Novel EGFR-directed therapy – a clinical study

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Inger Johanne Zwicky Eide

December 2022

List of abbreviations

CNS	Central nervous system
CR	Complete response
ctDNA	Circulating tumour DNA
DCR	Disease control rate
del19	Deletion in exon 19
DFS	Disease-free survival
DOH	Declaration of Helsinki
DoR	Duration of response
ECOG	Eastern Cooperative Oncology Group
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMA	The European Medicines Agency
ex20ins	Insertion in exon 20
FDA	The U.S. Food and Drug Administration
HR	Hazard ratio
iORR	Intracranial objective response rate
iPFS	Intracranial progression-free survival
NGS	Next-generation sequencing
NSCLC	Non-small cell lung cancer
ORR	Objective response rate
OS	Overall survival
PCR	Polymerase chain reaction
PD	Progressive disease
PDL1	Programmed death ligand 1
PFS	Progression-free survival
PR	Partial response
RECIST	Response evaluation criteria in solid tumours
RTK	Receptor tyrosine kinase
SBRT	Stereotactic body radiation therapy
SD	Stable disease
SRS	Stereotactic radiosurgery
TKI	Tyrosine kinase inhibitor
VEGF	Vascular endothelial growth factor
WBRT	Whole brain radiation therapy

Sammendrag av avhandlingen

På tross av betydelige fremskritt i behandling og diagnostikk de siste årene, og med dette økt overlevelse, er lungekreft fortsatt den kreftsykdommen som tar flest liv hvert år, både globalt og i Norge. I lungekreft av typen adenokarsinom, er det identifisert en rekke såkalte onkogene driver-mutasjoner. Dette er somatiske mutasjoner i deler av cellenes DNA som vanligvis involverer signalveier som regulerer celledeling og vekst. En onkogen driver-mutasjon kan føre til ukontrollert celledeling og dermed kreftutvikling. Typisk for flere av disse driver-mutasjonene er at de kan oppstå hos aldri-røykende personer. For de fleste mutasjonene som er identifisert som driver-mutasjoner, gjelder at man kun finner én av dem i svulsten. Ved å blokkere den intracellulære signalveien mutasjonen aktiverer, kan man bremse kreftsykdommen og i mange tilfeller oppnå til dels langvarige responser. Det er utviklet en rekke medikamenter som blokkerer de ulike signalveiene som kan være aktiverte, dette kalles målrettet behandling.

Mutasjoner i genet for epidermal vekstfaktor-reseptor (EGFR), forekommer i rundt 10-15 % av lunge-adenokarsinomer i den vestlige verden og enda hyppigere i Sørøst-Asia (40-50 %). De to vanligste typene mutasjoner i *EGFR* er delesjoner i genets ekson 19 (del19) og en punktmutasjon i ekson 21, L858R. Det er også identifisert en rekke mer sjeldent forekommende mutasjoner hvorav G719X i ekson 18, S768I i ekson 20 og L861Q i ekson 21 er blant de vanligste. G719X forekommer ofte sammen med en av de andre sjeldne mutasjonene. Alle disse *EGFR*-mutasjonene er driver-mutasjoner og fører til konstant aktivering av EGF-reseptoren som igjen aktiverer signalveier nedstrøms for reseptoren. Tyrosinkinasehemmere rettet mot EGF-reseptoren (EGFR-TKIer) blokkerer den aktiverte tyrosinkinasen på reseptoren og dermed de ukontrollert aktiverte signalveiene. Såkalte første- og andregenerasjonshemmere har i flere kliniske studier vist at de fleste pasientene oppnår sykdomsrespons på disse medikamentene og median progresjonsfri overlevelse (PFS) er i størrelsesorden 9-15 måneder for pasienter med avansert eller metastatisk *EGFR*-mutert lungekreft. De aller fleste pasientene vil tross dette utvikle resistens mot disse medikamentene. Den vanligste resistensmekanismen som oppstår hos 50-60% av pasientene, er en ny *EGFR*-mutasjon i ekson 20, kalt T790M. Osimertinib er en tredjegenerasjons EGFR-TKI som ble utviklet for å hemme effekten av T790M-mutasjonen, i tillegg til de vanlige sensitiviserende mutasjonene del19 og L858R. Kliniske studier har vist at osimertinib har høyere responsrate og lengre progresjonsfri overlevelse enn kjemoterapi for T790M-positive pasienter som har progrediert på behandling med andre EGFR-TKIer. Osimertinib er de seneste årene også utprøvd som første behandling ved *EGFR*-mutert avansert eller metastatisk sykdom og har vist seg mer effektiv enn førstegenerasjonshemmere når det kommer til progresjonsfri overlevelse og totaloverlevelse. Osimertinib har også større evne til å krysse blod-hjerne-barrieren enn de eldre medikamentene, og er derfor aktuelt for å behandle pasienter med hjernemetastaser.

