitinib-D3 (top-to-bottom).

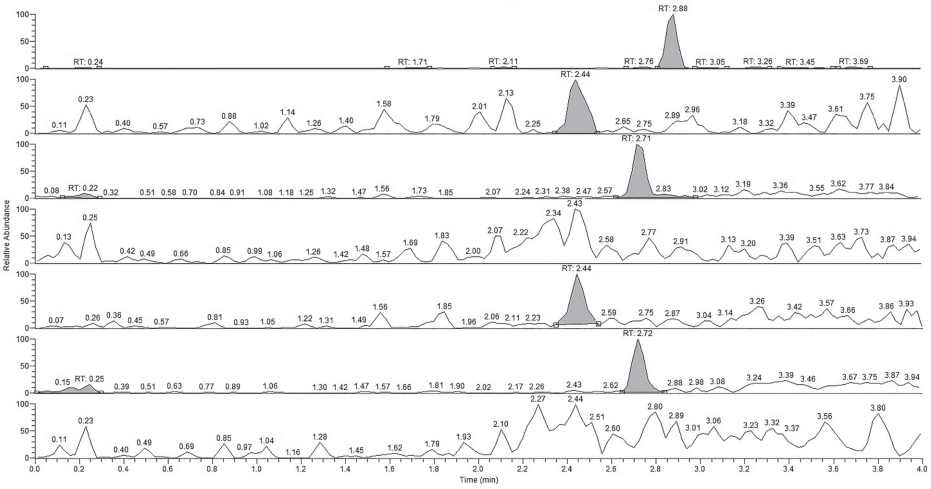

Figure 2: chromatograms of blank samples of alectinib, crizotinib, erlotinib, gefitinib, crizotinib-D^5, erlotinib-D^6 and gefitinib-D^3(top-to-bottom).

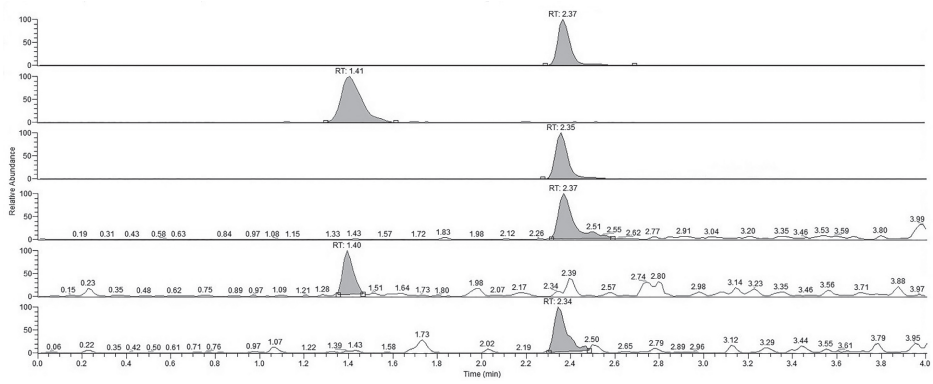

Figure 3: chromatograms of LLOQ and blank samples of osimertinib, its metabolite (AZ5104) and osimertinib-C13D3.
From top-to-bottom: osimertinib (LLOQ), AZ5104 (LLOQ), osimertinib-C13D3 (LLOQ), osimertinib (blank), AZ5104 (blank) and osimertinib-C13D3 (blank).

For osimertinib we additionally validated the quantification of the active, demethylated metabolite AZ5104 within the same assay. The addition of AZ5104 did not lead to any changes in sample preparation or the detection method. In Figure 3, the chromatograms of osimertinib, its active metabolite (AZ5104) and the internal standard used, osimertinib-$C_{13}D_3$, are shown. As seen with the other TKIs, the absolute abundance was higher for the LLOQ-samples, the ratio for osimertinib was 10.1, for AZ5104 15.2 and 202.1 for osimertinib-$C_{13}D_3$.

3.2 Method validation

3.2.1 Selectivity and carry-over

The results for selectivity and carry-over are shown in Table 2. For all TKIs six human EDTA plasma samples showed no interfering peaks. Blank TKI-responses were below 5.6% for all TKIs and ranged from 0.9% - 1.7% for alectinib, 1.2% - 5.6% for crizotinib, 0.5% - 1.4% for erlotinib and 1.0% - 4.9% for gefitinib. Carry-over was below 3.9% for all TKIs, and ranged from 2.6% - 3.9% for alectinib, 1.5% - 2.5% for crizotinib, 1.4% - 2.0% for erlotinib and 0.3% - 3.4% for gefitinib. For the internal standard blank responses were all below 0.3% and carry-over was below 0.3% for all TKIs. Selectivity and carry-over for AZ5104 also met all requirements (Appendix A).

Table 2: selectivity and carry-over of alectinib, crizotinib, erlotinib, gefitinib and the internal standard, and accuracy after a two-fold and four-fold dilution of a high concentration (1.5 × ULOQ).

Drug	Selectivity		Carry-over		Dilution	
	Accuracy (%) TKI (n=6)	Accuracy (%) IS (n=6)	Accuracy (%) TKI (n=5)	Accuracy (%) IS (n=5)	Accuracy (%) (two-fold) (n=5)	Accuracy (%) (four-fold) (n=5)
Alectinib	0.9 – 1.7	0.0 – 0.2	2.6 – 3.9	0.1 – 0.1	111.6 (±3.6)	112.2 (±2.8)
Crizotinib	1.2 – 5.6	0.0 – 0.2	1.5 – 2.5	0.1 – 0.3	107.3 (±2.1)	111.6 (±3.2)
Erlotinib	0.5 – 1.4	0.0 – 0.2	1.4 – 2.0	0.1 – 0.1	112.8 (±2.6)	108.2 (±2.3)
Gefitinib	1.0 – 4.9	0.0 – 0.3	0.3 – 3.4	0.1 – 0.2	106.5 (±1.8)	113.4 (±2.7)

TKI = tyrosine kinase inhibitor, IS = internal standard, n = number of samples
Selectivity was evaluated in blank samples and compared with the LLOQ of the corresponding TKI. Selectivity should be below 15%.
Carry-over was calculated by running a blank sample directly after the upper limit of quantification (ULOQ) and compare this with the LLOQ. Carry-over should be below 15% the TKI and below 1% for IS.
Dilution integrity should be within 15% from the nominal value (85% - 115%).

3.2.2 Calibration and linearity

The calibration curves were linear over the examined range for all four TKIs. The coefficient of determination varied between 0.9906 – 0.9990 for alectinib, 0.9924 – 0.9993 for crizotinib, 0.9922 – 0.9998 for erlotinib and 0.9903 – 0.9999 for gefitinib.

3.2.3 Precision and accuracy

Precision and accuracy data for all TKIs are shown in Table 3, and for AZ5104 in the Appendix A. Intra- and inter-day precision and accuracy for all TKIs and AZ5104 were within the pre-specified limits of 85% - 115% for QC_{LOW}, QC_{MED}, QC_{HIGH} and ULOQ, and 80% - 120% for LLOQ. Accuracies for the TKIs dilution integrity are shown in Table 2 and agreed with requirements mentioned above.

Table 3: intra- and inter-day accuracy and precision of alectinib, crizotinib, erlotinib and gefitinib in spiked human plasma samples.

Nominal concentration (ng/L)	Mean concentration (n=15) (ng/mL)	Intra-day precision (n=5) (%)	Inter-day precision (n=15) (%)	Intra-day accuracy (n=5) (%)	Inter-day accuracy (n=15) (%)
$LLOQ_A$ (100.0)	109.1	6.8	6.7	115.7	109.1
QC_{LOW_A} (200.0)	204.8	4.4	3.3	103.7	102.4
QC_{MED_A} (1000.0)	979.9	3.4	3.0	96.1	98.0
QC_{HIGH_A} (1500.0)	1495.1	4.5	4.2	102.4	99.7
$ULOQ_A$ (2000.0)	2027.0	3.0	2.9	103.6	101.4
$LLOQ_C$ (50.0)	55.6	4.4	5.4	116.3	111.1
QC_{LOW_C} (100.0)	108.2	5.4	4.0	110.2	108.2
QC_{MED_C} (500.0)	521.9	4.2	3.5	106.8	104.4
QC_{HIGH_C} (750.0)	782.5	2.6	3.3	108.1	104.3
$ULOQ_C$ (1000.0)	1035.9	4.2	4.1	106.9	103.6
$LLOQ_E$ (100.0)	106.6	4.0	4.5	111.3	106.6
QC_{LOW_E} (200.0)	206.7	3.6	3.4	106.1	103.3
QC_{MED_E} (1000.0)	1000.8	4.0	3.4	101.6	100.1
QC_{HIGH_E} (1500.0)	1507.2	4.5	3.6	101.8	100.5
$ULOQ_E$ (2000.0)	2012.5	3.7	2.4	101.4	100.6
$LLOQ_G$ (50.0)	55.1	5.5	5.8	115.3	110.2
QC_{LOW_G} (100.0)	107.8	2.7	3.6	110.3	107.8
QC_{MED_G} (500.0)	505.4	1.8	1.4	102.0	101.1
QC_{HIGH_G} (750.0)	753.4	0.9	1.5	102.3	100.5
$ULOQ_G$ (1000.0)	986.8	2.3	1.7	98.2	98.7

Abbreviations: LLOQ, lower limit of quantification; QC_{LOW}, low quality control; QC_{MED}, middle quality control; QC_{HIGH}, high quality control; ULOQ, upper limit of quantification; n = number of samples, ND = not determined.

A (subscript) = alectinib; C (subscript) = crizotinib; E (subscript) = erlotinib; G (subscript) = gefitinib.

3.2.4 Matrix effect

Matrix effect was evaluated using QC_{LOW} and QC_{HIGH}. For all TKIs the matrix effect was below 5%. The coefficient of variation (CV) was 4.8% (QC_{LOW}) and 2.8% (QC_{HIGH}) for alectinib, 3.9% (QC_{LOW}) and 4.2% (QC_{HIGH}) for crizotinib, 2.0% (QC_{LOW}) and 1.8% (QC_{HIGH}) for erlotinib, and 2.0% (QC_{LOW}) and 1.7% (QC_{HIGH}) for gefitinib. The results for matrix effect are shown in Table 4.

Table 4: matrix effect and freeze-thaw stability of alectinib, crizotinib, erlotinib and gefitinib in human plasma.

Drug (ng/ml)	Matrix effect (n = 6)	Freeze-thaw stability (n = 6)	Auto-injector stability (n = 5)
	Accuracy (var) (%)	Accuracy (var) (%)	Accuracy (var) (%)
Alectinib – QC_{LOW} (200.0)	51.2 (4.8)	102.6 (3.6)	103.6 (1.4)
Alectinib – QC_{MED} (1000.0)	-	102.9 (4.3)	97.4 (4.9)
Alectinib – QC_{HIGH} (1500.0)	55.8 (3.8)	102.8 (3.4)	99.3 (2.3)
Crizotinib – QC_{LOW} (100.0)	96.8 (3.9)	101.6 (1.7)	100.3 (4.2)
Crizotinib – QC_{MED} (500.0)	-	103.0 (1.7)	99.1 (1.6)
Crizotinib – QC_{HIGH} (1000.0)	104.8 (4.2)	102.5 (1.0)	101.6 (0.7)
Erlotinib – QC_{LOW} (200.0)	101.1 (2.0)	105.3 (1.3)	107.9 (2.5)
Erlotinib – QC_{MED} (1000.0)	-	105.1 (0.7)	101.0 (3.7)
Erlotinib – QC_{HIGH} (1500.0)	99.5 (1.8)	103.1 (4.1)	101.5 (2.1)
Gefitinib – QC_{LOW} (100.0)	101.3 (2.0)	108.5 (2.2)	104.7 (2.2)
Gefitinib – QC_{MED} (500.0)	-	102.8 (1.2)	101.5 (2.2)
Gefitinib – QC_{HIGH} (1000.0)	100.1 (1.7)	104.5 (0.2)	98.7 (2.0)

QC_{LOW} = low quality standard, QC_{MED} = middle quality standard, QC_{HIGH} = high quality standard, n = number of samples, var = variation.
Accuracy was evaluated compared to the nominal value.

3.2.5. Stability

3.2.5.1 Short- and long-term stability

Short- and long-term stability for alectinib, crizotinib, erlotinib and gefitinib in human EDTA and sodium citrate plasma was evaluated at four different temperatures and is shown in Table 5. In citrate plasma, alectinib, crizotinib, erlotinib and gefitinib were stable for at least 24 hours at room temperature (20°C). Osimertinib was not stable for 24 hours at room temperature and the concentration decreased to below 10% after 24 hours for all three QCs [20]. A similar trend was seen for AZ5104, which was also not stable for at least 24 hours at RT. At 4°C, all TKIs were stable for at least 24 hours in citrate plasma and gefitinib showed relatively the best stability at 4°C as it was stable for at least three weeks. Osimertinib proved to be less stable at 4°C in citrate plasma, and similar results were seen for AZ5104. However, AZ5104 proved to be stable for 24 hours at 4°C in EDTA-plasma (Appendix A).

When stored at -80°C, alectinib, crizotinib, erlotinib and gefitinib were stable for at least one month in EDTA plasma. When stored at -20°C alectinib, erlotinib and gefitinib were stable for at least three weeks in citrate plasma, while alectinib was stable for at least one day. Due to irregularities, the stability of crizotinib after three weeks at -20°C could not be determined. Osimertinib was stable at -80°C for at least six months [20]. AZ5104 also showed good stability when stored at -80°C, irrespective of the used matrix (Appendix A).

Additionally, the stability of all TKIs was evaluated in EDTA whole blood at room temperature and at 4°C. All TKIs were stable for at least four hours in whole blood at room temperature. Moreover, alectinib and erlotinib showed even better stability, as those were stable for at least 24 hours. When stored at 4°C, all TKIs were stable for at least 24 hours (Table 6). AZ5104 was not stable in whole blood when stored at RT but proved to be stable at 4°C for at least 8 hours.

Table 5: stability of alectinib, crizotinib, erlotinib and gefitinib in human plasma at various storage conditions.

Temperature (ºC)	Drug	Time (days)	Accuracy QC_{LOW} (%)	Accuracy QC_{MED} (%)	Accuracy QC_{HIGH} (%)
-80	Alectinib[a]	1	105.8	98.8	99.4
-80	Alectinib[a]	30	103.9	96.2	103.5
-80	Crizotinib[a]	1	105.1	99.3	103.4
-80	Crizotinib[a]	30	109.6	98.2	96.9
-80	Erlotinib[a]	1	106.7	97.3	99.9
-80	Erlotinib[a]	30	108.4	97.2	100.1
-80	Gefitinib[a]	1	105.6	99.5	101.7
-80	Gefitinib[a]	30	107.3	101.4	99.9
-20	Alectinib[b]	1	103.1	105.9	101.0
-20	Alectinib[b]	21	116.4	105.6	109.6
-20	Crizotinib[b]	1	110.1	109.5	105.3
-20	Crizotinib[b]	21	ND	ND	ND
-20	Erlotinib[b]	1	92.2	101.3	99.2
-20	Erlotinib[b]	21	104.8	106.9	111.7
-20	Gefitinib[b]	1	94.3	94.7	95.9
-20	Gefitinib[b]	21	94.3	93.5	97.5
4	Alectinib[b]	1	101.3	102.0	103.7
4	Crizotinib[b]	1	107.9	106.2	109.5
4	Erlotinib[b]	1	93.3	101.0	103.4
4	Gefitinib[b]	1	93.9	95.3	97.5
RT	Alectinib[b]	1	99.5	100.1	100.5
RT	Crizotinib[b]	1	101.3	107.5	99.9
RT	Erlotinib[b]	1	93.8	101.5	102.5
RT	Gefitinib[b]	1	94.7	93.7	98.0

Abbreviations: RT = room temperature; QC_{LOW} = low quality control; QC_{MED} = middle quality control; QC_{HIGH} = high quality control. ND = not determined.

Results are mean concentration compared with nominal value. All concentrations were analyzed in duplicate.

[a] Stability was evaluated in EDTA-plasma

[b] Stability was evaluated in citrate plasma

For every specific moment one plasma sample was analyzed in duplicate.

Table 6: stability of alectinib, crizotinib, erlotinib and gefitinib in EDTA whole blood at various storage conditions.

Temperature (°C)	Drug	Time (hours)	Accuracy QC_{LOW} (%)	Accuracy QC_{MED} (%)	Accuracy QC_{HIGH} (%)
RT	Alectinib	4	104.1	100.8	104.5
RT	Alectinib	8	99.7	98.9	107.7
RT	Alectinib	24	110.2	100.0	111.9
RT	Crizotinib	4	87.7	96.7	110.8
RT	Crizotinib	8	80.3	101.8	112.6
RT	Crizotinib	24	87.2	107.3	122.4
RT	Erlotinib	4	106.2	104.9	108.6
RT	Erlotinib	8	105.1	102.2	106.5
RT	Erlotinib	24	99.1	99.3	105.3
RT	Gefitinib	4	99.7	103.3	112.6
RT	Gefitinib	8	104.4	106.4	115.5
RT	Gefitinib	24	98.3	104.9	119.2
4	Alectinib	4	101.4	100.0	105.4
4	Alectinib	8	105.7	94.6	97.6
4	Alectinib	24	106.8	94.4	101.5
4	Crizotinib	4	91.5	108.3	105.7
4	Crizotinib	8	100.3	107.2	102.0
4	Crizotinib	24	99.0	110.5	103.9
4	Erlotinib	4	106.0	105.4	105.5
4	Erlotinib	8	106.1	101.8	98.1
4	Erlotinib	24	106.6	101.0	100.7
4	Gefitinib	4	106.2	109.5	106.8
4	Gefitinib	8	109.5	106.3	104.1
4	Gefitinib	24	109.3	107.0	110.7

Abbreviations: RT = room temperature; QC_{LOW} = low quality control; QC_{MED} = middle quality control; QC_{HIGH} = high quality control. Results are mean concentration compared with nominal value. All concentrations were analysed in duplicate.
For every specific moment one plasma sample was analysed in duplicate.

3.2.5.2 Stock stability

The stock solutions of alectinib, crizotinib, erlotinib and gefitinib were stable for three months when stored at -80°C, with accuracies of 99.6% for alectinib, 100.2% for erlotinib and 101.2% for gefitinib. The stock solution of crizotinib was not stable at -80°C, as the accuracy had increased to 125.5% after three months.

3.2.6 Clinical applicability

The analytical method described here is used to perform two studies evaluating EDTA plasma trough concentrations during steady state of patients who are treated with one of the TKIs as part of standard patient care (studies approved by the medical ethics review committee of the Maastricht University Medical Centre – approval number 2019-1080 and approval number 2018-0800). The range of plasma trough concentration measured at steady state was 184.93 – 783.9 for alectinib (n = 21), 216.7 – 340.4 ng/mL for crizotinib (n = 4), 361.8 – 1584.9 ng/mL for erlotinib (n = 6) and 101.3 – 437.6 ng/mL for osimertinib (n = 43). Until now, no patients using gefitinib were included. All measured plasma trough concentrations at steady state were within the validated calibration range, except for one osimertinib user who experienced an unusual high trough concentration (measured at 1130 ng/mL), and we reported this as >500 ng/mL due to the validated range. Incurred sample reanalysis for alectinib, crizotinib, erlotinib and gefitinib have not been performed yet, but will be performed when more blood samples are collected.

3.3 Comparison with previous studies

For each TKI several single drug assays have been published, but multi-drug assays are less frequently reported. Reis *et al*. described a method to quantify afatinib, crizotinib, erlotinib, nintedanib and osimertinib in sodium citrate and heparinized plasma [15]. Another multi drug assay, by Hayashi *et al.*, focused on the quantification of afatinib, erlotinib and gefitinib, but the type of plasma used in the method was not reported [17]. The method described by Veerman *et al*. was used to analyse alectinib, afatinib, crizotinib, and osimertinib in heparinized plasma [16]. All these studies have some similarities with our method, but none simultaneously analysed alectinib, crizotinib, erlotinib, gefitinib and osimertinib, which are currently the most commonly used ALK- and EGFR-TKIs to treat NSCLC patients in our hospital. In a recent study by Zhou *et al*. a simultaneous analytical method was developed and validated for the analysis of 12 TKIs, which also consisted of the five TKIs that are presented in this article. Compared to our method, a couple differences can be indicated. First, a salting-out liquid-liquid extraction (SALLE) was used, while we used a simple protein precipitation method with methanol. Using SALLE could leave residual protein dissolved in the plasma sample, which could be detrimental for the chromatographic column in the long term. Secondly, the run time in analytical method from Zhou *et al*. was 6.0 minutes, while our run-time was 3.8 minutes. Lastly, Zhou *et al*. used one internal standard (voriconazole) for quantification of each TKIs, where ideally a deuterated form is desirable, as both the TKI and the internal standard will be eluded at the same moment, and potential ion-suppressing or ion-enhancement will be comparable to both the TKI and the internal standard. However, if the TKI and its internal standard are not eluded at the same time, possible inaccuracies could occur, especially in patient samples, with other, sometimes unknown, substances [18]. We have used a deuterated form of the TKIs as much as possible if they were commercially available. Since our new method can be performed with the same equipment as our earlier developed method for osimertinib, it allows us to analyse all five TKIs (alectinib, crizotinib, erlotinib, gefitinib and osimertinib)

with a single-assay setup. This workflow is less time consuming. Laboratory technicians can save time, which can be used to perform other analyses for TDM purposes. The number of (expensive) laboratory equipment and the availability of laboratory technicians is frequently limited, while the intention is to offer a wide range of TDM analyses across various therapeutic fields. Subsequently, combining the analysis of multiple TKIs with a single run will be more cost-effective, as the mean preparation time per sample will be lower, compared to a situation where five different runs are performed for all individual TKIs, thereby saving time and costs.

In addition, in one centre only a small number of patients is treated with each individual TKI. Consequently, when single TKI assays are used, only a small number of samples can be evaluated in one run. Otherwise, or as an alternative, multiple samples need to be collected over a longer period of time, which could lead to delayed reporting results to physicians. Combining analysis of multiple TKIs in one assay enables us to perform a run more frequently. Improving reporting efficiency allows quicker dose adjustments when drug concentrations are outside the therapeutic window, for example.

HPLC was used for separation by Reis *et al*. and resulted in a run-time of 11 minutes [15]. Hayashi *et al*. and Veerman *et al*. used UHPLC which decreased the run-time to approximately 5 minutes [16, 17]. With our HPLC method, we achieved a run-time similar to that of published UHPLC methods. In addition, sample preparation was straightforward and efficient, without the need of the additional supernatant evaporation using nitrogen as described by Reis *et al*. and Hayashi *et al*. The sample preparation of osimertinib was slightly different, as it must be performed on dry-ice due to the limited stability of osimertinib and its metabolite at room temperature [20, Appendix A].

We decided to evaluate the stability of all TKIs extensively, to make sure the stability of TKIs is assured through the whole process of blood collection until quantification. During daily practice it may be necessary to store a blood sample temporarily at room temperature or in the fridge because of the daily working activities. All TKIs showed sufficient stability, either in EDTA whole blood (at RT and 4°C) as well as in plasma (at RT and 4°C) to ensure that all TKIs were stable through the whole process from blood collection to quantification.

We evaluated stability in EDTA-plasma and EDTA-whole blood, while others evaluated stability in heparinized plasma and sodium citrate anti-coagulated plasma [15, 16], while Zhou *et al*. used unspecified plasma [18]. Stability results are similar, independent of the used anticoagulant. Reis *et al*. described that crizotinib and erlotinib were stable for at least 24 hours at room temperature and for 60 days at -20°C [15]. In our study crizotinib and erlotinib were stable for at least 24 hours at room temperature and for at least 30 days when stored at -80°C. In the study by Veerman *et al*. alectinib and crizotinib were stable for at least 24 hours when stored at room temperature and for at least nine months when stored at -70°C [16]. These results were similar to our results, as both alectinib and crizotinib were

stable for 24 hours at room temperature. Furthermore, alectinib and crizotinib were stable for at least one month in our study. In the study by Zhou *et al*. alectinib, crizotinib, erlotinib and gefitinib showed sufficient stability. Furthermore, the limited stability of osimertinib was briefly discussed. This was similar to the stability we have observed, while specific details on osimertinib stability were described previously [15, 16, 18, 20, 21].

CONCLUSION

In this study an analytical method was developed and validated to simultaneously quantify alectinib, crizotinib, erlotinib and gefitinib in EDTA plasma. Although HPLC was used for separation, the run time was comparable to UHPLC methods. To our knowledge this is the first method validated for this selection of ALK- and EGFR-TKIs. Furthermore, the assay can be combined with our previously validated method for osimertinib using the same equipment, allowing the simultaneous quantification of the currently most used ALK- and EGFR-TKIs in the Netherlands among patients with NSCLC with a single-assay setup. Such a setup improves laboratory efficiency and enhances the reporting capabilities. The intended use of this assay in clinical practice for TDM may further support treatment optimization of these TKIs, by revealing under or over exposure, evaluating drug adherence or monitor drug-drug interactions with co-administered medications.

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APPENDIX A

Validation results for AZ5104, active metabolite of osimertinib

Table A1: selectivity and carry-over of AZ5104 and the internal standard.

	Selectivity		Carry-over	
Drug	**Accuracy (%)**	**Accuracy (%) IS**	**Accuracy (%)**	**Accuracy (%) IS**
AZ5104	0.0 – 0.2	0.1 – 0.3	0.7 – 1.9	0.1 – 0.3

IS = internal standard.
Selectivity was evaluated in blank samples and compared with the LLOQ of the corresponding TKI. Selectivity should be below 15%.
Carry-over was calculated by running a blank sample directly after the higher limit of quantification (HLOQ) and compare this with the LLOQ. Carry-over should be below 15% the TKI and below 1% for IS.

Table A2: intra- and inter-day accuracy and precision of AZ5104 in spiked human plasma samples.

Nominal concentration (ng/L)	Mean concentration (n=15) (ng/mL)	Intra-day precision (n=5) (%)	Inter-day precision (n=15) (%)	Intra-day accuracy (n=5) (%)	Inter-day accuracy (n=15) (%)
LLOQ (10.0)	9.26	12.2	12.2	82.1	92.6
QC_{LOW} (30.0)	29.27	5.2	5.2	94.5	97.6
QC_{MED} (100.0)	99.59	4.4	4.4	98.7	99.6
QC_{HIGH} (150.0)	154.93	3.9	3.9	107.1	103.3
ULOQ (200.0)	191.62	4.2	4.2	92.1	95.8

Abbreviations: ng = nanogram, L = litre, ml = millilitre, LLOQ = lower limit of quantification, QC_{LOW} = low quality control, QC_{MED} = middle quality control, QC_{HIGH} = high quality control, ULOQ = upper limit of quantification, ND = not determined.

Table A3: short-term stability of AZ5104 at room temperature (RT), at 4°C and in the auto-injector in different matrices.

Temp (°C)	Matrix	Time (hours)	Accuracy QC_{LOW} (%)	Accuracy QC_{MED} (%)	Accuracy QC_{HIGH} (%)
RT	Whole blood	4	79.0	81.1	84.7
RT	Whole blood	8	75.2	70.3	71.3
RT	Whole blood	24	48.9	38.0	37.2
RT	EDTA plasma	4	98.1	90.6	93.6
RT	EDTA plasma	8	80.5	85.8	84.2
RT	EDTA plasma	24	49.1	57.1	57.4
RT	Citrate plasma	4	62.8	72.4	74.8
RT	Citrate plasma	8	38.2	53.7	58.6
RT	Citrate plasma	24	6.6	14.4	20.7
RT	Serum	4	80.6	82.1	89.3
RT	Serum	8	69.4	64.5	72.1
RT	Serum	24	21.7	27.1	33.2
4	Whole blood	4	90.6	96.2	96.8
4	Whole blood	8	93.6	86.4	89.1
4	Whole blood	24	88.1	78.1	83.3
4	EDTA plasma	4	100.7	97.8	98.4
4	EDTA plasma	8	101.1	94.3	97.3
4	EDTA plasma	24	89.3	90.2	91.2
4	Citrate plasma	4	98.2	101.9	96.1
4	Citrate plasma	8	93.0	93.5	94.6
4	Citrate plasma	24	83.3	79.6	88.0
4	Serum	4	97.3	93.6	95.8
4	Serum	8	95.1	85.5	88.8
4	Serum	24	79.3	81.5	91.5
10 #	Citrate plasma	24	94.6	101.8	104.0

Abbreviations: RT = room temperature; QC_{LOW} = low quality control; QC_{MED} = middle quality control; QC_{HIGH} = high quality control. Results are mean concentration compared with nominal value. All concentrations were analyzed in duplicate.

Stability in auto-injector for 24 hours.

Table A4: long term stability of AZ5104 at -80°C in different matrices.

Temp (°C)	Matrix	Time (weeks)	Accuracy QC_{LOW} (%)	Accuracy QC_{MED} (%)	Accuracy QC_{HIGH} (%)
-80	EDTA plasma	2	96.0	93.5	96.7
-80	EDTA plasma	4	89.2	92.5	100.4
-80	Citrate plasma	2	96.8	108.4	103.2
-80	Citrate plasma	4	101.4	102.7	99.4
-80	Serum	2	100.8	86.0	98.0
-80	Serum	4	102.0	98.4	101.7

Abbreviations: RT = room temperature; QC_{LOW} = low quality control; QC_{MED} = middle quality control; QC_{HIGH} = high quality control. Results are mean concentration compared with nominal value. All concentrations were analyzed in duplicate.

2

Chapter 2.3

Development and validation of an HPLC-MS/MS method to simultaneously quantify brigatinib, lorlatinib, pralsetinib and selpercatinib in human K2-EDTA plasma.

J.L. Gulikers, A.J. van Veelen, E.M.J. Sinkiewicz, Y.M. de Beer, M. Slikkerveer, L.M.L. Stolk, V.C.G. Tjan-Heijnen, L.E.L. Hendriks, S. Croes, R.M.J.M. van Geel

Biomed Chromatogr. 2023 Mar 20;e5628.

ABSTRACT

A liquid chromatography-tandem mass spectrometry method was developed and validated to quantify the small molecule inhibitors (SMIs) brigatinib, lorlatinib, pralsetinib and selpercatinib, which are used in patients with oncogenic driven non-small cell lung cancer. Chromatographic separation was performed on a HyPURITY® C18 analytical column with a gradient elution using ammonium acetate in water and in methanol, both acidified with formic acid 0,1%. Detection and quantification were performed by a triple quad mass spectrometer with an electrospray ionization interface. The assay was validated over a linear range of 50 – 2,500 ng/mL for brigatinib, 25 – 1,000 ng/mL for lorlatinib, 100 – 10,000 ng/mL for pralsetinib, and 50 – 5,000 ng/mL for selpercatinib. All four SMIs were stable for at least seven days at cooling conditions (2 – 8 °C), and at least 24 hours at room temperature (15 – 25 °C) in K2-EDTA plasma. At freezing conditions (-20°C), all SMIs were stable for at least 30 days, except for the lowest quality control (QC_{LOW}) of pralsetinib. The QC_{LOW} of pralsetinib was stable for at least 7 days at -20°C. This method provides an efficient and simple way to quantify four SMIs with a single assay in clinical practice.

INTRODUCTION

With deeper understanding of the tumour biology in patients with non-small cell lung cancer (NSCLC), various oncogenes have been identified. The most common oncogenic drivers are mutations in epidermal growth factor receptor (EGFR) (10-26% in Caucasian patients) and Kirsten Rat sarcoma viral oncogene homolog (K-RAS) (±10%) [1-2]. Among the rarer oncogenic drivers are anaplastic lymphoma kinase (ALK) and RET fusions, which have a prevalence of 3-5% and ±1%, respectively [1]. Targeted therapies, mostly consisting of small molecule inhibitors (SMIs) targeting these specific oncogenic drivers, play an important role in the treatment of oncogenic driven metastatic NSCLC [1].

All targeted therapy is given in fixed-doses and regular quantification of drug exposure is not required. However, since interpatient variability with these therapies is large, therapeutic drug monitoring (TDM) could help to detect potential under- or overexposure and can be used for research purposes [3]. Previously, quantification methods for older targeted therapies that were part of standard of care were validated [4-7], but as more targeted agents become available, new quantification methods are needed. Lorlatinib and brigatinib are some of the newer ALK-directed SMIs, and recently, pralsetinib and selpercatinib were approved for RET fusion positive NSCLC [8-11]. While mono quantification assays have been developed for use in daily practice, it is not efficient to run different mono assays for each separate SMI, as the number of patients using individual SMIs is low. Therefore, it is more efficient to develop and use a combined quantification method for multiple SMIs as we have previously done for other EGFR- and other ALK-directed SMIs [5]. Separate assays combining the quantification of brigatinib and lorlatinib in human plasma [7, 12], and for the combination of pralsetinib and selpercatinib in mouse plasma have been developed [13]. However, an analytical method quantifying brigatinib, lorlatinib, pralsetinib, and selpercatinib in human plasma simultaneously is lacking.

In the current study we describe the development and validation of an analytical method to quantify brigatinib, lorlatinib, pralsetinib, and selpercatinib in human plasma using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).

METHODS

2.1 Chemical and reagents

Brigatinib (free base purity [FBP] = 99.98%), lorlatinib (FBP = 99.80%), pralsetinib (FBP = 98.01%), selpercatinib (FBP = 99.46%) were manufactured by MedChemexpress (New Jersey, USA) and purchased from Bio-connect (Huissen, The Netherlands). Erlotinib-D6.HCl (isotopic purity = 98%) was manufactured by TRC (Toronto, Canada) and purchased from Bio-connect. Brigatinib 13C6 (Chemical Purity 95.1%, isotopic purity= 99.1%) and lorlatinib 13CD3 (chemical purity = 97.4%, isotopic purity = 99.1% 13C and 99.1% D) were purchased

from Alsachim (Illkirch, France). Methanol (ULC/MS-CC/SFC grade) was purchased from Biosolve (Valkenswaard, the Netherlands). Acetonitrile (≥99.9%), 2-propanol (≥99.8%) and dimethylsulfoxide (DMSO, ≥99.0%) were obtained from Merck (Darmstadt, Germany). Ammonium-acetate (Normapur) was purchased from VWR (Leuven, Belgium). Sodium-citrate plasma (frozen, no additives) and K2-EDTA whole blood were purchased from Sanquin (Amsterdam, the Netherlands). K2-EDTA plasma was obtained by centrifugation of K2-EDTA whole blood for 5 minutes at 4000 RPM at room temperature (Hettich Centrifuge Rotanta 460 R).

2.2 Preparation of calibration standards and quality controls

For the calibration standards of lorlatinib, pralsetinib and selpercatinib, separately prepared stock solutions (1 mg/mL) were produced in DMSO. For the calibration standard of brigatinib, the stock solution (1 mg/mL) was produced in ethanol. The same was done for the preparation of the quality controls.

From this point, two solutions were made for each SMI. For the preparation of the calibration curve 50 µL of 1 mg/mL brigatinib, 20 µL lorlatinib, 100 µL selpercatinib, 200 µL pralsetinib and 630 µL methanol were pipetted in a glass tube (SOL1). 50 µL of 1 mg/mL brigatinib, 25 µL lorlatinib, 50 µL selpercatinib and 100 µL pralsetinib were pipetted in a 10 mL flask and substituted with methanol to a final volume of 10 mL (SOL2). The same proceedings were repeated for the preparation of the quality controls (SOLA and SOLB). The final concentrations in K2-EDTA plasma are shown in Table 1 and Table 2.

Table 1: concentrations used for the calibration curves of brigatinib, lorlatinib, pralsetinib, and selpercatinib.

Drug	Level 1 (ng/mL)	Level 2 (ng/mL)	Level 3 (ng/mL)	Level 4 (ng/mL)	Level 5 (ng/mL)	Level 6 (ng/mL)
Brigatinib	50	150	500	1250	1875	2500
Lorlatinib	25	75	250	500	750	1000
Pralsetinib	100	300	1000	5000	7500	10000
Selpercatinib	50	150	500	2500	3750	5000

Abbreviations: ng/mL = nanogram per millilitre

Table 2: concentrations used for the quality controls of brigatinib, lorlatinib, pralsetinib, and selpercatinib.

Drug	LLOQ (ng/mL)	QC_{LOW} (ng/mL)	QC_{MED} (ng/mL)	QC_{HIGH} (ng/mL)	ULOQ (ng/mL)
Brigatinib	50	100	1000	1750	2500
Lorlatinib	25	50	400	700	1000
Pralsetinib	100	200	4000	7000	10000
Selpercatinib	50	100	2000	3500	5000

Abbreviations: LLOQ, lower limit of quantification; QC_{LOW}, low quality control; QC_{MED}, middle quality control; QC_{HIGH}, high quality control; ULOQ, upper limit of quantification; ; ng/mL = nanogram per millilitre.

For each SMI, an appropriate internal standard (IS) was used. For brigatinib this was brigatinib-13C6 and for lorlatinib this was lorlatinib-13CD3. For pralsetinib and selpercatinib no specific isotope-labelled compounds were commercially available. Therefore, erlotinib-D6 was used as IS for pralsetinib and selpercatinib. The stock solutions of the ISs were prepared by reconstituting approximately 1 mg of each compound separately in 100 mL methanol (10 µg/mL). All stock solutions were stored at -80°C until analysis. Calibration standards consisted of six different concentrations, a zero sample and a blank sample (Table 1). The calibration standards were prepared by spiking both citrate and K2-EDTA plasma with solution 1 or 2. Both K2-EDTA and citrate plasma showed similar results and since (sodium) citrate plasma is commercially abundantly available, this plasma was further used for the calibration standards and quality control samples (QCs). The zero sample only consisted of all ISs, while the blank sample did not contain any SMI or IS. QCs were prepared in (sodium) citrate plasma at five different concentrations from solution A or B: lowest limit of quantification (LLOQ), QC_{LOW}, QC_{MED}, QC_{HIGH} and upper limit of quantification (ULOQ). QC_{LOW}, QC_{MED} and QC_{HIGH} were used for sample runs. The nominal concentrations of all QCs can be found in Table 2.

2.3 Instrumentation

For the analysis a Surveyor® Autosampler Plus with a quaternary MS Pump Plus and degasser (ThermoFischer, Breda, The Netherlands) as a chromatographic system was used. A TSQ Quantum-Access® triple quad mass spectrometer (ThermoFischer, Breda, the Netherlands) with an electrospray ionization interface (ESI) combined with Excalibur® software (version 2.2SP1) was used for detection and quantification. Chromatographic separation was performed on a HyPURITY® C18 analytical column (50 × 2.1 mm, 3 µm, ThermoFischer Scientific) combined with a drop-in guard (HyPURITY® C18, 10 × 2.1 mm, 3 µm).

2.4 Sample preparation

The solution for deproteinization was made by adding 250 µL of the stock solution of brigatinib 13C6, 125 µL of the stock solution of erlotinib-D6 and 50 µL of the stock solution of lorlatinib 13CD3 to 10 mL methanol. 150 µL of the deproteinization solution was added to a 20 µL plasma sample. The mixture was vortexed for two minutes and centrifuged at

11,300 g for five minutes. Subsequently, 100 µL supernatant was mixed with 400 µL water to ensure compatibility with the gradient used during chromatography.

2.5 Chromatographic condition and LC-MS/MS settings

Two mobile phases were used for achieving chromatographic separation. Mobile phase A consisted of 2 mM ammonium acetate in water (+ 0.1% formic acid). Mobile phase B consisted of 2 mM ammonium acetate in methanol (+ 0.1% formic acid). The starting gradient was set at 80% A and 20% B for 1.5 minutes. After 1.5 minutes the gradient linearly increased to 100% B in 6.5 minutes after which the gradient was reset to initial conditions and kept steady for 1 minute before a new sample was injected. The flow during the run was maintained at 500 µL/min. Column temperature was set at 60°C. Auto-sampler temperature was set at 10°C. The divert-valve was set in the waste-position for the first 1.5 minutes. A flush/needle-wash solution of water (25%), methanol (25%), acetonitrile (25%) and isopropanol (25%) was used to reduce carry-over.

MS detection was in multiple reaction monitoring-mode with the following settings: spray voltage 4500 V, Sheath gas pressure 60, Aux gas pressure 15, Capillary temperature 360°C, Collision gas pressure 1.5 mTorr. Transitions used for the different compounds were as follows: 584.1 – 483.9 (brigatinib), 407.0 – 227.8 (lorlatinib), 534.1 – 189.8 (pralsetinib), 526.01 – 121.9 (selpercatinib), 590.1 – 489.9 (brigatinib 13C6), 400.3 – 278.0 (erlotinib-D6) and 411.0 – 227.8 (lorlatinib 13CD3). The collision energy and tube lens value were 28 and 94 eV for brigatinib, 20 and 101 eV for lorlatinib, 34 and 108 eV for pralsetinib, 30 and 101 eV for selpercatinib, 27 and 92 eV for brigatinib-13C6, 29 and 91 eV for erlotinib-D6 and 21 and 101 eV for lorlatinib-13CD3.

2.6 Method validation

The validation was based on the most recent European Medicines Agency (EMA) guideline ‘bio-analytical method validation’ [14].

RESULTS

3.1 Method development

The chromatograms for the four SMIs (brigatinib, lorlatinib, pralsetinib, and selpercatinib) and the three ISs used (brigatinib-13C6, lorlatinib-13C2H3, and erlotinib-D6) are shown in Figure 1. As no commercially available IS was available for pralsetinib and selpercatinib, erlotinib-D6 showed to be the best fitting IS for those two drugs. The SMIs and ISs were spiked with the LLOQ concentration in (sodium) citrate plasma samples. Figure 2 shows the chromatograms of the SMIs and the ISs in blank samples. Because the chromatograms report relative abundance, the absolute abundances are described separately. The absolute abundances were considerably higher for the LLOQ chromatograms, and the ratio between the absolute abundances in the spiked sample compared to the blank sample was 143.2 for

brigatinib, 16.4 for lorlatinib, 10.8 for pralsetinib, 8.4 for selpercatinib, 307.2 for brigatinib-13C6, 927.4 for lorlatinib-13C2H3, and 726.0 for erlotinib-D6.

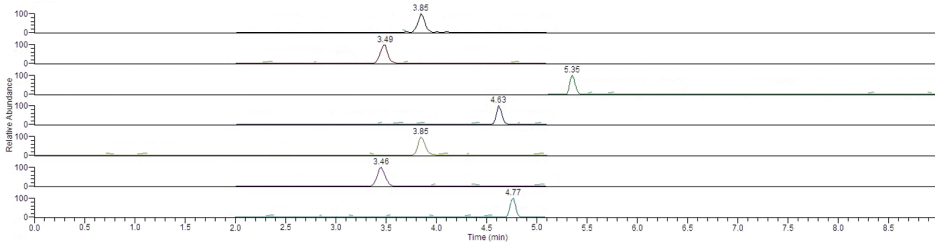

Figure 1: chromatogram of the LLOQ of brigatinib, lorlatinib, pralsetinib, selpercatinib, brigatinib (IS), lorlatinib (IS), and erlotinib-D6 (LLOQ = lower limit of quantification, IS = internal standard).

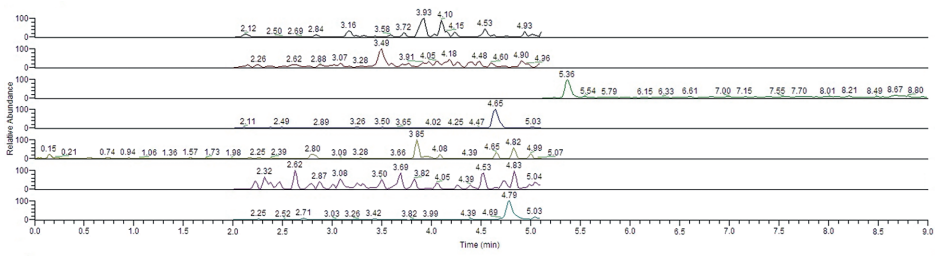

Figure 2: chromatograms of blanco sample of brigatinib, lorlatinib, pralsetinib, selpercatinib, brigatinib (IS), lorlatinib (IS), and erlotinib D6 (IS = internal standard).

3.2 Method validation

3.2.1 Selectivity and carry-over

No interfering peaks were seen in all six blank human samples for all four SMIs. The maximum blank SMI responses were 3.0% for brigatinib, 2.5% for lorlatinib, 9.9% for pralsetinib, and 13.1% for selpercatinib. Carry-over varied from 1.8% - 3.0% for brigatinib, 0.0% – 2.5% for lorlatinib, 11.0% – 12.2% for pralsetinib, and 11.4% - 13.1% for selpercatinib. For the ISs, blank responses were all below 0.4%, and carry-over was below 0.4%. Detailed results for selectivity and carry-over are shown in Table 3.

3.2.2 Calibration curve parameters

Calibration curves were made in citrate plasma and showed an adequate slope and intercept for all SMIs. The slopes varied from 0.000681516 – 0.000709749 for brigatinib, 0.00258897 – 0.0026564 for lorlatinib, 0.000269299 – 0.000281505 for pralsetinib, and 0.000167868 – 0.000181123 for selpercatinib. The intercept varied from -0.00421845 – 0.00170896 for brigatinib, -0.00970214 – -0.00514036 for lorlatinib, 0.00462435 – 0.0160506 for pralsetinib and 0.00243555 – 0.00403508 for selpercatinib.

Table 3: selectivity, carry-over, and dilution-effect for brigatinib, lorlatinib, pralsetinib, and selpercatinib in EDTA-plasma.

Drug	Selectivity		Carry-over		Dilution	
	Accuracy (%) TKI (n=6)	Accuracy (%) IS (n=6)	Accuracy (%) TKI (n=5)	Accuracy (%) IS (n=5)	Accuracy (%) (n=5) *	Accuracy (%) (n=5) #
Brigatinib	1.8 – 3.0	0.1 – 0.4	1.8 – 3.0	0.1 – 0.4	95.6	98.9
Lorlatinib	0.0 – 2.5	0.0 – 0.2	0.0 – 2.5	0.0 – 0.2	97.7	98.6
Pralsetinib	6.4 – 9.9	0.2 – 0.4	11.0 – 12.2	0.2 – 0.3	90.5	94.2
Selpercatinib	9.5 – 13.1	0.2 – 0.3	11.4 – 13.1	0.2 – 0.3	87.8	92.2

TKI = tyrosine kinase inhibitor, IS = internal standard, n = number of samples
Selectivity was evaluated in blank samples and compared with the LLOQ of the corresponding TKI. Selectivity should be below 15%.
Carry-over was calculated by running a blank sample directly after the upper limit of quantification (ULOQ) and compare this with the LLOQ. Carry-over should be below 20% the TKI and below 5% for IS and dilution integrity should be within 15% from the nominal value (85% - 115%).
* - two-fold dilution; # - four-fold dilution.

3.2.3 Precision and accuracy

Detailed results for the precision and accuracy of all SMIs in (K2-)EDTA plasma are shown in Table 4. Intra- and inter-day precision was below pre-specified requirements in EDTA plasma, with a maximum of 11.5% for brigatinib, 9.5% for lorlatinib, 16.7% for pralsetinib and 12.6% for selpercatinib (Table 4). Intra- and inter-day accuracy also met pre-specified requirements in EDTA plasma, and varied from 90.2% - 117.1% for brigatinib, 94.5% - 104.1% for lorlatinib, 90.5% - 99.5% for pralsetinib, and 94.2% - 104.0% for selpercatinib. Since the calibration curve was made in (sodium) citrate plasma, additional precision and accuracy measurements were performed in citrate plasma to compare this to the results in K2-EDTA-plasma. The results are shown in Appendix A1. Note that grossly similar results were obtained when citrate plasma was used instead of EDTA plasma, with only one deviating value (intra-day accuracy for the LLOQ of pralsetinib, 121.5%).

Table 4: intra- and inter-day accuracy and precision of brigatinib, lorlatinib, pralsetinib, and selpercatinib in spiked EDTA-plasma samples.

Nominal concentration (ng/mL)	Mean concentration (n=15) (ng/mL)	Intra-day precision (n=5) (%)	Inter-day precision (n=15) (%)	Intra-day accuracy (n=5) (%)	Inter-day accuracy (n=15) (%)
Brigatinib					
LLOQ (50.0)	52.1	9.6	12.2	117.1	104.2
QC_{LOW} (100.0)	95.3	5.5	5.7	90.2	95.3
QC_{LOW} (1000.0)	949.3	5.8	4.4	93.8	94.9
QC_{HIGH} (1750.0)	1672.2	4.3	3.3	94.4	95.6
HLOQ (2500.0)	2401.2	4.9	4.0	94.2	96.1
Mean	**-**	**6.0**	**5.9**	**97.9**	**97.2**
Lorlatinib					
LLOQ (25.0)	25.5	9.5	7.3	104.1	101.8
QC_{LOW} (50.0)	48.1	5.6	4.7	94.7	96.3
QC_{LOW} (50.0)	384.5	3.4	2.6	94.5	96.1
QC_{HIGH} (700.0)	677.1	3.2	2.4	95.3	96.7
HLOQ (1000.0)	971.6	2.8	2.6	95.9	97.2
Mean	**-**	**4.9**	**3.9**	**96.9**	**97.6**
Pralsetinib					
LLOQ (100.0)	99.3	16.7	13.6	90.7	99.3
QC_{LOW} (200.0)	189.1	7.1	5.3	91.4	94.5
QC_{MED} (4000.0)	3981.4	6.3	4.5	96.8	99.5
QC_{HIGH} (7000.0)	6802.1	7.1	5.1	94.7	97.2
HLOQ (10000.0)	9250.9	8.0	5.5	90.5	92.5
Mean	**-**	**9.0**	**6.8**	**92.8**	**96.6**
Selpercatinib					
LLOQ (50.0)	51.0	12.6	9.9	103.9	102.1
QC_{LOW} (100.0)	96.6	5.1	4.4	94.3	96.6
QC_{MED} (2000.0)	2042.8	4.6	4.0	104.0	102.1
QC_{HIGH} (3500.0)	3470.4	4.5	3.3	98.7	99.2
HLOQ (5000.0)	4736.7	5.6	3.3	94.2	94.7
Mean	**-**	**6.5**	**5.0**	**99.0**	**98.9**

Abbreviations: LLOQ, lower limit of quantification; QC_{LOW}, quality control low-level; QC_{MED}, quality control mid-level; QC_{HIGH}, quality control high-level; ULOQ, upper limit of quantification; n = number of samples; ng/mL = nanogram per millilitre, ND = not determined.

3.2.3 Matrix effect

Matrix effect was evaluated using QC_{LOW} and QC_{HIGH}. For all SMIs the matrix effect was below 5.3%, with one exception, namely the QC_{LOW} of brigatinib (18.0%). The coefficient of variation (CV) was 18.0% (QC_{LOW}) and 3.5% (QC_{HIGH}) for brigatinib, 2.8% (QC_{LOW}) and 1.8% (QC_{HIGH}) for lorlatinib, 3.6% (QC_{LOW}) and 1.2% (QC_{HIGH}) for pralsetinib, and 5.3% (QC_{LOW}) and 2.1% (QC_{HIGH}) for selpercatinib. The results for matrix effect are shown in Table 5.

Table 5: freeze-thaw stability (EDTA plasma), matrix effect (EDTA plasma) and auto-injector stability (EDTA and citrate plasma) of brigatinib, lorlatinib, pralsetinib, and selpercatinib.

Drug (ng/mL)	Freeze-thaw stability EDTA plasma (n=6)	Matrix effect EDTA plasma (n=6)	Auto-injector stability EDTA plasma (n=5)	Auto-injector stability citrate plasma (n=5)
	Accuracy (var) (%)	Accuracy (var) (%)	Accuracy (var) (%)	Accuracy (var) (%)
Brigatinib – LLOQ (50.0)	ND	ND	102.0 (16.7)	87.3 (40.7)
Brigatinib – QC_{LOW} (100.0)	108.7 (5.5)	18.0	91.8 (10.3)	105.1 (7.3)
Brigatinib – QC_{MED} (1000.0)	104.4 (6.3)	ND	97.1 (0.4)	97.9 (6.5)
Brigatinib – QC_{HIGH} (1750.0)	107.0 (3.1)	3.5	103.0 (1.7)	99.2 (4.0)
Brigatinib – HLOQ (2500.0)	ND	ND	99.7 (4.9)	98.9 (2.8)
Lorlatinib – LLOQ (25.0)	ND	ND	101.4 (6.0)	102.2 (5.3)
Lorlatinib – QC_{LOW} (50.0)	100.7 (4.5)	2.8	92.3 (4.2)	96.1 (2.7)
Lorlatinib – QC_{MED} (400.0)	103.9 (1.5)	ND	96.3 (1.3)	95.6 (4.0)
Lorlatinib– QC_{HIGH} (700.0)	104.4 (2.6)	1.8	95.2 (1.8)	96.8 (4.0)
Lorlatinib – HLOQ (1000.0)	ND	ND	95.2 (2.1)	95.4 (2.7)
Pralsetinib – LLOQ (100.0)	ND	ND	101.9 (8.8)	120.1 (13.3)
Pralsetinib – QC_{LOW} (200.0)	125.2 (8.3)	3.6	92.0 (2.3)	101.6 (2.6)
Pralsetinib – QC_{MED} (4000.0)	100.0 (1.5)	ND	96.1 (1.8)	95.2 (1.2)
Pralsetinib – QC_{HIGH} (7000.0)	99.6 (2.0)	1.2	95.4 (3.6)	94.9 (2.6)
Pralsetinib – HLOQ (10000.0)	ND	ND	89.0 (3.9)	91.9 (5.0)
Selpercatinib – LLOQ (50.0)	ND	ND	101.3 (7.4)	105.0 (6.8)
Selpercatinib – QC_{LOW} (100.0)	117.6 (1.6)	5.3	92.0 (3.3)	94.1 (3.6)
Selpercatinib – QC_{MED} (2000.0)	98.4 (1.1)	ND	100.0 (2.3)	94.5 (1.1)
Selpercatinib – QC_{HIGH} (3500.0)	98.3 (3.5)	2.1	97.0 (2.1)	93.5 (1.9)
Selpercatinib – HLOQ (5000.0)	ND	ND	91.9 (3.6)	91.5 (5.0)

Abbreviations: ng/mL = nanogram per milliliter, LLOQ = lower limit of quantification, QC_{LOW} = low quality standard, QC_{MED} = middle quality standard, QC_{HIGH} = high quality standard, HLOQ = higher limit of quantification, n = number of samples, var = variation, ND = not determined. Accuracy was evaluated compared to the nominal value.

3.2.4 Stability

Freeze-thaw stability was adequate for most EDTA QCs with post freeze-thaw concentrations between 98.3% and 109% of the expected concentration. Only the QC_{LOW} of pralsetinib and selpercatinib had a higher variation. These were 125.2% and 117.6%, respectively. Detailed results regarding the freeze-thaw stability are shown in Table 5. The short- and long-term stability in EDTA plasma is shown in Table 6. All four SMIs were stable at freezing temperature (-20 °C) for at least a month, with one exception, as the QC_{LOW} of pralsetinib varied more than 15% from the expected value (124.2%). All SMIs were stable for at least one day when stored at 2 – 8 °C or at RT, with one deviating QC, namely the QC_{LOW} of pralsetinib (121.1%).

Table 6: Stability of brigatinib, lorlatinib, pralsetinib, and selpercatinib in human EDTA plasma at various storage conditions.

Temperature	Drug	Time (days)	Accuracy QC_{LOW} (%)	Accuracy QC_{MED} (%)	Accuracy QC_{HIGH} (%)
-20	Brigatinib	1	103.4	97.1	97.3
-20	Brigatinib	7	94.7	98.5	96.5
-20	Brigatinib	30	107.8	99.3	100.5
-20	Lorlatinib	1	100.2	97.5	97.3
-20	Lorlatinib	7	104.0	98.4	97.8
-20	Lorlatinib	30	96.4	100.1	98.9
-20	Pralsetinib	1	112.6	98.8	96.8
-20	Pralsetinib	7	115.0	103.9	98.9
-20	Pralsetinib	30	124.2	107.0	101.0
-20	Selpercatinib	1	101.1	100.7	99.0
-20	Selpercatinib	7	109.4	106.4	99.3
-20	Selpercatinib	30	108.8	106.1	101.4
5	Brigatinib	1	109.5	95.3	99.8
5	Brigatinib	7	105.7	91.9	94.2
5	Lorlatinib	1	97.5	95.7	94.7
5	Lorlatinib	7	93.6	96.9	98.3
5	Pralsetinib	1	121.1	106.2	100.3
5	Pralsetinib	7	102.2	98.8	99.4
5	Selpercatinib	1	108.3	108.3	101.1
5	Selpercatinib	7	106.5	100.4	100.1
RT	Brigatinib	1	107.3	101.2	99.1
RT	Brigatinib	7	117.0	109.2	102.6
RT	Lorlatinib	1	100.1	98.8	99.2
RT	Lorlatinib	7	109.7	107.9	111.1
RT	Pralsetinib	1	100.7	110.9	109.5
RT	Pralsetinib	7	148.8	117.7	115.6

Table 6: Continued.

Temperature	Drug	Time (days)	Accuracy QC_{LOW} (%)	Accuracy QC_{MED} (%)	Accuracy QC_{HIGH} (%)
RT	Selpercatinib	1	103.0	108.9	106.9
RT	Selpercatinib	7	126.7	118.8	113.6

Abbreviations: RT = room temperature; QC_{LOW} = low quality control; QC_{MED} = middle quality control; QC_{HIGH} = high quality control. Results are mean concentration compared with the nominal value. All concentrations were analysed in duplicate.

The short-term stability in EDTA whole blood is shown in Table 7. Almost all QCs of lorlatinib and pralsetinib were stable for at least 7 days at RT and at 2 – 8 °C, except for the QC_{HIGH} of pralsetinib at 2 – 8 °C. For brigatinib, only the QC_{LOW} was stable for at least 7 days at RT and at 2 – 8 °C. The accuracy of the other QCs of brigatinib varied between 71.2% and 80.3% at 2 – 8 °C and between 77.1% and 82.4% at RT. For selpercatinib the accuracy of the QCs varied between 106.1% and 115.8% after 1 day at 2 – 8 °C and between 105.2% and 111.3% after 7 days at 2 – 8 °C. At RT the accuracy of the QCs varied between 115.9% and 119.6% after 1 day and between 110.3% and 116.7% after 7 days.

Table 7: Stability of brigatinib, lorlatinib, pralsetinib and selpercatinib in human K2-EDTA whole blood at various storage conditions.

Temperature (°C)	Drug	Time (days)	Accuracy QC_{LOW} (%)	Accuracy QC_{MED} (%)	Accuracy QC_{HIGH} (%)
5	Brigatinib	1	103.3	77.4	71.2
5	Brigatinib	7	103.5	80.3	75.8
5	Lorlatinib	1	96.9	95.7	90.1
5	Lorlatinib	7	92.9	95.8	89.5
5	Pralsetinib	1	94.5	85.4	83.1
5	Pralsetinib	7	88.5	87.2	82.6
5	Selpercatinib	1	115.5	106.1	115.8
5	Selpercatinib	7	109.8	105.2	111.3
RT	Brigatinib	1	104.0	79.0	77.1
RT	Brigatinib	7	96.2	81.4	82.4
RT	Lorlatinib	1	95.3	100.1	93.5
RT	Lorlatinib	7	101.6	101.6	95.7
RT	Pralsetinib	1	96.7	94.5	91.2
RT	Pralsetinib	7	105.9	95.4	93.9
RT	Selpercatinib	1	117.3	115.9	119.6
RT	Selpercatinib	7	111.7	110.3	116.7

Abbreviations: RT = room temperature; QC_{LOW} = low quality control; QC_{MED} = middle quality control; QC_{HIGH} = high quality control. Results are mean concentration compared with the nominal value. All concentrations were analysed in duplicate.

The stability of all SMIs in citrate plasma is shown in Appendix A2. In general, similar stability was seen as in EDTA-plasma, but slightly more deviating accuracies were observed when citrate plasma was used, especially for pralsetinib.

3.2.5 Clinical applicability

A similar HPLC-MS/MS method to the current study has previously been validated for a multidrug assay for the ALK- and EGFR-SMIs alectinib, crizotinib, erlotinib, gefitinib and osimertinib [5]. That method is currently used in an observational study to evaluate trough concentrations of these drugs in clinical practice (Maastricht University Medical Center medical ethics approval number 2019-1080). The currently developed testing method for brigatinib, lorlatinib, pralsetinib and selpercatinib has recently been added to this ongoing study in order to be able to also include patients treated with these SMIs. At this moment, patient enrolment is ongoing and K2-EDTA plasma trough concentrations during steady state will be measured and used for future incurred sample reanalysis. In addition to research purposes, this multidrug assay may be used for TDM in clinical practice.

3.3 Comparison with previous studies

For all investigated SMIs, single drug assays have been published in either heparinised mouse plasma, rat or human plasma, except for pralsetinib [15-18]. Furthermore, selpercatinib and lorlatinib have been validated using HPLC-MS/MS, but this was done using rat plasma and mouse serum, respectively [15, 18]. A multidrug assay for brigatinib and lorlatinib in combination with alectinib was validated in human sodium EDTA plasma using UPLC-MS/MS and pralsetinib and selpercatinib were combined in a multidrug assay validated in mouse lithium-heparin plasma using UPLC-MS/MS [12, 13]. A method to simultaneously quantify brigatinib, lorlatinib, pralsetinib and selpercatinib in human EDTA plasma has not been developed before.

Most studies used UPLC-MS/MS, whereas HPLC-MS/MS was used in this validation. In general, UPLC is considered to have a higher sensitivity and could therefore increase the efficiency of separation compared with HPLC. Running time of UPLC used previously for the reported SMIs was approximately 2 to 5 minutes, which is shorter than the running time reported here with HPLC (~9.5 minutes) [12, 13, 15, 18]. However, sample preparation for HPLC is simpler and less time consuming than for UPLC and is therefore easier to use in daily practice.

The combination of multiple SMIs in one analytical method improves the efficiency in the laboratory, as the sample preparation and quantification can be combined for multiple drugs, instead of performing an assay for all individual SMIs. Furthermore, the number of patients with these specific mutations for which the four SMIs in this study can be prescribed, is relatively low. The combination of multiple SMIs will make it possible to run a full assay quicker, which will increase the turn-around time in the laboratory and enables earlier reporting to the treating physician.

In daily practice, patient samples cannot always be processed and analysed immediately. Therefore, stability at various temperatures and time points were evaluated in both K2-EDTA plasma, sodium citrate plasma and EDTA whole blood. Previously, brigatinib and lorlatinib were reported to be stable at RT for 25 hours and at -30°C for 3 – 4 months, which was in line with our results at -20°C [12]. Stable results for brigatinib and lorlatinib at RT for at least 24 hours were noticed, while most QCs were even stable for at least seven days (apart from QC_{LOW} of brigatinib). Furthermore, both brigatinib and lorlatinib were stable for at least 30 days at freezing temperatures (-20°C). In EDTA whole blood, not all SMIs were stable for 24 hours at RT or cooling conditions. Stability after 4 hours at these conditions was not assessed, making it difficult to predict the stability at that time point. Therefore, immediate processing of the whole blood samples is advised. The obtained plasma from these samples can be analysed later, since we did show stability of the plasma samples at freezing conditions. The assessment of the long-term stability after several months will be performed in the future.

The QC_{LOW} of pralsetinib stored at freezing temperature differed more than 15% from the nominal value after 30 days, while all other eight QCs for pralsetinib met the pre-specified requirements at freezing conditions. In the study by Şentürk et al. both the QC_{LOW} and QC_{HIGH} of pralsetinib and selpercatinib were reported to be stable for 24 hours at RT and for 2 months at -30 °C [13]. This was supported by the results reported for selpercatinib in rat plasma [18]. Our results for the stability of pralsetinib are largely comparable with these previously published data, except for QC_{LOW} of pralsetinib. The QC_{LOW} of pralsetinib in our study differed 24.2% of the nominal value at freezing conditions, while Şentürk et al. reported a difference of 2.8% [13] (Table 6). Notably, Şentürk measured the stability after 2 months at -30 °C, while we determined this after 3 months at -20 °C. Analysis of the long-term stability of pralsetinib (and the other SMIs) is needed to further examine this.

CONCLUSION

In this study, an analytical method was developed and validated to simultaneously quantify brigatinib, lorlatinib, pralsetinib and selpercatinib in human K2-EDTA-plasma using HPLC-MS/MS. This method can help guide treatment in clinical practice and give the opportunity to perform clinical studies focusing on pharmacokinetic parameters of any of these four SMIs.

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APPENDIX A

Table A1: intra- and inter-day accuracy and precision of brigatinib, lorlatinib, pralsetinib, and selpercatinib in spiked citrate samples.

Nominal concentration (ng/mL)	Mean concentration (n=15) (ng/mL)	Intra-day precision (n=5) (%)	Inter-day precision (n=15) (%)	Intra-day accuracy (n=5) (%)	Inter-day accuracy (n=15) (%)
Brigatinib					
LLOQ (50.0)	51.2	16.7	15.2	117.8	102.4
QC_{LOW} (100.0)	92.3	8.3	8.5	85.2	92.3
QC_{MED} (1000.0)	924.5	5.8	5.0	91.0	92.5
QC_{HIGH} (1750.0)	1651.5	4.2	3.1	93.6	94.4
HLOQ (2500.0)	2363.2	3.0	2.7	92.9	94.5
Mean	-	**7.6**	**6.9**	**96.1**	**95.2**
Lorlatinib					
LLOQ (25.0)	25.7	5.4	4.5	104.4	102.9
QC_{LOW} (50.0)	48.1	5.9	5.0	93.3	96.3
QC_{MED} (400.0)	384.5	4.3	3.8	95.4	96.1
QC_{HIGH} (700.0)	677.1	3.7	3.1	95.3	96.7
HLOQ (1000.0)	967.9	3.9	2.9	95.8	96.8
Mean	-	**4.6**	**3.9**	**96.8**	**97.8**
Pralsetinib					
LLOQ (100.0)	111.3	15.0	14.4	121.5	111.3
QC_{LOW} (200.0)	195.8	7.2	5.8	93.9	97.9
QC_{MED} (4000.0)	3850.8	2.4	3.4	93.2	96.3
QC_{HIGH} (7000.0)	6566.7	3.7	4.3	90.3	93.8
HLOQ (10000.0)	9056.2	4.5	3.5	89.7	90.6
Mean	-	**6.6**	**6.3**	**97.7**	**98.0**
Selpercatinib					
LLOQ (50.0)	50.9	7.7	8.7	109.8	101.8
QC_{LOW} (100.0)	94.1	4.4	5.9	90.8	94.1
QC_{MED} (2000.0)	1920.2	3.4	4.0	94.0	96.0
QC_{HIGH} (3500.0)	3270.3	4.2	4.4	90.4	93.4
HLOQ (5000.0)	4510.8	5.0	4.4	87.9	90.2
Mean	-	**4.9**	**5.5**	**94.6**	**95.1**

Abbreviations: LLOQ = lower limit of quantification; QC_{LOW} = quality control low-level; QC_{MED} = quality control mid-level; QC_{HIGH} = quality control high-level; ULOQ = upper limit of quantification; n = number of samples; ng/mL = nanogram per millilitre, ND = not determined.

Table A2: stability of brigatinib, lorlatinib, pralsetinib, and selpercatinib in human citrate plasma at various storage conditions.

Temperature	Drug	Time (days)	Accuracy QC_{LOW} (%)	Accuracy QC_{MED} (%)	Accuracy QC_{HIGH} (%)
-20	Brigatinib	1	100.8	92.5	95.0
-20	Brigatinib	7	106.6	93.8	97.4
-20	Brigatinib	30	111.9	92.9	98.2
-20	Lorlatinib	1	97.6	93.5	94.6
-20	Lorlatinib	7	99.8	97.3	96.5
-20	Lorlatinib	30	105.6	89.1	99.2
-20	Pralsetinib	1	125.9	100.7	98.6
-20	Pralsetinib	7	125.1	100.1	102.4
-20	Pralsetinib	30	116.5	98.8	99.4
-20	Selpercatinib	1	107.2	101.7	99.9
-20	Selpercatinib	7	104.5	101.5	103.4
-20	Selpercatinib	30	103.3	98.3	99.5
5	Brigatinib	1	106.8	92.0	98.2
5	Brigatinib	7	98.7	95.2	100.9
5	Lorlatinib	1	104.3	93.2	100.0
5	Lorlatinib	7	102.2	96.2	101.5
5	Pralsetinib	1	112.6	103.8	102.0
5	Pralsetinib	7	127.6	103.8	99.5
5	Selpercatinib	1	104.3	104.7	104.4
5	Selpercatinib	7	106.9	106.5	101.7
RT	Brigatinib	1	104.5	96.0	97.7
RT	Brigatinib	7	120.4	106.3	106.1
RT	Lorlatinib	1	101.3	93.9	98.9
RT	Lorlatinib	7	107.3	109.0	109.0
RT	Pralsetinib	1	132.6	106.2	103.0
RT	Pralsetinib	7	136.1	113.3	111.5
RT	Selpercatinib	1	112.6	108.6	104.8
RT	Selpercatinib	7	117.7	112.7	110.9

Abbreviations: RT = room temperature; QC_{LOW} = low quality control; QC_{MED} = middle quality control; QC_{HIGH} = high quality control. Results are mean concentration compared with the nominal value. All concentrations were analysed in duplicate.

3

Chapter 3

OBSERVATIONAL STUDIES USING ELECTRONIC HEALTH RECORDS DATA EVALUATING TREATMENT OUTCOMES WITH OSIMERTINIB

Chapter 3.1

Exploring the impact of patient clinical features on osimertinib effectiveness in a real-world cohort of patients with EGFR mutated non-small cell lung cancer.

A. van Veelen, G.D.M. Veerman, M.V. Verschueren, J. Gulikers, A.J.W.M. Brouns, S. Dursun, V.C.G. Tjan – Heijnen, A.C. Dingemans, R.H.J. Mathijssen, E.M.W. van de Garde, P.C. Souverein, J.H.M. Driessen, L.E.L. Hendriks, R.M.J.M. van Geel, S. Croes.

Submitted

ABSTRACT

Introduction: Osimertinib is prescribed to patients with metastatic non-small cell lung cancer (NSCLC) and a sensitizing *EGFR* mutation. Limited data exists on the impact of patient characteristics or osimertinib exposure on effectiveness outcomes.

Methods: This was a Dutch, multicentre cohort study. Eligible patients were ≥18 years, with metastatic *EGFR*m+ NSCLC, receiving osimertinib. Primary endpoint was progression-free survival (PFS). Secondary endpoints included overall survival (OS) and safety. Kaplan-Meier analyses and multivariate Cox proportional hazard models were performed.

Results: In total, 294 patients were included. Primary *EGFR*-mutations were mainly exon 19 deletions (54%) and p.L858R point mutations (30%). Osimertinib was given in first-line (40%), second-line (46%) or beyond (14%), with median PFS 14.4 (95%CI 9.4–19.3), 13.9 (95%CI 11.3–16.1) and 8.7 months (95%CI 4.6–12.7), respectively. Patients with low BMI (<20.0 kg/m^2) had significantly shorter PFS/OS compared to all other subgroups. Patients with a high plasma trough concentration in steady state ($C_{min,SS}$; >271 ng/mL) had shorter PFS compared to a low $C_{min,SS}$ (<163 ng/mL) (aHR 2.29; 95%CI 1.13 – 4.63). A significant longer PFS was seen in females (aHR = 0.61, 95%CI 0.45 – 0.82) and patients with the exon 19 deletion (aHR = 0.58, 95%CI 0.36 – 0.92). A trend towards longer PFS was seen for *TP53* wild-type patients, while age did not impact PFS.

Conclusion: Patients with a primary *EGFR* exon 19 deletion had longer PFS, while a low BMI, male sex, and a high $C_{min,SS}$ were indicative for shorter PFS and/or OS. Age was not associated with effectiveness outcomes of osimertinib.

INTRODUCTION

For patients with non-small cell lung cancer (NSCLC) and a sensitizing epidermal growth factor receptor mutation (*EGFR*m+), several tyrosine kinase inhibitors (TKIs) have been approved resulting in considerably improved treatment outcomes [1]. Osimertinib is a third generation EGFR-TKI approved for the treatment of *EGFR*m+ NSCLC. In the metastatic setting, it has been approved in the first line or upon progression on first/second generation EGFR-TKI, if a patient developed the *EGFR* p.T790M-mutation. Recently, osimertinib has been approved in the adjuvant setting for patients with completely resected *EGFR*m+ stage IB-IIIA NSCLC [2 - 5]. Osimertinib is given as a flat dose of 80 milligram (mg) once daily (QD), irrespective of patient characteristics or individual drug exposure (indirectly measured by steady state plasma trough level ($C_{min,SS}$)).

The characteristics of patients treated in clinical practice often differ from patients included in clinical trials [6]. This may cause worse treatment outcomes, previously described as the efficiency – effectiveness gap [7]. Clinical trial data alone, often do not accurately reflect the effectiveness of a drug in the real-world setting, due to strict inclusion and exclusion criteria. Therefore, the effectiveness of osimertinib in the real world has been evaluated in multiple retrospective studies, in the first line treatment [8 - 14], second line treatment or beyond [15 - 27]. As first line studies were mainly performed in Asian patients, and 62% of all patients in the FLAURA-trial [3, 8, 10 - 14] were Asian, there is a lack of outcome data in Caucasian patients. The effect of some patient characteristics, such as primary *EGFR*-mutation or *TP53*-status, have been described before [22, 28]. However, for various other patient and treatment characteristics, such as age, body mass index (BMI) and plasma trough concentration ($C_{min,SS}$), limited information on their effect on osimertinib outcomes has been described [10, 29 - 32] while they have shown to significantly impact the effectiveness of other anti-cancer treatments [33, 34].

Therefore, in this study we aim to explore the impact of patient-specific clinical features on osimertinib treatment outcomes in a real-world setting, focusing on age, BMI and osimertinib $C_{min,SS,}$ in primarily Caucasian patients.

METHODS

Study design and patients

This study was performed in four centres in the Netherlands: two academic (Maastricht University Medical Centre and Erasmus Medical Centre) and two large teaching centres (St Antonius and Amphia hospital). All patients treated with osimertinib in regular care between 01-02-2016 and 01-03-2022 were selected. In addition, eligibility criteria were age 18 years or older, a diagnosis of advanced or metastatic *EGFR*m+ NSCLC and at least one response assessment after the start of osimertinib. The first prescription of osimertinib

determined the index date, and patients were followed until they die, were lost to follow-up, or reached the end of study (01-03-2022).

Data collection

Data on the use of osimertinib was extracted from the pharmacy information systems of the participating hospitals or patients were identified through participation in a clinical study (START-TKI, NCT05221372). Clinical data at index date (defined as start of osimertinib treatment) was retrieved from the electronic medical records and included demographic information, smoking status, disease characteristics (including location of metastases, and localisation in the central nervous system (CNS), grade (locally advanced or metastatic), type of primary *EGFR*-mutation, *TP53*-status), co-medication and prior received treatments. The *EGFR*-mutation was evaluated before the start of osimertinib treatment for patients that received osimertinib in the first line and re-evaluated for patients that received osimertinib in the second-line or later, after progression on a first- or second-generation EGFR-TKI. In the patients that received osimertinib in a later line, *EGFR*-mutation analysis was performed to evaluate for the presence of the T790M-mutation, which is required to receive reimbursement for osimertinib in the Netherlands. All evaluation CT scans were retrospectively evaluated and scored using the Response Evaluation Criteria in Solid Tumours (RECIST), version 1.1, by an experienced radiologist and/or pulmonologist (GV, AB, SD) [35]. Response evaluations were performed every 8 – 12 weeks with at least a chest CT. CNS involvement was evaluated in case of symptomatic presentation or on routinely performed scans. CNS involvement was scored as yes (CNS metastasis on MRI or CT scan), no (no CNS metastasis on MRI or CT scan) or unknown (no MRI or CT brain scan available). The quantification of osimertinib in plasma was done for research purposes. Plasma concentrations for osimertinib were included for analysis in this study if a) the patient did not receive a dose-reduction or –interruption of osimertinib to ensure the consistent use of 80 mg daily osimertinib over the whole treatment period, b) data regarding the exact moment of blood withdrawal and accurate time frame of osimertinib intake was available, c) blood withdrawal was performed at least 15 days after the start of osimertinib treatment, to ensure steady state concentrations, d) blood withdrawal was performed at least six hours after the last intake of osimertinib and e) the withdrawal took place at least three months prior to progression, as an increase of plasma trough concentration was seen shortly before, around and after progression which could bias the osimertinib plasma level (Figure A4). During the first six hours after osimertinib intake, osimertinib is absorbed from the gastrointestinal tract, and the maximum plasma concentration (C_{max}) has not been reached. After achieving the C_{max}, osimertinib is primarily eliminated, and the plasma concentration could be extrapolated to the $C_{min,SS}$ using the method described by Wang *et al*. [36].

Outcomes

The primary efficacy endpoint was progression free survival (PFS), which was defined as the time in months since the index date until the occurrence of progression of disease, according to RECIST v1.1 [35], or all-cause death. Patients were censored if the patient was lost-to-follow-up or the end of study was reached. Secondary outcomes were overall survival (time since index date until death, OS), best overall response, objective response rate (ORR), disease control rate (DCR), and safety. For safety, all adverse events that led to a hospital admission, dose reduction, interruption, or definitive stop of osimertinib were collected. Interruption of osimertinib treatment was defined as a stop of at least one week. ORR and DCR was scored for intracranial and extracranial response. The extracranial response was scored for all patients, while intracranial response was scored for all patients with a CNS metastasis at the start of osimertinib treatment and the possibility to select a CNS metastasis as lesion according to RECIST v1.1.

Statistical analysis

Patient demographics, disease specific information, other baseline characteristics and safety data were summarized using descriptive statistics. The Kaplan-Meier method was used to calculate the median PFS (mPFS) and OS (mOS) of the overall patient population. Furthermore, treatment outcomes were evaluated for specific subgroups (age, BMI, $C_{min,SS}$, treatment line, primary *EGFR*-mutation, and *TP53*-status at index date). The following subgroups were used in the Kaplan-Meier analyses and Cox proportional hazards models: age - <65 years, 65 – 69 years, 70 – 74 years and ≥75 years, furthermore <70 years vs. ≥ 70 years; BMI - <20.0 kg/m^2, 20.0 – 24.9 kg/m^2, 25.0 – 29.9 kg/m^2 and ≥ 30.0 kg/m^2; and for plasma trough concentration - <163 ng/mL, 163 – 271 ng/mL and >271 ng/mL. The subgroups for age and BMI were selected based on classifications commonly used in clinical research, e.g., subgroups of 5-unit points (years or kg/m^2). The classification for plasma trough concentration was selected based on the 25th and 75th percentile calculated from all plasma trough concentrations that were included in the analysis. Additionally, the plasma trough concentration was analysed as continuous variable, instead of a nominal value. This was not done for age and BMI, as no (inversely) proportional linear relation between parameter and outcome was expected or hypothesized. Multivariate Cox proportional hazards models were used to calculate hazard ratios (adjusted - aHR) for progression and all-cause mortality. HRs were adjusted for age, sex, primary *EGFR*-mutation, *TP53*-status, BMI, $C_{min,SS}$ and line of treatment, as those were known to have an impact on osimertinib treatment outcomes or were of special interest in our study. For the HRs: the lowest subgroup (age, BMI or $C_{min,SS}$) was used as reference group. As sensitivity analysis the cohort of patients was limited to only first-line users. All statistical analyses were performed using SAS 9.4 (SAS Institute).

RESULTS

Data from 294 real-world osimertinib users was available, which were all included in this study. An overview of all baseline characteristics and per treatment line, is shown in Table 1. In short, 118 (40%), 134 (46%), and 42 (14%) patients were treated in first, second and third line or beyond, respectively. Median age was 67 years (range: 27 – 89), median BMI was 24.6 (range: 17.6 – 67.1). 92.9% of all patients were former or never smoker and 89.8% were Caucasian. Exon 19 deletions (53.7%) and the p.L858R point mutations (29.6%) were the most frequent activating primary *EGFR*-mutations, while 26.2% had a definitive registration of a CNS metastasis (first line – 33.1%; second line – 23.1%, and third line or beyond - 66.7%). No patients used a strong cytochrome P450 3A4 (CYP3A4) inhibitor or inducer during osimertinib treatment. Median follow-up time for the full cohort was 21.5 months (range: 0.2 – 65.5 months). The median follow-up time was shorter for patients who used osimertinib as first-line treatment (11.7 months; range: 0.2 – 43.7 months), compared to patients who were treated with osimertinib in the second line (28.8 months; range: 0.7 – 65.5 months) or the third line or later (30.0 months; range: 1.6 – 40.6 months).

Sex, primary *EGFR*-mutation, and TP-53 status

Characteristics that are known to be associated with treatment outcomes of osimertinib, were also indicative of treatment outcomes in our cohort. Female patients had a lower risk of progression as compared to men on osimertinib (aHR = 0.61, 95% CI 0.45 – 0.82). This was also found for those with an exon 19 deletion as primary *EGFR*-mutation (compared to the group of patients with other *EGFR*-mutations): aHR = 0.58 (95% CI 0.36 – 0.92). Furthermore, patients with a *TP53*-mutation at baseline had a trend for a higher risk for a shorter PFS on osimertinib: aHR = 1.31 (95% CI 0.96 – 1.78).

Table 1: baseline characteristics of all patients (total) and stratified per treatment line.

	Total (N = 294)		**1L (N = 118)**		**2L (N = 134)**		**3L+ (N = 42)**	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Age (years)	66.6		66.9		67.0		64.0	
Sex (female)	193	65.6	73	61.9	94	70.1	26	31.9
Smoking								
Never	120	40.8	48	40.7	59	44.0	13	31.0
Former	153	52.0	60	50.8	67	50.0	26	61.9
Current	16	5.4	10	8.5	4	3.0	2	4.8
Unknown	5	1.7	-	-	4	3.0	1	2.4

Table 1: Continued.

	Total (N = 294)		1L (N = 118)		2L (N = 134)		3L+ (N = 42)	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Race								
Caucasian	264	89.8	104	88.1	119	88.8	41	97.6
African American	5	1.7	4	3.4	1	0.7	-	-
Asian	21	7.1	10	8.5	11	8.2	-	-
Hispanic	1	0.3	-	-	-	-	1	2.4
Other/Unknown	3	1.0	-	-	3	2.2	-	-
CNS metastases								
Yes	77	26.2	39	33.1	31	23.1	28	66.7
No	108	36.7	28	23.7	52	38.8	7	16.7
Unknown	109	37.1	51	43.2	51	38.1	7	16.7
Primary *EGFR*m								
Exon 19 deletion (1)	158	53.7	67	56.8	72	53.7	19	45.2
L858R (2)	87	29.6	24	20.3	47	35.1	16	38.1
1 or 2 + second mutation	35	11.9	20	16.9	9	6.7	6	14.3
Other	14	4.8	7	5.9	6	4.5	1	2.4
TP53-status								
Positive	134	45.7	60	50.8	55	41.4	19	45.2
Negative	138	47.1	44	37.3	73	54.9	21	50.0
Unknown	22	7.2	14	11.9	6	3.8	2	4.8
Age (years)								
<65	114	38.8	44	37.3	53	39.6	17	40.5
65 – 69	56	19.0	23	19.5	25	18.7	8	19.0
70 – 74	51	17.3	21	17.8	20	14.9	10	23.8
≥75	73	24.8	30	25.4	36	26.9	7	16.7
BMI (kg/m^2)								
<20.0	24	8.2	10	8.5	12	9.0	2	4.8
20.0 – 24.9	136	46.3	61	51.7	58	43.3	17	40.5
25.0 – 29.9	85	28.9	34	28.8	40	29.9	11	26.2
≥ 30.0	37	12.6	12	10.2	18	13.4	7	16.7
Missing	12	4.1	1	0.8	6	4.5	5	11.9

Abbreviations: N = number, 1L = first line treatment, 2L = second line treatment, 3L+ = third line treatment or beyond, % = percentage, CNS = central nervous system, EGFRm = epidermal growth factor receptor mutation, BMI = body mass index, kg = kilogram, m = meter

Outcome per treatment line

The mPFS were 14.4 months (95% CI = 9.4 – 19.3 months, first-line), 13.9 months (95% CI = 11.3– 16.1 months, second line) and 8.7 months (95% CI = 4.6 – 12.7 months, third line or beyond). The mOS since the start of osimertinib were 34.5 months (first line; 95% CI = 34.5 – NR), 28.0 months (second line; 95% CI = 23.6 – 39.1 months) and 18.9 months (third line; 95% CI = 13.6 – 25.1 months). Detailed results for the cohort of first line users are summarized in supplemental Table A1.

Outcome by age

Detailed baseline characteristics stratified by age group are listed in supplemental Table A2. Irrespective of treatment line, mPFS according to age groups was 11.5 months (<65 years; 95% CI = 8.2 – 13.9 months), 18.0 months (65 – 69 years; 95% CI = 13.5 – 21.4), 10.5 months (70 – 74 years; 95% CI = 5.9 – 19.1 months) and 13.1 months (≥75 years; 95% CI = 9.8 – 17.1 months). Compared to the youngest group there were no statistical differences in aHR, as can be seen in Table 2 and Figure A1A. The mOS was similar for three age groups: <65 years: 25.3 months (95% CI = 18.7 – 34.5), 70 – 74 years: 23.6 months (95% CI = 14.8 – 41.4 months) and ≥75 years: 25.5 months (95% CI = 20.4 – 30.9 months) but was increased in patients who were 65 – 69 years at the start of osimertinib: 42.3 months (95% CI = 26.2 – NR) (Table 3 and Figure A1B). For OS, patients between 65 years and 70 years at the start of osimertinib had a longer OS than patients that were younger than 65 at the start of osimertinib treatment (aHR = 0.52; 95% CI 0.29 – 0.92).

Outcome by BMI

Detailed baseline characteristics stratified by BMI subgroup are shown in Table A3. Irrespective of treatment line, mPFS was relatively short in the patients with a low BMI (8.1 months; 95% CI = 3.3 – 14.3 months) compared to the other three subgroups. The risk for progression was significant lower in two subgroups (20.0 – 24.9 kg/m^2 – aHR = 0.55, 95% CI 0.33 – 0.93 and 25.0 – 29.9 kg/m^2 – aHR = 0.40, 95% CI 0.23 – 0.71) compared to the lowest BMI subgroup (≤20.0 kg/m^2), while a trend for reduced risk of progression was seen for the highest BMI subgroup (≥30.0 kg/m^2, aHR = 0.57, 95% CI 0.31 – 1.06) (Table 2 and Figure A2A). All BMI subgroups showed a reduced risk of mortality (mOS) as compared to BMI <20.0 kg/m^2; aHR = 20.0 – 24.9 kg/m^2 – 0.45, 95% CI 0.23 – 0.87; 25.0 – 29.9 kg/m^2 – 0.41, 95% CI 0.21 – 0.82; ≥30.0 kg/m^2 – 0.38, 95% CI 0.17 – 0.86) (Table 3 and Figure A2B).

Outcome by $C_{min,SS}$

All patients with a dose reduction or interruption (due to toxicity) were excluded from the $C_{min,SS}$ analyses (n = 45). In patients for whom multiple $C_{min,SS}$ values were available over time, we observed that the $C_{min,SS}$ increased three months before, at and after progression (Figure A4). As these measurements could bias the osimertinib plasma level interpretation, determination of the mean $C_{min,SS}$ for each patient was done based on the available $C_{min,SS}$ measurements up to three months prior to first ever recorded radiological progression. If more than one measurement was available within the allowed sampling time frame, the

average $C_{min,SS}$ was used. Figure 1 shows the flowchart for the information regarding the $C_{min,SS}$ of all patients. Detailed baseline characteristics specified per $C_{min,SS}$ subgroup are shown in Table A4. In total, 25 patients (25.0%) had a low $C_{min,SS}$ (<163 ng/mL), 50 patients (50.0%) were in the middle group, and 25 patients (25.0%) had a high $C_{min,SS}$ (> 271 ng/mL). In patients with a high $C_{min,SS}$, mPFS was shortest, 8.8 months (95% CI 5.9 – 10.2 months), which was significantly worse compared to the group of patients with a low $C_{min,SS}$ (aHR = 2.29, 95% CI 1.13 – 4.63) (Table 2 and Figure A3A). A similar trend was seen for mOS, although no significant difference was found (aHR – 1.95, 95% CI 0.83 – 4.61, compared to patients with a low $C_{min,SS}$ (Table 3 and Figure A3B). Additionally, the results of $C_{min,SS}$ as continuous variable are shown in Table A5.

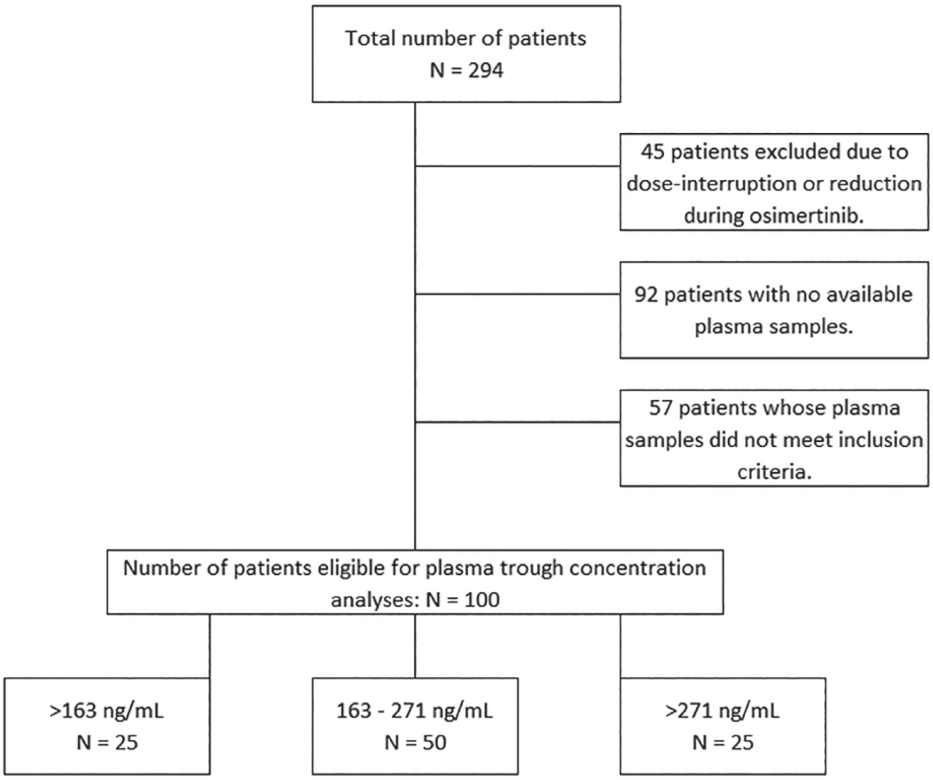

Figure 1: flowchart describing eligible patients for the plasma trough concentration evaluations.

Table 2: Adjusted hazard ratios for progression in patients treated with osimertinib in clinical practice, specified by age, BMI, and the plasma trough concentration.

Age (years)	Number of events	mPFS (months)	95% CI (months)		HR	95% CI		aHR	95% CI	
<65	79	11.5	8.2	13.9	ref	ref	ref	ref	ref	ref
65 – 69	33	18.0	13.5	21.4	0.70	0.46	1.05	0.68	0.45	1.03
70 – 74	36	10.5	5.9	19.1	1.08	0.73	1.61	0.97	0.64	1.48
≥75	44	13.1	9.8	17.1	0.92	0.63	1.34	0.80	0.53	1.19
BMI (kg/m²)										
<20.0	18	8.1	3.3	14.3	ref	ref	ref	ref	ref	ref
20.0 – 24.9	89	13.9	9.9	18.0	0.71	0.43	1.19	**0.55**	**0.33**	**0.93**
25.0 – 29.9	46	15.6	11.5	19.3	**0.54**	**0.31**	**0.93**	**0.40**	**0.23**	**0.71**
≥30.0	29	11.9	6.9	18.4	0.78	0.43	1.42	0.57	0.31	1.06
Unknown	10	8.2	2.8	17.8	0.92	0.40	2.08	0.66	0.28	1.57
Plasma trough concentration (ng/mL)										
<163	13	15.4	7.9	23.0	ref	ref	ref	ref	ref	ref
163 - 271	29	11.6	7.7	18.0	1.27	0.66	2.45	1.38	0.71	2.66
>271	22	8.8	5.9	10.2	1.92	0.96	3.83	**2.29**	**1.13**	**4.63**
Unknown	101	12.4	8.5	14.5	1.29	0.72	2.32	1.37	0.76	2.48

mPFS = median progression free survival, aHR = adjusted hazard ratio, 95% CI = 95% confidence interval, mOS = median overall survival, BMI = body mass index, kg = kilogram, m = metre, ng = nanogram, mL = millilitre. Cox proportional hazard model: adjusted for primary EGFR-mutation, TP53 status, line of treatment, sex, age, body mass index and plasma trough concentration.
aHRs and 95% CI in bold indicates a statistically significant different compared to the reference.

Table 3: Adjusted hazard ratios for mortality in patients treated with osimertinib in clinical practice, specified by age, BMI, and the plasma trough concentration.

Age (years)	Number of events	mOS (months)	95% CI (months)		HR	95% CI		aHR	95% CI	
<65	51	25.3	18.7	34.5	ref	ref	ref	ref	ref	ref
65 – 69	16	42.3	26.2	NR	**0.57**	**0.32**	**0.99**	**0.52**	**0.29**	**0.92**
70 – 74	22	23.6	14.8	41.4	1.15	0.69	1.89	0.91	0.53	1.57
≥75	29	25.5	20.4	30.9	1.04	0.66	1.66	0.95	0.58	1.55
BMI (kg/m²)										
<20.0	12	14.8	4.6	NR	ref	ref	ref	ref	ref	ref
20.0 – 24.9	52	28.4	21.8	42.3	0.58	0.31	1.09	**0.45**	**0.23**	**0.87**
25.0 – 29.9	31	26.2	21.9	38.2	0.55	0.28	1.07	**0.41**	**0.21**	**0.82**
≥30.0	15	23.6	19.8	NR	0.56	0.26	1.20	**0.38**	**0.17**	**0.86**
Unknown	8	10.9	5.6	NR	1.17	0.47	2.96	0.88	0.34	2.29
Plasma trough concentration (ng/mL)										
<163	8	28.9	15.4	NR	ref	ref	ref	ref	ref	ref
163 - 271	16	28.0	18.5	NR	1.20	0.51	2.82	1.13	0.56	3.11
>271	14	21.2	12.7	NR	1.94	0.81	4.64	1.82	0.75	4.42
Unknown	68	25.3	18.7	36.9	1.62	0.77	3.38	1.68	0.79	3.56

mPFS = median progression free survival, aHR = adjusted hazard ratio, 95% CI = 95% confidence interval, mOS = median overall survival, BMI = body mass index, kg = kilogram, m = metre, ng = nanogram, mL = millilitre. Cox proportional hazard model: adjusted for primary EGFR-mutation, TP53 status, line of treatment, sex, age, body mass index and plasma trough concentration.
aHRs and 95% CI in bold indicates a statistically significant different compared to the reference.

Severe adverse events

In total, 51 unique patients (17.3%) experienced a grade 3 adverse event that led to hospitalization, an interruption, a dose-reduction, or a definitive stop of osimertinib. Safety issues resulted in an interruption of osimertinib in 34 patients (11.6%), led to a dose reduction in 36 patients (12.2%), caused hospitalization of six patients (2.0%), and provoked a definitive stop of osimertinib in nine patients (3.1%). The most frequent reasons were increased laboratory values (mainly deviating liver enzymes), skin toxicity, and pneumonitis (Table 4).

Table 4: adverse events of osimertinib responsible for hospitalization, dose reductions, treatment discontinuation or definitive stop of osimertinib treatment.

	Hospitalization (N = 6)		Interruption (N = 32)		Dose-reduction (N = 36)		Stop (N = 9)	
	N =	%	N =	%	N =	%	N =	%
Cardiomyopathy	-	-	-	-	-	-	1	11.1
Diarrhoea	2	33.3	16	47.1	13	36.1	-	-
Deviant laboratory value	-	-	3	8.8	2	5.6	-	-
Fatigue	-	-	4	11.8	3	8.3	-	-
Nausea	-	-	4	11.8	4	11.1	1	11.1
Overall deterioration	-	-	1	2.9	4	11.1	-	-
Pain	-	-	-	-	1	2.8	1	11.1
Palpitations	-	-	1	2.9	-	-	1	11.1
Paronychia	-	-	4	11.8	6	16.7	2	22.2
Pneumonitis	4	66.7	4	11.8	2	5.6	4	44.4
Pruritus	-	-	1	2.9	1	2.8	-	-
QTc-prolongation	-	-	-	-	1	2.8	-	-
Skin toxicity	-	-	6	17.6	6	16.7	-	-
Thrombocytopenia	-	-	-	-	1	2.8	-	-

Abbreviations: N = number, QTc = QT-interval, % = percentage. One patient could potentially experience multiple adverse events at the same time.

DISCUSSION

In this Dutch multicentre cohort study the treatment outcomes of 294 patients with metastatic *EGFRm+* NSCLC that were treated with osimertinib were assessed. We found that age was not associated with mPFS or mOS, while a low BMI (<20 kg/m^2) and a high $C_{min,SS}$ (>271.0 ng/mL) were associated with a higher risk of shorter PFS (both) and OS (BMI). Additionally, no new safety issues were identified, compared to reports from previously performed randomized controlled trials (RCTs) and/or real-world data studies. Factors that were already known to be associated with effectiveness outcomes of osimertinib, such as primary *EGFR*-mutation (exon 19 deletion) and female sex, were also found to significantly increase mPFS with osimertinib in our cohort, and a trend was seen for *TP53* wild-type patients. While this agrees with previous research [22, 28], data regarding the *TP53* status was not available for all patients, which limits the number of patients with a mutation status that could be included in the analysis to evaluate the impact of the *TP53* status on effectiveness outcomes of osimertinib.

Compared to the mPFS of 18.9 months (95% CI = 15.2 – 21.4) in the FLAURA study, the mPFS of first line osimertinib users in our study was shorter (14.4 months, 95% CI = 9.4 – 19.3) [3]. This difference could be caused by a higher proportion of patients in our study that had CNS involvement (33% vs. 19%) or is due to the inclusion of real-life patients with uncommon *EGFR* mutations (other than solely exon 19 deletions or the p.L858R point mutation (Table A6; 22.8% vs. 0.0%). Meanwhile, the mPFS of osimertinib in the second line was shorter in the AURA3 study compared to our study (10.1 months [95% CI = 8.3 – 12.3] vs. 13.9 months [95% CI = 11.3 – 16.1) [2]. This could potentially be explained by the larger proportion of female patients in our study (70% vs. 62%). However, other factors, such as broader inclusion of patients with uncommon primary *EGFR* mutations and patients with CNS metastasis in our study would hypothetically reduce osimertinib treatment outcomes in second-line users. Given these issues, the observed difference in mPFS requires further clarification and could be subject for future studies, while a potential explanation for the observed difference could be the higher frequency of radiological imaging, which was performed more strictly (every six weeks) in the AURA3 trial compared to our study. A detailed overview of the results of our study, compared to the large clinical trials, as well as other large observational series, is shown in Table A6.

Similar to Yamamoto *et al*. (N = 132) [10], we observed that elderly patients derive benefit from osimertinib. Furthermore, this was also seen in a smaller French study (N = 43), evaluating the effectiveness of osimertinib in second line or later [32]. However, both studies included elderly (>75 years / ≥80 years, respectively) only, while we compared osimertinib treatment outcomes in different age groups. The mPFS was numerically better in the study by Yamomoto *et al.* compared with our study (19.4 months [10] vs. 14.4 months, 95% CI = 9.4 – 19.3 months) for all first-line users. Contrary to our study, they mainly included Asian patients, while our population was mostly Caucasian (90%). This difference

could potentially influence mPFS, as better absolute mPFS with osimertinib was seen in Caucasian patients in the FLAURA study [3]. However, this did not translate into a similar trend in clinical practice as the opposite was true when comparing data published by Yamamoto with our study (Table A7).

The number of overweight and obese patients is rising worldwide, and consequently the average BMI increases [37]. BMI has shown to be associated with shorter OS in patients with NSCLC (both underweight and morbid obese patients) as well as OS with immunotherapy (longer OS in patients with baseline BMI ≥30 kg/m^2) [33, 38]. We found that a low BMI (<20.0 kg/m^2) was associated with shorter mPFS and mOS. A potential explanation for the lower effectiveness outcomes of osimertinib in patients with low BMI could be the occurrence of cachexia, which is characterized by substantial weight loss, primarily related to loss of skeletal muscle mass and body fat but is also associated with worse survival outcomes [39, 40]. Unfortunately, we were unable to incorporate an indirect measure of cachexia in our analysis. Furthermore, the decrease in effectiveness outcomes in de low BMI subgroup could also be caused by the general effect on mortality that was seen in patients with a low BMI [41]. Patients with a low BMI have a higher probability for all-cause mortality, independent from other factors, such as comorbidity or mental health. This could potentially be caused by a higher risk of infection among elderly patients with a low BMI [42], which is in concordance with the population that was included in our study, as more than 65% of the patients in the low BMI subgroup was older than 70 years. The influence of BMI on treatment outcomes with osimertinib had previously been evaluated in a small retrospective study by Ono *et al* (n=47), using a cut-off of 21.5 kg/m^2 as threshold for low and high BMI and no difference was found between the two groups [29]. In our study, patients were divided into different BMI subgroups based on a classification that is used more routinely in clinical research. Furthermore, our study included considerably more patients (N=294, of whom 282 had a known BMI) (Table A7).

A low $C_{min,SS}$ (<163 ng/mL) seemed to be indicative of better osimertinib treatment outcomes, as mPFS in this subgroup was significantly better compared to patients with a high $C_{min,SS}$ (>271 ng/mL), but not compared to patients with a $C_{min,SS}$ between 163 and 271 ng/mL. A similar relation has recently been reported by Boosman *et al.* [30] and by Rodier *et al.* [31]. In the study by Boosman, patients with a $C_{min,SS}$ below 166 ng/mL were compared to patients with a $C_{min,SS}$ above 166 ng/mL. The threshold of 166 ng/mL in the study of Boosman *et al.* was selected based on the geometric mean as reported by the Food and Drug Administration (FDA) and is based on results from the AURA studies. However, the median $C_{min,SS}$ found by Boosman *et al.* was 211 ng/mL, and the median and mean $C_{min,SS}$ in our data were 216 ng/mL and 238 ng/mL, respectively. Therefore, we believe that the actual mean $C_{min,SS}$ is higher than originally reported by the FDA. This difference may be caused by limited osimertinib stability in plasma at room temperature, making adequate sample handling crucial and prone to deviations [43, 44]. Furthermore, interracial differences in CYP3A genotype and/or phenotype may potentially contribute

to the observed variation, as 90% of all patients in our study were Caucasian, while this was 32% in the AURA3 trial [2, 45 – 47]. Nevertheless, also Boosman *et al*. reported that patients with a $C_{min,SS}$ below 166 ng/mL had longer mPFS than patients with a $C_{min,SS}$ above 166 ng/mL, but this did not lead to statistical significance in the multivariate analysis. In the study by Rodier *et al*, a similar association between $C_{min,SS}$ and osimertinib effectiveness was found compared to our study. Patients with a high $C_{min,SS}$ (fourth quartile, >235 ng/mL) had a significant shorter mOS (Table A7). Similar to the analysis of Rodier and colleagues, we divided $C_{min,SS}$ values into quartiles and used the 25th and 75th percentile as threshold values for low and high exposure, respectively. We decided to compare multiple subgroups (low, middle, and high) as we were interested in evaluating the effect of the $C_{min,SS}$ over the whole range of $C_{min,SS}$ that was measured in our cohort, instead of using one previously defined hypothetical threshold value, as was done in both the study by Rodier *et al*., and the study by Boosman *et al*. $C_{min,SS}$ values were corrected for time of blood withdrawal and time of osimertinib intake. Blood samples that were collected within six hours of the last osimertinib intake were excluded, due to uncertainty in the extrapolation for the $C_{min,SS}$. This was contrary to the approach used in the other two studies [30, 31], where blood samples collected within six hours of the last osimertinib intake were incorporated as well, which could have impacted the accuracy of the extrapolation. Another study, by Agema *et al.* [48], found that patients with a plasma trough concentration higher than 259 ng/mL are more likely to experience severe toxicity. It should be noted that a substantial part of these patients was also included in our dataset (54%), although the focus of both studies differed (i.e., osimertinib toxicity vs. efficacy analysis). Boosman *et al*. hypothesized that higher cancer-induced inflammation (associated with poorer survival [49, 50]) could lead to lower CYP-activity, and therefore lower osimertinib clearance, resulting in higher plasma trough concentrations [30]. Unfortunately, we were unable to incorporate inflammation markers (such as c-reactive protein or the neutrophil-to-lymphocyte ratio) in our analyses, as these were not routinely registered. In addition, other factors that could not be included in our analyses, may contribute to the lower survival seen in patients with a high osimertinib $C_{min,SS}$. Cachexia for example, which is correlated with poor response and survival, leads to higher inflammation, reduced CYP-activity, and loss of body mass, thereby changing the body distribution of osimertinib and its $C_{min,SS}$ [51, 52]. While in a limited number of patients, Boosman *et al.* found no obvious effect of sarcopenia on the association between osimertinib effectiveness outcomes and its $C_{min,SS}$, [30]. The potential impact of cachexia on the effectiveness of osimertinib has not been evaluated extensively. Therefore, to elucidate which underlying factors could explain the paradoxical correlation between a high osimertinib $C_{min,SS}$ and low mPFS, cachexia may be of interest for further research. For the near future, the scientific basis to incorporate TDM as standard practice in the treatment with osimertinib is missing and more, prospective research is needed to elucidate a potential role for TDM in the treatment of osimertinib.

The added value of our study is the large cohort of 294 patients who were treated with osimertinib in clinical practice, with 118 patients receiving osimertinib as first line

treatment. And additionally, our study contains a large cohort of patients who received osimertinib in a later line, which leads to an extensive picture of osimertinib effectiveness outcomes in clinical practice. Also, all treatment responses were retrospectively reviewed and scored using RECIST 1.1, to ensure uniformity in treatment evaluation. Furthermore, all $C_{min,SS}$ values were accurately extrapolated using the method described by Wang *et al* [36]. All samples were collected during steady state, and samples obtained around progression were excluded, as an increase in $C_{min,SS}$ was observed around this time, which is shown in Appendix A. However, this study also has some limitations. As this was an observational study, not all subgroups consisted of a comparable number of patients, which impacts the certainty of the observed results. Furthermore, using data from patients that were treated with osimertinib in clinical practice, we were limited to the data that was registered for regular care. Therefore, not all characteristics of interest (extensive information on co-medication, inflammation, and cachexia parameters) could be included in our analyses. Additionally, 102 patients died during the study period, which equals 34.7%. The relatively low number of events make the results for the OS immature, and caution should be applied when drawing definitive conclusions. However, data regarding the primary outcome is clear, and an extensive data collection was performed to minimise missing data in other variables.

Results from this study can help clinicians to adequately inform patients with NSCLC in clinical practice. Furthermore, we identified meaningful effects of patient specific clinical features on osimertinib effectiveness , which can be used to develop or improve a reliable decision support system for NSCLC patients in real world practice. Previous research already resulted in the development and implementation of such a tool [53]. Additional information about the impact of patient specific clinical features (such as age and BMI), may be helpful in further tailoring this tool for patients treated with osimertinib, which then has to be tested and validated in a prospective study.

CONCLUSION

Osimertinib treatment outcome in clinical practice was not associated with age, while shorter mPFS and/or mOS were seen in patients with a low BMI (<20.0 kg/m^2), male sex, and a high $C_{min,SS}$ (>271 ng/mL). Patients with *EGFR* exon 19 deletion or *TP53* wild-type status had longer mPFS. Patient specific clinical features affecting the response to osimertinib identified from this real-world data analysis can eventually help clinicians to adequately inform patients with NSCLC about what may be expected from osimertinib treatment.

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APPENDIX A

Table A1: Adjusted hazard ratios for progression and mortality in patients treated with osimertinib in the first line in clinical practice, specified by age, BMI, and the plasma trough concentration.

	mPFS				mOS			
Age (years)	**(months)**	**aHR**	**95% CI**		**(months)**	**aHR**	**95% CI**	
<65	13.9	reference	reference	reference	43.7	reference	reference	reference
65 – 69	20.0	1.07	0.46	2.44	N/A	1.20	0.34	4.20
70 – 74	10.5	1.64	0.80	3.36	41.4	1.60	0.54	4.76
≥75	11.5	1.10	0.53	2.30	17.1	1.69	0.62	4.60
BMI (kg/m^2)								
<20.0	8.7	reference	reference	reference	14.3	reference	reference	reference
20.0 – 24.9	11.5	0.89	0.34	2.35	34.5	0.50	0.16	1.59
25.0 – 29.9	19.3	0.46	0.16	1.37	41.4	**0.16**	**0.04**	**0.67**
≥30.0	9.4	1.21	0.39	3.80	22.5	0.37	0.08	1.73
Unknown	-				-			
Plasma trough concentration (ng/mL)								
<163	17.1	reference	reference	reference	30.3	reference	reference	reference
163 - 271	15.2	1.11	0.29	4.18	N/A	0.36	0.04	3.74
>271	8.1	2.80	0.73	10.72	22.5	1.82	0.34	9.78
Unknown	14.6	1.20	0.41	3.55	41.4	1.23	0.33	4.56

mPFS = median progression free survival, aHR = adjusted hazard ratio, 95% CI = 95% confidence interval, mOS = median overall survival, BMI = body mass index.
Cox proportional hazard model: adjusted for primary EGFR-mutation, TP53 status, line of treatment, sex, age, body mass index and plasma trough concentration.
aHR and 95% CI in bold indicates a statistically significant difference compared to the reference.

Table A2: Baseline characteristics of all patients, stratified by age-group.