Artiklene i denne avhandlingen er basert på data fra to kliniske studier som begge involverte pasienter med avansert eller metastatisk *EGFR*-mutert lungekreft. Den første studien, TREM, var en enarmet fase 2-studie utført i Norden og Litauen. Til sammen 199 pasienter ble inkludert i perioden 2015-2017. Alle pasientene hadde progrediert på minst en tidligere linje med EGFR-TKI, men til forskjell fra de fleste andre studier med osimertinib i andrelinje eller senere linjer, ble pasienter både med og uten resistensmutasjonen T790M inkludert. Den andre studien, FIOL, var av lignende enarmet fase 2-design, men inkluderte pasienter som var tidligere ubehandlet. Alle de 100 inkluderte pasientene mottok førstelinjebehandling med osimertinib. I begge studiene inkluderte vi

pasienter både med de vanlige *EGFR*-mutasjonene og med sjeldne *EGFR*-mutasjoner. Alle pasientene ble fulgt med regelmessige radiologiske evalueringer med CT toraks og abdomen og MR eller CT av hjernen enten ved kjente eller mistenkte hjernemetastaser (TREM) eller på alle uavhengig av om det forelå spredning til hjernen (FIOL). I begge studiene fikk pasientene behandling med osimertinib inntil sykdomsprogresjon eller intolerable bivirkninger. Pasientene kunne også fortsette med behandlingen etter progresjon dersom behandlende lege vurderte at de hadde klinisk nytte av dette. I FIOL-studien ble blodprøver fortløpende analysert for sirkulerende tumor-DNA. Det primære endepunktet for begge studiene var objektiv responsrate.

I første artikkel presenterte vi data fra TREM-studien. Ved inklusjon i studien hadde 24 % av pasientene hjernemetastaser, 15 % hadde redusert funksjonsnivå (ECOG 2) og 55 % hadde mottatt mer enn én tidligere linje behandling for metastatisk sykdom. Videre hadde 60 % påvist T790M og 26 % var T790M-negative. Vi fant at pasienter med T790M hadde en høyere objektiv responsrate på osimertinib enn pasienter uten T790M (60 % versus 28 %, $p < 0,001$), men at varigheten av respons var lik for de to gruppene (mediant 11,8 måneder versus 10,7 måneder, $p = 0,229$). Median PFS var 8,9 måneder for alle pasientene samlet. For T790M-positive var median PFS 10,8 måneder mot 5,1 måneder for T790M-negative, $p = 0,007$. Det var ingen statistisk signifikant forskjell i PFS mellom dem som hadde hjernemetastaser ved inklusjon og dem som ikke hadde det blant de T790M-positive, men for pasienter som var T790M-negative var det signifikant kortere median PFS hos dem med hjernemetastaser versus dem uten hjernemetastaser (1,6 måneder versus 5,6 måneder, $p = 0,009$).

I andre artikkel så vi nærmere på pasienter med hjernemetastaser i TREM-studien. Vi regransket MR- og CT-bilder av hjernen på 42 av de 48 pasientene dette gjaldt. De fleste hadde mottatt lokalbehandling mot hjernemetastasene før inklusjon, hvorav 73 % helhjernebestråling. Vi fant at intrakranial PFS var lenger for T790M-positive med mediant 39,7 måneder mot 3,5 måneder for T790M-negative. Risiko for progresjon i hjernen var også større for T790M-negative uavhengig av om de hadde hjernemetastaser ved inklusjon enn for T790M-positive (17 % mot 6 % etter et år).

I tredje artikkel undersøkte vi effekten av osimertinib hos pasienter med sjeldne *EGFR*-mutasjoner. 10 pasienter fra TREM og 11 pasienter fra FIOL ble inkludert i denne analysen. Vi fant at median PFS var 5,5 måneder både for pasienter behandlet i førstelinje (FIOL) og i senere linjer (TREM). Imidlertid var det en signifikant forskjell i PFS for pasienter med kun en mutasjon versus hos dem som hadde en dobbeltmutasjon (G719X i kombinasjon med enten S768I eller L861Q), henholdsvis 13,7 måneder og 3,5 måneder, $p = 0,003$. I FIOL-kohorten gjorde vi også analyse av sirkulerende tumor-DNA i plasma rett før oppstart av osimertinib og etter to ukers behandling, og fant at alle utenom to pasienter hadde lavere nivå av tumor-DNA ved det siste tidspunktet, noe vi tolket som tegn til respons på osimertinib.

Resultatene fra disse tre artiklene viser at vår pasientpopulasjon med T790M-mutasjon har tilsvarende effekt av osimertinib som pasienter inkludert i en stor fase 3-studie, selv om det i vår studie ble inkludert pasienter med dårligere funksjonsstatus og som hadde fått flere linjer behandling. Videre fant vi også tegn til at utvalgte pasienter med T790M-negativ sykdom kan ha noe effekt av osimertinib. Når det gjelder pasienter med hjernemetastaser, tyder våre resultater på at T790M-positive pasienter har god effekt av osimertinib, mens det var dårligere utfall for dem med T790M-negativ sykdom. Vår analyse av pasienter med sjeldne *EGFR*-mutasjoner bestod av få

pasienter, men behandling av slike pasienter er lite undersøkt og våre data indikerer at pasienter med dobbeltmutasjoner har bedre nytte av osimertinib enn pasienter med enkeltmutasjoner.

Summary of the thesis

There have been substantial improvements in treatment and diagnostics of lung cancer the recent years, yet it is still the most common cause of cancer deaths both globally and in Norway. In the adenocarcinoma subtype of lung cancer, several oncogenic driver mutations have been identified. They are somatic mutations in regions of the cells' DNA involving signalling pathways regulating cell proliferation and growth. An oncogenic driver mutation leads to tumorigenesis by inducing uncontrolled cell division. These mutations might typically occur in never-smoking persons. They are usually mutually exclusive, and by blocking the intracellular pathway activated by the mutation, it is possible to achieve disease control and, in many cases, durable responses. Targeted therapy, i.e., drugs targeting the different aberrantly activated pathways are developed for several of these mutations.