	<65 years (N = 114)		65 – 69 years (N = 56)		70 – 74 years (N = 51)		≥75 years (N = 73)	
	N	%	N	%	N	%	N	%
Sex (female)	77	67.5	38	67.9	32	62.7	46	63.0
Smoking								
Never	49	43.0	24	42.9	15	29.4	32	43.8
Former	60	52.6	29	51.8	32	62.7	32	43.8
Current	5	4.4	2	3.6	2	3.9	7	9.6
Unknown	-	-	1	1.8	2	3.9	2	2.7
Race								
Caucasian	95	83.3	51	91.1	50	98.0	68	93.2
African American	3	2.6	1	1.8	-	-	1	1.4
Asian	15	13.2	2	3.6	1	2.0	3	4.1
Hispanic	-	-	1	1.8	-	-	-	-
Other	1	0.9	1	1.8	-	-	1	1.4
CNS metastasis								
Yes	36	31.6	17	30.4	16	31.4	8	11.0
No	39	34.2	21	37.5	18	35.3	30	41.1
Unknown	39	34.2	18	32.1	17	33.3	35	47.9
Primary *EGFR*m								
Exon 19 deletion (1)	69	60.5	33	58.9	22	43.1	34	46.6
L858R (2)	30	26.3	16	28.6	18	35.3	23	31.5
1/2 + second mutation	10	8.8	6	10.7	7	13.7	12	16.4
Other	5	4.4	1	1.8	4	7.8	4	5.5
***TP53*-status**								
Positive	61	53.5	31	55.4	21	42.0	21	28.8
Negative	48	42.1	22	39.3	25	50.0	43	58.9
Unknown	5	4.4	3	5.4	4	8.0	9	12.3
Line								
1	44	38.6	23	41.1	21	41.2	30	41.1
2	53	46.5	25	44.6	20	39.2	36	49.3
3	17	14.9	8	14.3	10	19.6	7	9.6
BMI (kg/m^2)								
<20.0	6	5.3	2	3.6	7	13.7	9	12.3
20.0 – 24.9	55	48.2	31	55.4	15	29.4	35	47.9
25.0 – 29.9	37	32.5	15	26.8	14	27.5	19	26.0
≥ 30.0	13	11.4	7	12.5	10	19.6	7	9.6
Missing	3	2.6	1	1.8	5	9.8	3	4.1

Abbreviations: N = number, % = percentage, CNS = central nervous system, EGFRm = epidermal growth factor receptor mutation, BMI = body mass index, kg = kilogram, m = meter

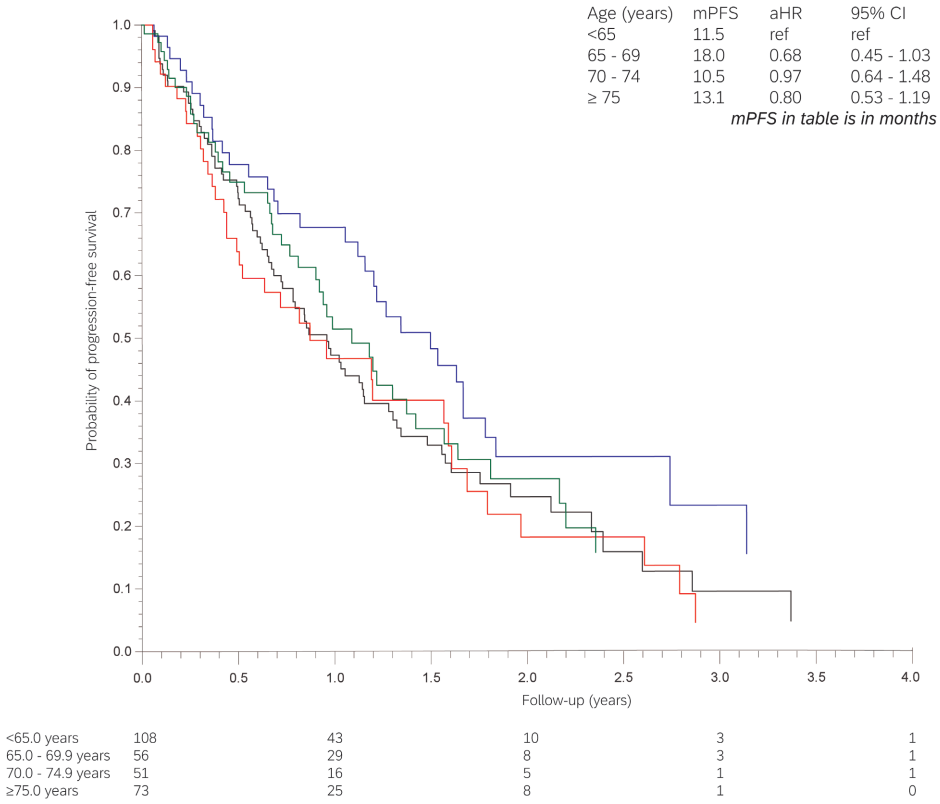
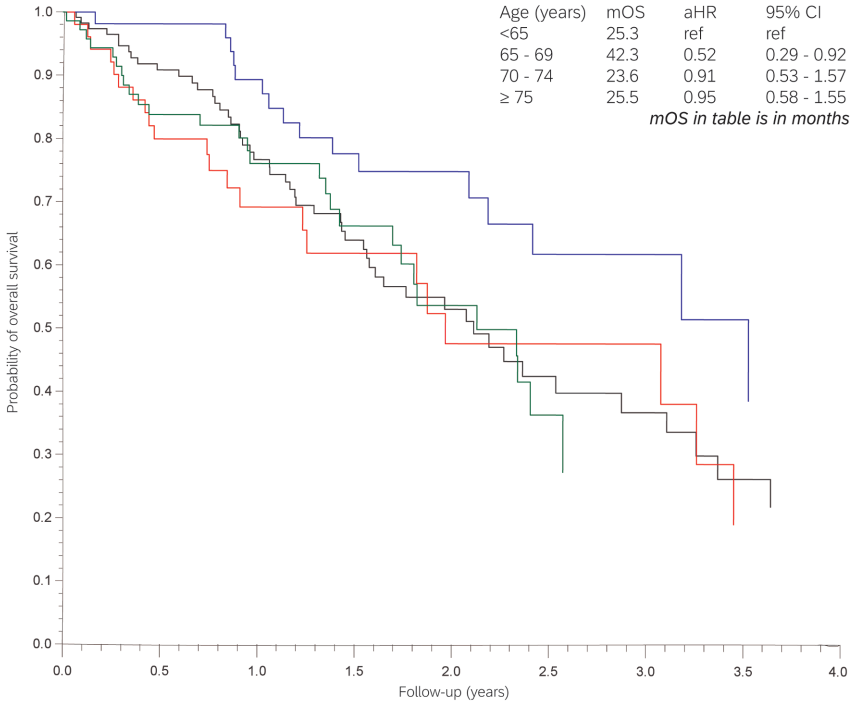


Figure A1A: progression free survival of osimertinib-users in clinical practice, stratified by age (<65 years – black, 69 – 69 years – blue, 70 – 74 – red, and ≥75 years – green).



<65.0 years	114	65	28	12	4
65.0 - 69.9 years	56	40	19	7	2
70.0 - 74.9 years	51	23	9	5	1
≥75.0 years	73	37	15	2	0

Figure A1B: overall survival of osimertinib-users in clinical practice, stratified by age (<65 years – black, 69 – 69 years – blue, 70 – 74 – red, and ≥75 years – green).

Table A3: Baseline characteristics of all patients, stratified by BMI-group.

	< 20.0 kg/m² (N = 24)		20.0 – 24.9 kg/m² (N = 136)		25.0 – 29.9 kg/m² (N = 85)		≥ 30.0 kg/m² (N = 37)		Unknown (N = 12)	
	N	%	N	%	N	%	N	%	N	%
Sex (female)	18	75.0	93	68.4	55	64.7	19	51.4	8	66.7
Smoking										
Never	10	41.7	60	44.1	33	38.8	16	43.2	1	8.3
Former	12	50.0	64	47.1	49	57.6	19	51.4	9	75.0
Current	2	8.3	11	8.1	3	3.5	-	-	-	-
Unknown	-	-	1	0.7	-	-	2	5.4	2	16.7
Race										
Caucasian	20	83.3	126	92.6	72	84.7	34	91.9	12	100.0
African American	-	-	2	1.5	3	3.5	-	-	-	-
Asian	3	12.5	8	5.9	8	9.4	2	5.4	-	-
Hispanic	-	-	-	-	1	1.2	-	-	-	-
Other	1	4.2	-	-	1	1.2	1	2.7	-	-
CNS metastasis										
Yes	4	16.7	41	30.1	20	23.5	10	27.0	2	16.7
No	9	37.5	45	33.1	31	36.5	13	35.1	10	83.3
Unknown	11	45.8	50	36.8	34	40.0	14	37.8	0	0.0
Primary *EGFR*m										
Exon 19 deletion (1)	15	62.5	76	55.9	47	55.3	16	43.2	4	33.3
L858R (2)	6	25.0	38	27.9	24	28.2	13	35.1	6	50.0
1/2 + second mutation	2	8.3	17	12.5	8	9.4	6	16.2	2	16.7
Other	1	4.2	5	3.7	6	7.1	2	5.4	0	0.0
***TP53*-status**										
Positive	11	45.8	63	46.3	43	51.2	15	40.5	2	16.7
Negative	9	37.5	62	45.6	37	44.0	20	54.1	10	83.3
Unknown	4	16.7	11	8.1	5	4.8	2	5.4	-	-
Line										
1	10	41.7	61	44.9	34	40.0	12	32.4	1	8.3
2	12	50.0	58	42.6	40	47.1	18	48.6	6	50.0
3	2	8.3	17	12.5	11	12.9	7	18.9	5	41.7
Age (years)										
<65	6	25.0	55	40.4	37	43.5	13	35.1	3	25.0
65 – 69	2	8.3	31	22.8	15	17.6	7	18.9	1	8.3
70 – 74	7	29.2	15	11.0	14	16.5	10	27.0	5	41.7
≥75	9	37.5	35	25.7	19	22.4	7	18.9	3	25.0

Abbreviations: N = number, % = percentage, CNS = central nervous system, EGFRm = epidermal growth factor receptor mutation, BMI = body mass index, kg = kilogram, m = meter

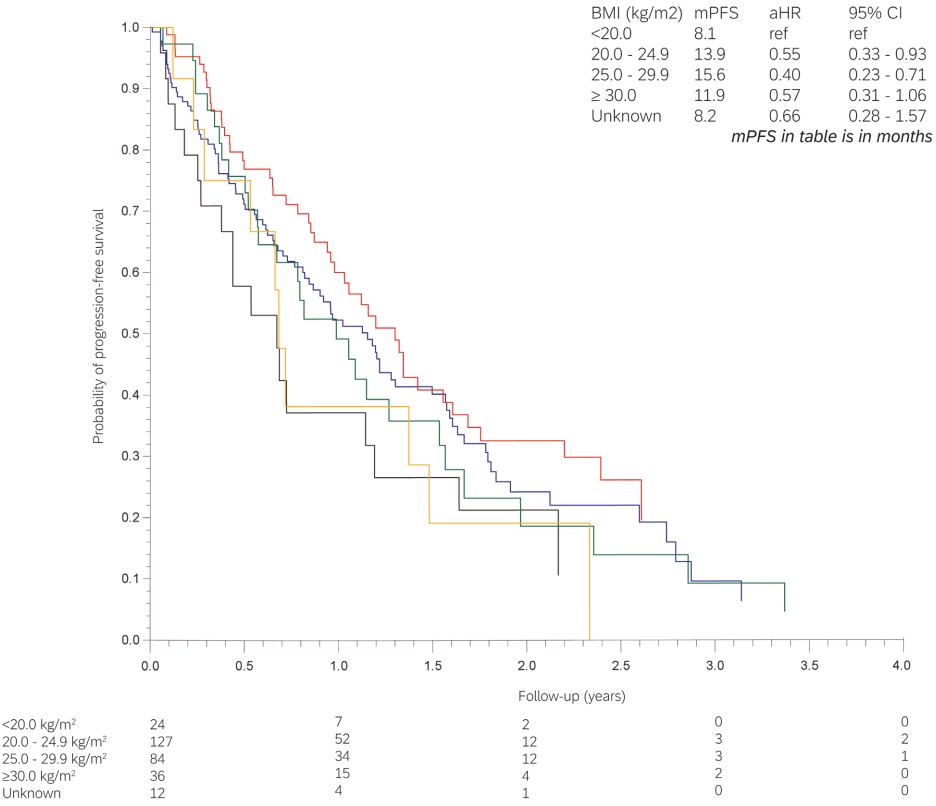


Figure A2A: progression-free survival of osimertinib-users in clinical practice, stratified by BMI (<20.0 kg/m² – black, 20.0 – 24.9 kg/m² – blue, 25.0 – 29.9 kg/m² – red, ≥30 kg/m² – green, and unknown - orange).

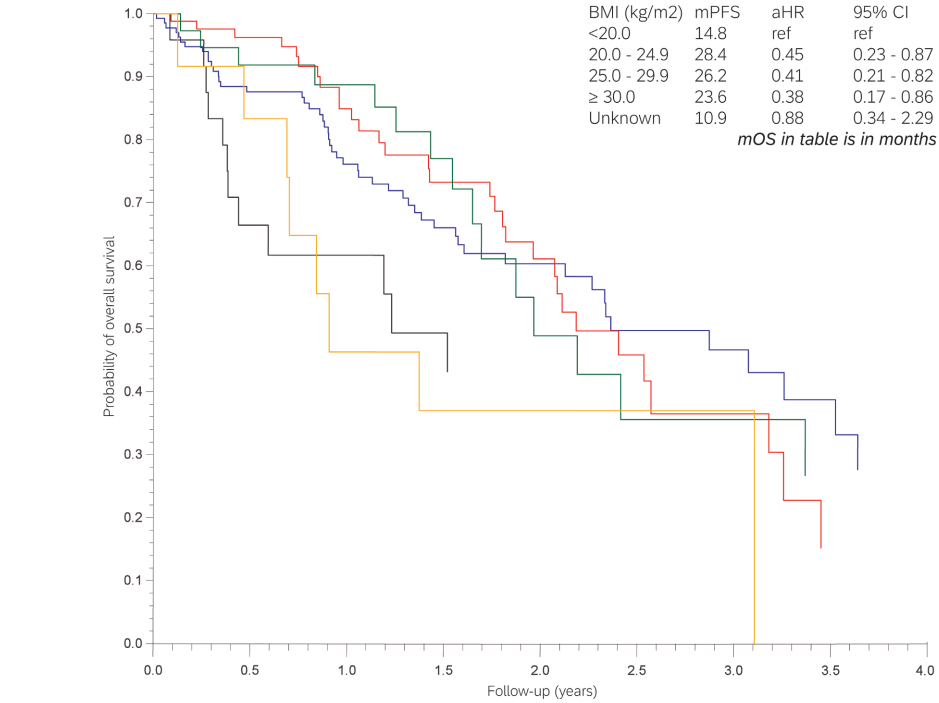


Figure A2B: overall survival of osimertinib-users in clinical practice, stratified by BMI (<20.0 kg/m² – black, 20.0 – 24.9 kg/m² – blue, 25.0 – 29.9 kg/m² – red, ≥30 kg/m² – green, and unknown - orange).

Table A4: Baseline characteristics of all patients, stratified by plasma trough concentration group.

	<163 ng/mL (N = 25)		163 – 271 ng/mL (N = 50)		>271 ng/mL (N = 25)		Unknown (N = 149)	
	N	%	N	%	N	%	N	%
Sex (female)	13	52.8	33	66.0	20	80.0	94	63.1
Smoking								
Never	8	32.0	22	44.0	12	48.0	57	38.3
Former	14	56.0	27	54.0	12	48.0	79	53.0
Current	3	12.0	0	0.0	1	4.0	10	6.7
Unknown	-	-	1	2.0	-	-	3	2.0
Race								
Caucasian	25	100.0	47	94.0	24	96.0	128	85.9
African American	-	-	1	2.0	-	-	4	2.7
Asian	-	-	2	4.0	1	4.0	14	9.4
Hispanic	-	-	-	-	-	-	1	0.7
Other	-	-	-	-	-	-	2	1.3
CNS metastasis								
Yes	8	32.0	12	24.0	8	32.0	36	24.2
No	11	44.0	14	28.0	9	36.0	62	41.6
Unknown	6	24.0	24	48.0	8	32.0	50	33.6
Primary *EGFR*m								
Exon 19 deletion (1)	12	48.0	30	60.0	11	44.0	82	55.0
L858R (2)	7	28.0	13	26.0	6	24.0	46	30.9
1/2 + second mutation	4	16.0	7	14.0	6	24.0	14	9.4
Other	2	8.0	-	-	2	8.0	7	4.7
***TP53*-status**								
Positive	16	64.0	24	48.0	10	40.0	68	45.9
Negative	9	36.0	26	52.0	15	60.0	61	41.2
Unknown	-	-	-	-	-	-	20	12.8
Line								
1	12	48.0	19	38.0	9	36.0	61	40.9
2	11	44.0	25	50.0	10	40.0	66	44.3
3	2	8.0	6	12.0	6	24.0	22	14.8
Age (years)								
<65	9	36.0	21	42.0	7	28.0	66	44.3
65 – 69	4	16.0	14	28.0	5	20.0	27	18.2
70 – 74	5	20.0	8	16.0	6	24.0	24	16.1
≥75	7	28.0	7	14.0	7	28.0	32	21.5

Table A4: Continued.

	<163 ng/mL (N = 25)		163 – 271 ng/mL (N = 50)		>271 ng/mL (N = 25)		Unknown (N = 149)	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
BMI (kg/m²)								
< 20.0	-	-	2	4.0	4	16.0	13	8.7
20.0 – 24.9	17	68.0	24	48.0	12	48.0	64	43.0
25.0 – 29.9	7	28.0	17	34.0	5	20.0	43	28.9
≥ 30.0	-	-	6	12.0	3	12.0	20	13.4
Unknown	1	4.0	1	2.0	1	4.0	9	6.0

Abbreviations: ng = nanogram, mL = millilitre, N = number, % = percentage, CNS = central nervous system, EGFRm = epidermal growth factor receptor mutation, BMI = body mass index, kg = kilogram, m = metre

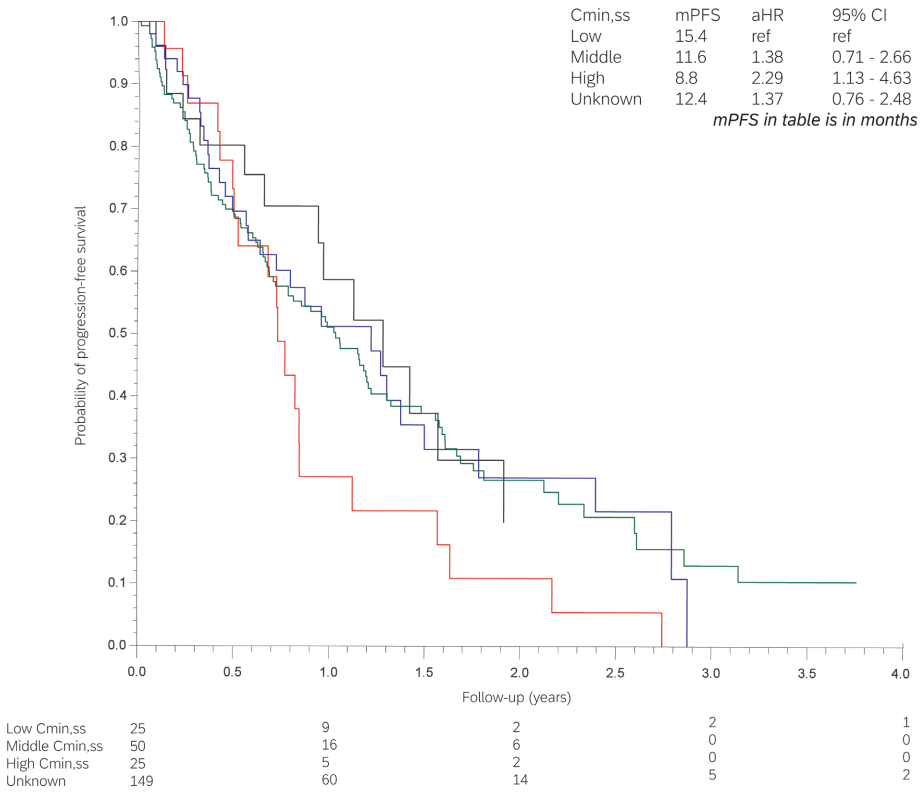


Figure A3A: progression-free survival of osimertinib-users in clinical practice, stratified by plasma trough concentration (<163 ng/mL – black, 163 – 271 ng/mL – blue, >271 ng/mL – red, and unknown - green).

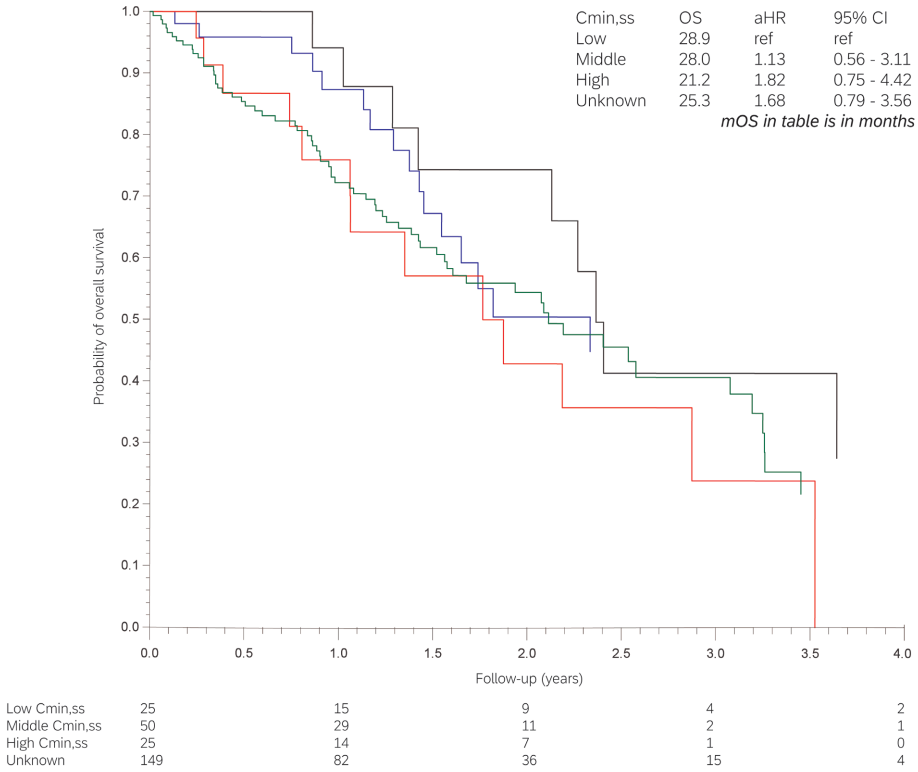


Figure A3B: overall survival of osimertinib-users in clinical practice, stratified by plasma trough concentration (<163 ng/mL – black, 163 – 271 ng/mL – blue, >271 ng/mL – red, and unknown - green).

Table A5: Adjusted hazard ratios for progression and mortality in patients treated with osimertinib in the first line in clinical practice for $C_{min,SS}$.

	Adjusted HR	95% CI	
PFS	1.001	0.999	1.003
OS	1.003	1.001	1.004

Abbreviations: HR = hazard ratio, CI = confidence interval, PFS = progression-free survival, OS = overall survival.

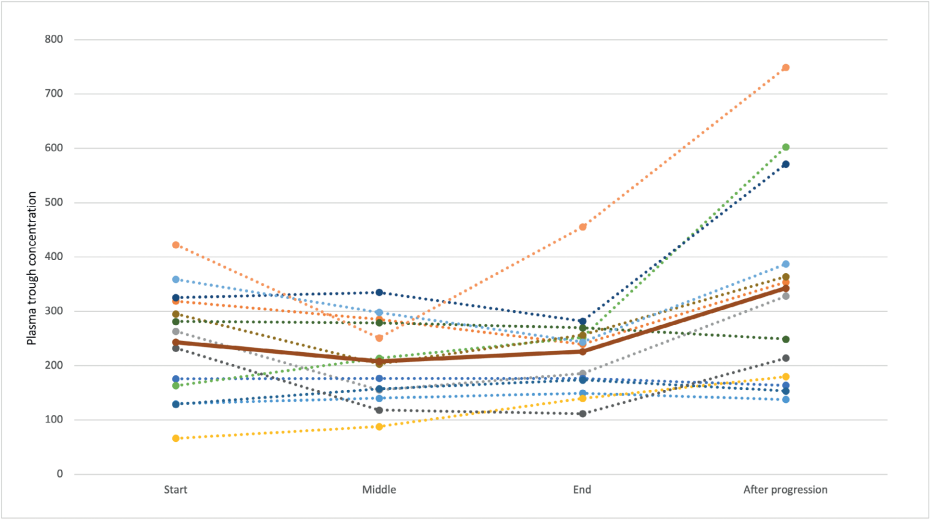


Figure A4: development of $C_{min,SS}$ over time, divided into start (I), middle (II), and end of treatment (III), and after progression (IV).
Legend: development of $C_{min,SS}$ of osimertinib for patients who had a measurement available in all four periods:
I – start, first three months of osimertinib treatment
II – middle, from three months after start until three months to progression
III – end, during three months before progression
IV – after progression, after progression.
Dotted lines are individual patients, solid line is the average of all 13 patients.

Table A6: overview of baseline characteristics and main results of studies evaluating treatment with osimertinib.

	This study	FLAURA [3]	FLOWER [9]	HOT2002 [10]	OSI-FACT [14]	This study	AURA3 [2]
Number of patients	118	279	126	132	538	134	279
Line of treatment	First	First	First	First	First	Second	Second
Type of study	Observational	RCT	Observational	Observational	Observational	Observational	RCT
Study design	Single cohort	Two cohorts	Single cohort	Single cohort	Single cohort	Single cohort	Two cohorts
Centres	Multicentre	Multicentre	Multicentre	Multicentre	Multicentre	Multicentre	Multicentre
Country	Netherlands	Multinational	Italy	Japan	Japan	Netherlands	Multinational
Age (years)	67	64	68	80	71	67	62
Sex (female)	66%	64%	64%	71%	66%	70%	62%
Caucasian	88%	36%	100%	0%	0%	89%	32%
EGFR-mutation (ex. 19 del)	57%	63%	50%	34%	49%	54%	68%
Uncommon *EGFR*-mutation	23%	0.0%	6%	9%	6%	11%	2%
TP53-status (positive)	46%	ND	ND	ND	ND	41%	ND
CNS-involvement	33%	19%	30%	21%	31%	23%	33%
ECOG score (0-1)	ND	100%	87%	86%	84%	ND	100%
DCR	96%	97%	96%	93%	94%	93%	93%
ORR	68%	80%	73%	75%	76%	62%	71%
mPFS (95% CI; months)	14.4 (9.4–19.3)	18.9 (15.2-21.4)	18.9 (11.2-26.7)	19.4 (15.9-23.9)	20.5 (18.6-NR)	13.9 (11.3–16.1)	10.1 (8.3-12.3)
mOS (95% CI; months)	34.5 (34.5-NR)	38.6 (34.5-41.8)	NR	NR	NR	28.0 (23.6-39.1)	26.8 (23.5-31.5)

Abbreviations: 1L = first line, 2L = second line, ex. 19 del = exon 19 deletion, TP53 = tumour protein P53, CNS = central nervous system, ECOG = Eastern Cooperative Oncology Group, DCR = disease control rate, ORR = objective response rate, mPFS = median progression-free survival, mOS = median overall survival, NR = not reached, ND = not determined, 95% CI.= 95% confidence interval.

Table A7: overview of baseline characteristics and main results of studies evaluating the impact of patient-specific clinical features on the treatment with osimertinib.

	This study	**Yamamoto *et al.***	**Auliac *et al.***	**This study**	**Ono *et al.***	**This study**	**Boosman *et al.***	**Rodier *et al.***
# of patients	294	132	43	294	47	249 / 100	145	87
Focus	Age	Age	Age	BMI	BMI	$C_{min,SS}$	$C_{min,SS}$	$C_{min,SS}$
Subgroups	≤65 / 65 – 69 / 70 – 74 / ≥75 (years)	75 – 79 / ≥80 (years)	No subgroups	<20 / 20.0 – 24.9 / 25.0 – 29.9 / ≥30 (kg/m^2)	≤21.5 / >21.5 (kg/m^2)	<163 / 163 – 271 / >271 (ng/mL)	<166 / ≥166 (ng/mL)	≤235 / >235 (ng/mL)
Type of study	Observational	Observational	Observational	Observational	Observational	Observational	Observational	Observational
Study design	Single cohort	Single cohort	Single cohort	Single cohort	Single cohort	Single cohort	Single cohort	Single cohort
Centres	Multicentre	Multicentre	Multicentre	Multicentre	Single centre	Multicentre	Single centre	Multicentre
Country	Netherlands	Japan	France	Netherlands	Japan	Netherlands	Netherlands	France
Age (years)	67	80	85	67	73	67	64	65
Sex (female)	66%	71%	91%	66%	66%	66%	75%	69%
Caucasian	88%	0%	95%	88%	0%	88%	ND	74%
EGFR-mutation (ex. 19 del)	57%	34%	60%	57%	64%	57%	57%	57%

Table A7: Continued.

	This study	Yamamoto et al.	Auliac et al.	This study	Ono et al.	This study	Boosman et al.	Rodier et al.
TP53-status (positive)	46%	ND	ND	46%	ND	46%	ND	ND
CNS-involvement	33%	20.5	ND	33%	34%	33%	31%	48%
ECOG score (0-1)	ND	86%	86%	ND	66%	ND	88%	67%
Conclusion	No significant impact of age on osimertinib effectiveness.	No significant impact of age on osimertinib effectiveness.	Similar effectiveness in octogenarian patients compared to RCTs.	Significant worse mPFS in patients with a low BMI (≤ 20 kg/m^2).	No significant impact of BMI on osimertinib effectiveness.	Significant worse mPFS in patients with a high $C_{min,SS}$ (> 271 ng/mL).	No significant impact of $C_{min,SS}$ on osimertinib effectiveness.	Significant higher risk of death in patients with a high (> 235 ng/mL) osimertinib $C_{min,SS}$.

Abbreviations: 1L = first line, 2L = second line, ex. 19 del = exon 19 deletion, TP53 = tumour protein P53, CNS = central nervous system, ECOG = Eastern Cooperative Oncology Group, mPFS = median progression-free survival, mOS = median overall survival, NR = not reached, ND = not determined, $C_{min,SS}$ = plasma trough concentration during steady-state.

Chapter 3.2

Incidence of bone metastases and skeletal related events in patients with epidermal growth factor receptor mutated non-small cell lung cancer treated with osimertinib.

A.J.W.M. Brouns, A. van Veelen, G.D.M. Veerman, C. Steendam, S. Dursun, C. van der Leest, S. Croes, A.C. Dingemans, L.E.L. Hendriks

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ABSTRACT

Introduction: Bone metastases are frequent in patients with epidermal growth factor receptor mutated (*EGFR+*) non-small cell lung cancer (NSCLC). Skeletal related events (SREs) are common in these patients, however no data on SRE in osimertinib treated patients are reported. We investigated the development of bone metastases and SREs in patients with *EGFR+* NSCLC treated with osimertinib.

Methods: Retrospective multicentre cohort study, including patients with metastatic *EGFR+* NSCLC who were treated with osimertinib between 02-2016 and 09-2021. Demographics, bone metastases related outcomes, SREs, treatment efficacy and overall survival (OS) were collected.

Results: In total, 250 patients treated with osimertinib (43% first line) were included. Fifty-one percent of patients had bone metastases at initiation of osimertinib. Sixteen percent of patients with bone metastases used bone targeted agents (BTAs). Median follow-up from initiation of osimertinib was 23.4 months (95% confidence interval [CI] 19.9-26.9 months). During osimertinib treatment, 10% developed new bone metastases or bone progression. Thirty-nine percent of patients with bone metastases had ≥1 SREs: 28% developed first SRE before osimertinib treatment, one percent after and 11% during. Median OS post bone metastasis was 30.8 months (95% CI 21.9-39.7). Median OS after first SRE was 31.1 months (95% CI 15.8-46.5).

Conclusion: Bone metastases and SREs are frequent before and during treatment with osimertinib in *EGFR+* NSCLC. Because of these findings and the long OS post bone metastases, we advocate prescription of BTAs in these patients and recommend adding bone specific endpoints in clinical trials.

INTRODUCTION

Bone metastases occur in 30-60% of patients with advanced non-small cell lung cancer (NSCLC) [1, 2]. Patients with bone metastases are at risk for skeletal related events (SREs), with subsequently a possible negative impact on quality of life (QoL) and overall survival (OS) [3 – 5]. Examples of SREs are a pathological fracture, spinal cord compression, necessity for radiation to bone (for pain or impending fracture) or surgery to bone [6]. Based on data of a nationwide registry (n=2,052) we have shown that at diagnosis of metastatic disease, 54% of patients with NSCLC and an epidermal growth factor receptor mutation (*EGFR+*) have bone metastases, which is the highest incidence compared to 33% in Kirsten rat sarcoma (*KRAS+*), 31% in anaplastic lymphoma kinase fusion (*ALK+*) and 32% in patients with *EGFR/KRAS/ALK* wild type [7]. However, in other mainly small retrospective series (n=137-209) no differences were observed [8, 9].

In patients with *EGFR+* advanced NSCLC, treatment with first- and second-generation EGFR-tyrosine kinase inhibitors (TKIs) results in superior progression free survival (PFS) compared to chemotherapy [10]. The incidence of SREs in this patient population is high (24-58%) [1, 11]. In a retrospective series (n=189), incidence and time to first SRE was similar between patients with *EGFR+*, *KRAS+* and *EGFR/KRAS* wild type NSCLC when treated with first/second generation EGFR-TKI (*EGFR+*) or chemotherapy, respectively [1]. However, patients with *EGFR+* NSCLC had a significantly longer post metastatic bone disease survival compared to the other patients (median 15 months [*EGFR+*], 9.0 months [*KRAS+*] and 3.2 months [*EGFR/KRAS* wild type]) [1]. Consequently, patients with an *EGFR* mutation are longer at risk for new SREs and live longer with SREs which might impact QoL. Nowadays, osimertinib is the preferred first-line treatment for patients with *EGFR+* NSCLC, with a median PFS of 18.9 months. The prevalence and incidence of SREs during osimertinib treatment is unknown [12].

Denosumab and bisphosphonates are bone targeted agents (BTAs), which inhibit normal osteoclast induced bone resorption. Bisphosphonates are ingested by osteoclasts during bone resorption, which causes cell death of the osteoclast. Denosumab binds to Receptor activator of Nuclear Factor κB Ligand (RANKL) and prevents the interaction with its receptor RANK, with reduction of bone resorption as result. Both denosumab and bisphosphonates are supposed to have (in)direct antitumor effects, but their precise role has to be elucidated [13]. BTAs prevent SREs or delay the time to SREs in solid tumours and multiple myeloma [14 – 16]. Although BTA use in breast cancer is associated with reduction of pain due to bone metastases, in lung cancer this evidence is less clear [17, 18].

It could be hypothesized that due to the superior efficacy of osimertinib, less bone metastases and consequently less SREs develop during osimertinib therapy, with as a result less need for the use of BTAs. Reporting of prevalence of bone metastases and/or SREs, and bone-specific outcomes in patients with *EGFR+* NSCLC in clinical trials evaluating EGFR-

TKIs, including osimertinib, is lacking [11]. Therefore, we performed this multicentre cohort study to evaluate bone metastases related outcomes in patients treated with osimertinib.

METHODS

In this multicentre cohort study, data from patients with *EGFR+* NSCLC in two tertiary referral university hospitals and one teaching hospital in the Netherlands (Maastricht University Medical Center+ [MUMC+], Erasmus Medical Centre Cancer Institute [Erasmus MC]) and Amphia hospital were analysed.

Patient selection and data collection

In MUMC+ all patients with metastatic *EGFR+* NSCLC treated with osimertinib as part of regular care between 02-02-2016 and 22-09-2021 were identified using dispensing data from the pharmacy. In Erasmus MC all patients with metastatic *EGFR+* NSCLC treated with osimertinib between 18-01-2017 and 22-09-2021, were retrieved from a prospective cohort study (START-TKI, NCT05221372). Patients were excluded if no follow-up data were available (at least one follow-up visit after initiation of osimertinib was required).

The in-and outpatient medical records of all patients were retrieved. The following data were collected: demographics, date of diagnosis of metastatic NSCLC, smoking status, histology, mutational status, presence of bone metastasis at diagnosis of metastatic NSCLC and development of bone metastases during the course of the disease, date of initiation of osimertinib treatment including treatment line, duration of osimertinib treatment and date of progression on osimertinib, presence of SREs and if applicable date and type of SRE, use of bone targeted agents and date of death or last follow-up. SRE at diagnosis of bone metastases was defined as an SRE within two months before and two months after diagnosis of bone metastases, SRE at initiation of osimertinib was defined as an SRE within two months before and two months after initiation of osimertinib. Dispensing data from the pharmacy were used to evaluate BTA prescription. Standard radiological evaluation was performed every two till three months by chest and upper abdomen computer tomography (CT) scans with iodine contrast. The last date of follow-up was 01-10-2021.

Medical ethical committee approval was obtained in accordance with local regulations (METC: 2021-2989 and START-TKI, MEC 2016-643, NCT05221372). The ethics committee waived the need for informed consent for 2021-2989, for the START-TKI study all patients provided informed consent.

Statistical analysis

Patient demographics and baseline characteristics are summarized using descriptive statistics. Categorical variables were compared using chi-square tests or Fisher Exact probability tests, continuous variables were compared using the Mann-Whitney U Test, Kruskall Wallis Test or Analysis of Variance (ANOVA). Survival analysis were performed by Kaplan Meier analysis. Incidence rates were calculated by dividing the number of events by the total follow-up time patients were at risk for developing the specific outcome. Statistical analyses were performed using SPSS (IBM statistics, version 20).

RESULTS

Patient characteristics

All patients treated with osimertinib (n = 64) in MUMC+ were included. One hundred eighty-six patients treated with osimertinib from Amphia Hospital and Erasmus MC were enrolled in the START-TKI study. As a result, 250 patients were included in this analysis. Patient characteristics are shown in Table 1.

Median follow-up from diagnosis of metastatic NSCLC was 43.0 months (95% confidence interval [CI]: 38.8-47.3 months). Median follow-up from initiation of osimertinib was 23.4 months (95% CI: 19.9-26.9 months). In 107 out of 250 patients (43%) osimertinib was administered as first line treatment.

Bone metastases

In total, 112 out of 250 (45%) patients had bone metastases at diagnosis of metastatic NSCLC. Fifteen out of 138 patients (11%) without bone metastases at diagnosis of metastatic NSCLC developed bone metastases before osimertinib treatment. As a result, 127 out of 250 patients (51%) were already diagnosed with bone metastases at initiation of osimertinib [Figure 1]. Thereafter, another 15 patients without bone metastases at initiation of osimertinib developed bone metastases (14 during and 1 after osimertinib treatment), resulting in total in 142 patients (57%) of the whole study population being diagnosed with bone metastases at the last follow-up.

Twenty five out of 250 patients (10%) developed bone progression or new bone metastases during osimertinib treatment with a median time to event of 6.4 months (95% CI 2.3-10.6 months). In three patients this was the first diagnosis of bone metastases. The incidence rate for bone progression or new bone metastasis formation after start of osimertinib was 7.0 per 100 person-years, with a 95% CI of 4.7 – 10.3.

Table 1: patient characteristics

Characteristics	Total N = 250		First line N = 107 *		Second line (or beyond) N = 143 *^		p-value
	N	%	N	%	N	%	
Sex (female)	165	67	71	66	94	66	NS
Mean age at diagnosis	65.1 (33 – 87)		67.2 (37 – 87)		63.6 (33 – 84)		<0.05
Never smoker	100	40	44	41	56	39	NS
WHO-PS							**NS**
0 – 1	180	72	80	75	100	70	
>2	54	22	26	24	28	20	
Unknown	16	6	1	1	15	11	
EGFR-mutation							**<0.001**
Exon 19 deletion [1]	60	24	57	53	3	2	
Exon 21 L858R [2]	28	11	25	23	3	2	
Two mutations	8	3	7	7	1	1	
Uncommon	17	7	16	15	1	1	
[1] or [2] + T790M	129	52	1	1	128	96	
Uncommon + T790M	8	3	1	1	7	5	
BM at diagnosis #	**112**	**45**	**55**	**51**	**57**	**40**	**NS**
BM at start osimertinib	127	51	56	52	71	50	NS
New BM or progression during osimertinib	25	10	10	10	15	11	NS
SRE at diagnosis $	21	15	11	20	8	6	<0.05
SRE in patients with BM	56	40	22	36	34	42	NS
First SRE before start osimertinib $	39	28	12	9	27	19	NS
First SRE during osimertinib $	15	11	8	6	7	5	NS
Type of SRE *							**<0.05**
Radiotherapy	45	80	17	30	28	50	
Pathologic fracture	4	7	2	4	2	4	
Surgery	6	11	3	5	3	5	
Spinal cord compression	1	2	0	0	1	2	
BTA use $	23	16	5	4	18	13	NS

Abbreviations: NS = not statistically significant; WHO-PS = World Health Organization – performance score; exon 21 L858R = single point mutation that substitutes leucine for arginine at position 858 in exon 21; T790M = point mutation that substitutes methionine for threonine at position 790 in exon 20; SRE = skeletal related event; BTA = bone targeted agent; BM = bone metastases.

* Percentages were calculated by subgroup

^ All patients received first- or second-generation EGFR-TKIs. 123 patients received osimertinib as second-line treatment.

Diagnosis of stage IV non-small cell lung cancer

$ Percentages were calculated by all patients with bone metastases (N = 142).

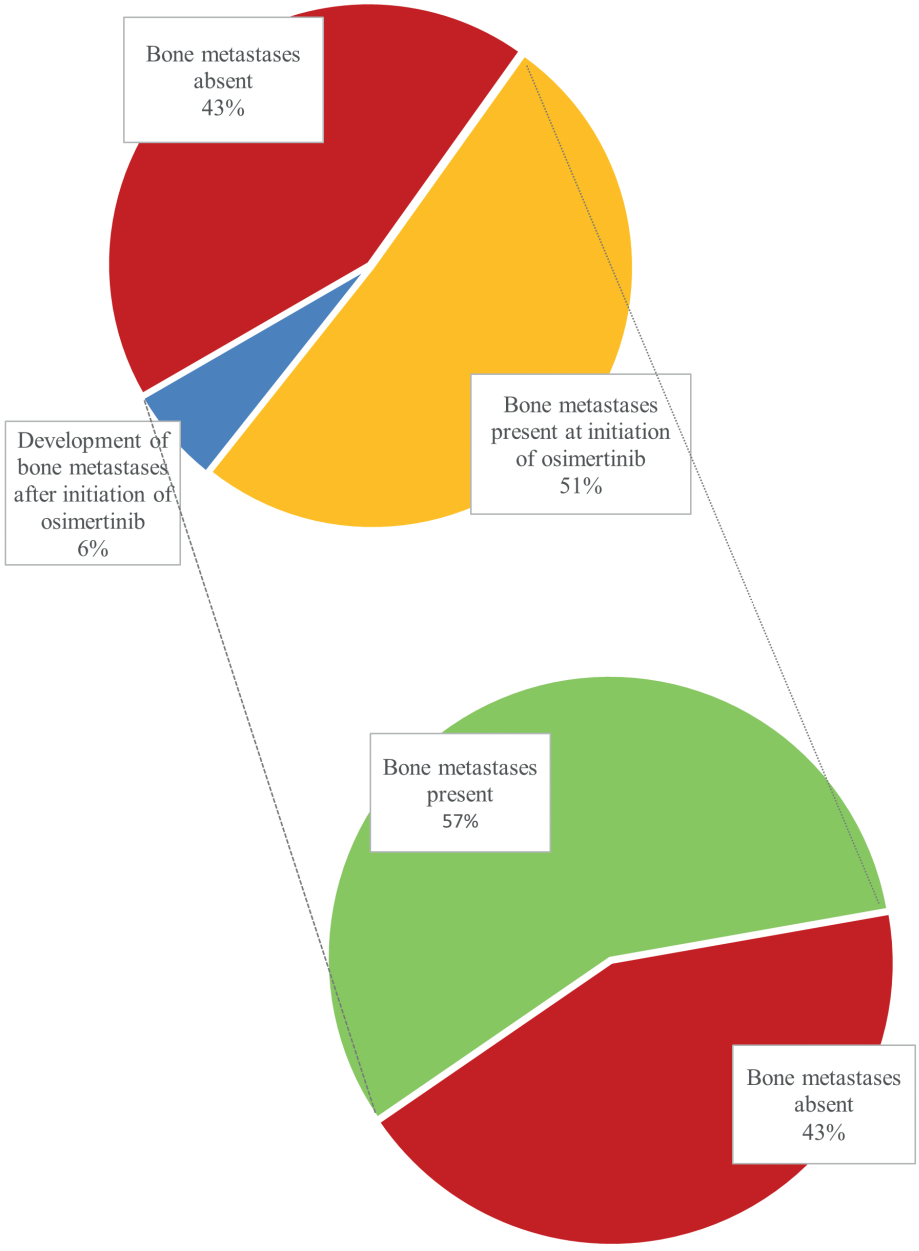

Figure 1: presence of bone metastases (time frame of development of bone metastases during NSCLC disease course).

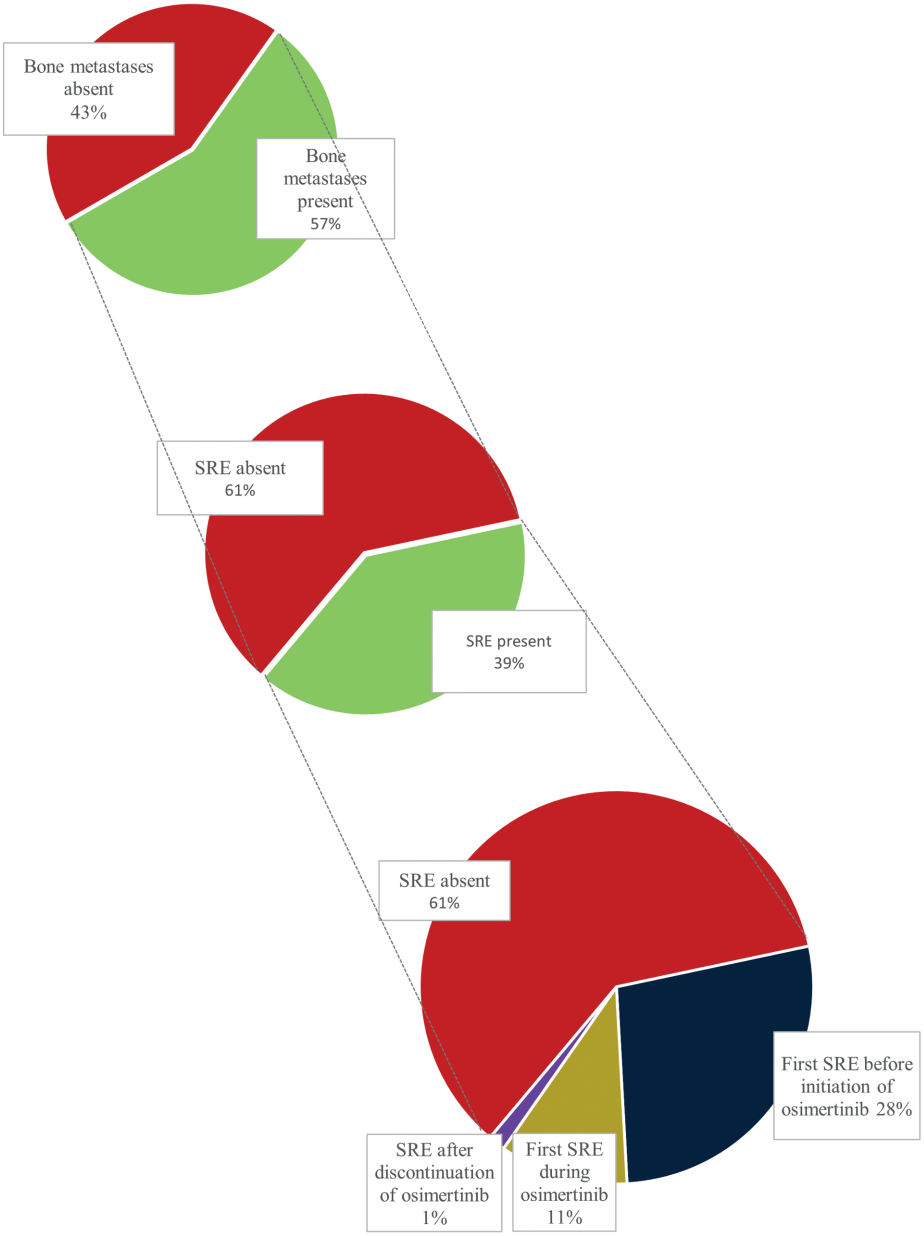

Figure 2: presence of skeletal related events

Legend: [Upper] - Bone metastases during NSCLC disease course, [Middle] - Presence of SRE in patients with bone metastases, [Lower] - Time frame of SRE development in patients with bone metastases during NSCLC disease course. Abbreviations: SRE = skeletal related events. SREs are presented as percentage of thestudy population with bone metastases, e.g., 39 patients have an SRE before initiation of osimertinib.

Skeletal related events

Of the 142 patients with bone metastases, 21 (15%) present with an SRE at diagnosis of advanced NSCLC and in total 56 (40%) developed one or more SREs during the course of their disease. Twenty eight percent of the patients developed their first SRE before, 11% during osimertinib treatment and 1% after discontinuation of treatment (Figure 2). The median time to first SRE for patients who did not have an SRE at advanced NSCLC diagnosis was 9.0 months (95% CI: 6.7-11.3). The incidence rate for an SRE was 10.1 per 100 person years, with a 95% CI of 7.7 – 13.0. In the group of patients with the first SRE during osimertinib treatment (15 out of 56 patients), the median time to SRE was 4.8 months (95% CI 2.1-7.6 months).

Overall survival

At data cut off, 106 out of 250 (42%) patients had deceased. The median OS from diagnosis of metastatic NSCLC was 48.5 months (95% CI 39.8 - 57.2 months) and was significantly shorter for patients with bone metastases during the course of their disease than for those without: 37.2 months (95% CI 33.3 - 41.1 months) versus 66.6 months (95% CI 55.9 - 77.2 months) ($p<0.0001$, Hazard Ratio [HR] 2.4 [95% CI 1.6 - 3.6 months]). The median OS for patients with bone metastases and SREs was not significantly different compared to those without SREs: 41.1 months (95% CI 27.3 - 54.9 months) versus 36.5 months (95% CI 29.4 - 43.5 months) ($p=0.585$, HR 1.1 [95% CI 0.7 - 1.8]).

The median OS from initiation of osimertinib treatment was 28.0 months (95% CI 23.8 - 32.2 months) and was significantly shorter for patients with bone metastases than for patients without bone metastases during the course of their disease: 23.6 months (95% CI 17.1 - 30.0 months) versus 38.3 months (95% CI 23.9 - 52.7 months) for patients without bone metastases ($p<0.0001$, HR 2.1 [95% CI 1.4 - 3.2]). The median OS for patients with bone metastases and SREs was not significantly different compared to those without SREs: 26.1 months (95% CI 18.2 - 34.1 months) versus 22.5 months (95% CI 14.7 - 30.3 months) ($p=0.939$, HR 1.0 [95% CI 0.6 - 1.6]). In Figure 3A and 3B the median overall survival for the study population with/without bone metastases, subdivided by the different treatment lines is shown.

The median OS after development of bone metastasis was 30.8 months (95% CI: 21.9 - 39.7 months). The median OS after development of the first SRE was 31.1 months (95% CI: 15.8 - 46.5 months).

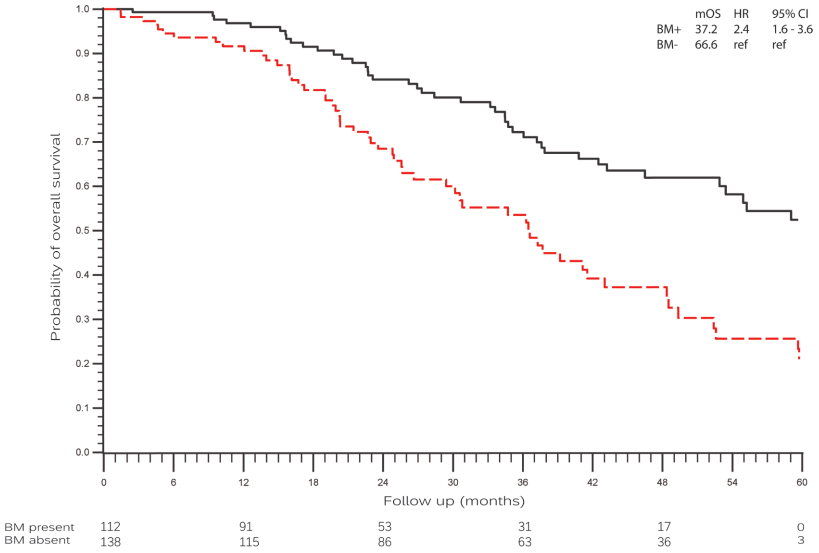

Figure 3A: overall survival from diagnosis of metastatic NSCLC.
[legend: black – BM-; red – BM+]
Abbreviations: BM+ = bone metastases present, BM- = bone metastases absent, HR = hazard ratio, CI = confidence interval.

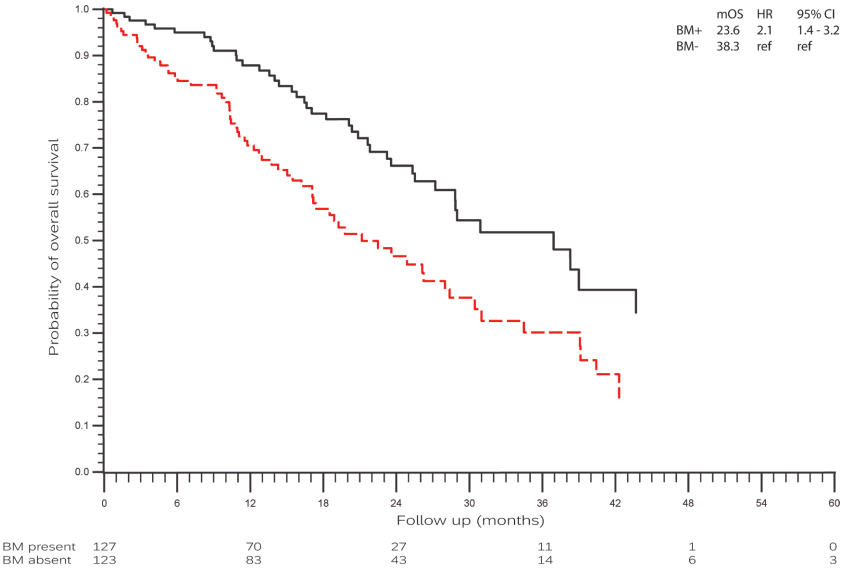

Figure 3B: overall survival from initiation of osimertinib.
[legend: black – BM-; red – BM+]
Abbreviations: BM+ = bone metastases present, BM- = bone metastases absent, HR = hazard ratio, CI = confidence interval.