Mutations in the epidermal growth factor receptor (EGFR) gene occur in 10-15% of lung adenocarcinomas in the Western world and even more frequently in Southeast Asia (40-50%). The two most common types of mutations in *EGFR* are deletions in exon 19 (del19) of the gene and a point mutation in exon 21, L858R. There are also several uncommon *EGFR*-mutations, of which G719X in exon 18, S768I in exon 20 and L861Q in exon 21 are among the most prevalent. G719X-mutations often co-occur with one of the other rare mutations. The different *EGFR*-mutations are driver mutations leading to constitutive activation of the EGF-receptor and its downstream signalling pathways. Tyrosine kinase inhibitors targeting the EGF receptor (EGFR-TKIs) block the tyrosine kinase on the receptor and thus the uncontrolled activated signalling pathways. Multiple clinical studies have demonstrated that first- and second-generation EGFR-TKIs induces tumour responses in around 60-80% of patients with advanced or metastatic *EGFR*-mutated lung cancer treated with these drugs. The median progression-free survival (PFS) is approximately 9-15 months. Despite this, most patients will develop resistance to these drugs. The most common resistance mechanism occurring in 50-60% of patients is a new *EGFR*-mutation in exon 20, T790M. Osimertinib is a third-generation EGFR-TKI developed to inhibit the effect of the T790M-mutation, in addition to the common sensitizing mutations del19 and L858R. Clinical studies have demonstrated superior response rate and longer median PFS for osimertinib compared to chemotherapy for T790M-positive patients who have progressed on prior treatment with older EGFR-TKIs. Recently, osimertinib has also been tested as the first treatment for *EGFR*-mutated advanced or metastatic disease and has proven more effective than first-generation inhibitors when it comes to PFS and overall survival. Osimertinib also has a greater ability to cross the blood-brain barrier than the older drugs, and is therefore effective treatment for brain metastases.

The articles in this thesis are based on data from two clinical studies, both of which involved patients with advanced or metastatic *EGFR*-mutated lung cancer. The first study, TREM, was a single-arm phase II-study conducted in the Nordic countries and Lithuania. A total of 199 patients were included in the period 2015-2017. All patients had progressed on at least one previous line of EGFR-TKI, but unlike most other studies with osimertinib in second-line or later lines, patients both with and without the resistance mutation T790M were included. The second study, FIOL, was of similar single-arm phase II-design, but included patients who were previously untreated. In both studies, we included patients with common and rare *EGFR*-mutations. Radiological evaluation with CT of the chest and abdomen was done regularly throughout the study period. MRI or CT of the brain was done either for known or suspected brain metastases (TREM) or for all, regardless of whether there was brain involvement (FIOL). In both studies, patients received treatment with osimertinib until

disease progression or intolerable side effects. The patients could also continue treatment after progression if regarded beneficial to the patient by the treating physician. In the FIOL-study, blood samples were consecutively analysed for circulating tumour DNA (ctDNA). The primary endpoint for both studies was objective response rate (ORR).

In the first article, we presented data from the TREM-study. At baseline, 24% of the patients had brain metastases, 15% had a reduced functional level (ECOG 2) and 55% had received more than one previous line of treatment for metastatic disease. Furthermore, 60% had detected T790M and 26% were T790M-negative. We found that patients with T790M had a higher objective response rate to osimertinib than patients without T790M (60% versus 28%, $p<0.001$), but that the duration of response was similar for the two groups (median 11.8 months versus 10.7 months, $p=0.229$). Median PFS was 8.9 months for all patients combined. For T790M-positives, the median PFS was 10.8 months versus 5.1 months for T790M-negatives, $p=0.007$. There was no statistically significant difference in PFS between those who had brain metastases at inclusion and those who did not among the T790M-positive, but for patients who were T790M-negative there was a significantly shorter median PFS in those with brain metastases versus those without brain metastases (1.6 months versus 5.6 months, $p=0.009$).

In the second article, we looked more closely at patients with brain metastases in the TREM-study. We re-examined MR and CT images of the brain of the 42 out of the 48 patients in whom this applied. Most had received local treatment to the brain metastases before inclusion, of which 73% received whole-brain irradiation. We found that intracranial PFS was longer for T790M-positive with a median of 39.7 months versus 3.5 months for T790M-negative. Risk of progression in the brain was also greater for T790M-negative regardless of whether they had brain metastases at inclusion than for T790M-positive (17% versus 6% after one year).

In the third article, we examined the effect of osimertinib in patients with rare *EGFR*-mutations. Ten patients from TREM and 11 patients from FIOL were included in this analysis. We found that the median PFS was 5.5 months both for patients treated in first line (FIOL) and in later lines (TREM). However, there was a significant difference in PFS for patients with only one mutation versus those with a double mutation (G719X in combination with either S768I or L861Q), 13.7 months and 3.5 months, respectively, $p=0.003$. In the FIOL cohort, we also analysed ctDNA in plasma before commencing osimertinib and after two weeks of treatment. We found that all but two patients had a lower level of ctDNA at the last time point, which we interpreted as a signal of response to osimertinib.

The results from these three articles show that our patient population with the T790M-mutation has a similar effect of osimertinib to patients included in a large phase III-study, even though our study included patients with poorer functional status and who had received several lines of treatment. Furthermore, we also found evidence that selected patients with T790M-negative disease may have some effect from osimertinib. Regarding patients with brain metastases, our results suggest that T790M-positive patients have a good effect of osimertinib, while there was a worse outcome for those with T790M-negative disease. Our analysis of patients with rare *EGFR*-mutations consisted of few patients, but the treatment of such patients has been little investigated, and our data indicate that patients with double mutations benefit more from osimertinib than patients with single mutations.

List of papers

Paper I

Osimertinib in T790M-positive and -negative patients with *EGFR*-mutated advanced non-small cell lung cancer (the TREM-study).
Eide IJZ, Helland Å, Ekman S, Mellemgaard A, Hansen KH, Cicenas S, Koivunen J, Grønberg BH, Brustugun OT. Lung Cancer. 2020 May;143:27-35.

Paper II

Intracranial effect of osimertinib in relapsed *EGFR*-mutated T790M-positive and -negative non-small cell lung cancer patients: results from a phase II study.
Eide IJZ, Grut H, Helland Å, Ekman S, Sørensen JB, Hansen KH, Grønberg BH, Cicenas S, Koivunen JP, Mellemgaard A, Brustugun OT. Acta Oncol. 2021 Dec;60(12):1565-1571

Paper III

Osimertinib in non-small cell lung cancer with uncommon *EGFR*-mutations: a post-hoc subgroup analysis with pooled data from two phase II clinical trials.
Eide IJZ, Stensgaard S, Helland Å, Ekman S, Mellemgaard A, Hansen KH, Cicenas S, Koivunen J, Grønberg BH, Sørensen BS, Brustugun OT. Transl Lung Cancer Res. 2022 Jun;11(6):953-963

1 Background

1.1 Epidemiology and prognosis

Lung cancer survival has increased steadily the past 10 years, yet it is still the most lethal cancer both on a worldwide basis as well as in Norway. Globally, lung cancer accounted for 11.4% of all new cases of cancer in 2020, with 2.2 million incident cases, making it one of the most common cancers together with prostate cancer in men, breast cancer in women and colorectal cancer in both sexes (1). Lung cancer has remained the primary cause of years of life lost among all cancers in the decade from 2007 to 2017, and the annual death toll reached 1.8 million in 2020, which corresponds to 18% of all cancer deaths (1, 2). In Norway, lung cancer constitutes the third most common cancer diagnosis with 3331 new cases in 2020 and the leading cause of cancer death with 2168 deaths (3). The incidence has been increasing for women the last 30 years, whereas for males there has been a tendency of a declining rate the recent years (Figure 1). This has led to a shift in distribution between the sexes; historically lung cancer was more prevalent in males, but the last few years there have been almost equal numbers for both sexes. The total incidence is expected to rise further the next 10-20 years, partly due to the observed increasing trend for women and partly due to larger population size and a more elderly population (4).

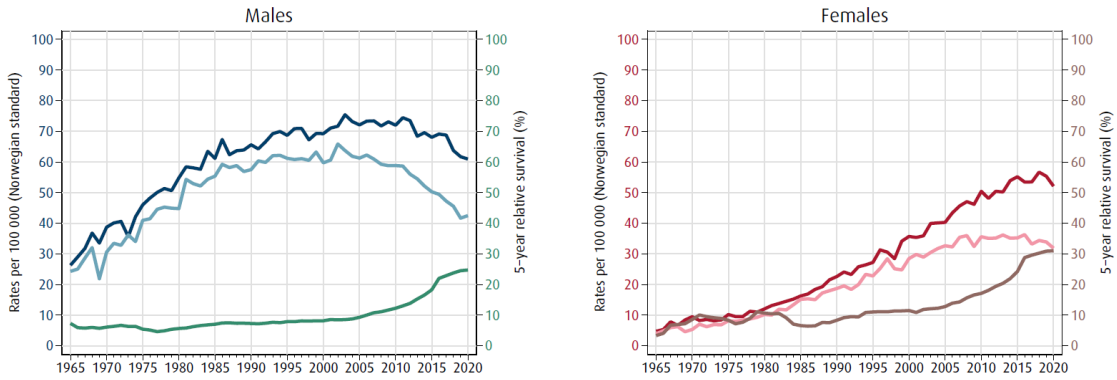
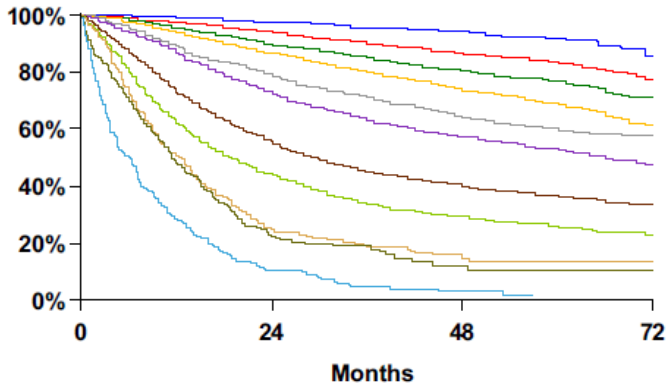


Figure 1. Trends in incidence (top curve), mortality (middle curve) and survival (bottom curve) in lung cancer in Norway 1965-2020. From Cancer in Norway 2020 - Cancer incidence, mortality, survival and prevalence in Norway. Cancer Registry of Norway, 2021 (3).