DISCUSSION

Baseline and cumulative incidence of bone metastases and SREs is high in patients with *EGFR+* metastatic NSCLC treated with first- and second-generation EGFR-TKIs and therefore better treatment options are necessary [11]. We found that the majority of patients (45%) already had bone metastases at first diagnosis of metastatic NSCLC and this percentage increased to 51% at initiation of osimertinib if patients were treated with osimertinib in second line and beyond. At diagnosis of metastatic *EGFR+* NSCLC 15% of patients with bone metastases were diagnosed with an SRE, the cumulative incidence increased to 39%. Consequently, both prevention of progression of existing bone metastases and SREs as well as prevention of new events is important. We found that during osimertinib treatment 10% of the patients developed new bone metastases or progression of existing bone metastases. In other series (including a systematic review evaluating EGFR-TKI trials (n=1,196) and several retrospective series evaluating patients (n=126-1081) treated with EGFR-TKI the percentage of patients with bone metastases at diagnosis of metastatic NSCLC was similar to our study (Table S1) [11, 19]. However, data about bone progression and development of SREs during EGFR-TKI treatment is scarce [11]. The percentage of patients who develop bone progression during osimertinib in our series is comparable to a smaller series (n=126) evaluating outcomes on first line osimertinib (10% vs. 12%) and with trials evaluating first- and second-generation EGFR-TKIs (11% vs. 3%-26%) (Table S1) [19 – 29]. The highest percentages of bone progression were found in two studies (n=38-53) in which regularly a 2-deoxy-2-[fluorine-18] fluoro-D-glucose positron emission tomography-computed tomography scan (FDG-PET-CT scan) was made during follow-up. This is not surprising as FDG-PET has a high sensitivity to detect bone metastases [20, 22, 30]. Another small series (n=101) in patients treated with osimertinib in second line (78% of patients) and beyond also reported a 22% bone progression rate. Radiological tumour assessment during follow-up was comparable to our series [31].

We are the first to report the incidence of SREs during osimertinib treatment (11% of the patients with bone metastases developed their first SRE during osimertinib treatment), which is more than half compared with the 25.9-28% observed in series (n=274-552) evaluating first- or second-generation EGFR-TKI [4, 32].

In our series, we show a relatively long median OS of 48.5 months, and although shorter, the majority of patients with bone metastases survived more than three years (median OS 37.2 months). Development of SREs did not considerably impact OS (median OS after first SRE was 31.1 months). Most SREs occur at diagnosis or develop during the first year after a diagnosis of bone metastases. Furthermore, previous SREs are a risk factor for development of new SREs, therefore the best systemic (i.e., EGFR-TKI) treatment is needed as patients live long with SREs [33]. BTAs are not specifically recommended in Dutch NSCLC or bone metastases guidelines [34, 35]. In clinical practice, BTAs are not frequently used in the treatment strategy of NSCLC, as is also reflected in the low percentage of use (only 16%

in patients with bone metastases) in our series. Data is also lacking on BTA use in other series evaluating *EGFR+* NSCLC. In series (n=114-10,982) evaluating patients with NSCLC unselected for oncogenic drivers, uptake of BTA use was also limited (15-38%) [36 – 38]. This low BTA usage is in contrast with the European Society for Medical Oncology (ESMO) guideline on bone health in which zoledronic acid or denosumab are recommended in patients with a life expectancy of >3 months and clinically significant bone metastases (level of evidence I, grade of recommendation B) [13]. The ESMO guideline on metastatic NSCLC advises denosumab or zoledronic acid in selected patients (not further specified) with bone metastases and a life expectancy of >3 months and considered to be at high risk for SREs (level of evidence I, grade of recommendation B, based on a study including also other solid tumours) [39]. In metastatic breast and prostate cancer, two solid malignancies with a similar favourable prognosis as *EGFR+* NSCLC, the majority of the patients with bone metastases received a BTA, which translated into a significant SRE reduction (relative risk, 95% CI 0.78-0.98) [14, 37].

Based on our data as well as the international guideline recommendations, we strongly recommend to prospectively evaluate and consider the use of BTA in this specific oncogenic driven subgroup with a favourable survival, to reduce the burden of SREs [39, 40]. Other arguments for the use of BTA are small, hypothesis generating, in vivo (n=62 - 129) and in vitro series which show synergy between bisphosphonates and EGFR-TKIs with effects on tumour suppression, PFS and OS post bone metastases [41 – 43]. This synergistic effect should be evaluated prospectively. Currently, one trial (NCT03958565) is enrolling patients with bone metastasized NSCLC to assess the percentage reduction of bone markers in urine or serum while treated with zoledronic acid or denosumab. This study population is subdivided in patients with any oncogenic driver treated with a TKI and in patients without actionable mutations treated with chemotherapy and/or immunotherapy. The incidence of SREs in both groups is a secondary outcome measurement.

This study has its limitations. First, part of the data was retrospectively collected. Nevertheless, bone metastases and SREs are relevant clinical events which are captured in the medical records. Second, not all patients underwent an FDG-PET-CT scan or bone scintigraphy in order to detect asymptomatic bone metastases. However, there was no underreporting of SREs as these per definition cause complaints. Third, we included all lines of osimertinib treatment as although osimertinib is the preferred first line treatment, not all patients worldwide have access to first line osimertinib, and data on osimertinib in second line and beyond remains therefore important [44, 45]. Finally, as it was a retrospective study, we could not evaluate the impact of SREs on patient reported outcomes. However, other studies already showed declines in patients' physical and emotional well-being, ability to perform basic functions of daily living and quality of life [46, 47].

CONCLUSION

To conclude, bone metastases and SREs are frequent events both before and during treatment with osimertinib in patients with *EGFR+* NSCLC. These findings together with the long OS after the occurrence of bone metastases and SREs advocate the prescription of BTAs in *EGFR+* NSCLC with bone metastases and the use of bone specific end points in clinical trials.

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APPENDIX A

Table A1: summary of reported bone metastases and SREs of EGFR-TKI-studies

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Sunaga (2007)[48]	Phase II, single-arm, multicenter study	21/21	Gefitinib 250 mg q.d. (100)	-	12.6	5/21 (24)	NR	NR	NR	NR
Inoue (2009)[49]	Phase II, single-arm study	29/29	Gefitinib 250 mg q.d. (100)	-	17.8	12/29 (41)	NR	NR	NR	NR
Rosell (2012) [Eurtac][10]	Phase III, open-label, multicenter RCT	173/173	Erlotinib 150 mg q.d. (50)	3-week cycles of chemotherapy[1] (50)	Erlotinib arm: 18.9 Chemotherapy arm: 14.4	Erlotinib arm: 28/86 (33) Chemotherapy arm: 29/87 (33)	NR	NR	NR	NR
Yoshimura (2013)[50]	Phase II, single-arm, study	27/27	3-weekly cycles of pemetrexed d1 500mg/m^2 and erlotinib/ gefitinib d2-16 dose NR (100)	-	11.4	16/27 (59)	NR	NR	NR	NR
Reguart (2014)[51]	Phase I-II, single-arm, multicenter study	25/25	Erlotinib 150mg q.d. + vorinostat 400mg q.d. (100)	-	NR	10/25 (40)	NR	NR	NR	NR

Table A1: Continued.

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Zwitter (2014)[20]	Phase II, single-arm, study	53/38	3-weekly cycles of gemcitabin 120mg/m^2 d1, cisplatin 75mg/m^2 d2, gemcitabin 1250mg/m^2 d4, erlotinib 150mg q.d. d5-15 (100)	-	NR	24/38 (63)	*EGFR+* group: "bone (10) most frequent site of PD." Number of pts with PD NR	*EGFR+* group: 10/38 (26)	NR	NR
Yoshimura (2015)[52]	Phase II, open-label, single-arm study	26/26	3-weekly cycles of pemetrexed d1 500mg/m^2 and gefitinib 250mg q.d. d2-16 (100)	-	19.7	8/26 (31)	NR	NR	NR	NR
Park (2016a) [Aspiration study][21]	Phase II, single-arm, multicenter study	207/207	Erlotinib 150mg q.d. (100)	-	11.3	NR	14/171 (8)	14/207 (21)	NR	NR
Park (2016b) [Lux-lung 7][53]	Phase IIB, open-label, multicenter RCT	319/319	Afatinib 40mg q.d.; dose escalation to 50mg q.d. allowed after 4 weeks without AE (50)	Gefitinib 250mg q.d. (50)	27.3	Afatinib arm: 80/160 (50) Gefitinib arm: 73/159 (46)	NR	NR	NR	NR

Table A1: Continued.

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Zwitter (2016)[22]	Phase II, open-label, single-arm, study	38/38	3-weekly cycles of gemcitabin 1250mg/m^2 d1+4, cisplatin 75mg/m2 d2, erlotinib 150mg q.d. d 5-15 (100)	-	35	24/38 (63)	"Bone (10) most frequent site of PD." Number of pts with PD NR.	10/38 (26)	NR	NR
Atagi (2016)[23]	Combined results of 2 phase II studies: JO22903 (single arm) and JO25567 study (randomized)	177/177	JO22903: erlotinib 150mg q.d. (56) JO25567: erlotinib 150mg q.d. (22)	JO22903: - JO25567: bevacizumab 15mg/kg 3-weekly cycles + erlotinib 150mg q.d. (22)	JO22903: 20.4 JO25567: at minimum 20	NR	20/125 (16)	20/177 (11)	NR	NR
Hirano (2016)[54]	Phase II, single-arm, multicenter study	11/11	Erlotinib 25mg q.d.; dose escalation to 150mg q.d. in case of PD (100)	-	NR	NR	1/8 (13)	1/11 (9)	NR	NR
Goss (2016) [Aura 2][25]	Phase II, open-label, multicenter single-arm study	199/199	Osimertinib 80 mg q.d. (100)	-	13.0	NR	9/65 (14)	9/199 (5)	NR	NR

Table A1: Continued.

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Mok (2017) [Aura 3][26]	Phase III, open-label, multicenter RCT	419/419	Osimertinib 80mg q.d. (67)	3-weekly cycles of pemetrexed 500mg/m^2 + carboplatin AUC 5 or cisplatin 75mg/m^2 (33)	8.3	NR	Osimertinib arm: 9/97 (9) Platinum/ pemetrexed arm: 6/101 (6)	Osimertinib arm: 9/277 (3) Platinum/ pemetrexed arm: 6/149 (4)	NR	NR
Soria (2018) [Flaura][12]	Phase III, multicenter, double-blind, RCT	556/556	Osimertinib 80mg q.d.(50)	Erlotinib 150mg q.d. or Gefitinib 250mg q.d. (50)	15	NR	Osimertinib arm: 11/NR Gefitinib or erlotinib arm: 11/NR	Osimertinib arm: 11/278 (4) Gefitinib or erlotinib arm: 11/278 (4)	NR	NR
Lim (2018)[55]	Phase II, single-arm, study	49/49	Gefitinib 250mg q.d. (100)	-	At minimum 6	9/49 (18)	NR	NR	NR	NR
Ahn (2019)[56]	Combined results of 2 phase II studies (AURA extension and AURA 2 trial), both single arm	411/411	Osimertinib 80mg q.d. (100)	-	NR	NR	28/NR	28/411 (7)	NR	NR
Zheng (2019)[27]	Phase II, single-arm study	10/10	Erlotinib 150mg q.d. or Gefitinib 250mg q.d. plus thoracic radiotherapy[4] (100)	-	12	9/10 (90)	2/7 (29)	2/10 (20)	NR	NR

Table A1: Continued.

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Cho (2019) [KCSG-Lu15-09][57]	Phase II, open-label, single arm, study	36/36	Osimertinib 80mg q.d.(100)	-	20.6	10/36 (28)	NR	NR	NR	NR
Noronha (2020)[28]	Phase III, open-label, study	350/350	3-weekly cycles of Gefitinib 250mg q.d. and pemetrexed 500mg/m^2 + carboplatin AUC 5 on d1, (up to four cycles), followed by 3-weekly cycles maintenance pemetrexed (50)	Gefitinib 250mg q.d. (50)	17	Gefitinib+ chemo arm: 24/174 (14) Gefitinib arm: 25/176 (14)	Gefitinib+chemo arm: 3/97 (3) Gefitinib arm: 7/136 (5)	Gefitinib+ chemo arm: 3/175 (2) Gefitinib arm: 7/175 (4)	NR	NR
Wu (2020) [Insight study][58]	Phase Ib/II, open-label, study	55/55	Teponitinib 500mg q.d. + gefitinib 250mg q.d. (66)	Pemetrexed 500mg/m^2+ cisplatin 75mg/m^2 or carboplatin AUC 5-6 on d1 ≤6 cycles or 4 cycles + pemetrexed maintenance (34)	21.8	Teponitinib plus gefitinib arm: 15/49 (23) Chemotherapy arm: 9/24 (38)	NR	NR	NR	NR

Table A1: Continued.

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Lagana (2020)[4]	Retrospective multicenter study	274/274	First-line gefitinib 250mg q.d. (67), erlotinib 150mg q.d. (16), afatinib 40mg q.d. (17), osimertinib 80mg q.d. (0.4)	-	23	274/274 (100)	NR	NR	77/274 (28)	NR
Luo (2021)[59]	Prospectively observed cohort study	417/417[3]	≥2nd line osimertinib 80mg q.d. (100)	-	49.2	76/154 (49)[5]	NR	NR	NR	NR
Dal Maso (2021)[31]	Retrospective multicenter study	139/139	≥2nd line osimertinib 80mg q.d. (73), any systemic treatment (27)	-	14.1	Osimertinib arm: 46/101 (46) Any systemic treatment arm: 8/38 (21)	Osimertinib arm: 22/71 (31) Any systemic treatment arm: 6/33 (18)	Osimertinib arm: 22/101 (22) Any systemic treatment arm: 6/21 (29)	NR	NR
Lorenzi (2021)[19]	Real-world. Prospective study	126/126	First-line osimertinib 80mg q.d. (100)	-	12.3	59/126 (47)	15/44 (34)	15/126 (12)	NR	NR

Table A1: Continued.

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Gen (2022)[60]	Retrospective cohort study	388/388	First-line gefitinib 250mg q.d./erlotinib 150mg q.d. (47), afatinib 40mg q.d. (14), osimertinib 80mg q.d. (39)	-	NR	160/388 (41)	NR	NR	NR	NR
Zeng (2022)[29]	Retrospective cohort study	1081/1081	First-line gefitinib 250mg q.d./erlotinib 150mg q.d. (86), afatinib 40mg q.d. (5), osimertinib 80mg q.d. (9)	-	35	485/1081 (45)	58/619 (9)	58/1081 (5)	NR	NR

Abbreviations: SREs = skeletal related events, EGFR-TKI = epidermal growth factor receptor tyrosine kinase inhibitors, y = year, pts = patients, EGFR+ = activating mutation in the epidermal growth factor receptor (EGFR), BM = bone metastasis, q.d. = once a day, NR = not reported, RCT = randomized controlled trial, AE = adverse events, AUC = area under the curve.

[1] Cisplatin 75 mg/m^2 on day 1 plus docetaxel (75 mg/m^2 on day 1) or gemcitabin (1250 mg/m^2 on days 1 and 8). In patients with contra-indications for cisplatin, carboplatin (AUC 6 with docetaxel 75 mg/m^2 or AUC 5 with gemcitabin 1000 mg/m^2) was allowed.

[3] Only 154 out of 417 patients received 1st/2nd generation TKI with subsequent osimertinib, the other 263 patients received 1st/2nd generation TKI without subsequent osimertinib (n=203) or no EGFR-TKI treatment (n=60)

[4] 54-60 Gray / 27-30 fractions / 5.5-6 weeks.

[5] Percentage bone metastases at initiation of osimertinib, percentage at baseline is not reported.

4

Chapter 4

OBSERVATIONAL STUDIES USING BRITISH PRIMARY CARE DATABASES

Chapter 4.1

Characteristics of patients with lung cancer in clinical practice and their potential eligibility for clinical trials evaluating tyrosine kinase inhibitors or immune checkpoint inhibitors.

A. van Veelen, S. Abtahi, P.C. Souverein, J.H.M. Driessen, O.H. Klungel, A.C. Dingemans, R.M.J.M. van Geel, F. de Vries, S. Croes.

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ABSTRACT

Introduction: In- and exclusion criteria of randomized clinical trials (RCTs) aim to include a homogeneous study-population. This study compared characteristics of lung cancer patients from phase III RCTs evaluating tyrosine kinase inhibitors (TKIs) or immune checkpoint inhibitors (ICIs) with characteristics of lung cancer patients in a real-world setting in the United Kingdom.

Methods: A retrospective study was conducted using the Clinical Practice Research Datalink GOLD. Patients (N = 9239) with a first ever lung cancer registration between 2014 and 2018 were identified. Eligibility for inclusion was assessed for twelve RCTs (evaluating TKIs or ICIs). Reasons for potential exclusion and the number of unmet criteria were assessed for each RCT independently. OS was assessed using Kaplan-Meier and Cox proportional hazards analyses.

Results: The proportion of potentially eligible patients was 74.3% and 51.9% for TKI and ICI RCTs, respectively. History of another malignancy, renal insufficiency or concomitant drug-use were main reasons for exclusion. OS was considerably longer for potentially eligible patients. Hazards ratios varied from 1.17 (95% confidence interval, 1.11–1.24) to 1.35 (1.20–1.42) across the RCTs.

Conclusion: This study showed that a considerable proportion of lung cancer patients in a real-world setting would have been ineligible for participation in phase III RCTs and that potentially ineligible patients experienced a shorter OS.

INTRODUCTION

Lung cancer is the most common cause of cancer related deaths among men worldwide and among women in more developed countries [1]. Lung cancer can be subdivided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). In the United Kingdom (UK), approximately 87% of patients with lung cancer have NSCLC and their 5-year survival rate is 9.5% [2]. Survival is strongly influenced by the disease stage at diagnosis, i.e., early-stage disease is associated with much better prognosis compared with diagnosis at an advanced-stage [3]. Around 75% of the patients with lung cancer in the UK are diagnosed with an advanced (stage III or IV) disease [4]. For those patients a curative approach is no longer available and systemic therapy is normally considered a cornerstone of treatment. Over the past 15 years, tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors (ICIs) have become available. The efficacy of TKIs and ICIs have been evaluated in large phase III randomized clinical trials (RCTs), with strict in- and exclusion criteria [5–16].

It is unclear whether patients who participated in those RCTs [5–16] are good representatives for patients with lung cancer in clinical practice. RCTs often exclude patients with a wide range of comorbidities, abnormal laboratory measurements or concomitant drug use. In real life, a considerable number of patients with lung cancer have chronic comorbidities such as cardiovascular disease, chronic obstructive pulmonary disease, and type 2 diabetes mellitus [17,18], often related to tobacco smoking [19–22]. Previous studies have shown that NSCLC patients who received chemotherapy or first-generation TKIs in clinical practice did not possess the eligibility criteria of the underlying RCTs that provided evidence for the efficacy of these treatments [23–26]. Substantial differences between the studied clinical trial population and the population seen in daily clinical practice may lead to less favourable treatment outcomes, as has been shown for chemotherapy treatment in patients with lung cancer [27].

Whereas the efficacy of osimertinib, alectinib, nivolumab, pembrolizumab, atezolizumab, and durvalumab in the treatment of selected NSCLC-patients is well-established [5–16], the representativeness of the patients included in the RCTs leading to market approval, has not been evaluated.

Therefore, the primary aim of this study was to compare the characteristics of patients included in phase III RCTs that evaluated TKIs or ICIs for treatment of lung cancer with the characteristics of patients with lung cancer in a real-world setting in the UK from 2014 through 2018. The secondary aim was to compare overall survival (OS) among real world lung cancer patients in the United Kingdom (UK) who would have been eligible for inclusion in these phase III RCTs with that of patients who did not meet those eligibility criteria.

METHODS

Data source

Data were obtained from the Clinical Practice Research Datalink GOLD, (CPRD (www.cprd.com)), hereafter referred to as CPRD. The CPRD contains computerized medical records from 674 primary care practices in the UK, representing 6.9% of the population in 2013 [28]. The CPRD features demographic information, prescription details, clinical events, preventive care provided, specialist referrals, hospital admissions and major outcomes since 1987, with on-going data collection. The clinical events in the CPRD are classified using Read codes, which is a hierarchical system used to specify medical conditions and clinical events [29]. The validity of lung cancer recording in the CPRD has been reported by others, as the concordance of lung cancer registration between CPRD and the cancer registry was ≥90% [30,31].

Literature search of clinical trials

We selected six newly approved drugs used to systemically treat patients with NSCLC: osimertinib and alectinib as TKIs, and pembrolizumab, nivolumab, atezolizumab and durvalumab as ICIs. A literature search was performed in PubMed to identify phase III RCTs published between 01-01-2014 and 31–12–2018 evaluating the efficacy of these drugs. The literature search was performed by using the different drug names in combination with 'randomized controlled trial', 'RCT', 'phase 3' or 'NSCLC'. For nivolumab and pembrolizumab, only RCTs evaluating first-line use were included as studies of generalizability as RCTs evaluating second-line use of nivolumab and pembrolizumab had been published before [25]. In total, twelve RCTs were identified (Table A1.1, Appendix A).

Study Cohort

A retrospective cohort study was conducted among patients aged ≥18 years with a first diagnosis of lung cancer between 01 January 2014 and 31 December 2018. Read codes used to identify lung cancer patients are shown in Appendix B. The list was composed by one researcher (AV) and verified by a pulmonary oncologist (AD). The date of the first lung cancer diagnosis during valid data collection defined the index date (and thereby the start of follow-up).

Inclusion and exclusion criteria

The in- and exclusion criteria of the selected RCTs were evaluated, listed, and grouped into comorbidities, medication use, and laboratory values (Appendix C). Codelists for all comorbidities and drugs were independently reviewed by a pharmacist (AV) and a physician (SA). In case of disagreement, a third author (PS) did an additional review. For laboratory measurements, the registered unit of measurement was assessed for all laboratory values and all measurements with an appropriate unit were included. When a laboratory value was registered with an inappropriate or without a unit of measurement, the registered value was not included in the analysis. The in- and exclusion criteria were

grouped according to eight sets of criteria (laboratory values, cancer, immune-related, cardiovascular, infections, psychiatric, drugs and other), as shown in Appendix C. For the evaluation of laboratory measurements, reference values were used to identify deviant values (Appendix D). In addition, if a laboratory value was not registered for a patient, it was assumed that the laboratory value was not deviant and potential exclusion would only be done when a deviant laboratory value was specifically registered. Some disease areas were only generally described in the protocols (e.g., immune-related diseases, serious infections, psychiatric diseases, and organ transplant). To identify these broad terms, a set of relevant medical conditions was compiled for every broad term and those specific conditions were used as exclusion criteria. The selections were verified by a pulmonary oncologist (AD). A list of all used exclusion criteria is shown in Appendix C. Each comorbidity, laboratory measurement, or concomitant drug use had to be registered in a specific timeframe (time-window of exposure) before the diagnosis of lung cancer (index date) to be considered as active (Appendix C). Study protocols of the included RCTs were reviewed to identify the time-window of exposure for the different exclusion criteria. When a time-window of exposure was not specified in the protocols, an appropriate time-window of exposure was selected by the researcher (AV). For each comorbidity the time-window of exposure was determined by the nature of the condition. For laboratory measurements and concomitant drug use a 3-month period was selected as time-window of exposure. The exclusion criteria per study and their corresponding time-window of exposure are shown in Appendix E.

Data analysis

Descriptive analyses were used to summarize patient characteristics, both for full study period and by calendar year. To assess the eligibility of patients with lung cancer in CPRD for potential inclusion in each RCT, the numbers and proportions of patients who met all criteria for each individual RCT were determined. Furthermore, the reason for potential study exclusion (restricted to the eight sets of criteria previously specified [Appendix C]) was assessed and the maximum number of unmet criteria were evaluated individually. For all lung cancer patients in CPRD, Kaplan-Meier analyses compared OS between those who met eligibility criteria for each individual RCT versus those did not meet eligibility criteria. Corresponding Kaplan-Meier curves compared OS in CPRD patients whose characteristics made them eligible or ineligible for inclusion in published RCTs (Table A1.1, Appendix A). In addition, Cox proportional hazards analyses estimated crude, and age-sex adjusted hazard ratios (HRs) for mortality of patients with lung cancer, comparing patients who would have been eligible for participation in RCTs (Table A1.1, Appendix A) to those who would have been ineligible [5–16]. A sensitivity analysis was performed in which we evaluated what the potential effect would be on potential study participation if the criteria for kidney function were less strict for the trials evaluating immunotherapy. All analyses were performed using the SAS software application (version 9.4: SAS Institute, Cary, NC, U.S.A.). This study was approved by the Independent Scientific Advisory Committee for MHRA database research.

RESULTS

The baseline characteristics of the study population are shown in Table 1. A total number of 9,239 adult patients with lung cancer were identified. The mean age of the included patients was 72.1 years, and more than half of the patients (51.0%) were male. Virtually all patients were previous or current smokers, as only 6.3% had never smoked before. Most patients had a body mass index (BMI) between 18.6 and 30.0 kg/m^2 (68.5% for the total population). A history of another malignancy within 5 years prior to the lung cancer diagnosis was the most frequent comorbidity responsible for potential study exclusion (10.2%). The most frequent types of other malignancies were breast, prostate, or colorectal cancer. A low estimated glomerular filtration rate (eGFR < 60 mL/min) recorded within the past 3 months was the most common deviant laboratory value (10.5%), and the most frequently concomitantly used drug was a systemic glucocorticoid (20.6%).

Table 1: baseline characteristics of lung cancer patients in CPRD GOLD between 2014 and 2018, overall and stratified by calendar year.

	Total		**2014**		**2015**		**2016**		**2017**		**2018**	
	N = 9,239		**N = 2,426**		**N = 2,114**		**N = 1,795**		**N = 1,510**		**N = 1,394**	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Sex												
No. of Males	4,710	51.0	1,290	53.2	1,080	51.1	901	50.2	745	49.3	694	49.8
Age (years)												
Mean (SD)	72.1	(10.5)	71.8	(10.8)	72.0	(10.3)	72.3	(10.5)	72.1	(10.3)	72.4	(10.2)
< 50.0 years	258	2.8	84	3.5	46	2.2	53	3.0	39	2.6	36	2.6
50.0 – 64.9 years	2,055	22.2	551	22.7	493	23.3	386	21.5	335	22.2	290	20.8
65.0 – 79.9 years	4,880	52.8	1,249	53.3	1,126	53.3	960	53.5	792	52.5	753	54.0
≥ 80.0 years	2,046	22.1	542	22.3	449	21.2	396	22.1	344	22.8	315	22.6
BMI (kg/m^2)												
≤ 18.5	611	6.6	155	6.4	150	7.1	98	5.5	111	7.4	97	7.0
18.6 – 25.0	3,490	37.8	947	39.0	834	39.4	665	37.0	532	35.2	512	36.7
25.1 – 30.0	2,836	30.7	728	30.0	633	29.9	540	30.1	481	31.9	454	32.6
30.1 – 35.0	1,260	13.6	326	13.4	266	12.6	255	14.2	228	15.1	185	13.3
> 35.0	514	5.6	117	4.8	122	5.8	124	6.9	76	5.0	75	5.4
Missing	528	5.7	153	6.3	109	5.2	113	6.3	82	5.4	71	5.1
Smoking status												
Current	3,462	37.5	942	38.8	805	38.1	639	35.6	550	36.4	526	37.7
Former	5,106	55.3	1,317	54.3	1,155	54.6	1,028	57.3	846	56.0	760	54.5
Never	582	6.3	145	6.0	132	6.2	109	6.1	103	6.8	93	6.7
Missing	89	1.0	22	0.9	22	1.0	19	1.1	11	0.7	15	1.1

Table 1: Continued.

	Total		2014		2015		2016		2017		2018	
	N = 9,239		N = 2,426		N = 2,114		N = 1,795		N = 1,510		N = 1,394	
	N	%	N	%	N	%	N	%	N	%	N	%
Cancer-related												
Previous malignancies [a]	939	10.2	243	10.0	219	10.4	185	10.3	147	9.7	145	10.4
Immune-related diseases												
Vasculitis [b]	57	0.6	15	0.6	17	0.8	11	0.6	10	0.7	<6	<0.5
Coeliac disease [b]	48	0.5	14	0.6	10	0.5	11	0.6	10	0.7	<6	<0.5
Crohn's disease [b]	58	0.6	19	0.8	10	0.5	12	0.7	11	0.7	6	0.4
Ulcerative colitis [b]	114	1.2	33	1.4	22	1.0	21	1.2	24	1.6	14	1.0
Grave's disease [b]	21	0.2	6	0.2	6	0.3	5	0.3	<6	<0.4	<6	<0.5
Multiple sclerosis [b]	26	0.3	9	0.4	<6	<0.3	<6	<0.4	9	0.6	<6	<0.5
Myasthenia gravis [b]	7	0.1	<6	<0.3	<6	<0.3	<6	<0.4	<6	<0.4	<6	<0.5
Ankylosing spondylitis [b]	21	0.2	<6	<0.3	8	0.4	<6	<0.4	<6	<0.4	<6	<0.5
Dermatomyositis [b]	<6	<0.1	<6	<0.3	<6	<0.3	<6	<0.4	0	0.0	<6	<0.5
Polymyalgia rheumatic [b]	189	2.0	44	1.8	39	1.8	39	2.2	36	2.4	31	2.2
Psoriatic arthritis [b]	36	0.4	8	0.4	7	0.3	7	0.4	6	0.4	8	0.6
Rheumatoid arthritis [b]	283	3.1	69	2.8	68	3.2	51	2.8	56	3.7	39	2.8
Psoriasis [b]	558	6.0	142	5.9	111	5.3	115	6.4	98	6.5	92	6.6
Sarcoidosis [b]	12	0.1	<6	0.3	<6	<0.3	<6	<0.4	<6	<0.4	0	0.0
Systemic lupus erythematous [b]	19	0.2	<6	0.3	<6	<0.3	10	0.6	<6	<0.4	<6	<0.5
Cardiovascular disease												
Heart failure [b]	460	5.0	111	4.6	95	4.5	101	5.6	71	4.7	82	5.9
Heart rhythm disturbances [bc]	77	0.8	16	0.7	16	0.8	16	0.9	15	1.0	14	1.0
Myocardial infarction [bd]	51	0.6	11	0.5	6	0.3	13	0.7	12	0.8	9	0.6
Poor controlled hypertension [d]	<6	<0.1	<6	<0.3	0	0.0	0	0.0	0	0.0	0	0.0
Unstable angina pectoris [d]	<6	<0.1	0	0.0	<6	<0.3	0	0.0	0	0.0	<6	<0.5
Serious infections												
Meningitis [e]	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Pneumonia [e]	118	1.3	23	0.9	23	1.1	30	1.7	22	1.5	20	1.4
Sepsis [e]	18	0.2	<6	<0.3	<6	<0.3	<6	<0.4	<6	<0.4	<6	<0.5
Hepatitis [f]	<6	<0.1	<6	<0.3	<6	<0.3	<6	<0.4	0	0.0	0	0.0
Psychiatric diseases												
Bipolar disorde[r b]	33	0.4	7	0.3	11	0.5	7	0.4	<6	<0.4	6	0.4
Dementia [b]	275	3.0	59	2.4	74	3.5	46	2.6	43	2.8	53	3.8
Schizophrenia [b]	64	0.7	21	0.9	16	0.8	7	0.4	12	0.8	8	0.6

Table 1: Continued.

	Total		2014		2015		2016		2017		2018	
	N = 9,239		N = 2,426		N = 2,114		N = 1,795		N = 1,510		N = 1,394	
	N	%	N	%	N	%	N	%	N	%	N	%
Other												
HIV/aids [b]	13	0.1	<6	<0.3	<6	<0.3	<6	<0.4	<6	<0.4	<6	<0.5
Organ transplant [b]	14	0.2	<6	<0.3	<6	<0.3	<6	<0.4	<6	<0.4	<6	<0.5
Substance abuse [g]	17	0.2	<6	<0.3	<6	<0.3	<6	<0.4	<6	<0.4	<6	<0.5
Pregnancy [f]	<6	<0.1	0	0.0	0	0.0	<6	<0.4	0	0.0	0	0.0
Deviant laboratory values [h]												
eGFR [d]	969	10.5	267	11.0	201	9.5	196	10.9	153	10.1	152	10.9
Alkaline phosphatase [d]	106	1.1	25	1.0	28	1.3	25	1.4	15	1.0	13	0.9
ALAT [d]	123	1.3	27	1.1	28	1.3	27	1.5	28	1.9	13	0.9
ASAT [d]	34	0.4	11	0.5	<6	<0.3	<6	<0.4	7	0.5	8	0.6
Total bilirubin [d]	116	1.3	29	1.2	21	1.0	22	1.2	25	1.7	19	1.4
Lymphocyte [d]	35	0.4	8	0.3	<6	<0.3	12	0.7	8	0.5	<6	<0.5
Neutrophils [d]	14	0.2	6	0.2	0	0.0	<6	<0.4	<6	<0.4	<6	<0.5
WBC [d]	<6	<0.1	<6	<0.3	<6	<0.3	<6	<0.4	<6	<0.4	<6	<0.5
Platelets [d]	26	0.3	12	0.5	6	0.3	<6	<0.4	<6	<0.4	<6	<0.5
Hemoglobin [d]	106	1.1	26	1.1	22	1.0	26	1.4	16	1.1	16	1.1
INR [d]	293	3.2	81	3.3	57	2.7	60	3.3	54	3.6	41	2.9
TSH [d]	341	3.7	84	3.5	75	3.5	72	4.0	54	3.6	56	4.0
Drug use												
Systemic glucocorticoids [i]	1,903	20.6	490	20.2	420	19.9	383	21.3	336	22.3	274	19.7
Other immunosuppressants												
Ciclosporin [d]	<6	<0.1	0	0.0	<6	<0.3	<6	<0.4	<6	<0.4	0	0.0
Everolimus [d]	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Sirolimus [d]	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Tacrolimus [d]	<6	<0.1	<6	<0.3	0	0.0	<6	<0.4	<6	<0.4	<6	<0.5
Strong CYP3A4-inhibitors												
Erythromycin [d]	131	1.4	46	1.9	43	2.0	18	1.0	13	0.9	11	0.8
Clarithromycin [d]	860	9.3	267	11.0	209	9.9	156	8.7	130	8.6	98	7.0
Itraconazole [d]	7	0.1	<6	<0.3	<6	<0.3	<6	<0.4	<6	<0.4	<6	<0.5
Ketoconazole [d]	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Ritonavir [d]	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

Table 1: Continued.

	Total		2014		2015		2016		2017		2018	
	N = 9,239		N = 2,426		N = 2,114		N = 1,795		N = 1,510		N = 1,394	
	N	%	N	%	N	%	N	%	N	%	N	%
Voriconazole [d]	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

Abbreviations: N = number; % = percentage; HIV = human immunodeficiency virus; aids = acquired immune deficiency syndrome; eGFR = estimated glomerular filtration rate; ALAT = alanine transaminase; ASAT = aspartate transaminase; WBC = white blood cell count; INR = international normalized ratio; TSH = thyroid-stimulating hormone.

[a] time-window of exposure for previous or concurrent malignancies and laboratory values differed between the twelve clinical trials, as can be seen in Appendix C in which all specific exclusion criteria are shown per trial. The results shown in this table are corresponding with the strictest threshold, which would exclude the most patients.
[b] time-window of exposure was ever before index date.
[c] for heart rhythm disturbances three specific conditions were used: complete left bundle branch block, second degree heart block and third-degree heart block.
[d] time-window of exposure was 3 months before index date.
[e] time-window of exposure was 1 month before index date.
[f] time-window of exposure was 1 year before index date.
[g] time-window of exposure was 5 years before index date.
[h] for some laboratory values specific threshold values were reported in the study protocols, and those were used to identify lung cancer patients in CPRD with deviant laboratory values. For other laboratory values reference values were used as threshold. The used threshold values were not exactly similar for all twelve studies. In this Table the results are shown for the strictest threshold value, which would exclude the highest number of patients. Specific threshold values for all laboratory variables can be seen in Appendix D.
[i] for systemic glucocorticoids included the following substances: dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, and triamcinolone.

Table 2 shows that the proportion of patients with lung cancer from CPRD who would have been eligible for inclusion in RCTs ranged from 49.1% to 78.1%. The mean proportion of patients that would have been eligible for RCTs evaluating TKIs was 74.3% (range: 72.4–78.1%) and 51.9% for ICIs (range: 49.1–54.3%). For some of the drugs (osimertinib, alectinib, nivolumab and pembrolizumab) more than one RCT was included (Table A1.1, Appendix A). Potential eligibility for RCTs evaluating the same drug was similar except for AURA3 (78.1%) and FLAURA (72.4%). A considerable number of patients with lung cancer in CPRD would have been excluded due to a single unmet criterion. RCTs evaluating TKIs had up to 4 unmet eligibility criteria. For RCTs evaluating ICIs the maximum number of unmet criteria was 6, with the exception of the nivolumab trials, for which the maximum number of unmet criteria was 5.

Table 2: proportion of lung cancer patients in CPRD GOLD eligible for enrolment in twelve randomized controlled trials of new treatment for non-small cell lung cancer and the number of unmet criteria when ineligible.

Name of trial	Eligible (%)		Number of unmet eligibility criteria (N and %)					
		Range	1	2	3	4	5	6
Osimertinib AURA3 [10]	78.1%	1 – 4	1758 (19.0)	233 (2.5)	29 (0.3)	3 (0.0)	-	-
Osimertinib FLAURA [16]	72.4% (61.1)	1 – 4	2166 (23.4)	340 (3.7)	42 (0.5)	5 (0.1)	-	-
Alectinib ALEX [13]	73.7% (61.3)	1 – 4	2129 (23.0)	277 (3.0)	20 (0.2)	1 (0.0)	-	-
Alectinib J-ALEX [9]	73.7% (61.3)	1 – 4	2129 (23.0)	277 (3.0)	20 (0.2)	1 (0.0)	-	-
Alectinib ALUR [11]	73.6% (61.2)	1 – 4	2132 (23.1)	280 (3.0)	22 (0.2)	1 (0.0)	-	-
Nivolumab CheckMate 017 [7]	53.9% (45.6)	1 – 5	2916 (31.6)	1059 (11.5)	229 (2.5)	44 (0.5)	10 (0.1)	-
Nivolumab CheckMate 057 [6]	54.3% (45.9)	1 – 5	2906 (31.5)	1045 (11.3)	224 (2.4)	39 (0.4)	9 (0.1)	-
Pembrolizumab KEYNOTE-024 [14]	49.1% (40.0)	1 – 6	3206 (34.7)	1120 (12.1)	285 (3.1)	67 (0.7)	19 (0.2)	4 (0.0)
Pembrolizumab KEYNOTE-189 [8]	52.2% (42.5)	1 – 6	3176 (34.4)	995 (10.8)	199 (2.2)	41 (0.4)	6 (0.1)	1 (0.0)
Pembrolizumab KEYNOTE-407 [12]	50.0% (40.7)	1 – 6	3229 (34.9)	1084 (11.7)	248 (2.7)	49 (0.5)	11 (0.1)	1 (0.0)
Durvalumab PACIFIC [5]	53.0% (43.1)	1 – 5	3137 (34.0)	942 (10.2)	218 (2.4)	45 (0.5)	3 (0.0)	-
Atezolizumab OAK [15]	50.7% (41.4)	1 – 6	3004 (32.5)	1142 (12.4)	327 (3.5)	63 (0.7)	16 (0.2)	2 (0.0)

Abbreviations: N = number, % = percentage.
Eligibility was based on the characteristics of lung cancer patients registered in the CPRD and compared with eligibility criteria used in clinical trials evaluating drugs used in the treatment of non-small cell lung cancer patients.

Table 3: hypothetical reason for exclusion of lung cancer patients in CPRD GOLD from the twelve clinical trials evaluating new drugs to treat non-small cell lung cancer patients.

Name of trial	Hypothetical reason for exclusion (%)							
	A	B	C	D	E	F	G	H
Osimertinib – AURA [10]	7.5	[a]	[a]	5.6	0.0	[a]	10.6	0.2
Osimertinib – FLAURA [16]	7.5	7.2	[a]	5.6	0.0	[a]	10.6	0.2
Alectinib – ALEX [13]	5.7	8.4	[a]	[a]	0.0	4.0	10.6	0.3
Alectinib – J-ALEX [9]	5.7	8.4	[a]	[a]	0.0	4.0	10.6	0.3
Alectinib – ALUR [11]	5.8	8.4	[a]	[a]	0.0	4.0	10.6	0.3
Nivolumab – CheckMate 017 [7]	6.2	7.2	11.4	[a]	1.5	4.0	26.7	0.2
Nivolumab – CheckMate 057 [6]	5.6	7.2	11.4	[a]	1.5	4.0	26.7	0.2
Pembrolizumab – KEYNOTE024 [14]	18.7	10.2	11.4	[a]	1.5	4.0	20.6	0.5
Pembrolizumab – KEYNOTE189 [8]	13.1	10.2	11.4	[a]	1.5	4.0	20.6	0.5
Pembrolizumab – KEYNOTE407 [12]	17.2	10.2	11.4	[a]	1.5	4.0	20.6	0.3
Durvalumab – PACIFIC [5	7.5	10.2	11.4	5.7	1.5	4.0	20.6	0.3
Atezolizumab – OAK [15]	7.4	10.2	11.4	6.1	1.5	[a]	26.7	0.3

Reason for exclusion summarized per set of criteria, in detail specified in Appendix B, with corresponding time-window of exposure for each criterion individually.
A = laboratory values; B = cancer-related ; C = immune related diseases; D = cardiovascular diseases; E = serious infections; F = psychiatric diseases ; G = concomitant drug-use ; H = other

[a] the corresponding set of criteria was not part of the exclusion criteria used for this specific study. Therefore, no lung cancer patients in CPRD would hypothetically be excluded because of this set of criteria.

Table 3 shows the proportion of lung cancer patients who would be excluded for each individual RCT´s set of in- and exclusion criteria. A history of malignancies or concomitant drug use were the most frequent criteria for potential exclusion. A history of a malignancy was applied as an exclusion criterion in all RCTs but one (AURA3) and led to exclusion of 7.2–10.2% of all patients in CPRD. In all RCTs, concomitant drug-use led to exclusion of > 10% of patients and was highest in the CheckMate 017 and 057 trials (26.7%). In addition, laboratory values, serious infections and other criteria were applied in all RCTs, which would also lead to considerable exclusion. The specific criteria per RCT are shown in Appendix E.

Table 4 and Appendix F show that mortality of lung cancer patients from CPRD was consistently lower for patients who would have been eligible for inclusion of the original RCTs versus patients who would have been ineligible. The age-sex adjusted HR varied between 0.74 (95% CI: 0.71 – 0.78; CheckMate 057 [6] to 0.85 (95% CI: 0.81 – 0.90; FLAURA [16])).

Table 4: hazard ratios for mortality of lung cancer patients in CPRD GOLD hypothetically eligible for study inclusion in twelve randomized controlled trials of new treatment for non-small cell lung cancer compared to hypothetically ineligible lung cancer patients in CPRD GOLD.

Trial	HR, unadjusted	95% CI	HR, age-sex adjusted	95% CI
Osimertinib – AURA3 [10]	0.75	0.71 – 0.80	0.79	0.74 – 0.84
Osimertinib – FLAURA [16]	0.83	0.78 – 0.87	0.85	0.81 – 0.90
Alectinib – ALEX [13]	0.83	0.79 – 0.88	0.84	0.80 – 0.89
Alectinib – J-ALEX [9]	0.83	0.79 – 0.88	0.84	0.80 – 0.89
Alectinib – ALUR [11]	0.83	0.79 – 0.88	0.84	0.79 – 0.89
Nivolumab – CheckMate 017 [7]	0.75	0.71 – 0.79	0.74	0.71 – 0.78
Nivolumab – CheckMate 057 [6]	0.75	0.71 – 0.78	0.74	0.71 – 0.78
Pembrolizumab – KEYNOTE-024 [14]	0.76	0.72 – 0.80	0.79	0.75 – 0.83
Pembrolizumab – KEYNOTE-189 [8]	0.76	0.72 – 0.80	0.78	0.74 – 0.82
Pembrolizumab – KEYNOTE-407 [12]	0.76	0.73 – 0.81	0.79	0.75 – 0.84
Durvalumab – PACIFIC [5]	0.77	0.73 – 0.81	0.79	0.75 – 0.84
Atezolizumab – OAK [15]	0.80	0.76 – 0.84	0.80	0.76 – 0.85

Abbreviations: HR = hazard ratio, CI = confidence interval.
HRs are calculated by comparing the mortality of eligible patients to the mortality of ineligible patients.

If the exclusion criterion for estimated creatinine clearance was relaxed to 10 mL per minute, for the studies in which immunotherapy were evaluated, the proportion of patients that would have been eligible increased. The increase was larger for the clinical trials which initially applied a stricter threshold value for the kidney function. The largest absolute increase was observed for the KEYNOTE-407 study as potential inclusion increased with 4.9% (from 50.0% to 54.9%) and varied from 0.4% to 4.9% for all immunotherapy trials.

DISCUSSION

We found that a considerable proportion of patients with lung cancer in a real-world setting would have been ineligible to participate in one of the phase III RCTs evaluating TKIs or ICIs from 2014 through 2018. Lung cancer patients would often be excluded based on 1 or 2 unmet eligibility criteria. Previous or concurrent malignancies, a decreased estimated glomerular filtration rate (eGFR) or concomitant systemic glucocorticoid use were the most frequent reasons for hypothetical exclusion. OS of real-world patients was considerably shorter among those who would have been ineligible for potential inclusion compared with those who would have been eligible.

The hypothetical study eligibility of patients with lung cancer in clinical practice has been evaluated previously for other treatments, such as chemotherapy, earlier generation TKIs or ICIs applied as second-line treatment [23 – 26]. Some studies used specific in- and exclusion criteria applied in a particular RCT [23, 24], while others used a more general, self-selected set of criteria, composed from more general criteria which are often used in RCTs [25, 26], such as the performance status (PS), the number of previous treatments and possible registered comorbidities. Hypothetical study inclusion for multiple chemotherapy RCTs and for RCTs evaluating TKIs or ICIs was generally below 50%, apart from the FLEX and NEXUS-studies [23, 24]. When the most stringent set of criteria were used in studies using self-selected criteria, only 30% of patients would have been eligible for potential participation [25, 26]. Although the exact proportion of patients that would have been eligible for RCT participation was higher in our study, a considerable proportion of patients would have been ineligible for hypothetical trial participation. Other studies concluded that PS was one of the most important reasons for patient exclusion [23 – 26]. PS is not registered in the CPRD and could therefore not be incorporated in our study. This could have led to a potential underestimation of the proportion of CPRD patients who would have been ineligible for RCT inclusion. A lower hypothetical study inclusion of patients in real-life has also been seen for other types of cancer [32 – 36]. In most of these studies a shorter progression-free survival and OS was reported for the patients who would have been ineligible [32 – 34, 36], which is in line with our findings.

A recent study evaluated the effect of broadening eligibility criteria for trial inclusion in NSCLC patients, which showed that considerably more patients could be safely included [37]. In the different clinical trials evaluating the efficacy of immunotherapy, varying threshold values were applied for estimated creatinine clearance, from 30 mL per minute till 60 mL per minute). As monoclonal antibodies (large protein structures) are not renally eliminated, it could be rationalized that immunotherapy can be given to patients with an impaired kidney function [38]. When we lowered the threshold value for the kidney function to 10 mL per minute the inclusion would increase up to 4.9% (KEYNOTE-407, from 50.0% to 54.9%). In the future, a more tailored set of criteria based on the (pharmacokinetic)

characteristics of the new drug could increase the number of potential patients eligible for study participation.

A strength of our study was the large number of included patients with a recording of lung cancer (N = 9,239). This number was considerably larger compared with other studies [23 – 26]. In addition, for each patient, an extensive medical history could be retrieved. Given the population-based nature of CPRD we believe that this is a reliable reflection of the patients who are diagnosed with lung cancer in the UK's clinical practice.

Several studies have previously evaluated the validity of the registration of cancer cases in CPRD. CPRD's lung cancer codes have a high degree of concordance with the cancer registry, as the agreement in lung cancer registration between CPRD and the national cancer registry of England is higher than 90% [30, 31]. However, all studies evaluating the concordance between CPRD, and the national cancer registry of England stated that a minority of patients will be missed when using solely CPRD data [30, 31, 39, 40]. In addition, a differential survival has been reported between patients registered in CPRD and patients registered in the cancer registry [39]. Patients who die shortly after their diagnosis are potentially less likely to be captured in CPRD. Given their medical inclusion. This could have caused an overestimation of the OS of the group of patients that would have been ineligible for RCT participation in our study. In addition, the classification of lung cancer patients in CPRD is lacking specific details. The RCTs included in our analyses solely evaluated drugs for the treatment of patients with NSCLC, whereas the Read codes used in CPRD do not differentiate between NSCLC and SCLC (Appendix B). Furthermore, information on disease stage is not available in CPRD. A proportion of patients in our cohort, therefore, do not match with the target patient population of the RCTs, as these focus on patients with locally advanced or metastatic NSCLC. However, in the UK the majority of patients with lung cancer (85%) are diagnosed with a non-small cell subtype [41] and approximately 75% are diagnosed with stage III or IV [4]. Therefore, the majority of patients registered in CPRD match with the target population of the pivotal RCTs. We believe that more detailed information on disease type and stage would have led to a decrease in the total number of included patients in our study as we were unable to differentiate between patients diagnosed with early-stage NSCLC and advanced NSCLC. Information about type and stage would have led to the exclusion of patients with an early-stage NSCLC. The inclusion of all patients with lung cancer could therefore have led to an overestimation of the proportion of patients that would have been eligible.

Another limitation of this study was possible misclassification of several in- and exclusion criteria. This could occur when a comorbidity or a laboratory value is not correctly registered or has changed over time without being properly updated. Only patients with a known registration of a comorbidity, deviant laboratory value or concomitant drug-use could be excluded. If no registration was available in CPRD it was assumed that the patient met the specific criteria. The effect of missing, or not-registered, data is unknown. However,

this could only have led to a higher proportion of patients being excluded, as all patients without information about a specific criterion were assumed to meet that specific criterion. Furthermore, clinical lab test values are not routinely collected in CPRD, whereas in RCTs, these are measured at baseline or during a screening period. In CPRD we selected a 3-month time-window of exposure to capture non-routinely collected lab-test values. If clinical lab test results that were not requested by the general practitioner but by consultants are being captured in CPRD is uncertain. Our choice for a three-month time-window was a trade-off between dealing with missing data and the assumption that the most recently recorded lab test value in the past 3 months would reflect baseline.

In all RCTs evaluating ICIs, patients were excluded when treated with ≥10 mg prednisone or an equivalent dose of another systemic glucocorticoid. We were not able to specify the prescribed daily dose of systemic glucocorticoids because this was only registered in 45% of all cases. Therefore, it was decided to exclude all patients with a glucocorticoid prescription within 3 months prior to the lung cancer diagnosis. As some patients will not have exceeded the threshold value of the equivalent glucocorticoid dosage, this approach may have led to erroneous exclusion of some patients. In addition, the prevalence of a chronic obstructive pulmonary disease (COPD) registration before the index date (lung cancer diagnosis) is almost 30% in this cohort, compared to 2% in the whole UK population. During a COPD exacerbation high dose glucocorticoids can be prescribed for a short period of time. The high number of COPD-patients in our cohort might explain the observed relatively high proportion of patients with a prescription for systemic glucocorticoid treatment. Furthermore, the situation in clinical practice is often more flexible than the data in a large database reflect. For instance, if a patient receives a systemic glucocorticoid or an antibiotic, which would be the only reason for exclusion, a physician could try to taper or stop the treatment with glucocorticoids, or select another antibiotic, which would make the patient eligible for clinical trial inclusion. This could have led to an underestimation of the eligibility rate of patients with lung cancer in the general population.

Although the limitations of various assumptions in our CPRD study may have led to uncertainties in the exact proportion of patients that would have been eligible, we believe that the analyses still give an insightful view on potential trial eligibility of patients with lung cancer in the general population. This study shows that a considerable proportion of patients in a real-world setting would have been ineligible for inclusion in RCTs evaluating TKIs or ICIs, and that OS was shorter for patients that would potentially have been ineligible for RCT participation compared with those that would have been eligible. Additional information about other criteria, such as PS, would lead to a higher degree of exclusion, while more specific information on drug-use, especially systemic glucocorticoids, would lead to the exclusion of a lower proportion of patients. Given the previously described efficacy-effectiveness gap for chemotherapy used in patients with stage IV NSCLC, further research is needed to determine the actual effectiveness of the evaluated TKIs and ICIs [27],

as a large part of the patients in clinical practice is not well represented by the patients in pivotal phase III RCTs.

CONCLUSION

This study showed that a considerable proportion of patients, diagnosed with lung cancer between 2014 and 2018 in a real-world setting, would have been ineligible for inclusion in phase III RCTs evaluating TKIs or ICIs for the treatment of stage III/IV NSCLC patients. OS of patients who would have been ineligible for inclusion in these RCTs was considerably shorter compared with patients that would have been eligible.

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APPENDIX A

Table A1: list of included randomized clinical trials evaluating new drugs to treat patients with non-small cell lung cancer published between 2014 – 2018.

Type of drug	Drug	Trial-name	NSCLC stage	Line of treatment	Date of publication
Tyrosine kinase inhibitors	Osimertinib	AURA3 [10]	IIIB/IV	Second	06-12-2016
	Osimertinib	FLAURA [16]	IIIB/IV	First	18-11-2017
	Alectinib	ALEX [13]	IIIB/IV	First	06-06-2017
	Alectinib	J-ALEX [9]	IIIB/IV/ recurrent	First	10-05-2017
	Alectinib	ALUR [11]	IIIB/IV	Third	14-04-2018
Immune checkpoint inhibitors	Nivolumab	CheckMate 017 [7]	IIIB/IV	First/second	31-05-2015
	Nivolumab	CheckMate 057 [6]	IIIB/IV	Second	27-09-2015
	Pembrolizumab	KEYNOTE-024 [14]	IV	First	09-10-2016
	Pembrolizumab	KEYNOTE-189 [8]	IV	First	16-04-2018
	Pembrolizumab	KEYNOTE-407 [12]	IV	First	25-09-2018
	Durvalumab	PACIFIC [5]	III	Adjuvant	13-12-2018
	Atezolizumab	OAK [15]	IIIB/IV	Second or third	12-12-2016

APPENDIX B

Med Code	Read Code	Read term
2587	B22z.11	Lung cancer
3903	B22z.00	Malignant neoplasm of bronchus or lung NOS.
13243	B22..00	Malignant neoplasm of trachea, bronchus and lung.
25886	B222100	Malignant neoplasm or upper lobe of lung.
12870	B221.00	Malignant neoplasm of main bronchus.
10358	B222.00	Malignant neoplasm of upper lobe, bronchus or lung.
12582	B224100	Malignant neoplasm of lower lobe of lung.
31188	B224.00	Malignant neoplasm of lower lobe, bronchus or lung.
21698	B221z00	Malignant neoplasm of main bronchus NOS.
31700	B222000	Malignant neoplasm of upper lobe bronchus.
38961	B22y.00	Malignant neoplasm of other sites of bronchus or lung.
33444	B221100	Malignant neoplasm of hilus or lung.
39923	B223100	Malignant neoplasm of middle lobe of lung.
31268	B223.00	Malignant neoplasm of middle lobe, bronchus or lung.
44169	B222z00	Malignant neoplasm of upper lobe, bronchus or lung NOS.
18678	B224000	Malignant neoplasm of lower lobe bronchus.
15221	B220.00	Malignant neoplasm of trachea.
42566	B224z00	Malignant neoplasm of lower lobe, bronchus or lung NOS.
17391	B221000	Malignant neoplasm of carina or bronchus.
36371	B225.00	Malignant neoplasm of overlapping lesion of bronchus & lung.
41523	B223000	Malignant neoplasm of middle lobe bronchus.
54134	B223z00	Malignant neoplasm of middle lobe, bronchus or lung NOS.
37810	B220z00	Malignant neoplasm of trachea NOS.
103946	B220100	Malignant neoplasm of mucosa of trachea.