Survival is closely linked to the stage of the disease (5) (Figure 2). The majority of lung cancer cases are in advanced stages when diagnosed which reflects the poor prognosis. Nevertheless, although the mortality rate is high, there has been a substantial improvement in survival in Norway over the past decade. In 2020, the 5-year relative survival was nearly double of that in 2010, with 30.7% for females and 24.6% for males (4). Some of the improvement might be explained by more precise diagnostics and hence an increasing proportion of patients with early stage-disease treated with curative intent. Still, less than 40% receive potentially curative treatment. There has also been an increase in survival among stage IV patients, maybe due to the introduction of better targeted therapies (6). Even so, when it comes to patients with stage IV disease, half of the patients survive less than 6 months and among the 25% of the patients with worst prognosis, the survival is less than 2 months and has stayed unchanged the last decade (4) (Figure 3).



Proposed	Events / N	MST	24 Month	60 Month
IA1	68 / 781	NR	97%	92%
IA2	505 / 3105	NR	94%	83%
IA3	546 / 2417	NR	90%	77%
IB	560 / 1928	NR	87%	68%
IIA	215 / 585	NR	79%	60%
IIB	605 / 1453	66.0	72%	53%
IIIA	2052 / 3200	29.3	55%	36%
IIIB	1551 / 2140	19.0	44%	26%
IIIC	831 / 986	12.6	24%	13%
IVA	336 / 484	11.5	23%	10%
IVB	328 / 398	6.0	10%	0%

Figure 2. Overall survival according to disease stages in the eighth edition of TNM. From Goldstraw et al, The IASLC Lung Cancer Staging Project: Proposals for Revision of the TNM Stage Groupings in the Forthcoming (Eighth) Edition of the TNM Classification for Lung Cancer. J Thorac Oncol. 2016 (5). With permission.

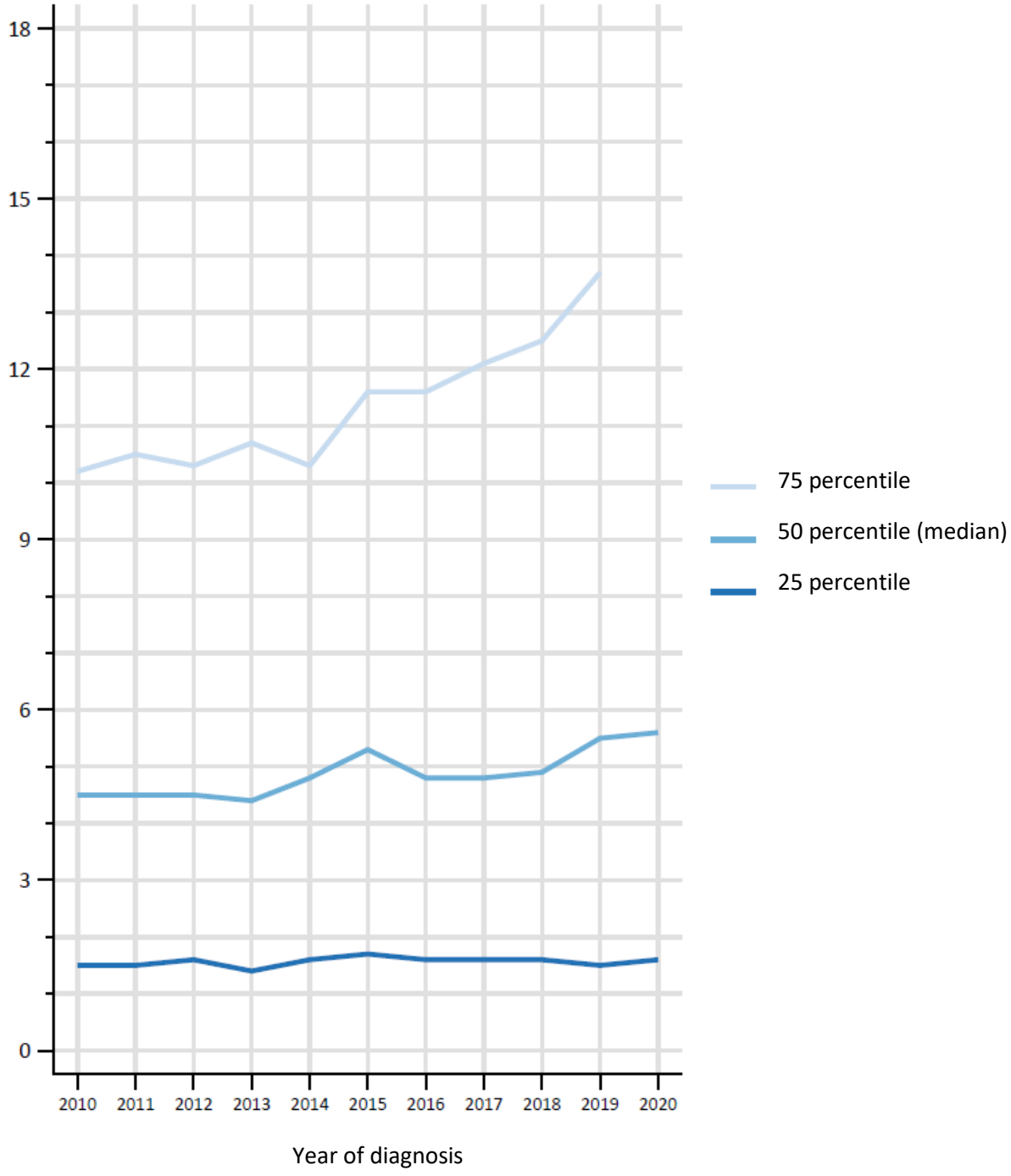


Figure 3. Overall survival in patients with stage IV. Adapted from «Årsrapport 2020 med resultater og forbedringstiltak fra Nasjonalt kvalitetsregister for lungekreft». Cancer Registry of Norway, 2021 (4).

1.2 Etiology

1.2.1 Tobacco

Smoking is the main risk factor for developing lung cancer. In the early 20th century, a sharp rise in incidence of lung cancer was noted (7), but the link between cigarette smoking and lung cancer was not established until 1950 when two independent case-control studies found that the lung cancer patients had a higher likelihood of being smokers than healthy individuals (7, 8). Richard Doll and A. Bradford Hill followed up with what is regarded as the first prospective cohort study, in which they studied British physicians and their smoking habits. They showed that smokers indeed had a higher risk of developing lung cancer, hence the association was confirmed (9).

The widespread and extensive global cigarette consumption, also referred to as the tobacco epidemic (10), has been the cause of millions of deaths, and although smoking prevalence has declined steadily the last thirty years globally (11) as well as in Norway (Figure 4) and cigarette smoking in the younger age groups in Norway is almost eliminated (12), the lung cancer incidence is expected to keep increasing due to the delay from tobacco exposure to manifest cancer.

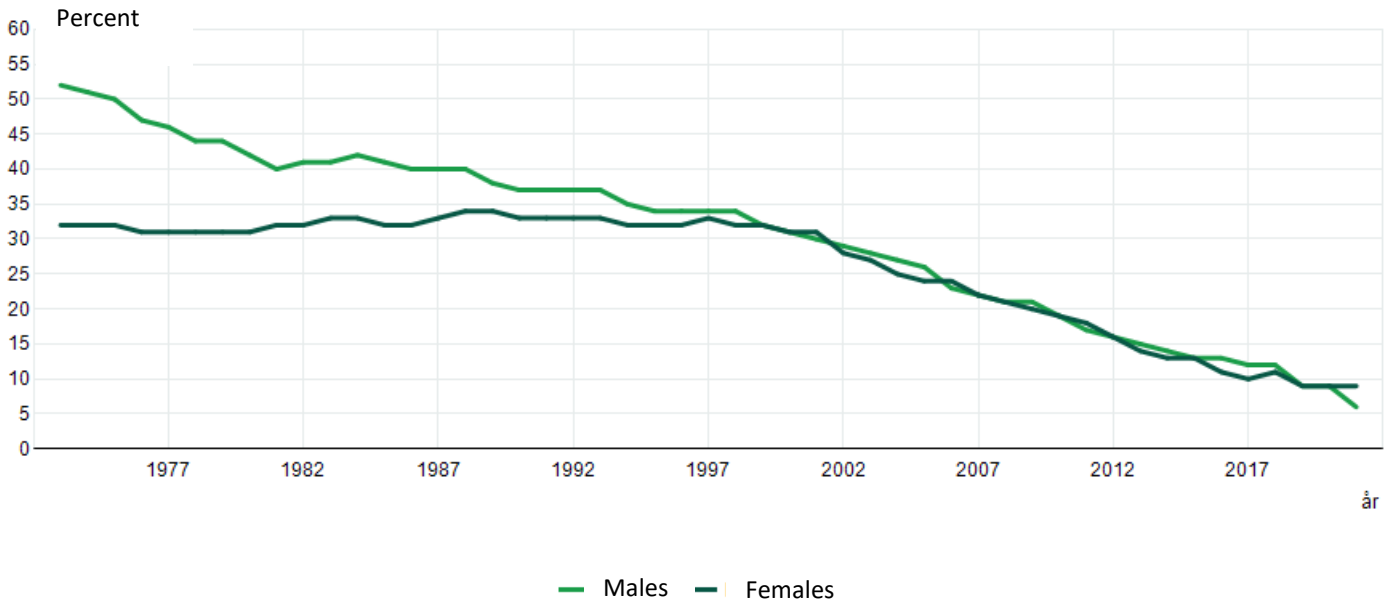


Figure 4. Percent daily smokers 1973-2021. From SSB.no

1.2.2 Lung cancer in never-smokers

Although smoking is by far the most important cause, around a third of lung cancer cases globally, with variable proportions in different geographic areas, arise in never-smokers (1, 13). Second-hand smoke is one of several other risk factors (14) and some studies have even demonstrated that exposure to passive smoking during childhood and early adulthood gives a significantly higher risk of lung cancer than passive exposure later in life (15, 16). Among other environmental factors associated with lung cancer are air pollution, radon and asbestos. Air pollution includes indoor particulate matter, i.e., from smoke from burning coal and fumes from cooking oil at high temperatures, which is widely used in parts of Asia (17). Outdoor air pollution including particulate matter and gases from traffic, has also been shown to increase the risk of lung cancer in never-smokers (18, 19).

Radon is a gas derived from the decay of uranium and is present in soil and rock. It emits alpha particles from which radiation can damage the respiratory epithelium and is known to cause lung cancer in uranium miners (20). Radon can also accumulate in houses, and a large meta-analysis concluded that radon in homes might account for 2% of lung cancer cases in Europe (20). Also, there is an additive effect of smoking and exposure to radon (20).