APPENDIX C

Table C1: the subdivision of all in- and exclusion criteria in eight different sets and the corresponding time-window of exposure for each criterion.

Criterion	Time-window of exposure
Laboratory values	
AP	Three months prior to index date
ALAT	Three months prior to index date
ASAT	Three months prior to index date
eGFR	Three months prior to index date
Hemoglobin	Three months prior to index date
International normalized ratio	Three months prior to index date
Lymphocytes	Three months prior to index date
Neutrophils	Three months prior to index date
White blood cells	Three months prior to index date
Platelets	Three months prior to index date
Total bilirubin	Three months prior to index date
Thyroid stimulation hormone	Three months prior to index date
Cancer related	
History of cancer [a]	Two/three/five years prior to index date [a]
Immune related disease	
Vasculitis	Ever before index date
Coeliac disease	Ever before index date
Crohn's disease	Ever before index date
Ulcerative colitis	Ever before index date
Grave's disease	Ever before index date
Multiple sclerosis	Ever before index date
Myasthenia gravis	Ever before index date
Ankylosing spondylitis	Ever before index date
Dermatomyositis	Ever before index date
Polymyalgia rheumatica	Ever before index date
Psoriatic arthritis	Ever before index date
Rheumatoid arthritis	Ever before index date
Psoriasis	Ever before index date
Sarcoidosis	Ever before index date
Systemic lupus erythematous	Ever before index date
Cardiovascular disease	
Heart failure	Ever before index date
Heart rhythm disturbances [b]	Ever before index date
Myocardial infarction	Three months prior to index date
Poor controlled hypertension	Three months prior to index date
Unstable angina pectoris	Three months prior to index date

Serious infections	
Meningitis	One month prior to index date
Pneumonia	One month prior to index date
Sepsis	One month prior to index date
Hepatitis	One year prior to index date
Psychiatric disease	
Bipolar mood disorder	Ever before index date
Dementia	Ever before index date
Schizophrenia	Ever before index date
Drugs	
Systemic treatment with strong CYP3A4-inhibitors [c]	Three months prior to index date
Systemic treatment with glucocorticoids [d]	Three months prior to index date
Systemic treatment with immunosuppressants [e]	Three months prior to index date
Other	
AIDS/HIV	Ever before index date
Organ transplant [f]	Ever before index date
Pregnancy	One year before index date
Substance abuse	Five years before index date

Abbreviations: AP = Alkaline phosphatase, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, eGFR = estimated glomerular filtration rate, CYP = cytochrome P450, AIDS = acquired immune deficiency syndrome, hiv = human immunodeficiency virus.

[a] in the twelve clinical trials different requirements were used for the history of other cancer types, and varied between two, three or five years before index date. The specific time period used for each study is shown in Appendix 3.
[b] for heart rhythm disturbances three specific conditions were used: complete left bundle branch block, second degree heart block and third-degree heart block.
[c] for systemic treatment with strong CYP3A4-inhibitors six drugs were included: erythromycin, clarithromycin, itraconazole, ketoconazole, ritonavir and voriconazole (all systemic).
[d] for systemic treatment with glucocorticoids six drugs were included: dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone.
[e] for systemic treatment with immunosuppressants the following drugs were included: ciclosporin, everolimus, sirolimus and tacrolimus (all systemic).
[f] for organ transplant four specific transplantations were used: heart, lung, kidney, liver.

APPENDIX

Reference value of laboratory values used as in- and exclusion criteria. [2]

Table D1.1: reference values for laboratory values using a normal value or upper limit of normal.

Laboratory value	Normal value
AP	< 120 U/L
ALAT	< 45 U/L (men) < 35 U/L (women)
ASAT	< 35 U/L (men) < 30 U/L (women)
Total bilirubin	3 – 21 umol/L
TSH	0.35 – 5.00 mU/L

AP = alkaline phosphatase, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, TSH = thyroid stimulating hormone, U = unit, L = litre, u = micro (10^{-6}), m = milli (10^{-3}).

Table D1.2: calculated threshold values based on reference values.

Laboratory value	Threshold value
AP	< 2.5 × ULN = < 300 U/L
$ALAT_1$	< 1.5 × ULN = 67.5 U/L (men) < 1.5 × ULN = 52.5 U/L (women)
$ALAT_2$	< 2.5 × ULN = 112.5 U/L (men) < 2.5 × ULN = 87.5 U/L (women)
$ALAT_3$	< 3.0 × ULN = 135.0 U/L (men) < 3.0 × ULN = 105.0 U/L (women)
$ASAT_1$	< 1.5 × ULN = 52.5 U/L (men) < 1.5 × ULN = 45.0 U/L (women)
$ASAT_2$	< 2.5 × ULN = 87.5 U/L (men) < 2.5 × ULN = 75.0 U/L (women)
$ASAT_3$	< 3.0 × ULN = 105.0 U/L (men) < 3.0 × ULN = 90.0 U/L (women)
Total $bilirubin_1$	< ULN = 21 umol/L
Total $bilirubin_2$	< 1.5 × ULN = 31.5 umol/L
TSH	within normal limits = 0.35 – 5.00 mU/L

AP = alkaline phosphatase, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, TSH = thyroid stimulating hormone, ULN = upper limit of normal, U = unit, L = litre, u = micro (10^{-6}), m = milli (10^{-3}).

APPENDIX E

List of all exclusion criteria used by the different clinical trials included in this study. [2]

Table E5.1: exclusion criteria used in AURA3-study.

Criterion	Used cut-off
Age	< 18 years
eGFR	< 50 mL/min
ALAT	> 2.5 × ULN
ASAT	> 2.5 × ULN
Total bilirubin	> 1.5 × ULN
Neutrophils	< 1.5 × 10^9/L
Platelets	< 100 × 10^9/L
Hemoglobin	< 90 g/L
Active serious infection	
Hearth rhythm disturbances	
Heart failure	
Uncontrolled hypertension	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors.	

Abbreviations: eGFR = estimated glomerular filtration rate, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, AIDS = acquired immunodeficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ml = millilitre, min = minute, ULN = upper limit of normal, L = litre, g = gram.

Table E5.2: exclusion criteria used in FLAURA-study.

Criterion	Used cut-off
Age	< 18 years
eGFR	< 50 mL/min
ALAT	> 2.5 × ULN
ASAT	> 2.5 × ULN
Total bilirubin	> 1.5 × ULN
Neutrophils	< 1.5 × 10^9/L
Platelets	< 100 × 10^9/L
Haemoglobin	< 90 g/L
Previous malignancy in two years prior to index date	
Active serious infection	
Hearth rhythm disturbances	
Heart failure	
Uncontrolled hypertension	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors.	

Abbreviations: eGFR = estimated glomerular filtration rate, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, AIDS = acquired immunodeficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ml = millilitre, min = minute, ULN = upper limit of normal, L = litre, g = gram

Table E5.3: exclusion criteria used in ALEX-study.

Criterion	Used cut-off
Age	< 18 years
eGFR	< 45 mL/min
ALAT	> 3.0 × ULN
ASAT	> 3.0 × ULN
Neutrophils	< 1.5 × 10^9/L
Platelets	< 100 × 10^9/L
Haemoglobin	< 90 g/L
Previous malignancy in three years prior to index date	
Hepatitis	
History of organ transplant	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors.	

Abbreviations: eGFR = estimated glomerular filtration rate, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, AIDS = acquired immune deficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ml = millilitre, min = minute, ULN = upper limit of normal, L = litre, g = gram.

Table E5.4: exclusion criteria used in J-ALEX-study.

Criterion	Used cut-off
Age	< 18 years
eGFR	< 45 mL/min
ALAT	> 3.0 × ULN
ASAT	> 3.0 × ULN
Neutrophils	< 1.5 × 10^9/L
Platelets	< 100 × 10^9/L
Haemoglobin	< 90 g/L
Previous malignancy in three years prior to index date	
Hepatitis	
History of organ transplant	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors.	

Abbreviations: eGFR = estimated glomerular filtration rate, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, AIDS = acquired immune deficiency syndrome, hiv = human immunodeficiency viruses, CYP = Cytochrome P450, ml = millilitre, min = minute, ULN = upper limit of normal, L = litre, g = gram.

Table E5.5: exclusion criteria used in ALUR-study.

Criterion	Used cut-off
Age	< 18 years
eGFR	< 45 mL/min
ALAT	> 3.0 × ULN
ASAT	> 3.0 × ULN
Neutrophils	< 1.5×10^9/L
Platelets	< 100×10^9/L
Haemoglobin	< 90 g/L
Previous malignancy in three years prior to index date	
Hepatitis	
History of organ transplant	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors.	

Abbreviations: eGFR = estimated glomerular filtration rate, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, AIDS = acquired immune deficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ml = millilitre, min = minute, ULN = upper limit of normal, L = litre, g = gram.

Table E5.6: exclusion criteria used in CheckMate 017-study.

Criterion	Used cut-off
Age	< 18 years
eGFR	< 40 mL/min
ALAT	> 1.5 × ULN
ASAT	> 1.5 × ULN
Total bilirubin	> ULN
Neutrophils	< 1.5 × 10^9/L
White blood cells	< 2.0 × 10^9/L
Platelets	< 100 × 10^9/L
Haemoglobin	< 90 g/L
Previous malignancy in two years prior to index date	
Active serious infection	
Active auto-immune disease	
Psychiatric condition	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors.	
Systemic treatment with glucocorticoids	
Systemic treatment with immunosuppressive drugs	

Abbreviations: eGFR = estimated glomerular filtration rate, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, AIDS = acquired immune deficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ml = millilitre, min = minute, ULN = upper limit of normal, L = litre, g = gram.

Table E5.7: exclusion criteria used in CheckMate 057-study.

Criterion	Used cut-off
Age	< 18 years
eGFR	< 40 mL/min
ALAT	> 1.5 × ULN
ASAT	> 1.5 × ULN
Total bilirubin	> 1.5 × ULN
Neutrophils	< 1.5 × 10^9/L
White blood cells	< 2.0 × 10^9/L
Platelets	< 100 × 10^9/L
Haemoglobin	< 90 g/L
Previous malignancy in two years prior to index date	
Active serious infection	
Active auto-immune disease	
Psychiatric condition	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors.	
Systemic treatment with glucocorticoids	
Systemic treatment with immunosuppressive drugs	

Abbreviations: eGFR = estimated glomerular filtration rate, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, AIDS = acquired immune deficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ml = millilitre, min = minute, ULN = upper limit of normal, L = litre, g = gram.

Table E5.8: exclusion criteria used in KEYNOTE-024 study

Criterion	Used cut-off
Age	< 18 years
eGFR	< 60 mL/min
AP	> 2.5 × ULN
ALAT	> 1.5 × ULN
ASAT	> 1.5 × ULN
Total bilirubin	> ULN
Neutrophils	< 1.5 × 10^9/L
Platelets	< 100 × 10^9/L
Haemoglobin	< 90 g/L
INR (unless anticoagulation)	> 1.5 × ULN
Previous malignancy in two years prior to index date	
Active serious infection	
Active auto-immune disease	
History of organ transplant	
Psychiatric condition	
Substance abuse	
AIDS/HIV	
Pregnancy	
Systemic treatment with glucocorticoids	
Systemic treatment with immunosuppressive drugs	

Abbreviations: eGFR = estimated glomerular filtration rate, AP = alkaline phosphatase, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, INR = international normalized ratio, AIDS = acquired immune deficiency syndrome, HIV = human immunodeficiency viruses, ml = millilitre, min = minute, ULN = upper limit of normal, L = litre, g = gram.

Table E1.9: exclusion criteria used in KEYNOTE-189 study.

Criterion	Used cut-off
Age	< 18 years
eGFR	< 50 mL/min
ALAT	> 2.5 × ULN
ASAT	> 2.5 × ULN
Total bilirubin	> 1.5 × ULN
Neutrophils	< 1.5 × 10^9/L
Platelets	< 100 × 10^9/L
Haemoglobin	< 90 g/L
INR (unless anticoagulation)	> 1.5 × ULN
TSH	Normal
Previous malignancy in five years prior to index date	
Active serious infection	
Active auto-immune disease	
Psychiatric condition	
Substance abuse	
AIDS/HIV	
Pregnancy	
Systemic treatment with glucocorticoids	
Systemic treatment with immunosuppressive drugs	

Abbreviations: eGFR = estimated glomerular filtration rate, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, INR = international normalized ratio, TSH = thyroid stimulating hormone, AIDS = acquired immune deficiency syndrome, HIV = human immunodeficiency viruses, CYP = cytochrome P450, ml = millilitre, min = minute, ULN = upper limit of normal, L = litre, g = gram.

Table E1.10: exclusion criteria used in KEYNOTE-407 study.

Criterion	Used cut-off
Age	< 18 years
eGFR	< 60 mL/min
ALAT	> 2.5 × ULN
ASAT	> 2.5 × ULN
Total bilirubin	> 1.5 × ULN
Neutrophils	< 1.5 × 10^9/L
Platelets	< 100 × 10^9/L
Haemoglobin	< 90 g/L
INR (unless anticoagulation)	> 1.5 × ULN
Previous malignancy in five years prior to index date	
Active serious infection	
Active auto-immune disease	
Psychiatric condition	
Substance abuse	
AIDS/HIV	
Pregnancy	
Systemic treatment with glucocorticoids	
Systemic treatment with immunosuppressive drugs	

Abbreviations: eGFR = estimated glomerular filtration rate, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, INR = international normalized ratio, AIDS = acquired immune deficiency syndrome, HIV = human immunodeficiency viruses, ml = millilitre, min = minute, ULN = upper limit of normal, L = litre, g = gram.

Table E1.11: exclusion criteria used in PACIFIC study.

Criterion	Used cut-off
Age	< 18 years
eGFR	< 50 mL/min
ALAT	> 2.5 × ULN
ASAT	> 2.5 × ULN
Total bilirubin	> 1.5 × ULN
Neutrophils	< 1.5 × 10^9/L
Platelets	< 100 × 10^9/L
Haemoglobin	< 90 g/L
Previous malignancy in five years prior to index date	
Active serious infection	
Active auto-immune disease	
History of organ transplant	
Heart failure	
Heart rhythm disturbances	
Myocardial infarction	
Unstable angina pectoris	
Psychiatric condition	
AIDS/HIV	
Pregnancy	
Systemic treatment with glucocorticoids	
Systemic treatment with immunosuppressive drugs	

Abbreviations: eGFR = estimated glomerular filtration rate, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, AIDS = acquired immune deficiency syndrome, HIV = human immunodeficiency viruses, ml = millilitre, min = minute, ULN = upper limit of normal, L = litre, g = gram.

Table E1.12: exclusion criteria used in OAK study.

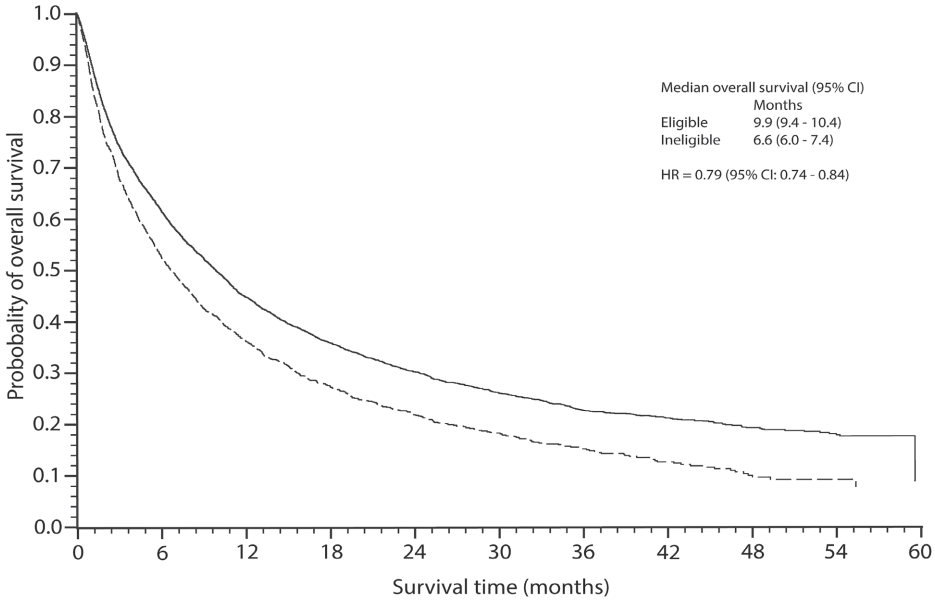
Criterion	Used cut-off
Age	< 18 years
eGFR	< 30 mL/min
ALAT	> 2.5 × ULN
ASAT	> 2.5 × ULN
Total bilirubin	> ULN
Neutrophils	< 1.5 × 10^9/L
Lymphocyte	< 0.5 × 10^9/L
White blood cells	< 2.5 × 10^9/L
Platelets	< 100 × 10^9/L
Haemoglobin	< 90 g/L
INR (unless anticoagulation)	> 1.5 × ULN
Previous malignancy in five years prior to index date	
Active serious infection	
Active auto-immune disease	
History of organ transplant	
Heart failure	
Heart rhythm disturbances	
Myocardial infarction	
Unstable angina pectoris	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors.	
Systemic treatment with glucocorticoids	
Systemic treatment with immunosuppressive drugs	

Abbreviations: eGFR = estimated glomerular filtration rate, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, INR = international normalized ratio, AIDS = acquired immune deficiency syndrome, HIV = human immunodeficiency viruses, CYP = cytochrome P450, mL = millilitre, min = minute, ULN = upper limit of normal, L = litre, g = gram.

APPENDIX F

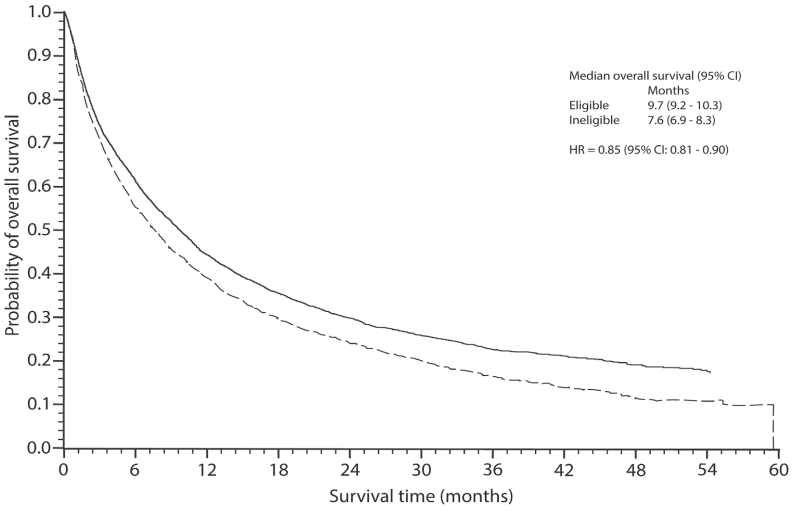
Overall survival of lung cancer patients in CPRD GOLD eligible for trial inclusion versus ineligible patients. [2]

Figure F1.1: Overall survival of lung cancer patients in CPRD GOLD eligible for inclusion in the AURA-trial (osimertinib) [10] versus ineligible patients.



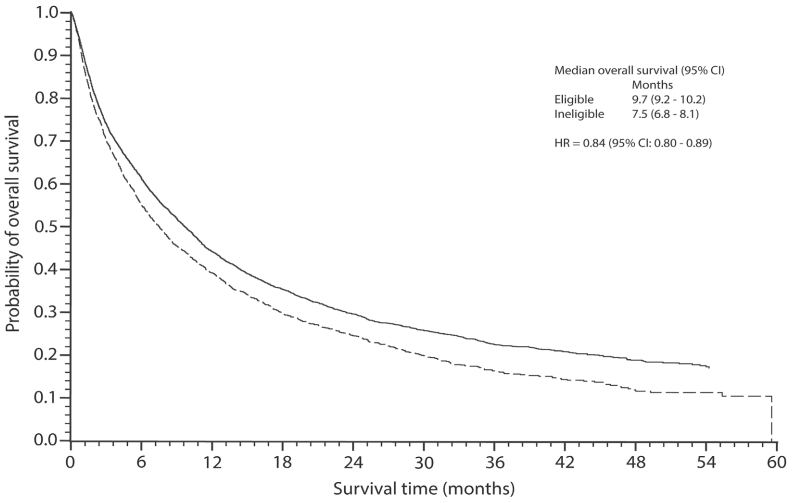
Legend: Solid line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **eligible** to participate in the AURA-trial (osimertinib) [10]. Dashed line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **ineligible** to participate in the AURA-trial (osimertinib) [10].

Figure F1.2: Overall survival of lung cancer patients in CPRD GOLD between 2014 and 2018 eligible for FLAURA-trial (osimertinib) [16] inclusion versus ineligible patients.



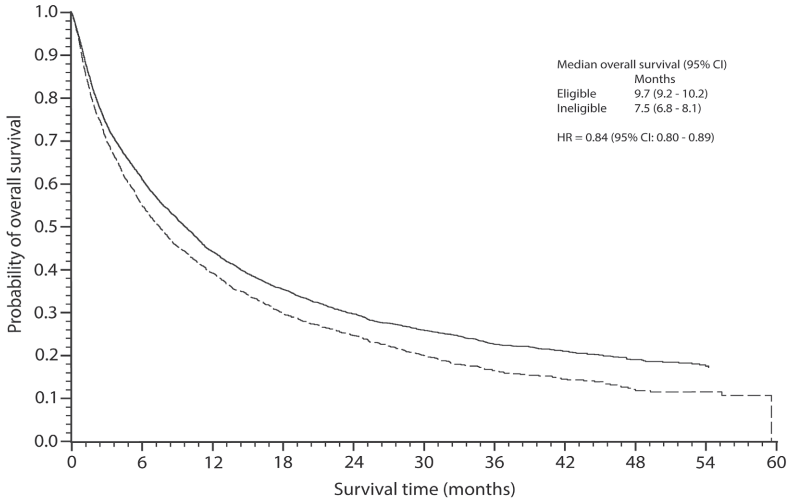
Legend: Solid line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **eligible** to participate in the FLAURA-trial (osimertinib) [16]. Dashed line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **ineligible** to participate in the FLAURA-trial (osimertinib)[16].

Figure F1.3: Overall survival of lung cancer patients in CPRD GOLD between 2014 and 2018 eligible for ALEX-trial (alectinib) [13] inclusion versus ineligible patients.



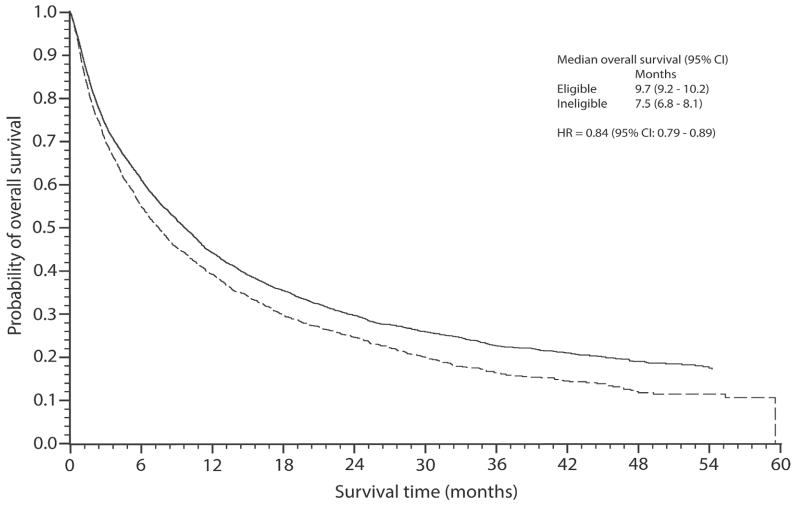
Legend: Solid line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **eligible** to participate in the ALEX-trial (alectinib) [13]. Dashed line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **ineligible** to participate in the ALEX-trial (alectinib) [13].

Figure F1.4: Overall survival of lung cancer patients in CPRD GOLD between 2014 and 2018 eligible for J-ALEX-trial (alectinib) [9] inclusion versus ineligible patients.



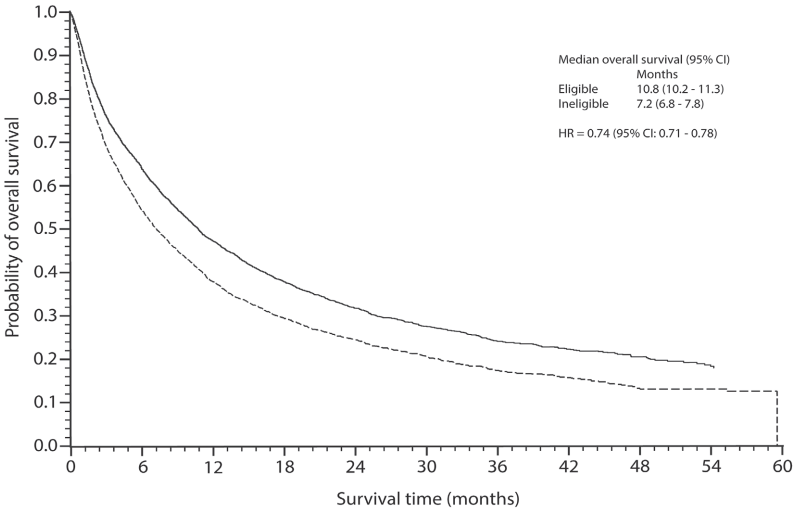
Legend: Solid line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **eligible** to participate in the J-ALEX-trial (alectinib) [9]. Dashed line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **ineligible** to participate in the J-ALEX trial (alectinib) [9].

Figure F1.5: Overall survival of lung cancer patients in CPRD GOLD between 2014 and 2018 eligible for ALUR-trial (alectinib) [11] inclusion versus ineligible patients.



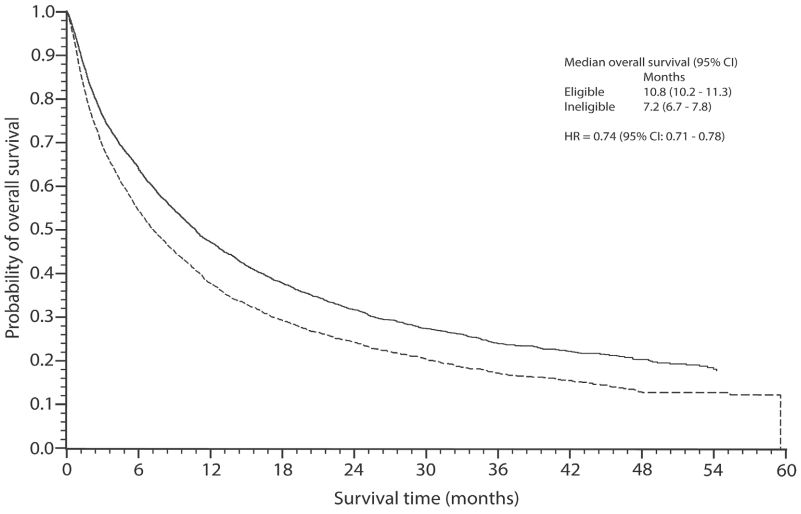
Legend: Solid line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **eligible** to participate in the ALUR-trial (alectinib) [11]. Dashed line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **ineligible** to participate in the ALUR-trial (alectinib) [11].

Figure F1.6: Overall survival of lung cancer patients in CPRD GOLD between 2014 and 2018 eligible for CheckMate 017-trial (nivolumab) [7] inclusion versus ineligible patients.



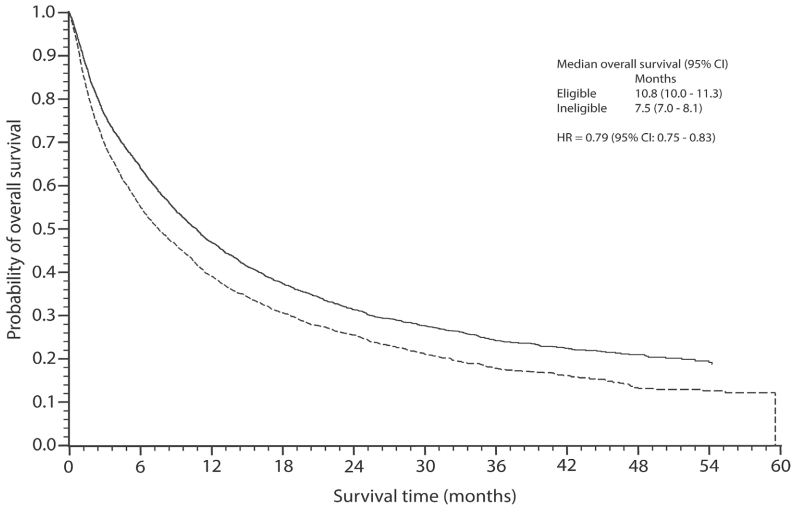
Legend: Solid line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **eligible** to participate in the CheckMate 017-trial (nivolumab) [7]. Dashed line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **ineligible** to participate in the CheckMate 017-trial (alectinib) [7].

Figure F1.7: Overall survival of lung cancer patients in CPRD GOLD between 2014 and 2018 eligible for CheckMate 057-trial (nivolumab) [6] inclusion versus ineligible patients.



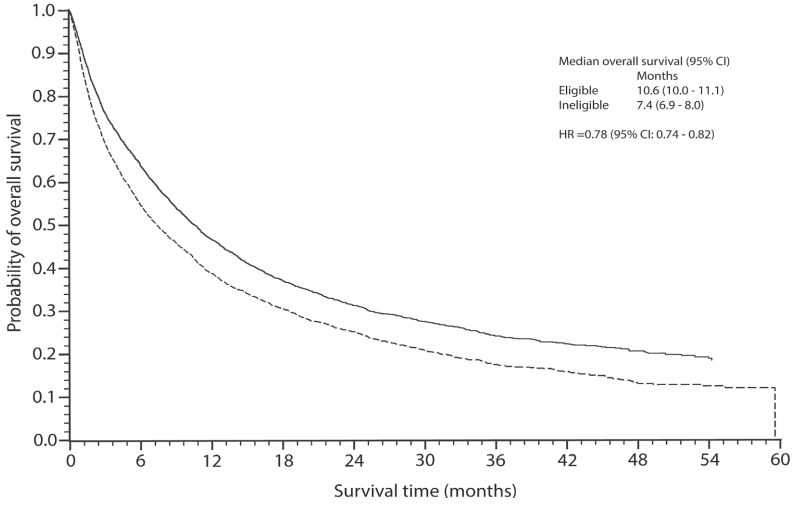
Legend: Solid line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **eligible** to participate in the CheckMate 057-trial (nivolumab) [6]. Dashed line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **ineligible** to participate in the CheckMate 017-trial (nivolumab) [6].

Figure F1.8: Overall survival of lung cancer patients in CPRD GOLD between 2014 and 2018 eligible for KEYNOTE-024-trial (pembrolizumab) [14] inclusion versus ineligible patients.



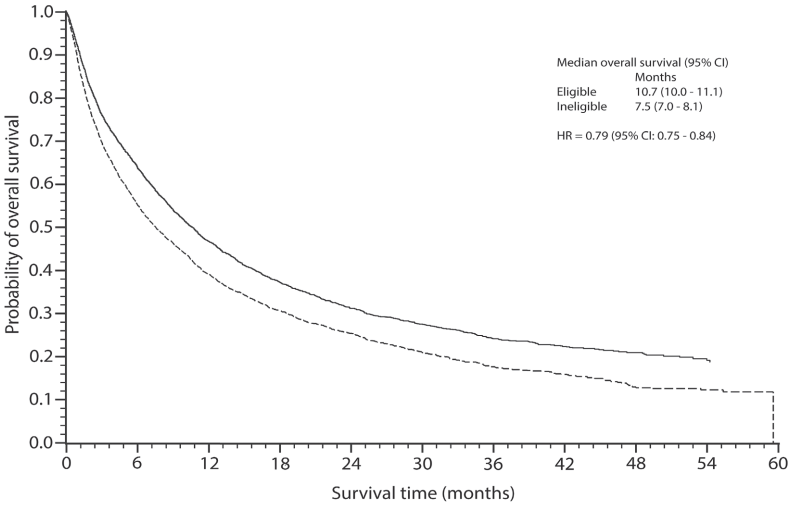
Legend: Solid line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **eligible** to participate in the KEYNOTE-024-trial (pembrolizumab) [14]. Dashed line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **ineligible** to participate in the KEYNOTE-024-trial (pembrolizumab) [14].

Figure F1.9: Overall survival of lung cancer patients in CPRD GOLD between 2014 and 2018 eligible for KEYNOTE-189-trial (pembrolizumab) [8] inclusion versus ineligible patients.



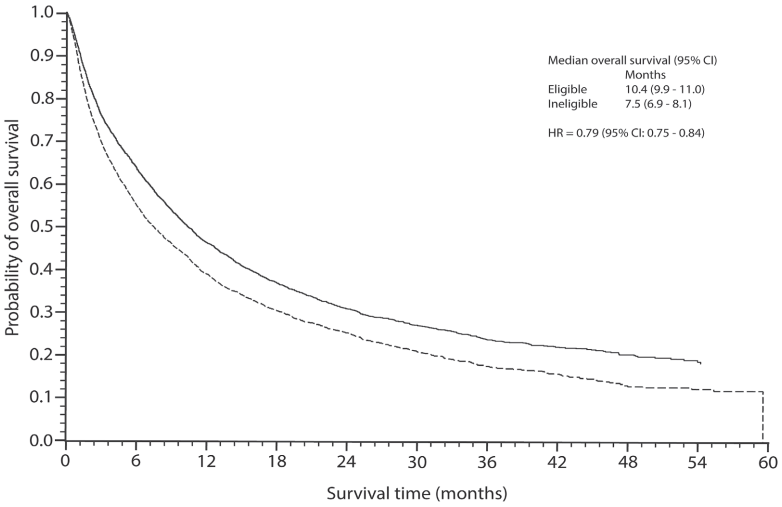
Legend: Solid line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **eligible** to participate in the KEYNOTE-189-trial (pembrolizumab) [8]. Dashed line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **ineligible** to participate in the KEYNOTE-189-trial (pembrolizumab) [8].

Figure F1.10: Overall survival of lung cancer patients in CPRD GOLD between 2014 and 2018 eligible for KEYNOTE-407-trial (pembrolizumab) [12] inclusion versus ineligible patients.



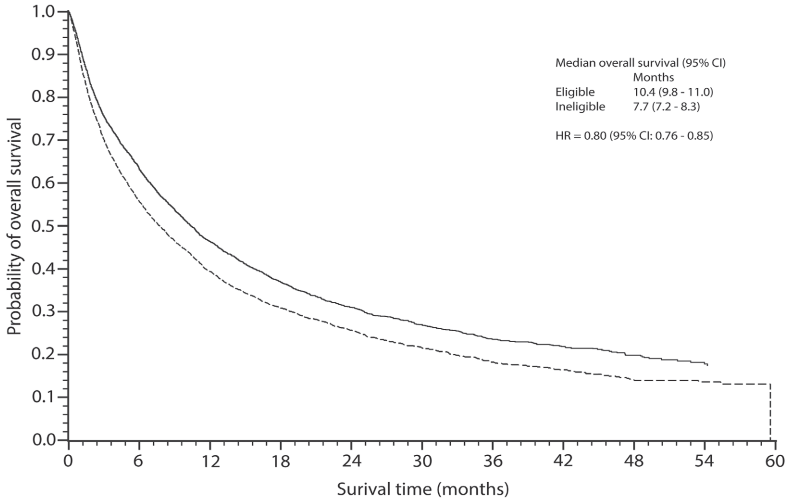
Legend: Solid line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **eligible** to participate in the KEYNOTE-407-trial (pembrolizumab) [12]. Dashed line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **ineligible** to participate in the KEYNOTE-407-trial (pembrolizumab) [12].

Figure F1.11: Overall survival of lung cancer patients in CPRD GOLD between 2014 and 2018 eligible for PACIFIC-trial (durvalumab) [5] inclusion versus ineligible patients.



Legend: Solid line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **eligible** to participate in the PACIFIC-trial (durvalumab) [5]. Dashed line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **ineligible** to participate in the PACIFIC-trial (durvalumab) [5].

Figure F1.12: Overall survival of lung cancer patients in CPRD GOLD between 2014 and 2018 eligible for OAK-trial (atezolizumab) [15] inclusion versus ineligible patients.



Legend: Solid line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **eligible** to participate in the OAK-trial (atezolizumab) [15]. Dashed line: Patients enrolled in CPRD GOLD (2014-2018) with lung cancer who were **ineligible** to participate in the OAK-trial (atezolizumab) [15].

Chapter 4.2

Comparison of characteristics of patients with lung cancer in UK primary care databases; Clinical Practice Research Datalink Aurum and GOLD.

J. Gulikers, A. van Veelen, J.H.M. Driessen, P.C. Souverein, V.C.G. Tjan - Heijnen, L.E.L. Hendriks, R.M.J.M. van Geel, S. Croes.

Submitted

ABSTRACT

Introduction: In recent years, the number of general practices contributing to the Clinical Practice Research Datalink (CPRD) database GOLD is decreasing. Therefore, for research questions addressing for instance novel treatments requiring up-to-date data, sample size will become an important consideration in study feasibility. In recent years, CPRD Aurum, a collection of general practices using EMIS software, has become an additional data source that is being used for CPRD studies. In order to establish whether Aurum is suited to act as data source for future studies in the field of lung cancer research, we aimed to compare characteristics between patients with lung cancer in Aurum and GOLD.

Methods: A retrospective study was performed comparing characteristics and overall survival (OS) of patients with lung cancer in Aurum and GOLD. To further evaluate similarity, hypothetical eligibility of these patients in Aurum and GOLD was compared for eleven randomized clinical trials (RCTs).

Results: Baseline characteristics registered in Aurum and GOLD were largely similar, with some clinically irrelevant differences for previous malignancies, deviant laboratory values and drug use. Median OS was 9.8 and 9.0 months for patients in Aurum and GOLD, respectively. Potential RCT eligibility varied between 49.4–79.5% and 49.1–78.1% for patients in Aurum and GOLD, respectively. Mortality rates and the comparison of the obtained HRs per hypothetical eligibility cohort per RCT were similar in Aurum and GOLD.

Conclusion: This study showed that data of patients with lung cancer in Aurum and GOLD are largely comparable, suggesting that Aurum is suitable for future epidemiological lung cancer research.

INTRODUCTION

The clinical practice research datalink (CPRD) collects electronic health data from general practitioner (GP) practices around the United Kingdom and is extensively used in observational studies. CPRD GOLD contains information of GP practices located in England, Wales, Scotland and Northern Ireland from 1987 onwards [1]. It includes primary care data from over 20.8 million patients as of February 2022, with an active patient population of approximately 3.1 million patients (14.9% of the total UK population) [2]. GOLD is considered a well-established database containing data of high quality and is widely used in medical research. However, due to a decreasing number of GPs using Vision software, the number of practices contributing to GOLD is decreasing. In 2013, 674 (8.3%) out of all GP practices in the UK were contributing to GOLD, but this has declined to 401 GP practices (4.9%) in May 2022 [1, 2]. Furthermore, the distribution of actively contributing GP practices has also changed over time, as the majority (84.0%) is now located in Scotland and Wales, while only a minority of GP practices (5.7%) is located in England. In 2017, CPRD introduced a new database, CPRD Aurum. Aurum collects data from practices using EMIS software and contains information on GP practices mainly located in England from 1995 onwards. In total, 1,358 GP practices are currently contributing to Aurum, which equals 16.6% of all GP practices in the UK. As of now, Aurum contains records from 40.9 million patients of which 13.4 million patients (32.8%) are currently actively enrolled in a participating practice [3, 4]. Data from both Aurum and GOLD can be linked to other databases in order to supplement primary care data with detailed information from hospitals (Hospital Episode Statistics [HES]) or to the National Cancer Registration and Analysis Service (NCRAS) to gain insight in cancer related topics such as tumour diagnosis and anti-cancer treatments [5-7].

Since a decreasing amount of GP practices is contributing to GOLD, GOLD will become less suitable to use in future observational cohort studies. Clinical research questions addressing novel treatments require up-to-date data and since a decreasing amount of GP practices is contributing to GOLD, the sample size needed for these studies will be become an recurring issue. On the other hand, since the number of GP practices contributing to Aurum is increasing, this will be more suited to study novel treatments. However, while there are many years of experience with using CPRD GOLD as a reliable database, with numerous studies reporting on data quality, less is known about the Aurum database. Therefore, evaluating the concordance of data registered in Aurum compared to GOLD in a time period in which GOLD was still in use by many practices will be of added value, before starting to use Aurum as primary study database, or to initiate subsequent lung cancer research with linkage to secondary databases. Since the release of Aurum, a few studies have addressed data similarity between Aurum and GOLD [8-11]. However, a population with a diagnosis of cancer has not been compared yet.

In this light, we evaluated baseline characteristics and overall survival (OS) of patients with lung cancer registered in Aurum and compared them to individuals with lung cancer registered in GOLD. As further attempt to evaluate the level of concordance of both data sets, an earlier performed study with GOLD data, was repeated using Aurum. In this study, the hypothetical eligibility of patients with lung cancer, for eleven selected, previously performed, pivotal randomized clinical trials (RCTs) for systemic therapy (i.e. targeted therapy and immuno-oncology therapy) in lung cancer was assessed [12]. Subsequently, the results of lung cancer populations in Aurum and GOLD were compared, in terms of eligibility percentages and simulated OS of potential eligible patients for those RCTs.

METHODS

Data sources

For this study data from both GOLD (release April 2019) and Aurum (release January 2021) was used. GOLD consists of primary care data from GP practices based in the UK using Vision® software and Aurum consists of primary care data from GP practices based mainly in England using EMIS Web® software. The primary care data includes information on demographics, diagnoses, symptoms, prescriptions, and laboratory tests, among others [1, 3]. This study is part of a protocol (#21_000413) approved by the CPRD Independent Scientific Advisory Committee.

Study population

All patients, aged 18 years or older with an incident diagnosis of lung cancer between 01-01-2014 and 31-12-2018 were included. The date of lung cancer diagnosis determined the index date. Diagnoses were based on the first registration of lung cancer using Read codes (GOLD) and SNOMED concept IDs (Aurum) for lung cancer (Appendix I and II) . All types of lung carcinoma were included, since both SNOMED and Read coding systems do not differentiate between different lung carcinomas in terms of type, stage, molecular status or histology of lung cancer. Information regarding whether the date of diagnosis was systematically based on date of biopsy or on imaging, is not included in either of the databases and was therefore impossible to retrieve.

Data extraction

Data on comorbidities was extracted using code lists consisting of Read (GOLD) and corresponding SNOMED concept IDs (Aurum). In short, GOLD Read codes were transferred to the SNOMED-format for Aurum and additional related SNOMED IDs were added to complete the Aurum code lists. Depending on the comorbidity, different time windows were used to determine presence of the comorbidities prior to the index date (i.e. 30 days, 90 days, 1 year, 5 years or ever before the index date (Appendix III)).

Only drug prescriptions up to 90 days before the index date were included to assess current use.

Laboratory values in GOLD and Aurum are stored in different ways. For GOLD, entities (numerical codes) are used which are linked to specific (laboratory) terms, and for Aurum laboratory values are stored using medcodeIDs which are also used to store diagnosis of morbidities. The laboratory value closest to the index date was used and only if this was registered within 90 days prior to index date (Appendix III). Similar to drug prescriptions, a 90 day period prior to the index date was thought to be still representative of the health status of the patient around the time of diagnosis.

Overall survival

Patients were followed from the index date until date of last data collection at the GP practice, transfer out of practice, end of study or date of death, whichever came first. Date of death was determined using the EMIS death date or in absence of an EMIS death date, CPRD death date and was determined for patients registered in Aurum. In GOLD, date of death was determined using CPRD death date.

Eligibility for clinical trials

Recently, potential eligibility rates for some previously performed lung cancer RCTs or anticancer targeted- and immunotherapies, were evaluated for patients with lung cancer registered in GOLD [12]. We aimed to repeat this potential eligibility assessment with the patient cohort in Aurum. In short, eligibility for RCTs was determined using the inclusion and exclusion criteria of eleven selected pivotal phase III RCTs that were published between 01-01-2014 and 31-12-2018 and evaluated systemic anti-cancer agents for the treatment of non-small cell lung cancer (NSCLC). These trials were chosen to reflect the new treatments that became available during this period. The included RCTs evaluated the tyrosine kinase inhibitors (TKIs) osimertinib (AURA3 and FLAURA) and alectinib (ALEX and ALUR) and the immune checkpoint inhibitors nivolumab (CheckMate 017 and 057), pembrolizumab (KEYNOTE-024, -189 and -407), durvalumab (PACIFIC), and atezolizumab (OAK) [13-23]. Exclusion criteria of each clinical trial included presence of certain comorbidities, co-medication use that could have an interaction with the drug under evaluation or diminish the function of the immune system and deviant laboratory values. Exclusion criteria per RCT are specified in Appendix V. Eligibility criteria did not include molecular gene status or disease stage, since this information is not available in our databases. Patients who met all criteria were classified as potentially eligible. Patients who did not meet all criteria were classified as ineligible. The criteria were not applied sequentially and a patient could be classified as non-eligible based on multiple exclusion criteria. For each RCT the proportion of patients registered in Aurum who were eligible for potential study participation was determined, as was described previously for the patients registered in GOLD [12]. Mortality of hypothetically eligible and ineligible patients was then compared for each RCT followed by a comparison of the mortality rates per RCT for Aurum to GOLD. Additionally, the reasons for ineligibility in RCTs were further specified for patients in Aurum.

Data analysis

Baseline characteristics for patients with a diagnosis of lung cancer in Aurum and in GOLD were described descriptively. For each RCT the proportion of potential eligible patients in Aurum was estimated and descriptively compared to the proportion of eligible patients in GOLD. Furthermore, the median OS (mOS) in Aurum was estimated and compared to GOLD, using Kaplan Meier analysis. Cox regression analyses was used to estimate the age and sex adjusted risk of mortality in Aurum vs GOLD.

Cox regression analysis was used to compare the risk of mortality between eligible and non-eligible patients separately for each RCT. The results were adjusted for age and sex. This was done for both Aurum and GOLD data, and thereafter results from these databases were compared using a test of interaction [24]. In short, for each RCT a hazard ratio for mortality was calculated for patients who are hypothetically eligible compared to non-eligible patients for a RCT. This hazard ratio was calculated for both the Aurum and GOLD cohort. In order to compare the calculated HR of Aurum to the HR of GOLD in for example the AURA3 study, the test of interaction was performed and the relative risk ratio was estimated according to the method described by Altman *et al.* [24]. The results are depicted as HR and 95% CI.

Sensitivity analysis

Since practices could migrate from Vision to EMIS software during the study period, it is possible that patients are included in both GOLD and Aurum. Therefore, additional cox regression analysis on the risk of mortality between eligible and non-eligible patients for each RCT was performed as sensitivity analysis. In this analysis, patients were excluded from the Aurum dataset if their index date was before the migration date of the practice. In order to select these patients, the data regarding migration of practices was provided by CPRD.

RESULTS

Baseline characteristics

Between 01-01-2014 and 31-12-2018, there were 34,831 patients with a diagnosis of lung cancer in Aurum and 9,239 patients with a diagnosis of lung cancer in GOLD.

The patients with lung cancer registered in Aurum and in GOLD were largely comparable in terms of demographics, comorbidities and drug use, but some deviations were observed (Table 1). There were more patients with previous malignancies registered in the last five years in Aurum (13.5%), compared to GOLD (10.2%). In terms of deviant laboratory values, as specified in Appendix III, the percentile difference was largest in deviant international normalized ratio (INR) values. These were more often found in GOLD (3.0%) than in Aurum (0.5%).

Table 1. Baseline characteristics of patients with lung cancer registered in CPRD Aurum and CPRD GOLD.

	Aurum	GOLD		
	N = 34831		N = 9239	
	N	%	N	%
Index date				
2014	8202	23.6	2426	26.3
2015	6440	18.5	2114	22.9
2016	6602	19.0	1795	19.4
2017	6770	19.4	1510	16.3
2018	6817	19.6	1394	15.1
Sex				
No. of Males	18291	52.5	4710	51.0
Age (years), mean (SD)	72.5 (10.8)		72.1 (10.5)	
≤ 50	1009	2.9	258	2.8
50 – 64.9	7452	21.4	2055	22.2
65 – 79.9	17923	51.5	4880	52.8
≥ 80	8447	24.3	2046	22.2
BMI (kg/m2), mean (SD)	**26.0 (5.5)**		**25.8 (5.6)**	
≤ 18.5	2107	6.1	611	6.6
18.5 – 25	13198	37.9	3490	37.8
25 – 30	10985	31.5	2836	30.7
30 – 35	4750	13.6	1243	13.5
> 35	2014	5.8	531	5.8
Missing	1777	5.1	528	5.7
Smoking status				
Current	13927	40.0	3462	37.5
Former	18250	52.4	5106	55.3
Non-smoker	2229	6.4	582	6.3
Missing	425	1.2	89	1.0
Cancer-related				
Previous malignancies [e]	4713	13.5	939	10.2
Immune-related diseases				
Ankylosing spondylitis [b]	91	0.3	21	0.2
Dermatomyositis [b]	20	0.1	5	0.1
Myasthenia gravis [b]	37	0.1	7	0.1
Multiple sclerosis [b]	102	0.3	26	0.3
Polymyalgia rheumatica [b]	813	2.3	189	2.1
Psoriatic arthritis [b]	135	0.4	36	0.4

Table 1. Continued.

	Aurum	GOLD		
	N = 34831		N = 9239	
	N	%	N	%
Rheumatoid arthritis [b]	2516	7.2	283	3.1
Coeliac disease [b]	125	0.4	48	0.5
Crohn's disease [b]	181	0.5	58	0.6
Ulcerative colitis [b]	417	1.2	114	1.2
Grave's disease [b]	59	0.2	21	0.2
Psoriasis [b]	1996	5.7	558	6.0
Sarcoidosis [b]	75	0.2	12	0.1
SLE [b]	62	0.2	19	0.2
Vasculitis [b]	230	0.7	57	0.6
Cardiovascular diseases				
Heart failure [b]	1711	4.9	460	5.0
Heart rhythm disturbances [b]	510	1.5	77	0.8
Myocardial infarction [a]	150	0.4	51	0.6
Poor controlled hypertension [a]	<5	0	<5	0
Unstable angina pectoris [a]	6	0	<5	0
Serious infections				
Meningitis [c]	<5	0	0	0
Pneumonia [c]	248	0.7	118	1.3
Sepsis [c]	35	0.1	18	0.2
Hepatitis [d]	31	0.1	<5	0.0
Psychiatric diseases				
Bipolar disorder [b]	215	0.6	33	0.4
Dementia [b]	1443	4.1	275	3.0
Schizophrenia [b]	331	1.0	64	0.7
Other				
HIV/AIDS [b]	65	0.2	13	0.1
Organ transplant [b]	61	0.2	14	0.1
Substance abuse [e]	11	<0.1	17	0.2
Pregnancy [d]	16	0.1	<5	<0.1
Deviant laboratory values				
Alkaline phosphatase [a]	411	1.2	106	1.2
ALAT [a]	499	1.4	123	1.3

Table 1. Continued.

	Aurum	GOLD		
	N = 34831	N = 9239		
	N	%	N	%
ASAT[a]	68	0.2	34	0.4
eGFR[a]	3255	9.4	969	10.5
Haemoglobin[a]	456	1.3	105	1.1
INR[a]	181	0.5	292	3.0
Neutrophils[a]	57	0.2	14	0.2
Platelets[a]	103	0.3	26	0.3
Total[b]ilirubin[a]	368	1.1	115	1.2
TSH[a]	742	2.1	215	2.3
White[b]lood counts[a]	14	<0.1	4	<0.1
Lymphocyte[a]	159	0.5	35	0.4
Drugs prescriptions				
Systemic corticosteroid [a]	7307	21.0	1903	20.6
Immunosuppressive drugs[a]				
Ciclosporine	15	<0.1	<5	0
Everolimus	0	<0.1	0	0
Sirolimus	0	<0.1	0	0
Tacrolimus	12	<0.1	<5	0
Strong CYP3A4-inhibitors[a]				
Erythromycin	358	1.0	131	1.4
Clarithromycin	2775	8.0	860	9.3
Itraconazole	18	0.1	7	0.1
Ketoconazole	0	0	0	0
Ritonavir	<5	<0.1	0	0
Voriconazole	<5	<0.1	0	0

Abbreviations: N = number, % = percentage, SD = standard deviation, BMI = body mass index, SLE = systemic lupus erythematosus, HIV = human immunodeficiency virus, AIDS = acquired immunodeficiency syndrome, ALAT = alanine transaminase, ASAT = aspartate transaminase, INR = international normalized ratio, TSH = Thyroid stimulating hormone, CYP3A4 = cytochrome P450 3A4, NA = not applicable.

[a] three months prior to index date, [b] ever prior to index date, [c] one month prior to index date, [d] one year prior to index date, [e] five years prior to index date

Eligibility for phase III clinical trials

The largest difference in potential trial eligibility between GOLD and Aurum for studies investigating TKIs was seen for the ALEX trial, where the percentage point difference was

3.1% (Table 2). For the studies investigating immune checkpoint inhibitors, the largest absolute difference was 2.3% for the PACIFIC trial. In general, a lower proportion of patients was eligible for RCTs investigating immune checkpoint inhibitors (CheckMate, KEYNOTE, PACIFIC and OAK studies) compared to RCTs with TKIs, but this trend was similar in both databases.

For patients registered in Aurum, the main reasons for ineligibility were previous malignancies and concomitant drug-use (Table 3). For RCTs investigating immune checkpoint inhibitors, 21.0-26.2% of the patients would be ineligible based on concomitant drug-use, including corticosteroids.

Table 2. Eligibility of CPRD Aurum and CPRD GOLD cohort in phase III randomized clinical trials (%).