Occupational exposure to asbestos is another established risk factor for lung cancer (21). As for radon, asbestos increases the risk many-fold in smokers compared to non-smokers (22).

Whether lung cancer also has a genetic basis is less well understood. However, some studies have demonstrated that first-degree relatives of individuals with lung cancer have an increased risk of developing the disease, even when adjusted for smoking habits (23)

1.3 Classification of lung cancer including driver mutations

Lung cancer is divided in two main groups based on histology. Around 15% of the cases are small-cell lung cancer, while non-small cell lung cancer (NSCLC) constitutes the remaining approximately 85%. NSCLC is further classified as adenocarcinomas, squamous carcinomas, large cell carcinomas or NOS (not otherwise specified). Historically, squamous cell carcinoma was the most prevalent type, but adenocarcinoma is now the most common among both men and women in Norway (24). This thesis will from now on focus on NSCLC, and in particular adenocarcinomas.

Oncogenic driver mutations are changes in the DNA of the cells which lead to aberrant cell signalling and hence dysregulation of cell growth and cell death which might result in cancer development. In adenocarcinomas, an increasing number of such driver mutations are identified (Figure 5), many of which are targetable with different drugs. These mutations are typically mutually exclusive, meaning that they rarely co-exist. The most common driver alterations in NSCLC include different mutations in the Kirsten rat sarcoma gene (*KRAS),* mutations in the gene encoding the epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*) and ROS proto-oncogen1 (*ROS1*) translocations and *BRAF* mutations. Given the increasing number of targeted therapies available, detection of druggable mutations has become an important part of the diagnostics of adenocarcinomas as detailed later in the thesis.

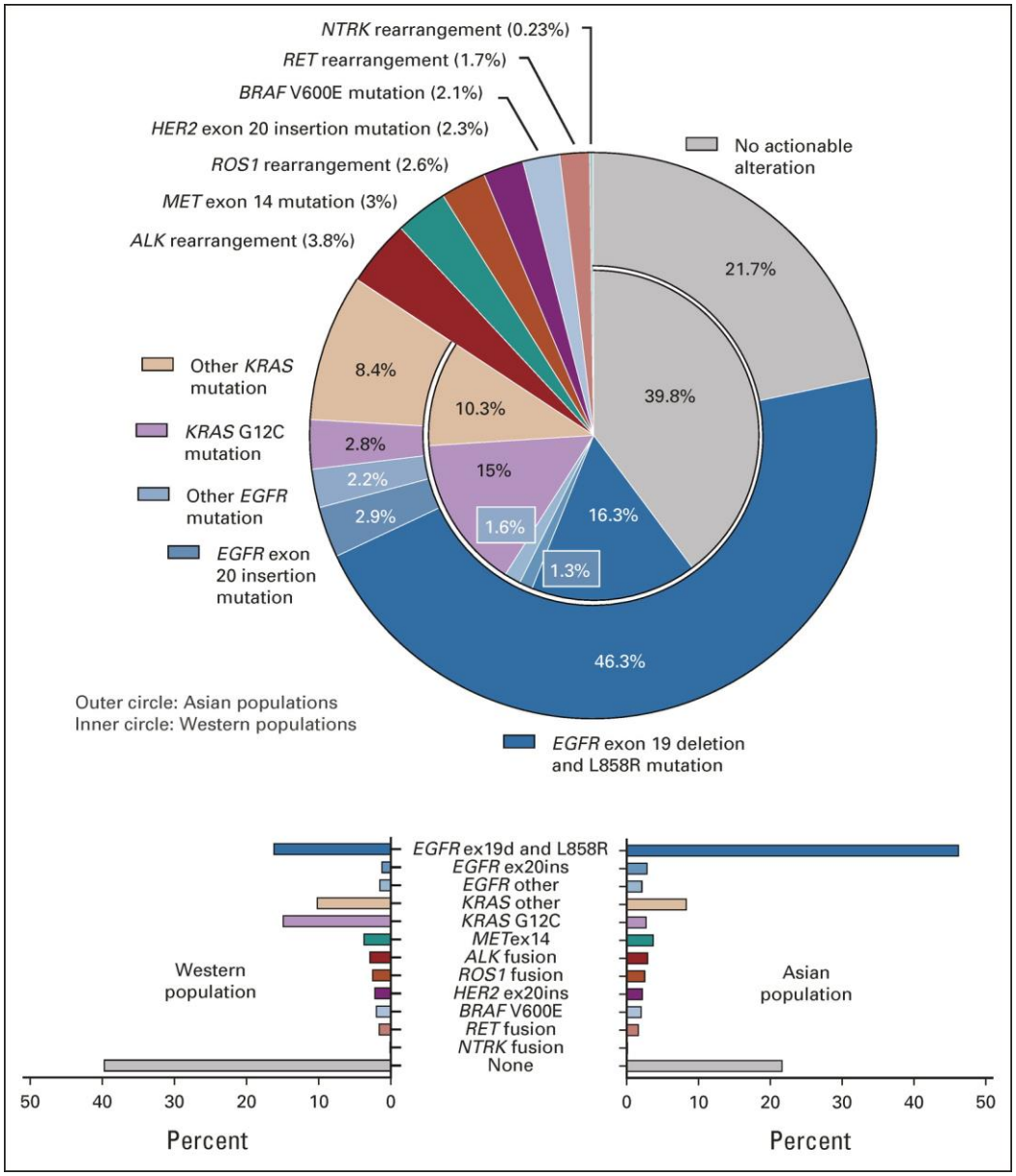


Figure 5. Frequency of targetable oncogenic driver molecular alterations in NSCLC (adenocarcinoma). From Tan et al., Targeted Therapies for Lung Cancer Patients With Oncogenic Driver Molecular Alterations. Journal of Clinical Oncology 2022 (25). With permission.