Name of trial	Drug investigated	Eligible proportion Aurum (%)	Eligible proportion GOLD (%) (8)	Percentage point difference Aurum and GOLD
AURA3	Osimertinib	79.5	78.1	1.4
FLAURA	Osimertinib	71.4	72.4	1.0
ALEX	Alectinib	71.6	73.7	3.1
ALUR	Alectinib	71.5	73.6	2.1
CheckMate 017	Nivolumab	52.4	53.9	1.5
CheckMate 057	Nivolumab	52.7	54.3	1.6
KEYNOTE-024	Pembrolizumab	49.4	49.1	0.3
KEYNOTE-189	Pembrolizumab plus chemotherapy	52.1	52.2	0.1
KEYNOTE-407	Pembrolizumab plus chemotherapy	50.2	50.0	0.2
PACIFIC	Durvalumab	50.7	53.0	2.3
OAK	Atezolizumab	50.9	50.7	0.2

Abbreviations: % = percentage.

Table 3. Proportion of patients in Aurum excluded for each randomized clinical trial by reason of exclusion.

Name of trial	Reason for exclusion (%)							
	A	B	C	D	E	F	G	H
Osimertinib – FLAURA	7.4	-	-	6.0	-	-	8.9	0.3
Osimertinib – AURA3	7.4	10.4	-	6.0	-	-	8.9	0.3
Alectinib – ALEX	5.7	11.7	-	-	-	5.4	8.9	0.5
Alectinib – ALUR	5.7	11.7	-	-	-	5.4	8.9	0.5
Nivolumab – CheckMate 017	6.0	10.4	11.3	-	0.8	5.4	26.2	0.3
Nivolumab – CheckMate 057	5.5	10.4	11.3	-	0.8	5.4	26.2	0.3
Pembrolizumab – KEYNOTE-024	14.7	13.5	11.3	-	0.8	5.4	21.0	0.5
Pembrolizumab – KEYNOTE-189	9.5	13.5	11.3	-	0.8	5.4	21.0	0.5
Pembrolizumab – KEYNOTE-407	13.2	13.5	11.3	-	0.8	5.4	21.0	0.5
Durvalumab – PACIFIC	7.4	13.5	11.3	6.0	0.8	5.4	21.0	0.5
Atezolizumab – OAK	5.4	13.5	11.3	6.4	0.8	-	26.2	0.5

A = Laboratory values; B = Cancer-related; C = Immune related diseases; D = Cardiovascular diseases
E = Serious infections; F = Psychiatric diseases; G = Concomitant drug-use; H = Other

Overall survival Aurum versus GOLD

Median OS of patients with lung cancer registered in Aurum was 9.8 months (95% CI 9.6 – 10.1) versus 9.0 months (95% CI 8.6 – 9.5) in GOLD (unadjusted HR = 0.94, 95% CI 0.91 – 0.97 and adjusted HR = 0.93, 95% CI 0.91 – 0.96) (Figure 1), implicating that the mOS in Aurum was significantly longer than in GOLD.

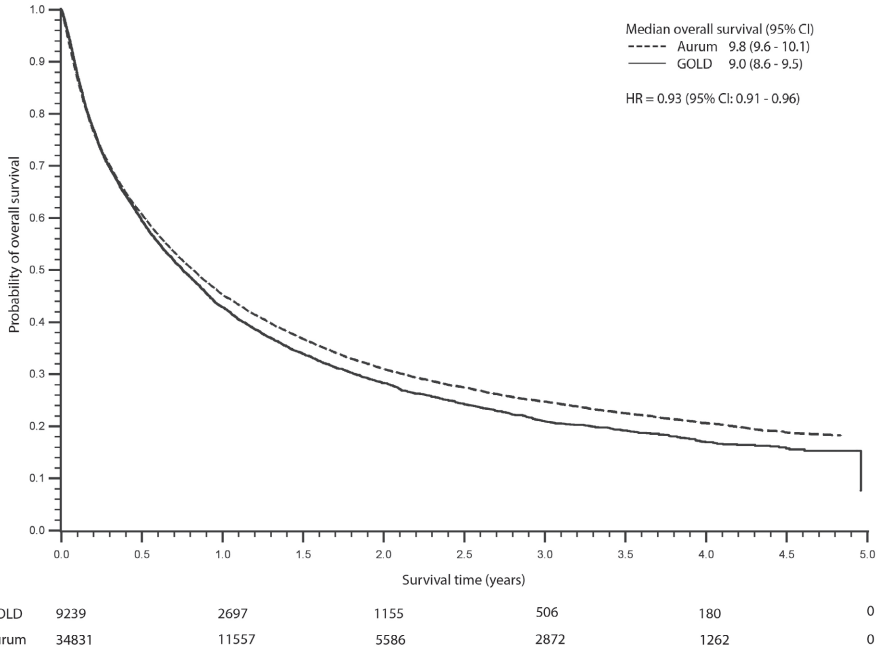


Figure 1. Overall survival GOLD cohort (continued line) and Aurum cohort (dotted line).

Mortality eligible patients vs ineligible patients

Mortality of RCT eligible patients compared to ineligible patients was consistent across all selected RCTs (Table 4). In all investigated RCTs, mortality was lower in the hypothetically eligible patients. For patients in Aurum the age and sex adjusted HR varied between 0.75 (CheckMate 017 and CheckMate 057, 95% CI 0.73 – 0.77) and 0.85 (FLAURA, 95% CI 0.83–0.87) when comparing mortality in eligible vs ineligible patients and in GOLD this varied between 0.77 (CheckMate 017 and CheckMate 057, 95% CI 0.73 – 0.81) and 0.89 (FLAURA, 95% CI 0.84 – 0.94). When comparing the HRs of mortality per RCT from Aurum to GOLD, no differences were found except for the OAK-study. The obtained ratios varied between 0.94 (95% CI 0.89 – 0.99) and 0.98 (95% CI 0.92 – 1.05), respectively (Table 4).

Table 4. Mortality of randomized clinical trial eligible versus ineligible patients and comparison of mortality rates in Aurum to GOLD

Name of trial	Aurum		GOLD		Ratio of mortality rates Aurum vs GOLD (95% CI)
	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	
AURA3	1.32 (1.27-1.36)	1.24 (1.20-1.29)	1.27 (1.19-1.35)	1.21 (1.14-1.29)	1.03 (0.96-1.10)
FLAURA	1.23 (1.19-1.27)	1.18 (1.14-1.22)	1.17 (1.10-1.23)	1.13 (1.07-1.20)	1.04 (0.99-1.10)
ALEX	1.22 (1.18-1.26)	1.19 (1.15-1.23)	1.17 (1.11-1.24)	1.16 (1.10-1.23)	1.02 (0.96-1.09)
ALUR	1.22 (1.18-1.26)	1.19 (1.15-1.23)	1.18 (1.11-1.25)	1.17 (1.10-1.23)	1.02 (0.96-1.09)
CheckMate 017	1.36 (1.32-1.40)	1.35 (1.31-1.39)	1.30 (1.23-1.37)	1.30 (1.23-1.37)	1.04 (0.98-1.10)
CheckMate 057	1.36 (1.32-1.40)	1.35 (1.31-1.39)	1.30 (1.24-1.37)	1.30 (1.23-1.37)	1.04 (0.98-1.10)
KEYNOTE-024	1.34 (1.30-1.37)	1.28 (1.24-1.32)	1.28 (1.21-1.35)	1.23 (1.17-1.30)	1.04 (0.98-1.10)
KEYNOTE-189	1.32 (1.28-1.36)	1.27 (1.24-1.31)	1.28 (1.21-1.35)	1.24 (1.18-1.31)	1.02 (0.96-1.09)
KEYNOTE-407	1.32 (1.28-1.36)	1.26 (1.23-1.30)	1.27 (1.21-1.34)	1.22 (1.16-1.29)	1.03 (0.97-1.10)
PACIFIC	1.34 (1.30-1.38)	1.28 (1.24-1.31)	1.27 (1.21-1.34)	1.23 (1.17-1.30)	1.04 (0.98-1.10)
OAK	1.29 (1.28-1.33)	1.27 (1.24-1.31)	1.21 (1.15-1.28)	1.19 (1.13-1.26)	1.07 (1.00-1.13)

Abbreviations: HR = hazard ratio, 95% CI = 95% confidence interval.

Sensitivity analysis

In total 4590 patients were excluded from the Aurum dataset, since they were enrolled in a practice that migrated within the study period. The mortality analysis performed with these patients did show highly similar results to the primary mortality analysis performed per RCT (Appendix VI).

DISCUSSION

The analysis of baseline characteristics and the eligibility study showed that Aurum and GOLD are largely comparable in terms of demographics, comorbidities and current drug use at the moment of lung cancer diagnosis. Although some differences were found in previous malignancies, psychiatric diseases, and use of (co)medication, these differences were considered not clinically relevant as these percentages were small in general.

The proportion of eligible patients for RCTs was comparable between Aurum and GOLD. In general, a lower proportion of patients was eligible for trials investigating immunotherapy (CheckMate, KEYNOTE, PACIFIC and OAK), and this finding was similar for both Aurum and GOLD. In these studies, concomitant use of immunosuppressive drugs (including corticosteroids) was prohibited, leading to a larger proportion of ineligible patients compared to RCTs investigating osimertinib and alectinib , in which concurrent use of immunosuppressive drugs was allowed.

The OS in CPRD Aurum was slightly higher compared to GOLD, but when comparing the calculated HRs of mortality per RCT of both databases, no differences were found, indicating large overall concordance between both databases. It is important to mention that comparing mean HRs could have some limitations, since HRs can vary over time and that it may not be collapsible [25, 26]. Regarding the latter one, the compared HRs were adjusted for age and sex and since these two factors were distributed equally in Aurum and GOLD, collapsibility was considered a non-meaningful issue.

The minor differences in laboratory values could be due to missing data, since extraction of this information was not registered under a universal number in Aurum, as was the case in GOLD, but had to be done using a manually constructed list. This might have led to an increased amount of hypothetically eligible patients for RCTs, since a patient was only classified as ineligible if a deviant laboratory value was registered and not if this was missing. Furthermore, some laboratory values, such as INR and estimated glomerular filtration rate (eGFR), might be influenced by variations in co-medications and/or differences in daily dosages. For instance, use of coumarin derivatives could not be equally distributed in both datasets. Additionally, an extensive and systematic search is needed to find all registrations linked to one laboratory outcome. Reports describing laboratory

values in Aurum are rare and validated methods to extract this data are still missing, as was published previously by Persson *et al.*[27].

To our knowledge, this is the first study to assess data on patients with lung cancer registered in Aurum and to compare this data to patients registered in GOLD. Other comparison studies performed in patients with psoriasis and chronic obstructive pulmonary disease (COPD) and antibiotic use, also did not find substantial differences in the data collected in Aurum in comparison to GOLD [8-10].

We chose to extract comorbidities and drug prescriptions from Aurum based on Read codes found in GOLD, to ensure that the same terms were used for each extraction. A similar method of converting Read code lists to SNOMED ID code lists has been described before by Gulliford *et al.* [9].

This study also has some limitations. First, the number of potential eligible and non-eligible patients could be over- or underestimated due to missing data as was earlier discussed for laboratory values, even though extensive searches were done to minimize this risk. As Trafford *et al.* described, when comparing the two databases, differences could occur due to differences in the way the databases are built-up and the data is stored [8]. Secondly, since the eligibility of the patients was tested on the whole lung cancer population registered in GOLD and Aurum, respectively, the reported proportion of hypothetically eligible patients might be different to the actual eligible proportion of patients. We could not differentiate between the major histological subtypes of NSCLC and small cell lung cancer (SCLC), because the subtypes are snot registered in these primary care databases. In the UK, 80-85% of the patients with lung cancer is diagnosed with NSCLC, therefore we can assume the same percentages are captured in GOLD and Aurum [28]. Thirdly, we noted that approximately 10% of the patients had previous malignancies. In the RCTs investigated, only primary lung cancer cases were eligible for enrolment. With the available information, we were not able to distinguish whether the diagnosed lung cancer was a primary or secondary malignancy. Fourthly, we did not have access to information on cancer characteristics such as gene mutation status and stage of the disease. Therefore, patients with other forms of lung cancer could have been wrongfully assigned to either the RCT eligible or to the non-eligible group. However, since the above mentioned information is unavailable in both databases, and the aim of the eligibility substudy was to be an additional uniformity check between lung cancer related data registered in Aurum and in GOLD, the results from the comparison itself can still be considered valid. Linkage to secondary databases such as the database of NCRAS, could prevent misclassification as it contains information on tumour characteristics, tumour stage and anti-cancer treatment. Future research is needed to further elaborate on this. Lastly, due to the transition of practices from Vision to EMIS, patients could have been registered in both Aurum and GOLD. However, it was not possible to identify these patients directly, since only data on the practice that migrated was available. We did exclude patients from Aurum that were

in a practice that previously used Vision software for GOLD in a sensitivity analysis, but did not find any noticeable differences compared to the results obtained in the complete Aurum lung cancer cohort.

In summary, the uniformity of data, and the completeness of information recorded of patients with lung cancer registered in CPRD Aurum is appropriate and reliable, and similar to the data quality that was retrieved from CPRD GOLD. Therefore, we conclude that the data of patients with lung cancer in Aurum is similar to the data of patients with lung cancer in GOLD. The Aurum database could therefore be considered suitable for future epidemiological research on lung cancer.

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APPENDIX A

List of Read codes and SNOMED IDs of lung cancer diagnosis in CPRD Aurum. [2]

Med code ID	Read code	SNOMED description ID	Term
4026111000006110		510696018	Primary malignant neoplasm of hilus of lung
155287019	B221100	155287019	Malignant neoplasm of hilus of lung
1773111000006110		510792012	Primary malignant neoplasm of lung
733371000006119	B22z.11	3288586014	Lung cancer
4163281000006110		173925017	Overlapping malignant neoplasm of bronchus and lung
723301000006110	B225.00	1219469018	Malignant neoplasm of overlapping lesion of bronchus & lung
288810010	B220100	288810010	Malignant neoplasm of mucosa of trachea
288813012	B221000	288813012	Malignant neoplasm of carina of bronchus
288819011	B222000	288819011	Malignant neoplasm of upper lobe bronchus
288820017	B222100	288820017	Malignant neoplasm of upper lobe of lung
288822013	B223.00	288822013	Malignant neoplasm of middle lobe, bronchus or lung
880061000006110	B223.99	880061000006110	Ca middle lobe bronchus/lung
288825010	B223z00	288822013	Malignant neoplasm of middle lobe, bronchus or lung NOS
288823015	B223000	288823015	Malignant neoplasm of middle lobe bronchus
4748061000006110		3443979013	Malignant neoplasm of right middle lobe of lung
288824014	B223100	288824014	Malignant neoplasm of middle lobe of lung
288826011	B224.00	288826011	Malignant neoplasm of lower lobe, bronchus or lung
880071000006115	B224.99	880071000006115	Ca lower lobe bronchus/lung
288829016	B224z00	288826011	Malignant neoplasm of lower lobe, bronchus or lung NOS
288827019	B224000	288827019	Malignant neoplasm of lower lobe bronchus
288828012	B224100	288828012	Malignant neoplasm of lower lobe of lung

Med code ID	Read code	SNOMED description ID	Term
403688010	B222.00	403688010	Malignant neoplasm of upper lobe, bronchus or lung
880051000006113	B222.99	880051000006113	Ca upper lobe bronchus/lung
288821018	B222z00	403688010	Malignant neoplasm of upper lobe, bronchus or lung NOS
11925881000006100		482515017	Malignant tumour of lung
11918131000006100		396221000006112	[X]Malignant neoplasm of bronchus or lung, unspecified
6243241000006110		6243241000006110	Malignant tumour of lung
6243261000006110		1228498010	CA - Lung cancer
288832018	B22y.00	482516016	Malignant neoplasm of other sites of bronchus or lung
403689019	B22z.00	482516016	Malignant neoplasm of bronchus or lung NOS
6245791000006110		482663014	Malignant tumour of trachea
6245821000006110		1228559015	CA - Cancer of trachea
6245811000006110		1228558011	Tracheal cancer
6245831000006110		3289017011	Malignant tracheal tumour
6245801000006110		482662016	Malignant tumour of trachea
6245841000006110		3289020015	Malignant tracheal tumour
721391000006116	B220.00	482662016	Malignant neoplasm of trachea
288811014	B220z00	482662016	Malignant neoplasm of trachea NOS
6363661000006110		1218028010	Ca main bronchus
155361017	B221.00	1210643012	Malignant neoplasm of main bronchus
288815017	B221z00	1210643012	Malignant neoplasm of main bronchus NOS
288808013	B22..00	2765453013	Malignant neoplasm of trachea, bronchus and lung
880031000006118	B22..98	880031000006118	Ca trachea/bronchus/lung NOS
880041000006111	B22..99	880041000006111	Ca trachea/bronchus/lung

APPENDIX B

List of Read codes of lung cancer diagnosis in CPRD GOLD.

Med code	Read code	Term
2587	B22z.11	Lung cancer
3903	B22z.00	Malignant neoplasm of bronchus or lung NOS
17391	B221000	Malignant neoplasm of carina of bronchus
33444	B221100	Malignant neoplasm of hilus of lung
18678	B224000	Malignant neoplasm of lower lobe bronchus
12582	B224100	Malignant neoplasm of lower lobe of lung
42566	B224z00	Malignant neoplasm of lower lobe, bronchus or lung NOS
12870	B221.00	Malignant neoplasm of main bronchus
21698	B221z00	Malignant neoplasm of main bronchus NOS
41523	B223000	Malignant neoplasm of middle lobe bronchus
39923	B223100	Malignant neoplasm of middle lobe of lung
31268	B223.00	Malignant neoplasm of middle lobe, bronchus or lung
54134	B223z00	Malignant neoplasm of middle lobe, bronchus or lung NOS
31188	B224.00	Malignant neoplasm of lower lobe, bronchus or lung
103946	B220100	Malignant neoplasm of mucosa of trachea
38961	B22y.00	Malignant neoplasm of other sites of bronchus or lung
36371	B225.00	Malignant neoplasm of overlapping lesion of bronchus & lung
15221	B220.00	Malignant neoplasm of trachea
37810	B220z00	Malignant neoplasm of trachea NOS
13243	B22..00	Malignant neoplasm of trachea, bronchus and lung
31700	B222000	Malignant neoplasm of upper lobe bronchus
25886	B222100	Malignant neoplasm of upper lobe of lung
10358	B222.00	Malignant neoplasm of upper lobe, bronchus or lung
44169	B222z00	Malignant neoplasm of upper lobe, bronchus or lung NOS

APPENDIX C

The subdivision of all in- and exclusion criteria in eight different sets and the corresponding time-window of exposure for each criterion.

Criterion	Time-window of exposure
Laboratory values	
AP	Three months prior to index date
ALAT	Three months prior to index date
ASAT	Three months prior to index date
eGFR	Three months prior to index date
Hemoglobin	Three months prior to index date
International normalized ratio	Three months prior to index date
Lymphocytes	Three months prior to index date
Neutrophils	Three months prior to index date
White blood cells	Three months prior to index date
Platelets	Three months prior to index date
Total bilirubin	Three months prior to index date
Thyroid stimulation hormone	Three months prior to index date
Cancer related	
History of cancer [a]	Two/three/five years prior to index date[a]
Immune related disease	
Vasculitis	Ever before index date
Coeliac disease	Ever before index date
Crohn's disease	Ever before index date
Ulcerative colitis	Ever before index date
Grave's disease	Ever before index date
Multiple sclerosis	Ever before index date
Myasthenia gravis	Ever before index date
Ankylosing spondylitis	Ever before index date
Dermatomyositis	Ever before index date
Polymyalgia rheumatica	Ever before index date
Psoriatic arthritis	Ever before index date
Rheumatoid arthritis	Ever before index date
Psoriasis	Ever before index date
Sarcoidosis	Ever before index date
Systemic lupus erythematous	Ever before index date
Cardiovascular disease	
Heart failure	Ever before index date
Heart rhythm disturbances [b]	Ever before index date
Myocardial infarction	Three months prior to index date
Poor controlled hypertension	Three months prior to index date
Unstable angina pectoris	Three months prior to index date

Criterion	Time-window of exposure
Serious infections	
Meningitis	One month prior to index date
Pneumonia	One month prior to index date
Sepsis	One month prior to index date
Hepatitis	One year prior to index date
Psychiatric disease	
Bipolar mood disorder	Ever before index date
Dementia	Ever before index date
Schizophrenia	Ever before index date
Drugs	
Systemic corticosteroid treatment [c]	Three months prior to index date
Immunosuppressive drugs [d]	Three months prior to index date
Strong CYP3A4-inhibitors [e]	Three months prior to index date
Other	
AIDS/HIV	Ever before index date
Organ transplant [f]	Ever before index date
Pregnancy	One year before index date
Substance abuse	Five years before index date

Abbreviations: AP = Alkaline phosphatase, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, eGFR = estimated glomerular filtration rate, CYP = cytochrome P450, AIDS = acquired immune deficiency syndrome, HIV = human immunodeficiency virus.

[a] in the eleven clinical trials different requirements were used for the history of other cancer types, and varied between two, three or five years before index date. The specific time period used for each study is shown in Appendix D.
[b] for heart rhythm disturbances three specific conditions were used: complete left bundle branch block, second degree heart block and third-degree heart block.
[c] for systemic corticosteroid treatment six drugs were included: dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone.
[d] for immunosuppressive drugs four drugs were included: ciclosporin, everolimus, sirolimus and tacrolimus.
[e] for strong CYP3A4-inhibitors six drugs were included: erythromycin, clarithromycin, itraconazole, ketoconazole, ritonavir and voriconazole.
[f] for organ transplant four specific transplantations were used: heart, lung, kidney, liver.

APPENDIX D

List of exclusion criteria per randomized controlled trial.

Table D1.1: exclusion criteria used in the AURA3 study.

Criterion	Used cut-off
Age	< 18 years
ALAT	> 2.5 ×ULN
ASAT	> 2.5 ×ULN
eGFR	< 50 mL/min
Haemoglobin	< 90 g/L
Total bilirubin	> 1.5 ×ULN
Neutrophils	< 1.5 $\times 10^9$/L
Platelets	< 100 $\times 10^9$/L
Active serious infection	
Hearth rhythm disturbances	
Heart failure	
Uncontrolled hypertension	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors	

Abbreviations: ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, eGFR = estimated glomerular filtration rate, AIDS = acquired immunodeficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ULN = upper limit of normal, mL = millilitre, min = minute, g = gram, L = litre,

Table D1.2: exclusion criteria used in FLAURA study.

Criterion	Used cut-off
Age	< 18 years
ALAT	> 2.5 ×ULN
ASAT	> 2.5 ×ULN
eGFR	< 50 mL/min
Haemoglobin	< 90 g/L
Total bilirubin	> 1.5 ×ULN
Neutrophils	< 1.5 $\times 10^9$/L
Platelets	< 100 $\times 10^9$/L
Previous malignancy in two years prior to index date	
Active serious infection	
Hearth rhythm disturbances	
Heart failure	
Uncontrolled hypertension	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors	

Abbreviations: ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, eGFR = estimated glomerular filtration rate, AIDS = acquired immunodeficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ULN = upper limit of normal, mL = millilitre, min = minute, g = gram , L = litre,

Table D1.3: exclusion criteria used in ALEX study.

Criterion	Used cut-off
Age	< 18 years
ALAT	> 3.0 ×ULN
ASAT	> 3.0 ×ULN
eGFR	< 45 mL/min
Haemoglobin	< 90 g/L
Neutrophils	< 1.5 $\times 10^9$/L
Platelets	< 100 $\times 10^9$/L
Previous malignancy in three years prior to index date	
Hepatitis	
History of organ transplant	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors	

Abbreviations: ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, eGFR = estimated glomerular filtration rate, AIDS = acquired immunodeficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ULN = upper limit of normal, mL = millilitre, min = minute, g = gram, L = litre

Table D1.4: exclusion criteria used in ALUR study.

Criterion	Used cut-off
Age	< 18 years
ALAT	> 3.0 ×ULN
ASAT	> 3.0 ×ULN
eGFR	< 45 mL/min
Haemoglobin	< 90 g/L
Neutrophils	< 1.5 $\times 10^9$/L
Platelets	< 100 $\times 10^9$/L
Previous malignancy in three years prior to index date	
Hepatitis	
History of organ transplant	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors	

Abbreviations: ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, eGFR = estimated glomerular filtration rate, AIDS = acquired immunodeficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ULN = upper limit of normal, mL = millilitre, min = minute, g = gram, L = litre

Table D1.5: exclusion criteria used in CheckMate 017 study.

Criterion	Used cut-off
Age	< 18 years
ALAT	> 1.5 ×ULN
ASAT	> 1.5 ×ULN
eGFR	< 40 mL/min
Haemoglobin	< 90 g/L
Total bilirubin	> ULN
White blood cells	< 2.0 $\times 10^9$/L
Neutrophils	< 1.5 $\times 10^9$/L
Platelets	< 100 $\times 10^9$/L
Previous malignancy in two years prior to index date	
Active serious infection	
Active auto-immune disease	
Psychiatric condition	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors	
Systemic treatment with glucocorticoids	
Systemic treatment with immunosuppressive drugs	

Abbreviations: ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, eGFR = estimated glomerular filtration rate, AIDS = acquired immunodeficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ULN = upper limit of normal, mL = millilitre, min = minute, g = gram, L = litre,

Table D1.6: exclusion criteria used in CheckMate 057 study.

Criterion	Used cut-off
Age	< 18 years
ALAT	> 1.5 ×ULN
ASAT	> 1.5 ×ULN
eGFR	< 40 mL/min
Haemoglobin	< 90 g/L
Total bilirubin	> 1.5 ×ULN
White blood cells	< 2.0 $\times 10^9$/L
Neutrophils	< 1.5 $\times 10^9$/L
Platelets	< 100 $\times 10^9$/L
Previous malignancy in two years prior to index date	
Active serious infection	
Active auto-immune disease	
Psychiatric condition	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors	
Systemic treatment with glucocorticoids	
Systemic treatment with immunosuppressive drugs	

Abbreviations: ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, eGFR = estimated glomerular filtration rate, AIDS = acquired immunodeficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ULN = upper limit of normal, mL = millilitre, min = minute, g = gram, L = litre,

Table D1.7: exclusion criteria used in KEYNOTE-024 study.

Criterion	Used cut-off
Age	< 18 years
ALAT	> 1.5 ×ULN
ASAT	> 1.5 ×ULN
AP	> 2.5 ×ULN
eGFR	< 60 mL/min
Haemoglobin	< 90 g/L
Total bilirubin	> ULN
Neutrophils	< 1.5 $\times 10^9$/L
Platelets	< 100 $\times 10^9$/L
INR (unless current use of anticoagulation)	> 1.5 ×ULN
Previous malignancy in two years prior to index date	
Active serious infection	
Active auto-immune disease	
History of organ transplant	
Psychiatric condition	
Substance abuse	
AIDS/HIV	
Pregnancy	
Systemic treatment with glucocorticoids	
Systemic treatment with immunosuppressive drugs	

Abbreviations: ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, AP = alkaline phosphatase, eGFR = estimated glomerular filtration rate, INR = international normalized ratio, AIDS = acquired immunodeficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ULN = upper limit of normal, mL = millilitre, min = minute, g = gram, L = litre

Table D1.8: exclusion criteria used in KEYNOTE-189 study.

Criterion	Used cut-off
Age	< 18 years
ALAT	> 2.5 ×ULN
ASAT	> 2.5 ×ULN
eGFR	< 50 mL/min
Haemoglobin	< 90 g/L
Total bilirubin	> 1.5 ×ULN
Neutrophils	< 1.5 $\times 10^9$/L
Platelets	< 100 $\times 10^9$/L
INR (unless current use of anticoagulants)	> 1.5 ×ULN
TSH	Normal
Previous malignancy in five years prior to index date	
Active serious infection	
Active auto-immune disease	
Psychiatric condition	
Substance abuse	
AIDS/HIV	
Pregnancy	
Systemic treatment with glucocorticoids	
Systemic treatment with immunosuppressive drugs	

Abbreviations: ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, AP = alkaline phosphatase, eGFR = estimated glomerular filtration rate, INR = international normalized ratio, TSH = thyroid stimulating hormone, AIDS = acquired immunodeficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ULN = upper limit of normal, mL = millilitre, min = minute, g = gram, L = litre,

Table D1.9: exclusion criteria used in KEYNOTE-407.

Criterion	Used cut-off
Age	< 18 years
ALAT	> 2.5 ×ULN
ASAT	> 2.5 ×ULN
eGFR	< 60 mL/min
Haemoglobin	< 90 g/L
Total bilirubin	> 1.5 ×ULN
Neutrophils	< 1.5 $\times 10^9$/L
Platelets	< 100 $\times 10^9$/L
INR (unless current use of anticoagulants)	> 1.5 ×ULN
Previous malignancy in five years prior to index date	
Active serious infection	
Active auto-immune disease	
Psychiatric condition	
Substance abuse	
AIDS/HIV	
Pregnancy	
Systemic treatment with glucocorticoids	
Systemic treatment with immunosuppressive drugs	

Abbreviations: ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, AP = alkaline phosphatase, eGFR = estimated glomerular filtration rate, INR = international normalized ratio, AIDS = acquired immunodeficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ULN = upper limit of normal, mL = millilitre, min = minute, g = gram, L = litre

Table D1.10: exclusion criteria used in PACIFIC study.

Criterion	Used cut-off
Age	< 18 years
ALAT	> 2.5 ×ULN
ASAT	> 2.5 ×ULN
eGFR	< 50 mL/min
Haemoglobin	< 90 g/L
Total bilirubin	> 1.5 ×ULN
Neutrophils	< 1.5 $\times 10^9$/L
Platelets	< 100 $\times 10^9$/L
Previous malignancy in five years prior to index date	
Active serious infection	
Active auto-immune disease	
History of organ transplant	
Heart failure	
Heart rhythm disturbances	
Myocardial infarction	
Unstable angina pectoris	
Psychiatric condition	
AIDS/HIV	
Pregnancy	
Systemic treatment with glucocorticoids	
Systemic treatment with immunosuppressive drugs	

Abbreviations: ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, AP = alkaline phosphatase, eGFR = estimated glomerular filtration rate, AIDS = acquired immunodeficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ULN = upper limit of normal, mL = millilitre, min = minute, g = gram, L = litre

Table D1.11: exclusion criteria used in OAK study.

Criterion	Used cut-off
Age	< 18 years
ALAT	> 2.5 ×ULN
ASAT	> 2.5 ×ULN
eGFR	< 30 mL/min
Haemoglobin	< 90 g/L
Total bilirubin	> ULN
Neutrophils	< 1.5 $\times 10^9$/L
Lymphocyte	< 0.5 $\times 10^9$/L
White blood cells	< 2.5 $\times 10^9$/L
Platelets	< 100 $\times 10^9$/L
INR (unless current use of anticoagulation)	> 1.5 ×ULN
Previous malignancy in five years prior to index date	
Active serious infection	
Active auto-immune disease	
History of organ transplant	
Heart failure	
Heart rhythm disturbances	
Myocardial infarction	
Unstable angina pectoris	
Psychiatric condition	
AIDS/HIV	
Pregnancy	
Systemic treatment with strong CYP3A4-inhibitors	
Systemic treatment with glucocorticoids	
Systemic treatment with immunosuppressive drugs	

Abbreviations: ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, AP = alkaline phosphatase, eGFR = estimated glomerular filtration rate, INR = international normalized ratio, AIDS = acquired immunodeficiency syndrome, HIV = human immunodeficiency viruses, CYP = Cytochrome P450, ULN = upper limit of normal, mL = millilitre, min = minute, g = gram, L = litre

APPENDIX E

Criteria for deviant laboratory values

Laboratory value	Normal value	Criteria deviant value
Alkaline phosphatase	< 120 U/L	< 2.5 × ULN = < 300 U/L
ALAT	< 45 U/L (men) < 35 U/L (women)	< 3.0 × ULN = 135.0 U/L (men) < 3.0 × ULN = 105.0 U/L (women)
ASAT	< 35 U/L (men) < 30 U/L (women)	< 3.0 × ULN = 105.0 U/L (men) < 3.0 × ULN = 90.0 U/L (women)
INR	1*	> 1.5 x ULN
Total bilirubin	3 – 21 umol/L	< 1.5 × ULN = 31.5 umol/L
TSH	0.35 – 5.00 mU/L	Exceeding normal limits = 0.35 – 5.00 mU/L

ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, INR = international normalized ratio, TSH = thyroid stimulating hormone, U = unit, L = litre, u = micro (10^{-6}), ULN = upper limit of normal, m = milli (10^{-3}).
*INR level could not be classified as deviant in case of anticoagulant use.

5

Chapter 5

PHARMACOKINETIC TREATMENT ENHANCEMENT

Chapter 5.1

Pharmacokinetic boosting of osimertinib with cobicistat in patients with non-small cell lung cancer: the OSIBOOST trial.

A. van Veelen, J. Gulikers, L.E.L. Hendriks, S. Dursun, J. Ippel, E.F. Smit, A.C. Dingemans, R.M.J.M. van Geel, S. Croes.

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ABSTRACT

Introduction: Exposure to osimertinib, a third-generation epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitor (TKI) for treatment of non-small cell lung cancer (NSCLC) and a sensitizing *EGFR* mutation, can be substantially below average. We evaluated whether plasma levels could be boosted by co-administration of cobicistat, a strong Cytochrome P3 450A-inhibitor.

Methods: This was a pharmacokinetic, proof-of-concept clinical trial (the OSIBOOST trial, NCT03858491). NSCLC-patients with osimertinib were eligible if their steady state osimertinib plasma trough concentration was low (≤195 ng/mL). On day 1, the area under the plasma curve ($AUC_{0\text{-}24,ss}$) of osimertinib and its metabolite (AZ5104) was calculated using a limited sampling strategy (four samples). Cobicistat co-treatment (150 mg, once daily) was started on day 2. Between day 22–26, a second AUC was determined. Cobicistat dose could be escalated if the osimertinib trough concentration was still ≤195 ng/mL, in the absence of toxicity. Primary endpoint was the increase in osimertinib exposure, secondary endpoint was toxicity. Cobicistat could be continued during the expanded access phase, with follow-up (2–4 months) of the boosting effect.

Results: The mean baseline osimertinib trough concentration for the eleven enrolled patients was 154 ng/mL. In all patients, cobicistat addition led to an increase in osimertinib exposure. Mean increase in total $AUC_{0\text{-}24,SS}$ (AUC osimertinib + AUC AZ5104) was 60%, (range 19%–192%). The boosting effect was consistent over time. No grade ≥2 toxicity was observed.

Conclusion: Pharmacokinetic boosting of osimertinib with cobicistat in patients with NSCLC is feasible without increasing toxicity, although the degree of boosting is variable.

INTRODUCTION

Approximately 10% of Caucasian patients, with adenocarcinoma of the lung, have a sensitizing epidermal growth factor receptor (*EGFR*) mutation [1]. Targeted therapy, especially tyrosine kinase inhibitors (TKIs), have revolutionized the treatment outcome of patients with oncogene addicted non-small cell lung cancer (NSCLC), with unprecedented 5-year overall survival (OS) of 40%–60% [2]. Osimertinib, a third generation EGFR-TKI, recommended at a flat dose of 80 mg once daily (QD), is used in *EGFR* mutation positive NSCLC-patients, both as first line treatment in patients with metastatic disease as well as in the adjuvant setting [3, 4, 5].

In clinical practice, therapeutic drug monitoring (TDM) can be used to monitor the exposure to increase efficacy or limit toxicity of treatment. For osimertinib, a correlation has been observed between area under the plasma concentration–time curve ($AUC_{0\text{-}24,SS}$) and the occurrence of rash or diarrhoea [6]. However, no relation was observed between systemic exposure and efficacy outcomes, although large variation was observed in osimertinib $AUC_{0\text{-}24,SS}$ and the maximal concentration ($C_{min,SS}$) after multiple dosing [6, 7, 8, 9].

Although central nervous system (CNS) penetration of osimertinib is good, increasing osimertinib exposure, especially in patients with sub-average blood levels, could theoretically further improve responsiveness of metastases in the CNS, which is a common metastatic site in EGFR mutated NSCLC [10, 11]. Increasing the osimertinib exposure can be achieved by doubling the daily dose [9], but this is expensive. Because osimertinib is mainly metabolized by Cytochrome P450 3A (CYP3A), co-administration with a strong CYP3A-inhibitor could potentially be an affordable method to increase osimertinib exposure [12]. Previous research demonstrated that co-administration of osimertinib with itraconazole caused a 24% increase in osimertinib $AUC_{0\text{-}24,SS}$ [13]. However, itraconazole is not the most potent CYP3A-inhibitor available [14, 15] and has additional pharmacological properties and therefore off-target effects. Cobicistat is a strong CYP3A inhibitor, lacks off target effects and has previously been studied extensively as booster for antiretroviral therapies [16, 17]. Given its highly potent CYP3A inhibiting property and favourable safety profile, cobicistat may be an excellent candidate drug to use as booster in the oncology setting as well. Until now, the experience with cobicistat in the oncology setting is extremely limited [18, 19]. Although one study evaluated a similar approach with ritonavir in erlotinib patients [20], the boosting capacity of cobicistat on osimertinib exposure is unknown.

Therefore, in this pharmacokinetic, proof-of-concept study (OSIBOOST trial), we evaluated if, and to what extent, cobicistat could increase osimertinib exposure, and whether the boosting effect was stable over time.

METHODS

Patients

Patient eligibility criteria included a) using osimertinib as part of their regular treatment plan, without any signs of progression or if treatment beyond progression was deemed appropriate by the treating physician because of continuing clinical benefit; b) 18 years or older; c) World Health Organization (WHO) performance status (PS) of 2 or lower; d) able and willing to sign informed consent; e) able and willing to undergo whole blood sampling for pharmacokinetic analysis and f) steady state plasma trough concentration ($C_{min,SS}$) of osimertinib ≤195 ng/mL. The plasma trough concentration of osimertinib was determined previously during routine care before study participation. The threshold $C_{min,SS}$ was selected based on the population mean observed in the two participating centres, which was 224 ng/mL (data not published). Exclusion criteria were: a) concurrent use of a drug that is known to strongly inhibit or induce CYP3A4/CYP3A5 (see Appendix A for specific drugs); b) concurrent use of a drug that is metabolized by CYP3A4/CYP3A5 and has a small therapeutic window (see Appendix A for specific drugs; c) concurrent use of products that are known to influence CYP3A4/CYP3A5-activity (e.g. grapefruit(juice), St. John's wort); d) impairment of gastrointestinal function that may alter absorption of osimertinib or cobicistat (ulcerative disease, uncontrolled nausea of vomiting, malabsorption syndrome or small bowel resection); e) pregnancy or breast feeding and f) chronic liver disease, with a Child-Pugh score class C.

Trial design

This was a pharmacokinetic, proof-of-concept study in two comprehensive cancer centres in the Netherlands, Maastricht University Medical Centre (MUMC+) and the Netherlands Cancer Institute / Antoni van Leeuwenhoek Hospital (NKI/AVL) (the OSIBOOST trial). In this study, cobicistat was selected as CYP3A-inhibitor, given its high potency, lack of off-target effects, and based on the wide experience with cobicistat as boosting agent for antiretroviral therapies.

Patients were asked to visit the hospital twice for pharmacokinetic (PK) blood sampling. The second PK visit was scheduled 22–26 days after the first PK visit. Cobicistat use started the day after the first PK visit and continued up to and including the day of the second PK visit. After the second PK day, patients a) could opt to stop cobicistat treatment, b) could continue cobicistat treatment on expanded access basis if substantial boosting was observed and the treating physician approved continuation after shared decision making with the participant or c) were asked to participate in a subsequent part of the study, in which the cobicistat dose was escalated in a stepwise manner to 150 mg, twice daily (BID) or four times a day (QID). Dose escalation of cobicistat was solely performed in patients who still had an osimertinib plasma trough concentration ≤195 ng/mL on the second PK-visit and if the previous cobicistat dose did not cause additional toxicity. An overview of the design of the study is schematically shown in Figure B1 in Appendix B.

The study was conducted in accordance Good Clinical Practice guidance. The study protocol (NCT03858491 / EudraCT number 2018–004290-28) was reviewed and approved by an independent ethics committee (METC19-013). This study was funded by the Netherlands Organisation for Health Research and Development (ZonMw).

Procedures

On both PK days, blood samples were collected on pre-specified time points, which were used to plot the plasma concentration–time curve of osimertinib to calculate the $AUC_{0\text{-}24,SS}$. EDTA whole blood samples were collected for pharmacokinetic analysis at four different time points: pre-dose, 0.5–1.5 h post-dose, 2.5–3.5 h post-dose and 7–8 h post-dose, which were similar to the moments used in the phase II and III AURA registration studies of osimertinib. Blood samples drawn for osimertinib measurement were transported and processed immediately, as the stability of osimertinib in blood (plasma) at room temperature is limited [15]. Osimertinib and metabolite AZ5104 concentrations were determined in a pharmaceutical laboratory in the MUMC+, using a previously described and validated assay [21]. In addition, an electrocardiogram was evaluated, as well as haematology, renal and liver function tests (sampled pre-dose).

Outcomes

The primary outcome was the change in total $AUC_{0\text{-}24,SS}$ for osimertinib and AZ5104. AZ5104 was incorporated in the pharmacokinetic analyses, as it was shown to be potent against mutated *EGFR in vitro* [9]. However, it is complex to estimate the exact contribution of AZ5104 to the *in vivo* antitumor effect. Therefore, we pragmatically decided to weigh the $AUC_{0\text{-}24,SS}$ of AZ5104 similar to the $AUC_{0\text{-}24,SS}$ of osimertinib and calculate a total $AUC_{0\text{-}24,SS}$ (osimertinib + AZ5104). The AUC constructed for the first PK day was used as baseline, and change was calculated as: $(AUC_{SECOND} – AUC_{FIRST}) / AUC_{FIRST}$. Secondary outcomes included information on CYP3A4 and CYP3A5 genotype, adverse events (AEs, registered according to CTCAE v5.0 [22]) and osimertinib plasma trough concentration(s) after study participation (in patients that continued cobicistat) as a surrogate marker of $AUC_{0\text{-}24,SS}$ 2–4 months after study participation to evaluate whether the effect of cobicistat lasted and was consistent with results seen on the second PK day. For CYP3A4 genotype several alleles were evaluated: *1A, *1B, *1G, *6, *8, *11, *13, *16, *17, *18, *20, *22 and *26. Furthermore, for CYP3A5 alleles *1 - *7 were evaluated.

Pharmacokinetic and statistical analysis

For the $AUC_{0\text{-}24,SS}$ curve we assumed that the concentration of osimertinib 24 h after the intake of osimertinib was similar to the concentration measured immediately pre-dose. The $AUC_{0\text{-}24,SS}$ was estimated using the trapezoidal method [23]. Results are shown in percentages, concentrations or presented descriptively. As this was a pharmacokinetic, proof-of-concept study, no formal statistical tests were performed.

RESULTS

Patients

In total 11 patients were enrolled, with a mean age of 67.4 years. Four patients were male, and one patient was a current smoker. All patients had WHO PS 0–1. Five patients had exon 19 deletion as primary *EGFR*-mutation, four exon 21 L858R, and two an uncommon *EGFR*-mutation. Furthermore, the T790M mutation was identified in nine patients. One patient was regularly treated in the first line with osimertinib, and ten patients in a later treatment line. Ten patients were treated with 80 mg once daily, while one patient received 160 mg daily, but still had a $C_{min,SS}$ ≤195 ng/mL. Detailed baseline characteristics are shown in Table 1.

Table 1: baseline characteristics and boosting of osimertinib exposure in patients simultaneously treated with cobicistat (150 mg, QD) for osimertinib AUC alone and the total AUC (osimertinib plus AZ5104) during steady state.

Patient	Sex	Primary EGFR-mutation	T790M	Previous EGFR-TKI treatment	Baseline trough concentration (ng/mL)	Boost $AUC_{0\text{-}24,SS}$ - osimertinib	Boost $AUC_{0\text{-}24,SS}$ – total
#1	Female	Exon 19 deletion	Yes	Erlotinib	219	22%	19%
#2	Male	L858R	Yes	Erlotinib	151	21%	21%
#3	Male	Exon 19 deletion	Yes	Erlotinib	134	39%	35%
#4	Female	L858R	No	Erlotinib	118	37%	35%
#5	Male	L858R	Yes	Erlotinib	162	50%	44%
#6	Female	Exon 19 deletion	Yes	Erlotinib Gefitinib	185	50%	46%
#7	Male	Exon 19 deletion	Yes	Erlotinib	107	56%	52%
#8	Female	Other	Yes	-	150	77%	68%
#9	Female	Exon 19 deletion	No	Gefitinib	156	77%	75%
#10	Female	L858R	Yes	Erlotinib	155	77%	76%
#11	Female	Other	Yes	Afatinib	114	215%	192%

Abbreviations: EGFR = epidermal growth factor receptor, TKI = tyrosine kinase inhibitor, ng = nanogram, mL = millilitre, AUC = area-under-the-curve, 0-24, ss = from 0 – 24 hours during steady state.

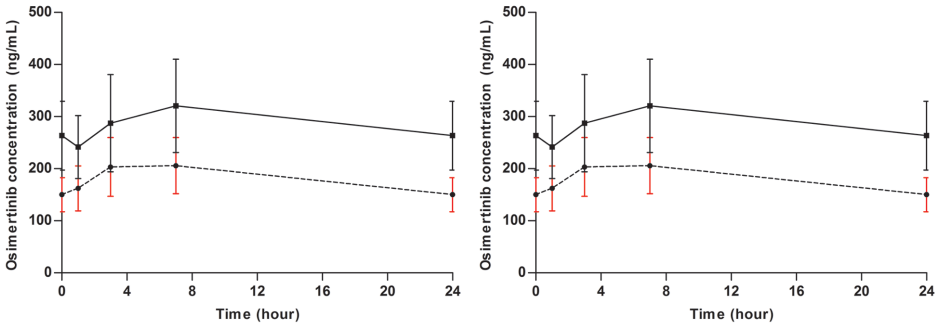

Figure 1: mean plasma concentration of osimertinib (left) and AZ5104 (right) on both PK days.
Legend: mean plasma concentration on PK Day I (dotted line) and PK Day II (solid line), with variation shown in red and black, respectively.

Pharmacokinetic analyses

The mean baseline osimertinib $C_{min,SS}$ was 154 ng/mL, which was measured during steady state for all patients, during the screening phase before study participation. The mean duration between the start of osimertinib and the first plasma trough concentration measurement was 64 days (range: 15–224 days). During the intervention period, all patients experienced an increase in osimertinib + AZ5104 exposure. The mean total $AUC_{0-24,SS}$ increase was 60% (range 19%–192%), which seemed to be larger in women (73%; range 19%–192%) as compared to men (38%; range 21%–52%), with one patient experiencing a considerably large increase in osimertinib exposure of 192% (*see* Table 1 and Appendix C).

The increase in total $AUC_{0-24,SS}$ was mainly driven by an increase of the osimertinib $AUC_{0-24,SS}$, as the absolute $AUC_{0-24,SS}$ of AZ5104 remained similar, while the $AUC_{0-24,SS}$ of osimertinib increased after co-administration of cobicistat (Figure 1). Among all patients, no major deviations in treatment compliance were observed, and no interfering CYP3A-treatments were started during the study period.

After co-administration with cobicistat, three patients had osimertinib plasma trough levels that were still below 195 ng/mL. Therefore, the cobicistat dose was escalated to 150 mg BID in these patients. One patient experienced a decrease in osimertinib exposure upon escalation to cobicistat 150 mg, BID, compared to cobicistat 150 mg, QD (+52% [QD] to + 39% [BID]), relative to osimertinib baseline exposure. The other two patients experienced an increase in osimertinib exposure (+21% [QD] to + 27% [BID], and + 35% [QD] to + 55% [BID], respectively, relative to baseline). The dosing frequency of cobicistat was further increased in one patient that experienced a decrease in osimertinib exposure. The exposure of osimertinib further decreased with cobicistat 150 mg, QID (+1%, relative to baseline exposure). In general, trough values ($C_{min,SS}$) of osimertinib correlated well with the total $AUC_{0-24,SS}$ *(R^2 = 0.926),* which is shown in Figure D1 in Appendix D.

Pharmacogenetics

Information about CYP3A4/CYP3A5 genotype was available for 7/11 patients. The evaluation of pharmacogenetics was done after study participation (informed consent was obtained in concordance with an approved amendment to the original trial protocol) and some patients were unable to supply an additional blood sample because they were meanwhile treated in another hospital or were lost to follow-up. Six patients carried the CYP3A4*1B/*1B variant, and one patient had the CYP3A4*1B/*1G polymorphism. Therefore, all patients were extensive CYP3A4 metabolizers. Furthermore, all seven patients were CYP3A5 non-expressers (CYP3A5*3/*3 in six patients, and CYP3A5*2/*3 in one patient). Both the extensive CYP3A4 metabolizer phenotype and the CYP3A5 non-expressor phenotype are the most frequently (>85–90%) found phenotypes in Caucasians. For these seven patients, genetic polymorphisms could therefore not explain any variation seen in osimertinib exposure and the total boosting effect of cobicistat.

Safety

No serious or unexpected AEs were observed. All reported AEs (n = 20) were of grade 1, of which 14 AEs were potentially related to osimertinib (ten = possible, one = probable, three = related) (see Table 2).

Table 2: adverse events reported in patients during simultaneous treatment of osimertinib and cobicistat 150 mg, QD.

Patient	AE	Specify	Grade	Relation to osimertinib
#1	Deviating laboratory value	AF, ASAT, LD, and monocytes	1	Possible
#1	Rash		1	Probable
#3	Cough		1	Possible
#3	Diarrhoea		1	Related
#3	Rhagades		1	Related
#5	Deviating laboratory value	ASAT, gGT and LD	1	Possible
#6	Deviating laboratory value	CK	1	Related
#6	Rhagades		1	Possible
#7	AV-block		1	Possible
#7	Deviating laboratory value	Creatinine, CK and urea	1	Possible
#8	Diarrhoea		1	Possible
#8	Deviating laboratory value	AF and potassium	1	Possible
#10	Pain	Headache	1	Possible
#11	Pain	Due to earlier fracture	1	Possible

Abbreviations: AF = alkaline phosphatase, ASAT = aspartate amino transaminase, AV = atrioventricular, CK = creatinine kinase, gGT = gamma glutamyl transferase, LD = lactate dehydrogenase.
This table shows all adverse events that were related to the osimertinib treatment (possible/probable/related).

Follow-up after study

In total, nine patients opted to continue cobicistat after the study intervention period, and six patients were willing to give one or two additional blood sample(s) during the expanded access phase. The measured plasma trough concentrations were extrapolated to an AUC, based on the correlation between $C_{min,SS}$ and $AUC_{0\text{-}24,SS}$ seen at the two study PK visits. In five patients, the extrapolated AUC was comparable (mean difference = 21%) to the total $AUC_{0\text{-}24,SS}$ seen on the last study visit. However, in one patient, a considerable increase in the plasma trough concentration, and consequently the extrapolated AUC, was noticed (increase = 376%). This could not be explained by adjustments in co-medication or changes in treatment adherence. As no possibly osimertinib-related AEs were reported for this patient it was decided to continue simultaneous treatment with osimertinib and cobicistat.

DISCUSSION

In this study, the boosting capacity of cobicistat on osimertinib exposure was evaluated in patients with NSCLC who had a low osimertinib plasma trough concentration, i.e., ≤195 ng/mL. In all patients, treatment with cobicistat led to an increase in the total $AUC_{0\text{-}24,SS}$ of osimertinib + AZ5104, without adding significant toxicity. The increase in osimertinib exposure was stable in general, even after long-term continuation of osimertinib plus cobicistat 150 mg QD, in most patients. Furthermore, a large increase in osimertinib exposure in one patient was noticed during the study (+192%) period and in one patient during the follow-up (+376%, after extrapolation of the plasma trough concentration). Potential causes for interpatient differences in osimertinib boosting including CYP3A-genotypes and changes in co-medication were excluded. We were unable to find a plausible explanation for these large increases and decided to continue treatment as long as the combination treatment was well tolerated. Both patients continued cobicistat addition to osimertinib for at least six months, after study participation, without any safety concerns.

Dose escalation of cobicistat (to 150 mg BID or QID) led to inconsistent results. In two patients the increase of the cobicistat dosage to 150 mg BID let to a further increase in osimertinib exposure, relative to the increase seen with cobicistat 150 mg QD. However, in one patient, cobicistat dose escalation resulted in a decrease in osimertinib exposure, which was even more so when the dose was further escalated to 150 mg QID. Unfortunately, we were unable to identify the cause of this paradoxical effect as changes in adherence and use of co-medication with potential CYP3A influencing effects were ruled out.

In contrast to the extensive number of studies investigating the use of cobicistat in patients with acquired immunodeficiency syndrome (AIDS), the use of cobicistat to boost the exposure to anti-cancer drugs has only been described in two cases [18, 19]. A patient with renal cell carcinoma was treated with axitinib and experienced low axitinib plasma trough concentrations. Because solely increasing the dosage of axitinib or combining the

therapy with the intake of grapefruit juice did not lead to the desired outcome, cobicistat was used to boost axitinib exposure. Eventually, adequate exposure was achieved when combining axitinib (10 mg, QID), with cobicistat (150 mg, QID). In this case-report, the effect of cobicistat was mainly seen on the maximum axitinib concentration, while the plasma trough concentration of axitinib remained relatively constant [18]. Another study was planned to evaluate the boosting capacity of cobicistat in patients treated with crizotinib. However, due to limited patient accrual, as a consequence of the marketing authorization for alectinib, only one patient was included. In this patient the combination with cobicistat, 150 mg QD, led to an increase in crizotinib exposure of 78%. No information was available about the consistency of the boosting effect of cobicistat, as only one patient was included, and no follow-up crizotinib exposure measurement was performed [19]. Our clinical trial is the first formal clinical trial in which a group of patients with cancer is treated with cobicistat to improve the exposure to an anti-cancer drug, including follow-up trough concentration measurements.

Osimertinib has two active metabolites, AZ5104 and AZ7550. *In vitro* studies demonstrated that AZ5104 may have a slightly higher potency for mutated *EGFR*s as compared to osimertinib, while the potency of AZ7550 is thought to be lower for mutated *EGFR*s compared to osimertinib. As both metabolites are formed to a similar extent (approximately 10% of the $AUC_{0\text{-}24,SS}$ of osimertinib), we decided to incorporate AZ5104 in these analyses, and ignore the minimal contribution of AZ7550 to the total effect [9]. However, it is rather complex to estimate how much osimertinib and its metabolites contribute to the anti-tumour effect *in vivo*. In addition to the potency of the metabolite, other factors could contribute to the antitumor activity, such as body distribution, tumour tissue penetration and protein binding. Lack of this information makes it difficult to make a reliable estimation of the exact effect of AZ5104 *in vivo* compared to the effect of osimertinib itself. Therefore, we arbitrarily allocated similar importance (1:1) to the $AUC_{0\text{-}24,SS}$ of osimertinib and the $AUC_{0\text{-}24,SS}$ of AZ5104, which was shown in Table 1 as total $AUC_{0\text{-}24,SS}$. A different allocation of importance of osimertinib and AZ5104 would have led to slightly different results of the boosting capacity of cobicistat. However, as the effect of cobicistat was mainly seen in the $AUC_{0\text{-}24,SS}$ of osimertinib itself, we believe a different allocation of importance for osimertinib and AZ5104 would not have led to other conclusions.

In this study, cobicistat increased osimertinib exposure in all patients, and in most patients a sufficient effect (plasma trough concentration >195 ng/mL) was achieved with cobicistat 150 mg QD co-administration. A larger boosting effect was seen in women compared to men. This apparent difference may potentially be explained by the higher CYP3A activity in women in general [24], as a higher CYP3A baseline activity offers an opportunity for a more pronounced inhibitory effect of cobicistat.

Consequently, the osimertinib boosting results of our study could have multiple potential implications for clinical practice. In patients with low osimertinib exposure, cobicistat

could be used to increase osimertinib exposure in a cheap and safe manner, as cobicistat has no physiological off-target effects. While the penetration of osimertinib in the CNS is considerably better compared to first- and second-generation EGFR-TKIs [25], the CNS remains a common metastatic and progression site for *EGFR*-mutated NSCLC [5, 10]. Therefore, in patients experiencing CNS (oligo-) progression, dose escalation might be considered to increase osimertinib exposure and anti-tumour activity in the CNS [11, 26]. As the price of 150 mg cobicistat is approximately 200 times less than doubling the osimertinib dose, the use of cobicistat may be a viable option to increase (cerebral) osimertinib exposure. A more general approach, of boosting osimertinib exposure purely based on the plasma trough concentration, to improve osimertinib effectiveness is less evident, as a definitive exposure–response relation seems absent for osimertinib. Especially boosting in patients with initially high osimertinib exposure may be less ideal, as it could this could lead to a higher level of toxicity (≥259 ng/mL) [27]. However, inhibiting CYP3A-activity could theoretically increase the anti-tumour activity of osimertinib, as intratumoral CYP3A activity would be inhibited, which is increased in NSCLC-patients [28].

Furthermore, in patients with an average or relatively high osimertinib exposure, addition of cobicistat may enable the use of a lower osimertinib dose, while maintaining similar exposure. However, the magnitude of osimertinib boosting in this study may not be representative for all osimertinib users. Given the low osimertinib exposure at baseline, CYP3A4 activity in our study population may be higher compared to patients with relatively high osimertinib exposure. Although other factors are involved, this may implicate that the boosting effect of cobicistat may be less pronounced in patients with average to high osimertinib exposure. Therefore, evaluating the effect of cobicistat in patients with a higher initial osimertinib exposure will be interesting, to further develop a strategy for osimertinib boosting by cobicistat in clinical practice.

However, the variation seen in osimertinib boosting by cobicistat so far makes it challenging to compose a one-fits-all approach. A similar variation was seen in a study by Boosman *et al.*, which evaluated the boosting capacity of ritonavir on erlotinib exposure [28]. More research is warranted to evaluate whether the boosting method can be fine-tuned using TDM guidance. Future research could therefore also focus on evaluating whether the approach presented in this study could be used for other (expensive) targeted small-molecule inhibitors. Any drug that is predominantly metabolized by CYP3A4/5 and is still under patent could be a viable option and potentially lead to a more tailored treatment in clinical practice with possibly considerable cost-savings.

CONCLUSION

In this study concomitant use of cobicistat successfully increased the osimertinib exposure ($AUC_{0\text{-}24,SS}$, osimertinib + AZ5104). Cobicistat addition was well tolerated and its boosting effect on osimertinib was constant during the follow-up.

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APPENDIX A

List of strong inhibitors or inducers of CYP3A4 and substrates with a narrow therapeutic window, which were prohibited during the study period.

Inducers of CYP3A4

- Carbamazepine
- Efavirenz
- Enzalutamide
- Hypericum
- Mitotane
- Nevirapine
- Phenobarbital
- Phenytoin
- Primidone
- Rifabutin
- Rifampicin

Inhibitors of CYP3A4

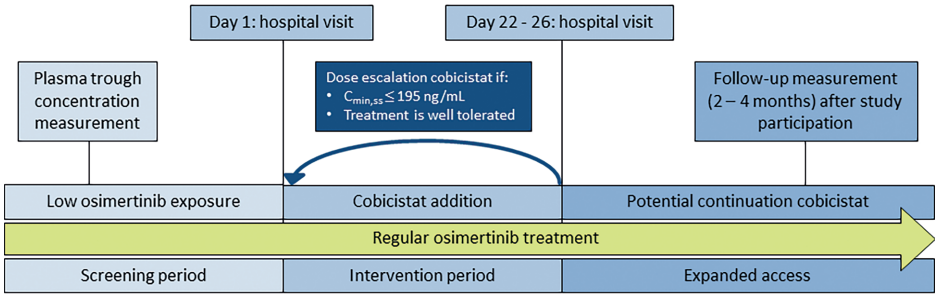
- Clarithromycin
- Cobicistat
- Erythromycin
- Itraconazole
- Ketoconazole
- Ritonavir
- Voriconazole

Substrates with narrow therapeutic window

- Cyclosporine

APPENDIX B

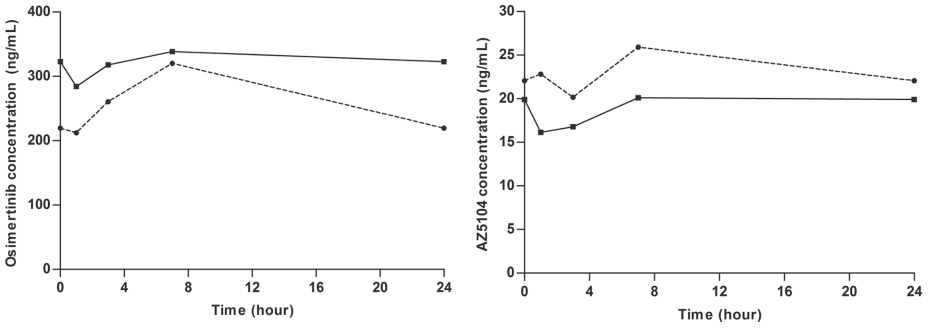
Figure B1: schematic overview of the OSIBOOST trial for patients with low initial osimertinib exposure ($C_{min,SS}$ ≤ 195 ng/mL).



APPENDIX C

Results of osimertinib boosting with cobicistat for each individual patient.

Figure C1: plasma concentration of osimertinib of patient #1 before and after simultaneous treatment with cobicistat 150 mg QD.

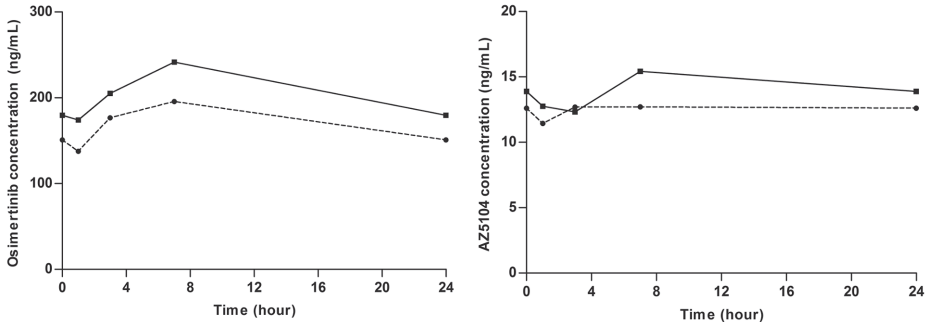


Legend: plasma concentration of osimertinib on PK day I (dotted line) and PK day II (solid line) of patient #1 (left) and plasma concentration of AZ5104 on PK day I (dotted line) and PK day II (solid line) of patient #1 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II reflects the osimertinib exposure after three weeks of co-administration with cobicistat.

AUC_{osi} – baseline	6436
AUC_{AZ5104} – baseline	565
AUC_{osi} – boosted	7838
AUC_{AZ5104} – boosted	465

*All AUCs are $AUC_{0\text{-}24,SS}$;
*Unit = ng*hour/mL.

Figure C2.1: plasma concentration of osimertinib of patient #2 before and after simultaneous treatment with cobicistat 150 mg QD.

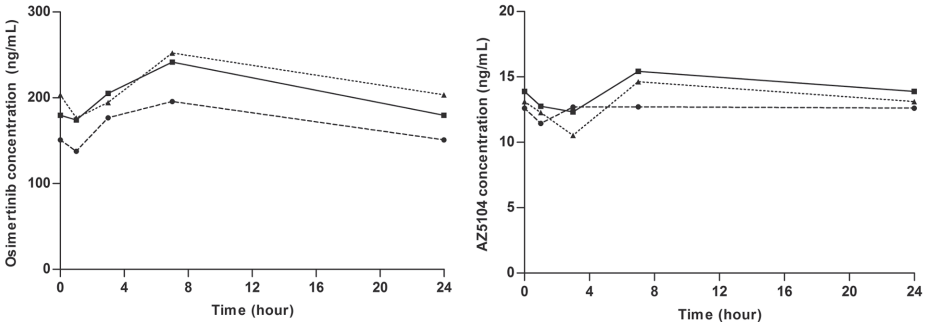


Legend: plasma concentration of osimertinib on PK day I (dotted line) and PK day II (solid line) of patient #2 (left) and plasma concentration of AZ5104 on PK day I (dotted line) and PK day II (solid line) of patient #2 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II reflects the osimertinib exposure after three weeks of co-administration with cobicistat.

AUC_{osi} – baseline	4147
AUC_{AZ5104} – baseline	302
AUC_{osi} – boosted	5030
AUC_{AZ5104} – boosted	343

*All AUCs are $AUC_{0\text{-}24,SS}$;
*Unit = ng*hour/mL.

Figure C2.2: plasma concentration of osimertinib of patient #2 before and after simultaneous treatment with cobicistat 150 mg QD and BID.

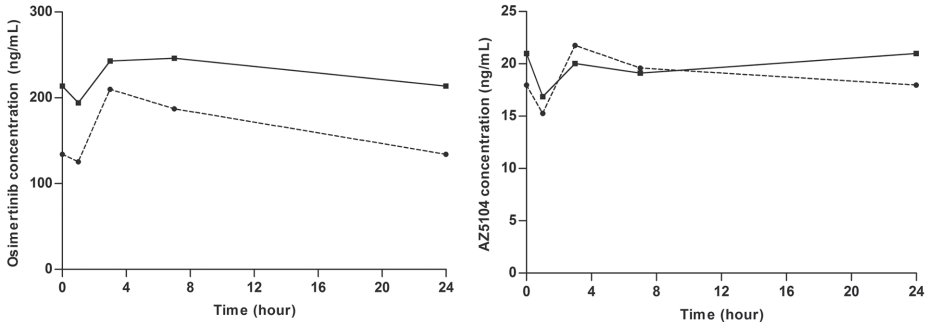


Legend: plasma concentration of osimertinib on PK day I (dotted line, circle), PK day II (solid line) and PK day III (dotted line, triangle) of patient #2 (left) and plasma concentration of AZ5104 on PK day I (dotted line, circle), PK day II (solid line) and PK day III (dotted line, triangle) of patient #2 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II/III reflects the osimertinib exposure after three weeks of co-administration with cobicistat (once and twice daily).

AUC_{osi} – baseline	4147
AUC_{AZ5104} – baseline	302
AUC_{osi} – boosted	5325
AUC_{AZ5104} – boosted	322

*All AUCs are $AUC_{0\text{-}24,SS}$;
*Unit = ng*hour/mL.

Figure C3: plasma concentration of osimertinib of patient #3 before and after simultaneous treatment with cobicistat 150 mg QD.

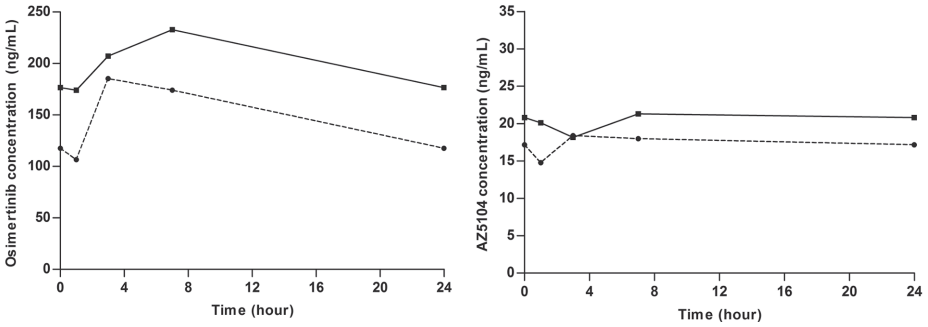


Legend: plasma concentration of osimertinib on PK day I (dotted line) and PK day II (solid line) of patient #3 (left) and plasma concentration of AZ5104 on PK day I (dotted line) and PK day II (solid line) of patient #3 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II reflects the osimertinib exposure after three weeks of co-administration with cobicistat.

AUC_{osi} – baseline	3988
AUC_{AZ5104} – baseline	455
AUC_{osi} – boosted	5527
AUC_{AZ5104} – boosted	475

*All AUCs are $AUC_{0\text{-}24,SS}$;
*Unit = ng*hour/mL.

Figure C4.1: plasma concentration of osimertinib of patient #4 before and after simultaneous treatment with cobicistat 150 mg QD.

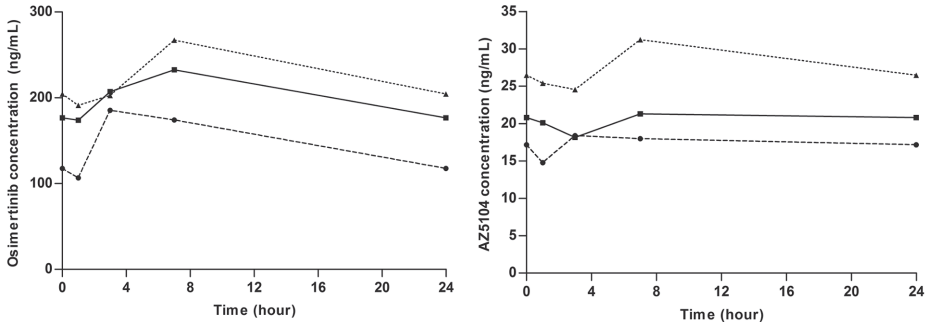


Legend: plasma concentration of osimertinib on PK day I (dotted line) and PK day II (solid line) of patient #4 (left) and plasma concentration of AZ5104 on PK day I (dotted line) and PK day II (solid line) of patient #4 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II reflects the osimertinib exposure after three weeks of co-administration with cobicistat.

AUC_{osi} – baseline	3602
AUC_{AZ5104} – baseline	420
AUC_{osi} – boosted	4937
AUC_{AZ5104} – boosted	495

*All AUCs are $AUC_{0\text{-}24,SS}$;
*Unit = ng*hour/mL.

Figure C4.2: plasma concentration of osimertinib of patient #4 before and after simultaneous treatment with cobicistat 150 mg QD and BID.

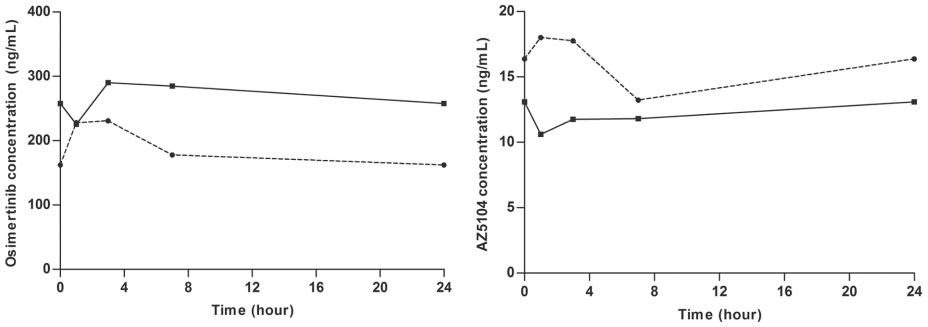


Legend: plasma concentration of osimertinib on PK day I (dotted line, circle), PK day II (solid line) and PK day III (dotted line, triangle) of patient #4 (left) and plasma concentration of AZ5104 on PK day I (dotted line, circle), PK day II (solid line) and PK day III (dotted line, triangle) of patient #4 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II/III reflects the osimertinib exposure after three weeks of co-administration with cobicistat (once and twice daily).

AUC_{osi} – baseline	3602
AUC_{AZ5104} – baseline	420
AUC_{osi} – boosted	5556
AUC_{AZ5104} – boosted	679

*All AUCs are $AUC_{0\text{-}24,SS}$;
*Unit = ng*hour/mL.

Figure C5: plasma concentration of osimertinib of patient #5 before and after simultaneous treatment with cobicistat 150 mg QD.

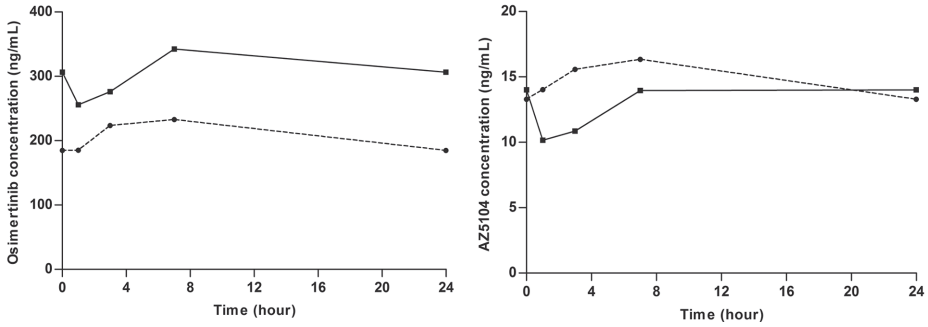


Legend: plasma concentration of osimertinib on PK day I (dotted line) and PK day II (solid line) of patient #5 (left) and plasma concentration of AZ5104 on PK day I (dotted line) and PK day II (solid line) of patient #5 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II reflects the osimertinib exposure after three weeks of co-administration with cobicistat.

AUC_{osi} – baseline	4361
AUC_{AZ5104} – baseline	367
AUC_{osi} – boosted	6520
AUC_{AZ5104} – boosted	293

*All AUCs are $AUC_{0\text{-}24,SS}$;
*Unit = ng*hour/mL.

Figure C6: plasma concentration of osimertinib of patient #6, before and after simultaneous treatment with cobicistat 150 mg QD.

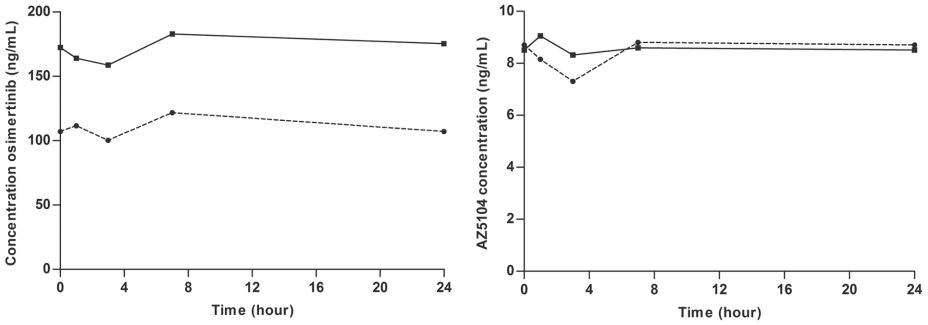


Legend: plasma concentration of osimertinib on PK day I (dotted line) and PK day II (solid line) of patient #6 (left) and plasma concentration of AZ5104 on PK day I (dotted line) and PK day II (solid line) of patient #6 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II reflects the osimertinib exposure after three weeks of co-administration with cobicistat.

AUC_{osi} – baseline	5055
AUC_{AZ5104} – baseline	359
AUC_{osi} – boosted	7565
AUC_{AZ5104} – boosted	320

*All AUCs are $AUC_{0\text{-}24,SS}$;
*Unit = ng*hour/mL.

Figure C7.1: plasma concentration of osimertinib of patient #7, before and after simultaneous treatment with cobicistat 150 mg QD.

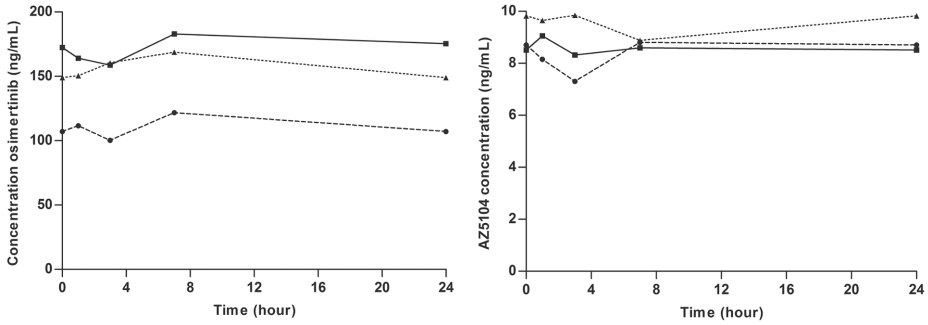


Legend: plasma concentration of osimertinib on PK day I (dotted line) and PK day II (solid line) of patient #7 (left) and plasma concentration of AZ5104 on PK day I (dotted line) and PK day II (solid line) of patient #7 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II reflects the osimertinib exposure after three weeks of co-administration with cobicistat.

AUC_{osi} – baseline	2710
AUC_{AZ5104} – baseline	205
AUC_{osi} – boosted	4222
AUC_{AZ5104} – boosted	205

*All AUCs are $AUC_{0\text{-}24,SS}$;
*Unit = ng*hour/mL.

Figure C7.2: plasma concentration of osimertinib of patient #7, before and after simultaneous treatment with cobicistat 150 mg QD and BID.

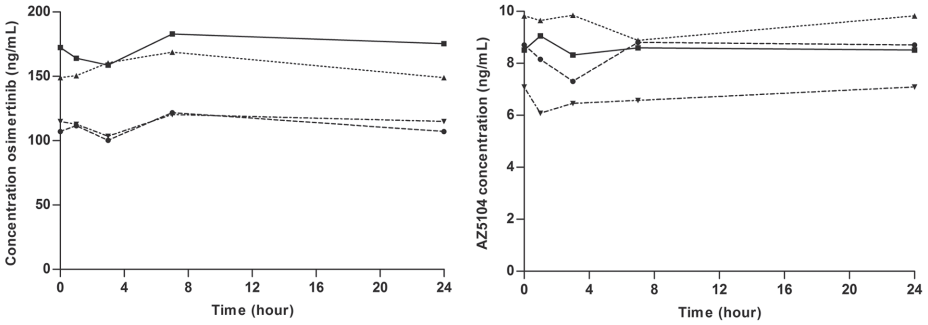


Legend: plasma concentration of osimertinib on PK day I (dotted line, circle), PK day II (solid line) and PK day III (dotted line, triangle) of patient #7 (left) and plasma concentration of AZ5104 on PK day I (dotted line, circle), PK day II (solid line) and PK day III (dotted line, triangle) of patient #7 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II/III reflects the osimertinib exposure after three weeks of co-administration with cobicistat (once and twice daily).

AUC_{osi} – baseline	2710
AUC_{AZ5104} – baseline	205
AUC_{osi} – boosted	3819
AUC_{AZ5104} – boosted	226

*All AUCs are $AUC_{0\text{-}24,SS}$;
*Unit = ng*hour/mL.

Figure C7.3: plasma concentration of osimertinib of patient #7 before and after simultaneous treatment with cobicistat 150 mg QD, BID and QID.

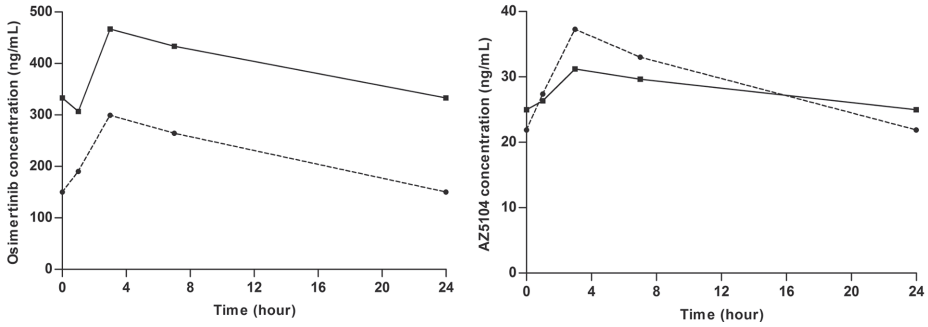


Legend: plasma concentration of osimertinib on PK day I (dotted line, circle), PK day II (solid line), PK day III (dotted line, triangle) and PK day IV (dotted line, upside down triangle) of patient #7 (left) and plasma concentration of AZ5104 on PK day I (dotted line, circle) and PK day II (solid line), PK day III (dotted line, triangle) and PK day IV (dotted line, upside down triangle) of patient #7 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II/III/IV reflects the osimertinib exposure after three weeks of co-administration with cobicistat (once daily, twice daily and four times a day).

AUC_{osi} – baseline	2710
AUC_{AZ5104} – baseline	205
AUC_{osi} – boosted	2776
AUC_{AZ5104} – boosted	161

*All AUCs are $AUC_{0\text{-}24,SS}$;
*Unit = ng*hour/mL.

Figure C8: plasma concentration of osimertinib of patient #8 before and after simultaneous treatment with cobicistat 150 mg QD.

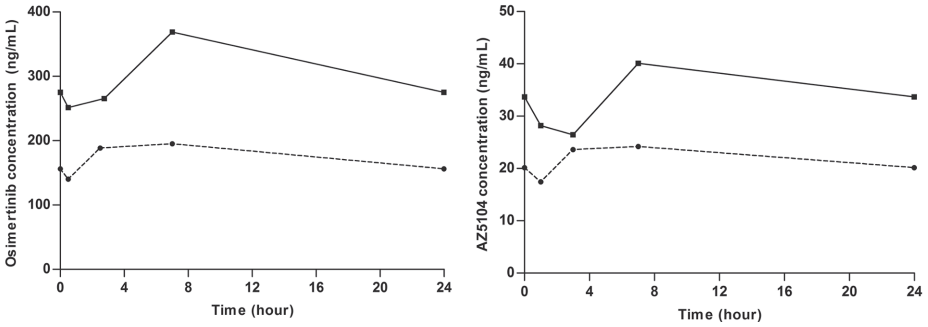


Legend: plasma concentration of osimertinib on PK day I (dotted line) and PK day II (solid line) of patient #8 (left) and plasma concentration of AZ5104 on PK day I (dotted line) and PK day II (solid line) of patient #8 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II reflects the osimertinib exposure after three weeks of co-administration with cobicistat.

AUCosi – baseline	5308
AUCAZ5104 – baseline	697
AUCosi – boosted	9408
AUCAZ5104 – boosted	669

*All AUCs are AUC0-24,SS;
*Unit = ng*hour/mL.

Figure C9: plasma concentration of osimertinib of patient #9 before and after simultaneous treatment with cobicistat 150 mg QD.

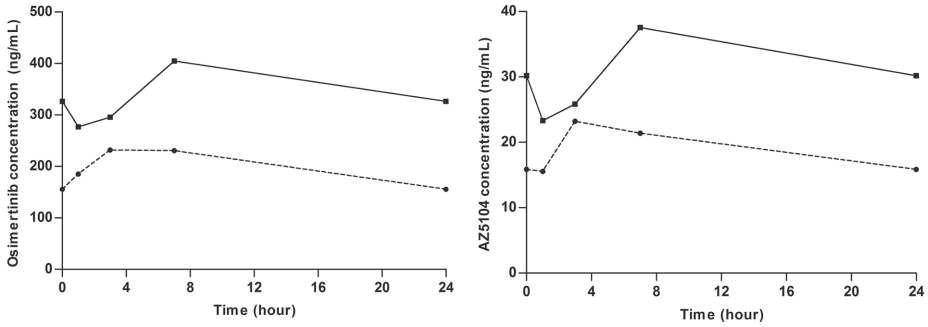


Legend: plasma concentration of osimertinib on PK day I (dotted line) and PK day II (solid line) of patient #9 (left) and plasma concentration of AZ5104 on PK day I (dotted line) and PK day II (solid line) of patient #9 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II reflects the osimertinib exposure after three weeks of co-administration with cobicistat.

AUC_{osi} – baseline	4251
AUC_{AZ5104} – baseline	532
AUC_{osi} – boosted	7535
AUC_{AZ5104} – boosted	846

*All AUCs are $AUC_{0\text{-}24,SS}$;
*Unit = ng*hour/mL.

Figure C10: plasma concentration of osimertinib of patient #10 before and after simultaneous treatment with cobicistat 150 mg QD.

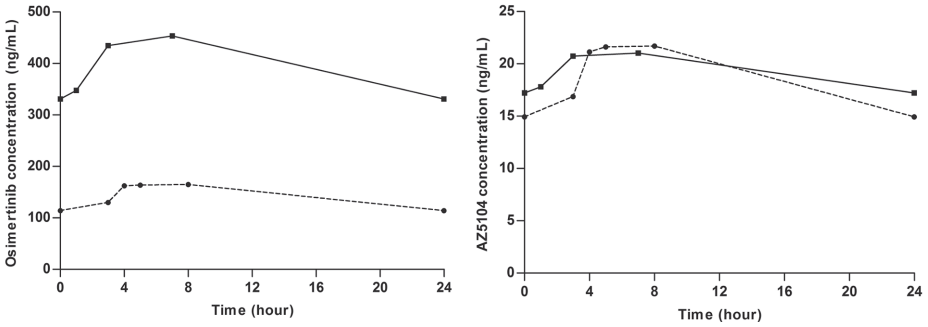


Legend: plasma concentration of osimertinib on PK day I (dotted line) and PK day II (solid line) of patient #10 (left) and plasma concentration of AZ5104 on PK day I (dotted line) and PK day II (solid line) of patient #10 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II reflects the osimertinib exposure after three weeks of co-administration with cobicistat.

AUC_{osi} – baseline	4794
AUC_{AZ5104} – baseline	460
AUC_{osi} – boosted	8489
AUC_{AZ5104} – boosted	779

*All AUCs are $AUC_{0\text{-}24,SS}$;
*Unit = ng*hour/mL.

Figure C11: plasma concentration of osimertinib of patient #11 before and after simultaneous treatment with cobicistat 150 mg QD.



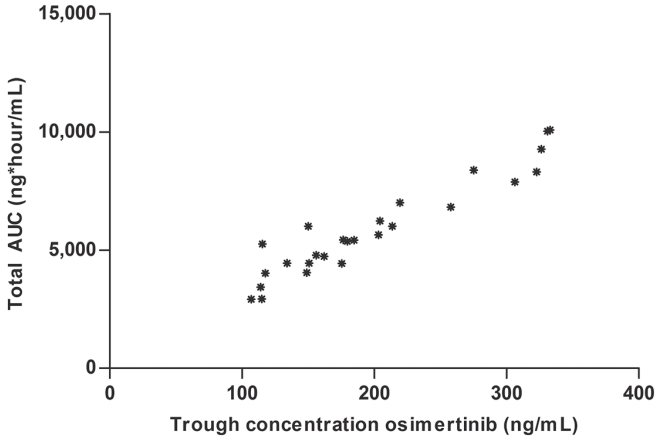
Legend: plasma concentration of osimertinib on PK day I (dotted line) and PK day II (solid line) of patient #11 (left) and plasma concentration of AZ5104 on PK day I (dotted line) and PK day II (solid line) of patient #11 (right). PK day I represents baseline osimertinib exposure during steady state, and PK day II reflects the osimertinib exposure after three weeks of co-administration with cobicistat.

AUC_{osi} – baseline	3033
AUC_{AZ5104} – baseline	398
AUC_{osi} – boosted	9566
AUC_{AZ5104} – boosted	465

*All AUCs are $AUC_{0\text{-}24,SS}$;
*Unit = ng*hour/mL.

APPENDIX D

Figure D1: relation between plasma trough concentration of osimertinib and the calculated AUC



Legend: correlation between the plasma trough concentration of osimertinib and the calculated total $AUC_{0\text{-}24,SS}$ for all patients on the study visit days (R^2 = 0.926).

6

Chapter 6

GENERAL DISCUSSION

GENERAL DISCUSSION & FUTURE PERSPECTIVES

The work presented in this thesis aimed to evaluate treatment optimization of patients with non-small cell lung cancer (NSCLC), focusing on efficacy, safety, and costs. In this chapter the findings of this thesis will be discussed and put into perspective in relation to other published work. Additionally, recommendations for clinical guidelines will be presented, as well as opportunities for future research. The chapter is divided into three subchapters, namely a) therapeutic drug monitoring (TDM), b) real-world data, and c) pharmacokinetic treatment enhancement.

Therapeutic drug monitoring

TDM can be used for multiple intentions, however its function can be summarised as optimizing pharmacotherapy by maximizing therapeutic efficacy, while minimizing adverse events, for drugs whose desired effects are better predicted by its blood concentration than its fixed dose. In **Chapter 2,** analytical methods to quantify tyrosine kinase inhibitors (TKIs) that are used in the treatment of epidermal growth factor receptor (*EGFR*) mutated NSCLC are described. This was done for osimertinib (**Chapter 2.1**), alectinib, crizotinib, erlotinib, gefitinib (**Chapter 2.2**), brigatinib, lorlatinib, pralsetinib and selpercatinib (**Chapter 2.3**). The analytical methods were developed to apply or investigate the role of TDM for those drugs. Additionally, the analytical methods can be used to perform drug-drug interaction (DDI) or pharmacokinetic boosting studies, which are presented in this thesis (**Chapter 3.1 and Chapter 5.1**) or are currently ongoing.

All three analytical methods were developed and validated according to the guideline of the European Medicines Agency (EMA) [1]. During the development of the osimertinib assay (**Chapter 2.1**), challenges regarding the short-term stability of osimertinib at room temperature were encountered. Osimertinib was instable in human serum (<2 hours), alkalized serum (<2 hours), acidified serum (<4 hours) and heparinized plasma (<4 hours), while the stability was better in EDTA plasma and EDTA whole blood. Similar issues with the stability of osimertinib at room temperature were reported by Veerman *et al.* [2]. However, previous studies had presented sufficient short-term stability (loss of <15% within six hours) for osimertinib at room temperature [3 - 6]. No stability issues were seen for the other TKIs, as described in **Chapter 2.2** and **Chapter 2.3**. During routinely performed blood analysis in clinical practice, it is critical to have knowledge about the stability of the drug in (different) blood components. During transport of a blood sample to the clinical laboratory, it will be present in whole blood, while it will be stored in plasma at freezing condition (-80 °C) and processed and analysed at room temperature (20 °C). A delay can occur in any of these processes in clinical practice, due to other clinical commitments/duties and unforeseen events. Therefore, it is critical to precisely establish the stability of the drug in each phase from blood extraction to the eventual quantification. This ensures a correct quantification of the drug, but also provides information on how to respond when a deviation occurred. Our results warranted extra caution for osimertinib,

during transport, but especially during plasma preparation and quantification, which we have advised to be performed on dry ice. In addition to detailed information regarding the stability of the drug, to be able to employ TDM, it is important to have complete and accurate information about the last administration of the drug and the timing of blood drawing. This information can be used to extrapolate plasma concentrations from blood samples which are not drawn at the exact specified time, using an algorithm as described by Wang *et al.* [7]. Furthermore, it is essential that the blood drawing took place after the moment when the plasma concentration reaches its peak (T_{max}), as until then the absorption speed (k_a) outweighs the elimination speed (k_e), making an accurate extrapolation impossible.

Both analytical methods described in **Chapter 2.2 and 2.3** comprises multiple TKIs (i.e., alectinib, crizotinib, erlotinib, and gefitinib and brigatinib, lorlatinib, pralsetinib, and selpercatinib respectively) that can be quantified in one single run. In addition, osimertinib quantification (**Chapter 2.1**) can be combined with the analytical method described in **Chapter 2.2**, to ensure the quantification of five TKIs with a single assay set-up. Combination of multiple TKIs in one analytical method improves the efficiency in the laboratory and reduces costs, as the workflow is less consuming and laboratory technicians can allocate their time more efficiently. Combining multiple TKIs in one assay is especially useful for an indication such as NSCLC, for which the number of patients using one specific TKI is relatively small in one centre. In addition, combining multiple TKIs in one assay provides a quicker reporting of the results to the treating physician, as a run can be performed more frequently.

Future perspectives

The analytical methods described in this thesis can be used to apply TDM in clinical practice and to evaluate the potential role that TDM may play in the future for the specific TKIs. For some TKIs a target threshold has been established, such as crizotinib ($C_{min,SS}$ >235 ng/mL) and alectinib ($C_{min,SS}$ >435 ng/mL) [8]. However, for the other TKIs that can be quantified with our analytical methods the role of TDM is less evident. In a recent review, the potential role of TDM in the treatment with antineoplastic drugs was discussed [9] and was scored as exploratory for afatinib, brigatinib, lorlatinib and osimertinib, as no target trough concentration has been determined for any of those TKIs. Furthermore, for drugs (e.g., pralsetinib, selpercatinib) that have been approved more recently, the role of TDM is not elucidated yet. Further investigating the role of TDM in the treatment with those drugs could be done in future research. This may also be done for TKIs that are close to reaching clinical practice, and have shown impressive antitumor activity in clinical studies, such as the KRAS-inhibitors sotorasib and adagrasib [10, 11]. Their clinical potential, combined with the high prevalence of *KRAS* G12C mutations in patients with NSCLC, will most likely lead to a substantial proportion of patients using sotorasib or adagrasib routinely in clinical practice in the future [12]. If a target concentration can be established early, this could further optimise the treatment with these drugs. On the other side, the validated analytical methods can be used for further clinical studies with

drug exposure (area-under-the-curve during steady-state; $AUC_{0\text{-}24,SS}$) as one of the main parameters or outcomes of interest. One example of such a clinical study is described in **Chapter 5.1**, which will be discussed in more detail below.

Real-world data

Besides TDM, studies using real-world data can also be used for treatment optimisation, as data from randomised clinical trials (RCTs) can be complemented with data from clinical practice. Treatment results in clinical practice may differ from RCT results, which is referred to as the efficacy-effectiveness gap. This has been previously shown for drugs used in the treatment of NSCLC-patients [13]. Differences in treatment effectiveness outcomes can be caused by the strict in- and exclusion criteria which are used for RCTs, leading to including a homogeneous group of patients [14]. Furthermore, the use of randomisation ensures two comparative groups of patients, in which the differences in outcomes are caused by the intervention (investigational drugs vs. standard of care). These characteristics give RCTs a strong internal validity. However, the potential to extrapolate the study results to the clinical practice, the external validity, may be hampered by the homogeneous group of included patients [15 - 17]. This has been shown previously in observational studies. Some observational studies used the exact set of criteria of a clinical trial to evaluate the eligibility of clinical practice patients [18, 19], while other studies used a more general set of criteria [20, 21]. Although this set of criteria in the latter was not directly retrieved from one specific RCT, the used criteria were all frequently applied in various RCTs, such as performance status, the presence of central nervous system (CNS) metastases or organ function. Real-world data can be used to complement data from RCTs. While studies using real-world data have some limitations (i.e., bias, due to confounding, misclassification, or missing data), It can be used to identify subgroups that are more likely to derive benefit from a specific treatment or are more prone to experience toxicity leading to treatment interruption or definitive treatment discontinuation.

Chapter 3.1 presents a real-world study which evaluated the effect of different patient characteristics on treatment outcomes with osimertinib, with focus on the impact of age, body mass index (BMI) and the plasma trough concentration of osimertinib ($C_{min,SS}$). In this study we found that the primary *EGFR* mutation, sex, BMI and $C_{min,SS}$ significantly influence the treatment effectiveness outcomes (i.e., median progression free survival [mPFS]) with osimertinib. Furthermore, a negative trend was seen for *TP53* mutation status, while age did not significantly influence the mPFS with osimertinib. Furthermore, while mPFS of osimertinib as first-line treatment was lower compared to the results from the FLAURA RCT [22], mPFS of osimertinib as second-line treatment was higher compared to the AURA3 RCT data [23]. Previous studies hinted towards a relation between primary *EGFR* mutation and *TP53* mutation status and mPFS with osimertinib [24, 25]. We observed similar effects, providing further evidence of exon 19 deletion and *TP53* wild type being indicators of better mPFS with osimertinib. Two previous studies focused on the effect of age and BMI [26, 27]. Similar results were found for age, as osimertinib reached similar mPFS in elderly patients,

compared to the results in the pivotal RCT [22]. Furthermore, the comparison between younger and older subgroups in our study, showed that age did not significantly impact mPFS for osimertinib. For BMI, our results differed from previous work. Ono *et al.* reported no significant difference between the low (<21.5 kg/m^2) and high BMI (≥21.5 kg/m^2) groups [27]. However, in our study a low BMI (<20 kg/m^2) negatively impacted effectiveness of osimertinib as a significant effect was seen for mPFS and median overall survival (mOS) in the multivariate analysis compared to higher BMI (≥ 20 kg/m^2) subgroups.

In addition, patients with a high $C_{min,SS}$ (>281 ng/mL) generally experienced worse PFS than patients with a low $C_{min,SS}$ (<171 ng/mL). The relation between osimertinib exposure ($AUC_{0\text{-}24,SS}$) and effectiveness outcomes was first described in a report by Brown *et al.* [28]. Herein a pharmacokinetic model was developed, and the conclusion was that no relation between osimertinib exposure and probability of objective response, duration of response or best percentage change in target lesion size was seen. However, an increased risk of adverse events (rash, diarrhoea, and QTC-prolongation) was seen with a higher osimertinib exposure (based on higher osimertinib dosages) [28]. While a similar effect was seen for the relation between exposure and mPFS by Boosman *et al*., a contrary effect was seen in this study as no relationship between exposure ($C_{min,SS}$ as a surrogate marker of $AUC_{0\text{-}24,SS}$) and toxicity was observed (p = 0.91). Compared with our study, a similar result of lower PFS in patients with high $C_{min,SS}$ was observed, although it reached statistical significance in our study. In another study, that focused mainly on the tolerability of osimertinib, a correlation was found between osimertinib exposure ($C_{min,SS}$) and the occurrence of severe – grade 3 or 4 – toxicity [29]. Herein, the probability of experiencing severe adverse events was seen in patients with a $C_{min,SS}$ >259 ng/mL.

Information regarding expected treatment outcomes for specific subgroups can be used by treating physicians to treat and inform their patients in clinical practice. In our study, some observed relations between patient characteristics (i.e., primary *EGFR* mutation and *TP53* mutation status) and the treatment outcomes on osimertinib could be logically explained, it feels counterintuitive that a higher drug exposure would lead to worse treatment outcomes. The correlation seen between a shorter PFS and an above average increased $C_{min,SS}$ may be caused by intensified cancer-related inflammation, which could affect the clearance of hepatic metabolised drugs, such as osimertinib, as stated by Boosman *et al.* [30]. Furthermore, other biological processes, such as cachexia, could impact the relation between osimertinib exposure and treatment outcomes. Cachexia leads to higher inflammation, reduced CYP-activity, and loss of body mass, which may change the tissue distribution of osimertinib. All these processes could contribute to higher osimertinib $C_{min,SS}$. Therefore, as cachexia in itself is correlated with poor response to treatment and survival as well [31, 32]. In our study, no data was included that could demonstrate the occurrence of cachexia (loss of body weight, inflammation markers, or CT-scans to evaluate the body composition). Therefore, we were unable to adjust for cachexia, and this could be a known confounding factor in our study.

In addition, to complementing data from clinical trials, observational research can be used to implement or propose changes for the guideline, or the general consensus, to optimise the treatment of a specific patient group. This could be done based on the results of **Chapter 3.2**, where bone-related outcomes and treatments were evaluated for patients with NSCLC treated with osimertinib. In this study, it was concluded that bone metastases and skeletal related events (SREs) occur frequently in *EGFR* mutated NSCLC patients, with a negative impact on overall survival (OS). The occurrence of bone metastases in our study is comparable with the results from previous studies [33, 34]. The use of bone targeting agents (BTAs) is currently low in the NSCLC population with bone metastases / SREs. Besides NSCLC, breast and prostate cancer are other cancer subtypes in which the bone is a common site for metastasis formation. However, the BTA prescription is higher in those populations and the use of BTA leads to a reduction of SREs in those populations [35, 36]. As the OS of NSCLC patients with bone metastases is increasing, and SREs can negatively impact OS, as well as the quality of life, the use of BTAs should be strongly considered in NSCLC patients with bone metastases. A potential reason for the discrepancy in BTA prescription between cancer subtypes could be that initially the OS of patients with NSCLC was limited. Given the short survival of patients with NSCLC the possibility of an SRE occurring after the diagnoses of BM was small. However, the OS of patients with NSCLC has increased over time, especially in patients with a target mutation, as several new effective drugs have become available. This development increases the possibility of the occurrence of an SRE. Therefore, the use of a BTA is warranted in patients with bone metastases to improve OS and quality of life. BTA use should be more prominently recommended in the guidelines and prescribed in clinical practice for NSCLC patients.

As was mentioned previously, the population in a RCT differs from the population in clinical practice. However, the representativeness of the RCT population evaluating newer treatment options for patients with NSCLC had not been investigated previously.

In **Chapter 4.1**, potential trial eligibility of British patients diagnosed with lung cancer in clinical practice was evaluated. In this study, we concluded that a large proportion of patients would not have been eligible to participate in a clinical trial, and that this ineligibility rate was largest for RCTs evaluating immunotherapy. Most frequent reason for potential exclusion was a deviating laboratory value, a history of cancer or simultaneous drug-use. Additionally, patients that would have been eligible for inclusion experienced better OS than potential ineligible patients. This was similar to results that were described in earlier research [18 – 21, 37], showing similar tendencies for the representativeness of lung cancer patients diagnosed in clinical practice. These results might impact how decision-makers use data from different types of studies. While RCTs remain the gold standard for causal relationships, due to the randomisation, the strict in- and exclusion criteria and the blinding of patients (and physicians), the high internal validity of RCTs can affect the potential extrapolation of treatment results to the clinical practice population [13, 38, 39]. The discrepancy between treatment outcomes seen in RCTs and in clinical

practice could eventually have impact on policy makers as well, although currently observational data is scarcely used in decision making [39].

While Clinical Practice Research Datalink (CPRD) GOLD has been used for multiple years, a new database (Aurum) was launched in 2017 by CPRD [41], which covers an extensive proportion of the English population (19.8%) [42]. Data for CPRD GOLD is retrieved from general practices, using Vision software, while general practices using EMIS-software can contribute to CPRD Aurum [43]. For CPRD GOLD, different validation studies have been performed with secondary data sources to validate specific outcomes or diseases [44 - 48], but validation of CPRD Aurum data or comparison with secondary, non-CPRD, data sources has been done scarcely [49 - 51]. In **Chapter 4.2**, the baseline characteristics of lung cancer patients diagnosed in clinical practice in CPRD Aurum were compared to lung cancer patients in CPRD GOLD. In addition, the OS of those patients was evaluated. The lung cancer populations in CPRD GOLD and CPRD Aurum were largely comparable, and only minor differences were found. The differences were seen in previous malignancies, deviant laboratory values and simultaneous drug use. While some minor differences occur in individual variables, the proportion of potential eligible patients for RCT participation was similar for both databases. Therefore, the conclusion was in line with previous studies comparing CPRD GOLD and CPRD Aurum, namely that the populations in both databases were largely similar [50, 51]. Therefore, CPRD Aurum could be used in the future to perform medical research in patients with lung cancer, as the number of (actively) enrolled patients is considerably larger compared to CPRD GOLD, as the coverage of CPRD GOLD has diminished over time [42, 43, 52].

Future perspectives

Future research could focus on further elucidating potential characteristics that could influence effectiveness outcomes with osimertinib, as well as clarifying the correlation we have seen between plasma trough concentration and PFS. We were unable to include variables which could serve as indication for inflammation or cachexia, and future studies could be performed to incorporate data on those variables. As the number of patients per centre is relatively low in the Netherlands, collaboration of multiple hospitals could increase the number of patients that could be included in the study, and thereby the accuracy of the analysis. In this thesis, we collaborated with three other Dutch hospitals, to increase the number of patients. Future research could further build on this and evaluate potential options to work together as Dutch hospitals to further optimise the treatment in patients with NSCLC. This could be especially valuable for the academic hospitals, as well as some other large teaching hospitals, as those hospitals cover a large proportion of care delivered to patients with NSCLC. Ideally, such initiatives should not be limited to centres in the Netherlands, and if possible, centres from multiple countries could participate in building a database for patients with NSCLC and their treatment outcomes. Creating a database with centres from multiple countries contributing data will come with challenges,

such as uniformity and costs. However, other examples have shown that a shared database could be feasible.

While collaboration with Dutch (or other) hospitals could be considered for studies using electronic health records, studies using database research may also focus on incorporation data from multiple data-sources. While **Chapter 4.1** only evaluated potential trial eligibility for British lung cancer patients in general, future research could focus on trial eligibility of patients receiving the specific drug in clinical practice, with known histological subtype or confirmed mutations in their tumour tissue. The current data source used, CPRD GOLD, did not enable such an analysis. However, this could be performed if data from CPRD GOLD is linked to more cancer-specific data. CPRD GOLD is a general practitioner database in the United Kingdom, with a long history of data collection, which has been frequently used for medical research [53]. However, detailed information about second-line care, such as data from hospitals (hospital episode statistics; HES) [54], the cancer registry [55] and the Systemic Anti-Cancer Therapy (SACT) database [56], and death registration (ONS) is not always accurately registered in CPRD GOLD. Linkage of CPRD to other databases could be done in future research, with focus on treatment-related outcomes or data quality in general. Evaluating real-world treatment outcomes with osimertinib, or any of the other new anticancer drugs. In short, linking different databases in the UK could give detailed overview of potential trial eligibility, and additionally the treatment outcomes (PFS and OS) with anticancer drugs in the clinical practice.

The significant higher number of new patients, and subsequently the patients that will be treated with recently approved oncology agents, gives new opportunities for future research. One opportunity could be the evaluation of potential (rare) adverse events that occur with anticancer drugs. One example being the development of type I diabetes (TIDM) after the start of immunotherapy. While some case-reports have been published on this subject [57 - 61], a population-based cohort study is lacking to evaluate the incidence of TIDM after immunotherapy initiation in patients with NSCLC. Using a large primary care database, combined with linkage to cancer-related databases, to precisely identify the patients treated with cancer immunotherapy and subsequent health outcomes, could be evaluated in a future study. Furthermore, more studies evaluating the quality of data in CPRD Aurum, such as incorporating the details of cancer-related data (i.e., subtype, stage, mutation-specific data), could be initiated. In the future, identifying subgroups that are more prone to rare adverse events or could further improve treatment optimization in patients with NSCLC.

Pharmacokinetic treatment enhancement

The exposure of most anticancer drugs varies within the population that is routinely treated with the specific drug. Variation in osimertinib exposure has also been reported, and was also shown in **Chapter 3.1**, with a relatively wide $C_{min,SS}$ range. Large variation in drug exposure might imply that a subset of patients experiences sub-optimal exposure. Increasing osimertinib exposure may be warranted in those patients to ensure the maximal therapeutic effect. Furthermore, the CNS is a common metastatic site in patients with NSCLC, and increasing osimertinib peripherally, may increase CNS exposure to osimertinib as well. A simple method to increase osimertinib exposure, is doubling the daily osimertinib dose. However, this makes the treatment unaffordable, as it is currently not reimbursed in a dosage higher than 80 mg per day and paying it out-of-pocket is very expensive. Therefore, other approaches that increase osimertinib exposure are interesting to explore. Osimertinib is a small molecule, that is mainly metabolized by Cytochrome P450 3A (CYP3A) enzymes [62]. Inhibition of the CYP3A-enzymes leads to a decreased metabolism rate of osimertinib, and therefore an increased exposure. Certain drugs are known to strongly inhibit CYP3A. For some drugs inhibition of CYP3A is an off-target effect, such as clarithromycin [63], while others are more, or specifically, known for their strong CYP3A-inhibiting characteristics, such as ritonavir and cobicistat. Ritonavir and cobicistat are both applied as boosting agent to improve the bioavailability of anti-HIV-drugs, although ritonavir is known to have antiretroviral effects, as protease inhibitor. The broader mechanism of action of ritonavir leads to additional side-effects, especially if it would be primarily used as CYP3A-inhibiting agent, without the need for the antiretroviral effect. Contrary, cobicistat was mainly developed to inhibit metabolic enzymes, such as CYP3A [64, 65] and lacks the side effects for which ritonavir is known. Adding cobicistat to the osimertinib treatment, would hypothetically increase the exposure to osimertinib. While this approach has been widely applied in other therapeutic areas, especially in the treatment of patients with acquired immunodeficiency syndrome (AIDS), the experience with CYP3A boosting in oncology patients is limited [66 - 68].

In **Chapter 5.1** we present a proof-of-concept study, in which we evaluated the capacity of cobicistat to boost osimertinib exposure in patients who were regularly treated with osimertinib and experienced a low $C_{min,SS}$ during the first months of treatment. We observed an increase in osimertinib exposure in all patients (n = 11). However, the inter-patient variability in boosting effect was large, ranging from 19% to 192%. In addition, we did not observe any unexpected or severe (common terminology criteria of adverse events [CTCAE] grade 2 or higher) adverse events.

In addition to our findings, two case reports have been published, which evaluated the effect of cobicistat (≥ 150 mg QD) on axitinib and crizotinib exposure, respectively [67, 68]. In both patients, the exposure of the anticancer drug was increased. The maximum concentration of axitinib increased considerably (approximately ten times higher), while the $C_{min,SS}$ doubled [67]. The $AUC_{0-24,SS}$ of crizotinib increased 78% after the two weeks.

However, the number of patients was rather small. In the study by Boosman *et al.*, the boosting capacity of ritonavir on erlotinib exposure was evaluated [66]. Herein patients received a 50% reduced dose of erlotinib (75 mg OD) in combination with ritonavir (200 mg OD). Subsequent exposure analyses demonstrated a large interpatient boosting variability, as erlotinib exposure ($AUC_{0\text{-}24,SS}$) ranged from -69% to +240% of full dose erlotinib single agent exposure [66]. We believe cobicistat is a more appropriate option than ritonavir to use as boosting agent, as ritonavir has additional off-target effects that lead to more adverse events, while the CYP3A-inhibiting capacities of ritonavir and cobicistat are grossly similar [69].

Hypothetically, using a cheap boosting agent to increase osimertinib exposure in patients that experience subtherapeutic plasma concentrations may be an efficient and cost-effective manner to improve treatment outcomes. Especially, when the alternative is doubling the dose of the expensive TKI osimertinib. However, as mentioned earlier in this chapter, no definitive concentration target for osimertinib has been established, so using cobicistat population-wide to increase osimertinib exposure to improve treatment outcomes is currently not evident. However, using cobicistat as addition to osimertinib treatment might have additional positive implications. As mentioned before, the CNS is a common metastatic site in patients with NSCLC [70]. Higher systemic exposure to osimertinib could also result in higher concentrations of osimertinib in the CNS to either treat existing metastases or prevent formation of CNS metastases. Furthermore, the additional inhibition of p-glycoprotein (ABCB1) and breast-cancer resistance protein (ABCG2) may improve osimertinib penetration through the blood-brain barrier, thereby further increasing the osimertinib exposure in the CNS [71]. Controlling CNS metastases or preventing growth of new metastases is crucial in the treatment of patients with NSCLC. The development or presence of CNS metastases is often accompanied with symptoms that influence the ability to perform everyday activities and have an additional negative impact on quality of life and OS [72, 73]. Furthermore, a recent study by Piper – Vallillo *et al*. described that dose escalation of osimertinib in patients with CNS (oligo)progression, has potential benefit. CNS control lasted approximately 3 – 6 months and seemed more effective in patients with leptomeningeal metastases [74]. In addition to the higher presence in the CNS, cobicistat could also increase the intratumoral concentration of osimertinib, as the CYP3A4 activity can be increased in tumour cells [75]. Using a strong CYP3A4 inhibitor could therefore not only lead to a higher systemic osimertinib exposure, and subsequently an increased osimertinib concentration in the CNS, but also to an improvement of intratumoral exposure, which is the most crucial location for the drug to be active. So, while a population-wide application of cobicistat is not appropriate (yet), it may be applied in individual patients who can profit from a better systemic or local osimertinib exposure, to optimise the related treatment outcomes.

Future perspectives

Besides improving the treatment outcomes directly associated with the anticancer treatment, the use of cobicistat could also have implications for the costs associated with osimertinib treatment and therefore to cost-efficiently optimise the treatment of patients with NSCLC. Like most new oncology drugs, osimertinib is expensive as the price of one year of treatment is approximately €75,000 in the Netherlands and over $100,000 in the USA [76, 77]. The costs of the treatment of patients with cancer has significantly increased over the last decades and will probably further increase in the coming years [78]. In patients that warrant a higher daily dose than the reimbursed standard dose (80 mg), adding cobicistat to the regimen would be a good starting point to improve osimertinib exposure, without the costs that are associated with a double dose of osimertinib. However, this is not the only approach that could be used to control or decrease the costs associated with osimertinib. In **Chapter 5.1** only patients with a relatively low initial exposure to osimertinib (based on their $C_{min,SS}$ ≤195 ng/mL) were included to evaluate the boosting capacity of cobicistat. Patients with average or above average initial exposure were not included but could be the target population of a study evaluating a new, cost-saving approach. It could be hypothesized that the effect of cobicistat in patients that experience higher initial exposure would be lower. The higher starting exposure to osimertinib could be caused by a lower CYP3A-activity, leading to higher steady state concentrations. If the CYP3A activity is lower the effect of cobicistat, a CYP3A inhibiting drug, could potentially be lower, as CYP3A would play a smaller part in the metabolism of osimertinib. However, the contrary could also be speculated. If the CYP3A activity is lower in patients with high osimertinib exposure, using cobicistat could more likely lead to a complete inhibition of CYP3A, while residual CYP3A activity could linger in patients that have a high CYP3A activity. A complete inhibition of CYP3A could result in a larger effect of cobicistat. It is therefore useful to further evaluate the effect of cobicistat in patients, who initially experience higher osimertinib exposure. If it is clear which effect of cobicistat could be expected in all patients, it could be evaluated as a cost-saving approach. Theoretically, the dosage of osimertinib could be decreased and cobicistat could be used to boost the osimertinib exposure, thereby maintaining its initial exposure. The future study should focus on evaluating this new creative approach that would be applicable in clinical practice. Simply halving the dose of osimertinib and supplementing this with cobicistat boosting would be inefficient for osimertinib, as the 40 mg tablets and 80 mg tablets of osimertinib are priced similarly. Therefore, a more creative approach should be evaluated, for example having osimertinib-free days. Based on the results of **Chapter 5.1**, it could be stated that all patients could skip at least one daily dose in a week, as the minimal boosting with cobicistat was 19%, which could correct for the 'missed' dose during the week. Potentially, having more osimertinib-free days could be possible, based on the individual results for each patient. In a previous study by Boosman *et al.* it was questioned whether decreasing the dosing frequency would be feasible for osimertinib, due to the turnover time of new *EGFRs* [30]. We believe that the long half-life of osimertinib (approximately 44 hours) and the inclusion of patients with average to high initial osimertinib exposure (based on $C_{min,SS}$) provides the opportunity to evaluate this

potential cost-saving approach in the future [23], as we believe sufficient osimertinib is available to bind new *EGFRs* due to his long half-life. The effect of such an approach should be evaluated in clinical practice to definitively determine the suitability to be performed in clinical practice.

Furthermore, a boosting strategy, like we evaluated in **Chapter 5.1**, could be explored for other CYP3A- hepatic and/or intestinal metabolized anticancer agents to control the overall costs associated with oncology therapy. However, not every expensive drug, used in the treatment of patients with NSCLC, would be feasible to be boosted with cobicistat, i.e. monoclonal antibodies as they are catabolized to peptides and amino acids by circulating phagocytic cells or by their target antigen-containing cells [79]. Furthermore, the price of the treatment must be relatively high to observe an effect on the budget impact of that specific treatment. If those conditions apply, a similar boosting approach could potentially be of interest for that drug.

Other approaches to control the costs associated with the treatment of anticancer drugs are warranted [78]. An approach that has been hinted at by Boosman *et al.* [30, 80] based on the results reported by Sonobe *et al*. [81] is to evaluate the possibility to lower the daily dosage of osimertinib. It was hypothesized that EGFR-directed TKIs seem to be dosed higher than necessary based on the low inhibitory concentration (IC_{50}) values *in vitro*. While this may be useful to improve the safety of osimertinib, it is unknown whether clinical efficacy of osimertinib will be maintained. Furthermore, it would unfortunately not have a big effect on the budget impact of osimertinib, as tablets of 40 mg of osimertinib have a similar price in the Netherlands compared to the tablets of 80 mg. Prospective studies further evaluating other treatment regimens for osimertinib are needed. Based on the $C_{min,SS}$ plasma trough concentration achieved in patients treated with the standard dose of osimertinib (80 mg QD) and the IC_{50} concentrations found in *in vitro* studies, it could be argued that a lower daily dose of osimertinib would be sufficient [29, 30, 82, 83]. However, a lot of unknown variables could compromise the potential of this hypothesis. First, it is unknown whether the $C_{min,SS}$ is the best pharmacokinetic variable to predict anticancer effect. Secondly, it is not clear whether the intratumoral drug concentration is similar to the plasma concentration. Osimertinib is distributed throughout the whole body, and locally concentrations can differ based on the specific characteristics of the drug (distribution volume, lipophilicity) and the tumour micro-environment. Furthermore, as mentioned previously, increased intratumoral CYP3A4 activity could lower the local concentration of osimertinib [75]. Additionally, it is unknown whether achieving the IC_{50} in blood is sufficient to provide optimal intratumoral osimertinib exposure.

Concluding remarks

Altogether, several approaches could be used to optimise the treatment of patients with NSCLC, focusing on efficacy, safety and/or costs. In this thesis we evaluated different options (TDM, observational studies, and pharmacokinetic treatment enhancement) and while some studies gave clear options to optimise the treatment of patients with NSCLC, other areas may need more work to come to a definitive approach for treatment optimization. This could be the topic of future research, to further optimise the treatment of patients with NSCLC, or maybe even patients with other (oncological) diseases.

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Chapter 7

IMPACT

IMPACT

In this chapter of the thesis, the most important findings of our work, the potential scientific impact of our research, and the relevance for patients, physicians and society are discussed. At the end, the dissemination of our work is addressed.

Aims and conclusion of this thesis

In this thesis we have evaluated several options that can be applied to optimise the systemic anti-cancer treatment of patients with non-small cell lung cancer (NSCLC). This was done by focusing on three different areas. First, we validated three analytical methods which can be used to quantify drug concentrations of nine tyrosine kinase inhibitors (TKIs). Secondly, multiple retrospective observational studies were performed. Two studies focused on treatment outcomes (effectiveness and safety) with osimertinib in clinical practice using data from electronic health records. Another two studies were done to evaluate to what extent patients included in clinical trials are a good representation of the general population with lung cancer. And thirdly, we evaluated the effect of cobicistat as booster of the osimertinib exposure in patients with NSCLC. This strategy could potentially be used to improve treatment effectiveness, such as progression-free survival (PFS) or overall survival (OS) on one hand but might also reduce the costs associated with osimertinib treatment on the other hand. We will discuss the results, and scientific/societal impact individually for each topic.

Therapeutic drug monitoring (TDM)

Three different analytical methods were developed and validated [2 - 4], which can be used to quantify the plasma drug concentration of nine TKIs. TKIs are drugs that are used to treat patients with advanced or metastatic NSCLC, or as adjuvant treatment, and acts on a specific target. The reason for developing the different analytical methods was twofold. Firstly, it can be used to evaluate the potential role for TDM in the treatment of patients with NSCLC, searching for a minimum effective concentration or a maximum level to avoid severe toxicity. Secondly, the quantification of drug concentrations enables evaluating intervention research opportunities, such as the osimertinib boosting proof-of-concept clinical trial, which is presented in this thesis. All analytical methods complied with the guideline of the European Medicines Agency (EMA) and can therefore be used in clinical practice to quantify drug concentrations in human plasma. However, limited stability of osimertinib at room temperature was found, especially in human serum and plasma (heparin) and we recommend performing sample preparation for osimertinib samples on dry ice, to ensure accurate quantification [2]. Similar instability was not seen for any other TKI, which were all stable in whole blood and EDTA plasma for at least 24 hours at room temperature [3, 4].

For some TKIs (crizotinib and alectinib) clinical target concentrations in plasma are proposed [5], but for most TKIs the potential role that TDM can play is not fully elucidated.

Based on our results [6], combined with previously published work, no concrete osimertinib target plasma trough concentration during steady state ($C_{min,SS}$) could be found that predicts treatment outcomes (progression-free or overall survival). Future research should focus on further elucidating the potential role of TDM in the treatment with osimertinib, as well as other TKIs that are frequently used in patients with NSCLC. This applies to TKIs that are already approved and reimbursed, but also for TKIs that are nearing market introduction. Ideally, a collaboration of multiple Dutch centres in which TKI care is performed would be preferred to ensure the inclusion of a large(r) number of patients for measuring plasma trough concentrations. For this thesis, we have collaborated with some centres in the Netherlands, and future efforts should focus to build on this.

Real-world data

During the development of new systemic anti-cancer treatment options, randomised (placebo) controlled trials (RCTs) are generally large international multicentre studies that are prospectively performed to establish the efficacy and safety of a new drug. RCTs have a strict set of in- and exclusion criteria. This results in a patient population that is homogeneous, and therefore is well fitted to precisely establish the treatment outcomes for the new drug compared to the standard treatment (at that time). However, extrapolation of treatment results to subsets of the population in clinical practice is sometimes hampered, as the clinical trial population is not representative of the patient population that is treated with the drug in the real world. Retrospective observational studies, which evaluate the effectiveness and safety outcomes of patients treated in clinical practice, can be used to complement data from RCTs. We performed a study evaluating the treatment outcomes (PFS, OS, objective response rate [ORR], disease control rate [DCR], and safety) for NSCLC patients that were regularly treated with osimertinib, with a special focus on age, body mass index (BMI) and $C_{min,SS}$ [6]. Herein, we found that PFS was worse in patients with a low BMI (<20 kg/m^2) and patients with a high $C_{min,SS}$, while age did not significantly influence PFS. Furthermore, female patients and patients with the exon 19 deletion as primary epidermal growth factor receptor (*EGFR*) mutation experienced significantly better PFS, while a trend for better PFS was seen in patients with *TP53* wild type. The second study with data from electronic health records focused on bone specific treatment outcomes of osimertinib users. This study concluded that bone metastases are frequently occurring in patients treated with osimertinib and can be accompanied by serious skeletal events (SREs). The use of bone targeting agents (BTAs), which can be used to prevent SREs, is relatively limited in patients with NSCLC [7]. A broader use of BTAs could decrease the number of SREs, and subsequently improve the quality of life of NSCLC patients treated with osimertinib. While survival of patients with lung cancer was previously limited, the development of treatment options during the last decades have improved survival considerably, especially in patients with EGFR mutated NSCLC. In this subgroup of patients, survival rate approaches the survival seen in patients with advanced/metastatic breast cancer or prostate cancer, where BTAs are prescribed more frequently. The results from these two observational studies can be used by treating physicians to make treatment

decisions or inform patients in clinical practice more precisely, as previous reported correlations were confirmed in our study, and new, potentially predictive, parameters were found. In the first study we observed decreased PFS in patients with a high $C_{min,SS}$, which does not align with the general consensus (lower exposure – worse outcomes). We hypothesized that underlying processes could be responsible for this effect. One such process could be cachexia, which is characterized by weight loss, increased inflammation, and lower liver (and intestinal) enzyme CYP3A-activity. All those factors may influence the body distribution of osimertinib and its $C_{min,SS}$. Unfortunately, parameters to measure cachexia were not included in our analyses, and therefore, could be the topic of further research.

In addition to studies using electronic health records, we also performed two studies with data from large databases [8, 9]. As mentioned previously, the RCT population is often not a good representation of the total target population in clinical practice. We evaluated the potential eligibility of patients diagnosed with lung cancer in clinical practice for large RCTs in Clinical Practice Research Datalink (CPRD) GOLD [8]. Subsequently, a similar study was performed in a more recently launched database (CPRD Aurum), and results from both studies were compared [9]. Both CPRD GOLD and CPRD Aurum are two British, primary care databases, that can be used for medical research. We concluded that a considerable proportion of patients diagnosed with lung cancer in clinical practice would have been ineligible for RCT participation. Our research adds to previously published studies that RCT and clinical practice populations differ substantially. As a consequence, previous research has also shown that treatment outcomes in clinical practice are lower than the efficacy seen in RCTs [10]. Recognition of the differences between the RCT and clinical practice population and potential implications for expected treatment outcomes is crucial for treating physicians. Future research could focus on further elucidating the efficacy-effectiveness gap of recently emerged immunotherapy or targeted therapy used in patients with NSCLC. This could be further improved by linking CPRD-databases to cancer specific databases in the United Kingdom (cancer registry, systemic anti-cancer treatment dataset). This will enable us to more precisely identify patients that may benefit from a specific treatment, since disease status, histology of the primary tumour, and driver mutation data are better categorized.

Pharmacokinetic treatment enhancement

The use of boosting agents is widely applied in other disease areas, (i.e., patients with acquired immune deficiency syndrome), however, in cancer patients, it has been scarcely reported. In our study, we evaluated the boosting capacity of cobicistat in patients that were regularly treated with osimertinib. We demonstrated that osimertinib exposure can be boosted with cobicistat, a strong CYP3A4 inhibitor. The mean increase in osimertinib exposure was 60%, with a range from 19% - 192% [11]. Boosting the exposure to osimertinib, by the addition of cobicistat, could hypothetically be used in different patient subgroups. Firstly, it can be applied in patients that would benefit from higher intratumoral or intracranial exposure to osimertinib. While a target concentration has

not been established for osimertinib, it could be hypothesized that increased exposure to osimertinib could potentially benefit patients with brain metastases. An increased total exposure to osimertinib, as well as the effect of cobicistat on transporter enzymes in the blood brain barrier, may increase the exposure to osimertinib in the central nervous system (CNS). This could potentially lead to a better control of existing metastases or prevent the growth of new intracranial metastases. Another study has indirectly shown benefit of increasing the systemic osimertinib exposure, by doubling the daily osimertinib dose, in patients with CNS-metastases who experienced progression [12]. As a much more affordable alternative, the addition of cobicistat might also increase intratumoral and/or osimertinib brain exposure, thereby possibly increasing the effectiveness of osimertinib [13]. Further studies may evaluate if the addition of cobicistat leads to improved CNS control with osimertinib.

Another possible application of cobicistat is reducing the high costs associated with the treatment of osimertinib. An approach in which a lower average daily dose of osimertinib is used, which is supplemented by the boosting effect of cobicistat, could result in a considerable cost saving. As of now, the effect of cobicistat is only evaluated in patients with low exposure to osimertinib, while the effect of cobicistat in patients with higher exposure to osimertinib is unknown. Hypothetically, lower CYP3A4 activity could be the potential reason for higher exposure to osimertinib in those patients, within the whole population of NSCLC patients that is regularly treated with osimertinib. Subsequently, if the CYP3A4 activity is lower in patients with a higher osimertinib exposure, the effect of cobicistat on osimertinib exposure could be diminished. A future study should focus on evaluating the effect of cobicistat in all patients treated with osimertinib, in combination with further elucidating a potential cost-saving approach using cobicistat. However, simply lowering the daily dose of osimertinib will not lead to cost savings, as 40 and 80 milligram Tablets of osimertinib are priced similarly. A study in which the weekly cumulative dose of osimertinib is lowered, and supplemented with the co-treatment with cobicistat, could be performed in the future. In addition to further elucidating an approach to improve osimertinib effectiveness, as well as cost-efficiency, other targeted agents could be selected for which a similar approach would potentially yield benefit (therapeutic or financial). Drugs that are primarily metabolized by CYP3A4 and are still patented, which is often accompanied with high drug prices, may be selected for future boosting studies.

Dissemination of our knowledge

To share the results of our studies with other researchers and healthcare professionals, we have published (most of) the articles in scientific journals and are in the process of publishing the ones that are not published yet. Furthermore, we have presented the results of our study at international conferences (European Lung Cancer Congress 2022, European Society for Medical Oncology 2022, International Society for Pharmacoepidemiology 2020) and at scientific meetings in the Netherlands and our own hospital. Lastly, the results are

also published at the website of the Netherlands Organisation for Health Research and Development, which have subsidized our work.

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7

Summary

ENGLISH SUMMARY

Non-small cell lung cancer (NSCLC) is the most frequent form of lung cancer, accounting for approximately 85% lung cancer diagnoses. The understanding of the biology of NSCLC has improved over the last decades, and several driver mutations, which play a role in the oncogenesis of NSCLC, have been identified. The discovery of specific driver mutations led to the development and market introduction of targeted agents. Additionally, immunotherapy has become widely available, which stimulates the immune system to increase the body's own response against the tumour. The new systemic treatment options have shown added therapeutic value. However, after market introduction several approaches can be evaluated to optimise the (new) treatment regimens. In this thesis we evaluated different methods for this optimization. We focused on three areas, namely: a) investigating the possibility and added value of therapeutic drug monitoring (TDM) of tyrosine kinase inhibitors (TKIs) and related analytical methods that are necessary to implement TDM; b) supplement data from randomized clinical trials (RCTs) with retrospective observational data and c) improving exposure and cost-efficiency of osimertinib therapy using pharmacokinetic enhancement (boosting). An extensive introduction of the different topics that were included in this thesis, such as NSCLC, TDM, different types of research (clinical and observational), and systemic anti-cancer treatment optimisation are presented in **Chapter 1**.

In total, three different analytical methods were developed and validated, which are described in **Chapter 2**. In **Chapter 2.1,** the analytical method for osimertinib is presented, while in **Chapter 2.2** and **Chapter 2.3** two multi-TKI assays are reported that can be used to quantify alectinib, crizotinib, erlotinib and gefitinib (**Chapter 2.2**) and brigatinib, lorlatinib, pralsetinib, and selpercatinib, respectively (**Chapter 2.3**). All three methods used high pressure liquid chromatography for compound separation and tandem mass spectrometry for quantification.

Osimertinib is a TKI that is used as first- and second-line treatment in patients with locally advanced or metastatic epidermal growth factor receptor (*EGFR*) mutated NSCLC, or as adjuvant treatment in patients with resected *EGFR* mutated NSCLC. The analytical method was developed and validated following the guidelines from the European Medicines Agency (EMA) (**Chapter 2.1**). All pre-specified requirements were met. However, >15% reduced osimertinib concentrations were found after two hours in human serum and citrate plasma. Stability of osimertinib was slightly better in EDTA-plasma and EDTA-whole blood at room temperature (>4 hours). Due to the limited osimertinib stability at room temperature we highly recommend performing plasma preparation on dry ice, to ensure adequate quantification of osimertinib.

In **Chapter 2.2** a method was developed to quantify four TKIs that are used in *EGFR* mutated (erlotinib and gefitinib) or anaplastic lymphoma kinase (*ALK*) mutated (alectinib and crizotinib) NSCLC patients. All validation parameters met the pre-specified requirements

as defined in the EMA guideline. The analytical method that was developed in Chapter 2.2 can be combined with the assay that was developed for osimertinib, which enables the quantification of five TKIs with a single assay setup.

In **Chapter 2.3** a third analytical method was developed, which enables the quantification of an additional four TKIs. Two of those (brigatinib and lorlatinib) are used in patients with *ALK* mutated NSCLC, while the other two (pralsetinib and selpercatinib) can be used in patients with rearranged during transfection (*RET*) mutated NSCLC. Accuracy and precision were within the pre-specified range, as were other parameters, while short- and long-term stability did not show any deviations. All three assays can be used in clinical practice to quantify drug concentrations.

In **Chapter 3 and 4,** four observational studies are presented. The two studies in **Chapter 3** used data from electronic health records in three (**Chapter 3.1**) and two (**Chapter 3.2**) Dutch hospitals, respectively, while the studies in **Chapter 4** were performed using data from two large primary care databases (Clinical Practice Research Database [CPRD] GOLD and Aurum) in the United Kingdom.

In **Chapter 3.1** we evaluated the treatment outcomes (both effectiveness and safety) of patients that were regularly treated with osimertinib. To be eligible for study inclusion, patients had to be 18 years or older, with at least one available CT-scan for response evaluation. In total, 294 patients were included, of which 118 patients used osimertinib as first-line treatment, 134 as second line treatment and 42 patients received osimertinib in the third line or beyond. The median progression free survival (mPFS) in our first line cohort was shorter than the reported mPFS in the FLAURA-study (14.6 vs. 18.9 months), while the mPFS in second-line cohort surpassed the mPFS described in the AURA3-trial (13.7 vs. 10.1 months). In our study, mPFS was significantly better in female patients, patients with an exon 19 deletion as primary *EGFR* mutation, patients with a body mass index (BMI) between 20 – 30 kg/m^2 and in patients with a low $C_{min,SS}$ (<171 ng/mL) (compared to patients with a high $C_{min,SS}$ (>281 ng/mL). A trend towards better mPFS was seen in patients with *TP53* wild-type tumours, while age at start of osimertinib treatment did not significantly influence mPFS.

NSCLC is often diagnosed in a later disease stage, as indicated by the approximate 50% of patients who have metastatic disease at diagnosis. Metastases are often found in the bone, and those metastases negatively impact the overall survival (OS) and quality of life. Approximately 50% of all patients with bone metastases experience a skeletal-related event (SREs), such as symptomatic fracture, surgery of radiation to bone, or spinal cord compression. In **Chapter 3.2** we describe a study that focused on the development of bone metastases and SREs in patients with *EGFR* mutated NSCLC. In this study we found that bone metastases and SREs are frequent in patients with *EGFR* mutated NSCLC, while most patients experienced their first SRE before initiation of osimertinib. After the development

of bone metastases, the median OS was 30.8 months. Use of bone targeting agents (BTAs) is low in the patients with bone metastases but is recommended in patients with bone metastases due to the relatively long OS in patients with *EGFR* mutated NSCLC.

Patients included in randomized controlled trials (RCTs) are often highly selected and unrepresentative of the general patient population with NSCLC. This is because of the strict inclusion and exclusion criteria that are applied in RCTs. The strict criteria and the use of randomization provides a very clean observation of the added therapeutic value of the new drug or treatment strategy. However, the external validity decreases due to the homogeneous patient population, which could affect the effectiveness outcomes in clinical practice. In **Chapter 4.1** we evaluate the potential trial eligibility of British lung cancer patients, diagnosed in clinical practice, for 12 RCTs in advanced NSCLC, focussing on TKIs or immunotherapy, which were performed between 2014 and 2018. For this study a large primary care database from the United Kingdom was used (CPRD GOLD). In total 9,239 lung cancer patients were included. For RCTs evaluating TKIs, the mean proportion of eligible patients was 74.3%, and 51.9% for RCTs evaluating immunotherapy. History of another malignancy, renal insufficiency or concomitant drug-use were the most frequent reasons for exclusion. For all RCTs, median OS was better in the group of potential eligible patients compared to the ineligible individuals.

In October 2017, CPRD launched a new database, called Aurum. General practices using specific health care software (EMIS) can contribute data to Aurum. In the last five years, the number of general practices using EMIS-software has increased considerably. This has led to a situation that approximately 20% of the English population is actively enrolled in CPRD Aurum as patients, supplemented by historical data. Simultaneously, the number of practices using the Vision software, which is necessary to contribute to CPRD GOLD, has considerably decreased, especially in England, which was the primary source for contributing practices. While there are many years of experience with using CPRD GOLD as a reliable database, with numerous studies reporting on data quality, less is known about the CPRD Aurum database. Therefore, we evaluated the differences and similarities between CPRD GOLD and CPRD Aurum in **Chapter 4.2**. Herein, we build on the results from **Chapter 4.1** and performed a similar study evaluating the potential eligibility of lung cancer patients in clinical practice, using the CPRD Aurum database. In addition, as a further manner of data quality validation of this database, we compared the baseline characteristics and OS from lung cancer patients registered in CPRD GOLD and Aurum. In this study we found that lung cancer patients registered in both CPRD Aurum are largely comparable with lung cancer patients in CPRD GOLD, since only minor differences were found in baseline characteristics, such as previous malignancies, deviant laboratory values and concomitant drug use. These minor differences did not impact the potential eligibility of lung cancer patients in clinical practice, as similar inclusion rates were found for all selected RCTs. Lastly, no substantial difference was found in OS between lung cancer patients in GOLD and Aurum (9.0 vs. 9.8 months). We determined that the quality of data,

and the completeness of information recorded of patients with lung cancer in CPRD Aurum is appropriate and reliable, and similar to the data quality that was retrieved from CPRD GOLD. Therefore, we conclude that the data of patients with lung cancer in both databases are an accurate representation of the English patient population with lung cancer in clinical practice and CPRD Aurum can be used for future research, as the current coverage of English lung cancer patients is very extensive in CPRD Aurum.

In **Chapter 5.1** we evaluated if, and to what extent cobicistat could boost the exposure to osimertinib. Cobicistat is a drug specifically designed to inhibit CYP3A4, which is the most important enzyme responsible for the metabolism of osimertinib, and many other TKIs. We hypothesized that the addition of cobicistat would increase the exposure to osimertinib and might be applied in patients that would potentially benefit from higher osimertinib exposure. Furthermore, increasing osimertinib exposure with a cobicistat, a relatively cheap drug, could provide the opportunity to develop a cost-saving approach. In this exploratory pilot study, we included 11 patients that were routinely treated with osimertinib, and experienced low osimertinib exposure (i.e., $C_{min,SS}$ ≤195 ng/mL). At the first day of the study, baseline exposure was evaluated ($AUC_{0-24,SS}$). The next day, co-treatment with cobicistat started. After three weeks, a second $AUC_{0-24,SS}$ was determined. In all patients, an increase of total $AUC_{0-24,SS}$ (combined for osimertinib and its most prominent and active metabolite, AZ5104) was noticed, with a mean increase of 60% (19% – 192%). The boosting effect of cobicistat was stable over time, at least during several months, and no severe adverse events were observed in any patient. All adverse events that occurred were scored as CTCAE (common terminology criteria for adverse events) grade 1.

In **Chapter 6**, the result of our work is discussed and put into context, while options for potential future research were also described. In **Chapter 7** the impact of our work (both clinical as societal) is presented.

Nederlandse Samenvatting

NEDERLANDSE SAMENVATTING

Niet-kleincellig longcarcinoom (NSCLC) is de meest voorkomende vorm van longkanker. In totaal heeft 85% van de patiënten die gediagnosticeerd wordt met longkanker NSCLC. In de afgelopen jaren is het inzicht in de biologische processen die van belang zijn bij het ontstaan van NSCLC toegenomen. Hierbij zijn verschillende driver mutaties, die een rol kunnen spelen bij de vorming van de tumor, ontdekt. De ontdekking van deze driver mutaties heeft ook geleid tot de ontwikkeling en de marktintrede van verschillende nieuwe geneesmiddelen, die zeer specifiek kunnen aangrijpen op de tumor. Naast deze gerichte behandelingen, is er ook een tweede groep geneesmiddelen beschikbaar gekomen, namelijk immuuntherapie. Geneesmiddelen die binnen deze groep vallen zijn in staat om het lichaamseigen immuunsysteem van de patiënt te stimuleren om hiermee de tumor te bestrijden. Al deze nieuwe geneesmiddelen zijn in klinische studies vergeleken met een eerder beschikbare behandeloptie en hebben hierin aangetoond van toegevoegde therapeutische waarde te zijn. Nadat een nieuw geneesmiddel op de markt komt, zijn er verschillende methodes die bestudeerd kunnen worden om de behandeling te optimaliseren. In dit proefschrift hebben we gekeken naar een aantal opties: a) onderzoek naar de mogelijkheid en de toegevoegde waarde van het toepassen van *therapeutic drug monitor*ing (TDM) van tyrosine kinase remmers (TKI's) en de analytische methoden die noodzakelijk zijn om de TKI concentraties te bepalen; b) het aanvullen van data afkomstig uit gerandomiseerd onderzoek met retrospectief observationeel onderzoek; en c) het verhogen van de blootstelling en mogelijk de kosteneffectiviteit van de behandeling met osimertinib door gebruik te maken van het farmacokinetisch verbeteren van deze behandeling (boosten). Een uitgebreide introductie van de verschillende onderwerpen die de revue passeren in dit proefschrift, zoals NSCLC, TDM, de verschillende typen onderzoek (klinisch en observationeel), en de systemische behandelingen die ingezet kunnen worden bij NSCLC worden besproken in **Hoofdstuk 1**.

In totaal zijn er drie verschillende analytische methoden ontwikkeld en gevalideerd. Deze worden alle drie beschreven in **Hoofdstuk 2**. In **Hoofdstuk 2.1** wordt de analytische methode die gebruikt kan worden voor de kwantificatie van osimertinib besproken. In zowel **Hoofdstuk 2.2** en **Hoofdstuk 2.3** wordt een analytische methode gepresenteerd die in staat is om meerdere TKI's te bepalen. In **Hoofdstuk 2.2** is dit een methode voor de bepaling van alectinib, crizotinib, erlotinib en gefitinib, terwijl het in **Hoofdstuk 2.3** een analysemethode is voor brigatinib, lorlatinib, pralsetinib en selpercatinib. In alle drie de methoden wordt er gebruikt gemaakt van *high-pressure liquid chromatography* voor de scheiding van de componenten, terwijl de kwantificatie uitgevoerd wordt met massa spectrometrie.

Osimertinib is een TKI die ingezet kan worden als eerste- of tweedelijns behandeling bij patiënten met lokaal gevorderd of gemetastaseerd epidermale groeifactor receptor (*EGFR*) gemuteerd NSCLC. Daarnaast kan het ook in een vroeger stadium ingezet worden, als adjuvante behandeling, bij patiënten met *EGFR* gemuteerd NSCLC. De analytische methode was ontwikkelend en gevalideerd volgens de richtlijnen die hiervoor vanuit de *European*

Medicines Agency (EMA) zijn opgesteld (**Hoofdstuk 2.1**). Alle vooraf gestelde eisen werden behaald. Echter, een afname van de osimertinib hoeveelheid van >15% werd gevonden na twee uur in menselijk serum en citraatplasma, wanneer het monster bewaard werd bij kamertemperatuur. De stabiliteit van osimertinib in EDTA-plasma en EDTA-volbloed bij kamertemperatuur was beter (>4 uur). Vanwege de beperkte stabiliteit van osimertinib bij kamertemperatuur adviseren we om het opwerken van de bloedmonsters uit te voeren op droogijs, om een adequate kwantificatie van osimertinib te waarborgen.

Hoofdstuk 2.2 beschrijft de ontwikkeling van een methode om de plasmaconcentratie te bepalen van vier TKI's, die ingezet kunnen worden bij patiënten met *EGFR* gemuteerd (erlotinib of gefitinib) of *anaplastic lymphoma kinase* (*ALK*) gemuteerd (alectinib en crizotinib). Voor deze methode werden ook alle vooraf gestelde eisen gehaald, en werden er geen problemen rondom de stabiliteit gevonden. De analytische methode die in **Hoofdstuk 2.2** is beschreven, kan gecombineerd worden met de methode uit **Hoofdstuk 2.1**, waardoor vijf TKI's bepaald kunnen worden met *één* assay.

In **Hoofdstuk 2.3** is een derde analysemethode beschreven, die de mogelijkheid geeft om nog eens vier TKI's te kwantificeren. Twee van deze TKI's kunnen ingezet worden bij patiënten met *ALK* gemuteerd NSCLC (brigatinib en lorlatinib), terwijl de twee andere voorgeschreven kunnen worden aan patiënten met *RET* gemuteerd NSCLC (pralsetinib en selpercatinib). De accuraatheid en precisie waren binnen de gestelde eisen, net zoals de andere parameters, en er werden geen afwijkingen gevonden in de stabiliteit voor de korte en lange termijn. Alle drie de methoden die beschreven worden in **Hoofdstuk 2** kunnen ingezet worden om de geneesmiddelconcentraties te bepalen in de klinische praktijk.

In Hoofdstuk 3 en 4 worden vier observationele onderzoeken gepresenteerd. De twee studies uit Hoofdstuk 3 zijn gedaan met data uit de patiëntendossiers van respectievelijk drie (**Hoofdstuk 3.1**) en twee (**Hoofdstuk 3.2**) Nederlandse ziekenhuizen, respectievelijk. In de twee studies die beschreven staan in Hoofdstuk 4 is gebruik gemaakt van data afkomstig van twee grote databases uit het Verenigd Koninkrijk, waarin data uit huisartsenpraktijken is vastgelegd (*Clinical Practice Research Datalink* [CPRD] GOLD en Aurum).

In **Hoofdstuk 3.1** hebben we gekeken naar de behandeluitkomsten (effectiviteit en veiligheid) van patiënten die in de klinische praktijk behandeld werden met osimertinib. Om geïncludeerd te kunnen worden dienden de patiënten ouder te zijn dan 18 jaar en minimaal een CT-scan te hebben die gebruikt kon worden voor de responsbepaling. In totaal werden 294 patiënten geïncludeerd, waarvan 118 patiënten osimertinib in de eerste lijn ontvingen, 134 patiënten in de tweede lijn, en 42 patiënten in de derde of latere lijn. De mediane progressie-vrije overleving (mPFS) in het cohort van eerste lijn gebruikers was korter dan de mPFS die in de grote fase III studie gepresenteerd werd (14,6 maanden vs. 18,9 maanden – FLAURA). De mPFS in tweede lijn gebruikers was langer dan de mPFS in de AURA3 studie (13,7 vs. 10,1 maanden). In onze studie was de mPFS significant beter bij

vrouwen patiënten met een exon 19 deletie als primaire *EGFR*-mutatie, patiënten met een *body mass index* (BMI) tussen de 20,0 – 30,0 kg/m^2, en bij patiënten met een lage dalspiegel (<171 ng/ml) (vergeleken met patiënten met een hoge dalspiegel [>281 ng/ml]). Een trend richting een betere mPFS werd gezien in patiënten met een wild-type *TP53*, terwijl de leeftijd geen invloed had op de behandeluitkomsten met osimertinib.

NSCLC wordt vaak gediagnosticeerd in een laat ziektestadium, aangezien er bij ongeveer 50% van de patiënten al sprake is van een gemetastaseerde vorm bij diagnose. Metastasen worden vaak aangetroffen in het skelet, en deze kunnen een negatieve impact hebben op de algehele overleving (OS) en de kwaliteit van leven. Bij ongeveer 50% van de patiënten met een botmetastase leidt dit ook tot een *skeletal-related event* (SRE), zoals een symptomatische fractuur, een operatie of bestraling van het bot, of verdrukking van het ruggenmerg. In **Hoofdstuk 3.2** beschrijven we een studie waarin gekeken is naar de ontwikkeling van botmetastasen en SRE's in patiënten met *EGFR* gemuteerd NSCLC. In deze studie werd gevonden dat botmetastasen en SRE's frequent voorkomen bij patiënten met *EGFR* gemuteerd NSCLC, terwijl de meeste patiënten hun eerste SRE al ervaren voordat osimertinib gestart is. Na de ontwikkeling van botmetastasen is de mediane OS 30,8 maanden. Het gebruik van geneesmiddelen die aangrijpen op het skelet is laag bij NSCLC patiënten met botmetastasen, terwijl het wel aanbevolen wordt om deze geneesmiddelen voor te schrijven, helemaal gezien de relatief lange OS bij deze groep patiënten.

Patiënten die deelnemen aan gerandomiseerd, gecontroleerd onderzoek (RCT) zijn vaak sterk geselecteerd en geen goede afspiegeling van de algehele populatie NSCLC patiënten. Dit wordt veroorzaakt door de strikte inclusie en exclusiecriteria die binnen RCT's gehanteerd worden. Deze strikte criteria en de toepassing van randomisatie zorgt ervoor dat in de RCT een betrouwbare schatting gemaakt kan worden van de toegevoegde therapeutische waarde van een nieuw geneesmiddel. Dit heeft echter ook effect op de externe validatie, die lager wordt door de homogene populatie binnen de RCT. Dit kan vervolgens leiden tot afwijkende behandeluitkomsten in de klinische praktijk ten opzichte van de uitkomsten die in de RCT's behaald zijn. In **Hoofdstuk 4.1** hebben we onderzocht welk gedeelte van de populatie in de klinische praktijk in aanmerking zou komen voor studiedeelname. Dit hebben we gedaan voor 12 afzonderlijke RCT's waarin de effectiviteit van een TKI of immuuntherapie beoordeeld werd en die tussen 2014 en 2018 waren uitgevoerd. Voor dit onderzoek werd gebruik gemaakt van een grote database uit het Verenigd Koninkrijk, waarin data uit Britse huisartsenpraktijken wordt verzameld. In totaal konden 9.239 patiënten met longkanker geïncludeerd worden. Voor de klinische studies waarin de effectiviteit van een TKI werd beoordeeld, was de gemiddelde geschiktheid van patiënten in de klinische praktijk 74,3%. Voor studies van immuuntherapie lag dit lager, namelijk 51,9%. Een andere maligniteit in de geschiedenis, een verstoorde nierfunctie, of het gelijktijdig gebruik van te vermijden comedicatie waren de meest frequente redenen dat patiënten niet deelhadden kunnen nemen aan het onderzoek. Een beeld dat voor alle afzonderlijke RCT's terugkwam, was dat de groep van patiënten die includeerbaar waren geweest een

betere OS hadden, dan de groep patiënten die niet voldeed aan de in- en exclusiecriteria van het onderzoek.

In oktober 2017 werd er vanuit CPRD een nieuwe database gelanceerd, namelijk Aurum, die qua opbouw vergelijkbaar was met CPRD GOLD, een oudere database. Echter, de huisartsenpraktijken die bij kunnen dragen aan de database verschillen, aangezien praktijken die gebruik maken van Vision softwaredata konden aanleveren voor GOLD, en praktijken met EMIS-software Aurum vullen. In de afgelopen vijf jaar is het gebruik van EMIS sterk toegenomen, en op dit moment is ongeveer 20% van de Engelse populatie actief gedekt binnen Aurum. Gelijktijdig is het aantal praktijken dat gebruik maakt van Vision sterk afgenomen, in het bijzonder in Engeland. Met GOLD is er de afgelopen jaren veel ervaring opgedaan, en zijn ook verschillende validatie studies uitgevoerd. De ervaring met Aurum is tot op heden nog zeer beperkt, helemaal in patiënten met longkanker. Om die reden hebben wij gekeken naar de overeenkomsten en verschillen tussen CPRD GOLD en Aurum, welke beschreven worden in **Hoofdstuk 4.2**. In dit onderzoek gaan we verder op de resultaten van **Hoofdstuk 4.1**, en hebben we een gelijkaardige studie uitgevoerd en gekeken naar de geschiktheid van potentiële deelname aan klinisch onderzoek. Aanvullend hierop, als manier om de kwaliteit van data verder te evalueren, hebben we ook gekeken naar de baseline karakteristieken en OS van longkankerpatiënten in Aurum, en deze vergeleken met longkankerpatiënten in CPRD GOLD. In dit onderzoek vonden we dat longkankerpatiënten in Aurum vergelijkbaar waren met longkankerpatiënten in CPRD GOLD, en er alleen kleine verschillen waren in baseline karakteristieken, zoals eerdere maligniteiten, afwisselende laboratorium waarden en gelijktijdig gebruik van geneesmiddelen. Deze kleine verschillen hadden geen impact op de groep patiënten die deel had kunnen nemen aan de RCT's, aangezien vergelijkbare proporties van geschikte patiënten werden gevonden. Als laatste werd er ook geen substantieel verschil gevonden in de OS van longkankerpatiënten in beide databases (9,8 maanden – Aurum, vs. 9,0 maanden – GOLD). We vonden dat de kwaliteit van de data, en de volledigheid van de geregistreerde informatie van longkankerpatiënten in CPRD Aurum vergelijkbaar is met de informatie en data in GOLD. Om die reden werd geconcludeerd dat beide databases een goede afspiegeling zijn van de Engelse patiëntenpopulatie en dat CPRD Aurum geschikt is om in de toekomst gebruikt te worden voor onderzoek bij longkankerpatiënten. Dit is in het bijzonder van toegevoegde waarde aangezien een groter aantal patiënten actief opgevolgd worden in Aurum, en er dus grotere aantallen patiënten behandeld zullen worden met de geneesmiddelen die relatief kortgeleden op de markt geïntroduceerd zijn.

In **Hoofdstuk 5.1** hebben we geëvalueerd of, en in welke mate cobicistat in staat is om de blootstelling aan osimertinib te verhogen. Cobicistat is een geneesmiddel dat specifiek ontworpen en ontwikkeld is, om ingezet te worden als CYP3A remmer. CYP3A4 is het voornaamste enzym dat betrokken is bij het metabolisme van osimertinib. De onderzoekshypothese was dat het combineren van osimertinib en cobicistat zou leiden tot een verhoging van de osimertinib blootstelling en mogelijk toegepast zou kunnen

worden bij patiënten die voordeel (zouden kunnen) hebben van een hogere osimertinib blootstelling. Daarnaast kan het verhogen van de blootstelling aan osimertinib met cobicistat, een goedkoop geneesmiddel, potentieel ingezet kunnen worden om de kosten van de osimertinib behandeling te verlagen. In deze exploratieve studie hebben we elf patiënten geïncludeerd die routinematig osimertinib ontvingen. Alle elf patiënten hadden initieel een lage blootstelling, gebaseerd op een dalspiegel meting gedurende steady state ($C_{min,SS}$ <195 ng/ml). Op de eerste dag werd de 24-uurs blootstelling van osimertinib bepaald ($AUC_{0-24,SS}$). De volgende dag werd de behandeling met cobicistat gestart. Na drie weken gecombineerd gebruik van cobicistat en osimertinib kwamen de patiënten opnieuw naar het ziekenhuis voor een nieuwe bepaling van de $AUC_{0-24,SS}$. Bij alle patiënten werd een totale $AUC_{0-24,SS}$ bepaald, gedefinieerd als de som van de $AUC_{0-24,SS}$ van osimertinib en haar belangrijkste metaboliet, AZ5104. De gemiddelde toename in blootstelling was 60%, met een spreiding van 19% - 192%. Het effect van cobicistat was stabiel over tijd, voor ten minste zes maanden, en er werden geen ernstige bijwerkingen gerapporteerd, aangezien alle bijwerkingen gescoord werden als graad 1.

In **Hoofdstuk 6** worden de resultaten van ons werk bediscussieerd en geplaatst binnen het eerder gepubliceerd onderzoek. Daarnaast worden ook mogelijkheden voor toekomstig onderzoek besproken. In **Hoofdstuk 7** wordt de potentiële impact van ons onderzoek besproken, zowel voor de kliniek, als voor de samenleving.

Appendices

LIST OF ABBREVIATIONS
LIST OF PUBLICATIONS
AUTHOR AFFILIATIONS
DANKWOORD
CURRICULUM VITAE

LIST OF ABBREVIATIONS

A	Alectinib
ABCB1	ATP-binding cassette sub-family B member 1
ABCG2	ATP-binding cassette super-family G member 2
AE	adverse event
aHR	adjusted hazard ratio
AIDS	acquired immunodificiency syndrome
ALAT	alanine transaminase
ALK	anaplastic lymphoma kinase
ANOVA	analysis of variance
AP	alkaline phosphatase
ASAT	aspartate transaminase
AUC	area-under-the-curve
$AUC_{0\text{-}24,ss}$	area-under-the-curve, 0 – 24 hours, during steady state conditions
BID	twice a day
BM	brain metastasis
BM^{+}	brain metastasis, present
BMI^{-}	brain metastasis, absent
BMI	body mass index
BRAF	proto-oncogene B-Raf
BTA	bone targeting agent
C	Crizotinib
[C]	concentration
$[C_0]$	concentration at T = 0
CK	creatinine kinase
C_{max}	maximum concentration
$C_{min,SS}$	maximum concentration during steady state
$C_{min,SS}$	plasma trough concentration during steady state
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
CPRD	clinical practice research datalink
CT	computed tomography scan
CTCAE	common terminology criteria for adverse events
CV	coefficient of variation
CYP	Cytochrome P450
CYP3A4	Cytochrome P450 3A4
DCR	disease control rate
DMSO	dimethyl sulfoxide
Dr.	doctor
E	erlotinib
ECOG	Eastern cooperative oncology group

EDTA	Ethylenediaminetetraacetic acid
eGFR	estimated glomerular filtration rate
EGFR	epidermal growth factor receptor
EHR	electronic health record
EMA	European medicines agency
Erasmus MC	Erasmus Medical Centre
ESI	electrospray ionization
ESMO	European society for medical oncology
et al.	and others
eV	electrovolt
ex. 19 del	exon 19 deletion
FBP	free base purity
FDG-PET-CT	2-deoxy-2-[fluorine-18] fluoro-D-glucose positron emission tomography-computed tomography scan
g	g-force
G	gefitinib
gGT	gamma-glutamyltransferase
GP	general practice
h	hour
HER2	human epidermal growth factor receptor 2
HES	hospital episode statistics
HIV	human immunodeficiency viruses
HPLC	high pressure liquid chromatography
HR	hazard ratio
IC_{50}	half maximal inhibitory concentration
ICI	immune checkpoint inhibitor
i.e.	that is
INR	international normalized ratio
IS	internal standard
ISR	incurred sample reanalysis
k_e	absorption rate constant
k_a	elimination rate constant
kg	kilogram
KRAS	Kirsten rat sarcoma viral oncogene homolog
L	litre
LC	liquid chromatography
LD	lactate dehydrogenase
LLOQ	lower limit of quantification
m	metre
M	molar
MET	mesenchymal epithelial transition factor receptor
METC	medical ethical committee

mg	milligram
min	minute
mL	millilitre
mm	millimetre
mM	millimolar
µg	microgram
µL	microliter
µm	micrometre
mOS	median overall survival
mPFS	median progression free survival
MRI	magnetic resonance imaging
MRM	multi reaction monitoring
MS/MS	mass spectrometry / mass spectrometry
MTD	maximal tolerated dose
MUMC+	Maastricht University Medical Centre +
N	number
NA	not achieved
NCRAS	national cancer registration and analysis service
ND	not determined
ng	nanogram
NKI/AvL	Netherlands Cancer Institute / Antoni van Leeuwenhoek hospital
NR	not reached
NRG-1	neuregulin 1
NS	not significant
NSCLC	non-small cell lung cancer
NTRK	neurotropic tyrosine receptor kinase
ONS	office for national statistics
ORR	objective response rate
OS	overall survival
$_{osi}$	osimertinib
OSIM	osimertinib
PAZO	pazopanib
PD-1	programmed death receptor 1
PD-L1	programmed death – ligand 1
PFS	progression-free survival
pH	potential of hydrogen
PK	pharmacokinetic
Prof.	professor
PS	performance status
Pts	patients
QC	quality control
QC_{LOW}	quality control low

QC_{MED}	quality control middle
QC_{HIGH}	quality control high
QC_1	stock solution for quality controls 1
QC_2	stock solution for quality controls 2
QC_3	stock solution for quality controls 3
QD	per day
QID	twice a day
QoL	quality of life
QTc	QT complex interval
RANKL	receptor activator of nuclear factor kappa-B ligand
RCT	randomised controlled trial
RECIST	response evaluation criteria in solid tumours
RET	rearranged during transfection
RT	room temperature
SACT	systemic anti-cancer therapy
SALLE	salting out liquid-liquid extraction
SCLC	small cell lung cancer
SD	standard deviation
SLE	systemic lupus erythematosus
SRE	skeletal related events
TDM	therapeutic drug monitoring
T_{max}	time where the maximum concentration is reached
TKI	tyrosine kinase inhibitor
TP53	tumour protein P53
TSH	thyroid stimulation hormone
u	unit
UK	United Kingdom
ULN	upper limit of normal
ULOQ	upper limit of quantification
UPLC	ultra high-pressure liquid chromatography
USA	United States of America
Var	variation
WBC	white blood cell count
WHO	world health organisation
y	years
ZonMw	Netherlands organisation for health research and development
%	percentage
1L	first line
2L	second line
3L+	third line or beyond
95% CI	95% confidence interval

LIST OF PUBLICATIONS

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AUTHOR AFFILIATIONS

Shahab Abtahi	Department of Clinical Pharmacy & Toxicology, Maastricht University Medical Center, Maastricht, The Netherlands. CARIM school for Cardiovascular Disease, Maastricht University Medical Centre, Maastricht, The Netherlands. Department of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University, The Netherlands.
Yvo de Beer	Department of Clinical Pharmacy & Toxicology, Maastricht University Medical Center, Maastricht, The Netherlands.
Anita Brouns	Department of Respiratory Medicine, Zuyderland, Geleen, The Netherlands Department of Pulmonary Diseases, Maastricht University Medical Center, Maastricht, The Netherlands. GROW – School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, The Netherlands.
Sander Croes	Department of Clinical Pharmacy & Toxicology, Maastricht University Medical Center, Maastricht, The Netherlands. CARIM school for Cardiovascular Disease, Maastricht University Medical Centre, Maastricht, The Netherlands.
Anne-Marie Dingemans	Department of Pulmonary Diseases, Maastricht University Medical Center, Maastricht, The Netherlands. GROW – School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, The Netherlands. Department of Respiratory Medicine, Erasmus Medical Center, Rotterdam, The Netherlands.
Annemariek Driessen	Department of Clinical Pharmacy & Toxicology, Maastricht University Medical Center, Maastricht, The Netherlands. CARIM school for Cardiovascular Disease, Maastricht University Medical Centre, Maastricht, The Netherlands. Department of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University, The Netherlands. NUTRIM, Maastricht University Medical Center, Maastricht, The Netherlands.
Safiye Dursun	Department of Pulmonary Diseases, Maastricht University Medical Center, Maastricht, The Netherlands.

Ewoudt van der Garde	Department of Clinical Pharmacy, St. Antonius Hospital, Utrecht/Nieuwegein, The Netherlands Department of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University, The Netherlands.
Robin van Geel	Department of Clinical Pharmacy & Toxicology, Maastricht University Medical Center, Maastricht, The Netherlands. CARIM school for Cardiovascular Disease, Maastricht University Medical Centre, Maastricht, The Netherlands.
Judith Gulikers	Department of Clinical Pharmacy & Toxicology, Maastricht University Medical Center, Maastricht, The Netherlands. CARIM school for Cardiovascular Disease, Maastricht University Medical Centre, Maastricht, The Netherlands.
Rob ter Heine	Department of Pharmacy, Radboud Institue for Health Sciences, Radboud University Medical Center, Nijmegen, The Netherlands.
Lizza Hendriks	Department of Pulmonary Diseases, Maastricht University Medical Center, Maastricht, The Netherlands. GROW – School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, The Netherlands.
Juanita Ippel	Department of Thoracic Oncology, Netherlands Cancer Institute, Amsterdam, The Netherlands.
Olaf Klungel	Department of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University, The Netherlands.
Cor van der Leest	Department of Respiratory Medicine, Amphia Hospital, Breda, The Netherlands.
Ron Mathijssen	Department of Medical Oncology, Erasmus Medical Center, Rotterdam, The Netherlands.
Marthe Paats	Department of Respiratory Medicine, Erasmus Medical Center, Rotterdam, The Netherlands.
Roy Schoufs	Department of Clinical Pharmacy & Toxicology, Maastricht University Medical Center, Maastricht, The Netherlands.
Elishia Sinkiewicz	Department of Clinical Pharmacy & Toxicology, Maastricht University Medical Center, Maastricht, The Netherlands.
Marielle Slikkerveer	Department of Clinical Pharmacy & Toxicology, Maastricht University Medical Center, Maastricht, The Netherlands.

Egbert Smit	Department of Thoracic Oncology, Netherlands Cancer Institute, Amsterdam, The Netherlands. Department of Pulmonology, Leiden University Medical Center, Leiden, The Netherlands.
Christi Steendam	Department of Pulmonary Medicine, Erasmus Medical Center, Rotterdam, The Netherlands.
Leo Stolk	Department of Clinical Pharmacy & Toxicology, Maastricht University Medical Center, Maastricht, The Netherlands.
Patrick Souverein	Department of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University, The Netherlands.
Vivianne Tjan – Heijnen	Department of Medical Oncology, Maastricht University Medical Center, Maastricht, The Netherlands. GROW – School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, The Netherlands.
Ard van Veelen	Department of Clinical Pharmacy & Toxicology, Maastricht University Medical Center, Maastricht, The Netherlands. CARIM school for Cardiovascular Disease, Maastricht University Medical Centre, Maastricht, The Netherlands. Department of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University, The Netherlands.
Marijn Veerman	Department of Respiratory Medicine, Erasmus Medical Center, Rotterdam, The Netherlands. Department of Medical Oncology, Erasmus Medical Center, Rotterdam, The Netherlands.
Marjon Verschueren	Department of Clinical Pharmacy, St. Antonius Hospital, Utrecht/Nieuwegein, The Netherlands Department of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University, The Netherlands.
Frank de Vries	Department of Clinical Pharmacy & Toxicology, Maastricht University Medical Center, Maastricht, The Netherlands. CARIM school for Cardiovascular Disease, Maastricht University Medical Centre, Maastricht, The Netherlands. Department of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University, The Netherlands. MRC Lifecourse Epidemiology Unit, Southampton General Hospital, University of Southampton, Southampton, United Kingdom.

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CURRICULUM VITAE

Ard van Veelen werd geboren op 1 augustus 1994 te Rotterdam. In 2012 rondde hij het Gymnasium af, aan de Gereformeerde Scholengemeenschap Randstad (GSR), met het profiel Natuur en Techniek. Aansluitend daarop begon hij de bachelor Farmacie, aan de Universiteit van Utrecht, gevolgd door de gelijknamige master. Voor deze master heeft Ard stage gelopen bij het Zorginstituut Nederland, waar hij onder begeleiding van dr. Amr Makady gekeken heeft in welke mate observationele data een rol spelen bij vergoedingsbeslissingen in Europa. In 2018 werd de master Farmacie cum laude afgerond. Aansluitend daarop startte Ard met zijn promotieonderzoek, waarvan het resultaat nu voor u ligt. Dit onderzoek heeft hij gedaan onder de begeleiding van dr. Sander Croes, dr. Robin van Geel, dr. Patrick Souverein, emeritus prof. dr. Frank de Vries, en in een later stadium, prof. dr. Vivianne Tjan – Heijnen. In dit promotieonderzoek is gekeken naar mogelijkheden om de behandeling van patiënten met niet kleincellig longcarcinoom (NSCLC) te optimaliseren.