1.4 Diagnosis and staging

The most common staging system for cancer is the TNM-system and the current version for lung cancer is TNM8 (5, 26). T represents tumour size, N the extent of involvement of regional lymph nodes and M the presence or absence of metastases. Accurate staging is important to guide treatment decisions. Furthermore, the stage of the disease is the factor with most impact on prognosis (Figure 2). Lung cancer is also classified as stage I-IV corresponding to different combinations of T-, N-, and M-status, where I is localized disease and IV represents metastatic disease.

The diagnosis of lung cancer is based on different modalities and the diagnostic work up aims to uncover the type of lung cancer, stage and extent of disease, the general condition of the patient and relevant comorbidities. These factors combined will guide treatment decisions and prognosis.

Symptoms are often vague or even absent, but might include dyspnea, cough, hemoptysis, pain and weight loss. Patients with brain metastases might present with neurological symptoms, headache or nausea.

The performance status of the patient should be described by the Eastern Cooperative Oncology Group (ECOG) performance status scale where 0 is no disability or no restriction in physical activity and 4 denotes a bedbound patient with no ability of self-care. Similar to stage of the disease, the performance status correlates with prognosis (27) and patients in poor performance status are generally considered not to benefit from toxic cancer treatment.

Radiological assessments include a CT scan of thorax and abdomen to reveal the extent of the disease. Further, if the primary examinations indicate that the patient could be a candidate for curative treatment, an FDG PET/CT scan should be performed to rule out obscure metastatic lesions, and also an MRI of the brain for accurate staging (28).

Histopathological diagnosis is crucial and is often obtained with a biopsy from a primary or metastatic lesion. The diagnosis can also be made with a cytological specimen. For instance, fine-needle aspiration of a pathological mediastinal lymph node, often performed guided by ultrasound

(EBUS), or cytological smear from pleural effusion can contribute to diagnosis and also to staging by confirming malignancy in effusions or lymph nodes.

The sample is classified by morphology and by the immunohistochemistry profile of the tissue. Immunohistochemistry is done by examining the protein expression on the cell surfaces with different antibodies. With this technique it is possible to discern between adenocarcinoma and squamous cell carcinoma and is especially useful if morphology is indeterminate as to which histology subtype is present. Adenocarcinomas are typically positive for TTF-1- and napsin A-protein expression and negative for p40, whereas the opposite is true for squamous carcinomas. Furthermore, a typical profile can give the organ of origin of the tumour in question, for instance to decide whether a pulmonary tumour is a primary lung tumour or a metastasis from a different cancer. In addition, when the diagnosis is established, immunohistochemistry is used to examine different predictive biomarkers. Of these, the presence and degree of expression of programmed death ligand-1 (PDL1) is crucial to guide the use of immunotherapy and is recommended to be done in all cases of NSCLC. PDL1 is graded as absent (<1% of tumour cells positive), low (1-49%) or high (50-100%). Immunohistochemistry is further utilized for predictive testing for protein products of driver alterations, such as ALK or ROS1.

To inform treatment with targeted therapies, it is pivotal to do molecular testing to detect any driver mutation. This has traditionally been done with a single-mutation approach with different methods such as the polymerase chain reaction (PCR) method, IHC or fluorescence in situ hybridisation (FISH) depending on the alteration in question. However, with the increasing number of drivers identified and with new drugs targeting them being developed, it has become more relevant to test for several mutations simultaneously by using next-generation sequencing (NGS) and multiplex gene panels where many genes or part of many genes can be examined in one procedure (29, 30). Testing is usually performed on the tissue biopsy or cytology specimen, but if there is not enough material available, mutations can also be detected in blood samples by analysing circulating tumour DNA (ctDNA).

1.5 Liquid biopsies

Shedding of DNA from cells into the circulation happens either when the cells destruct through apoptosis or necrosis, or by active secretion. So-called cell-free DNA in blood was first described in 1948 (31). It originates from normal healthy cells or from cancer cells (32). In the latter case,

circulating DNA derived from tumours (ctDNA) may be detectable in plasma as a fraction of the cell-free DNA and can be used to identify molecular alterations from the tumour in question, hence the term liquid biopsy (33, 34). In metastatic NSCLC, liquid biopsies can be used as upfront tumour genotyping at primary diagnosis or as a re-biopsy at resistance for detection of emerging resistance mutations (35). Among the advantages of liquid biopsy over tissue biopsy, is the ability to overcome the challenge of tumour heterogeneity with a single tissue sample, especially at the time of resistance to targeted therapy (36). Furthermore, it is a non-invasive method with less procedure related risk than tissue biopsy, and as such a feasible method of monitoring the status of the disease over time with repeated samples (37). Also, a tissue biopsy is not always possible to obtain and then a liquid biopsy might be a good alternative to be able to do genotyping. However, liquid biopsies are not informative on histology or some biomarkers like PDL1. Another challenge with blood-based testing is the fact that not all tumours shed DNA to the circulation, or the amount of ctDNA might be too small to detect (35). The rate of false negatives has been reported to be up to around 30% (38-40). Nevertheless, as methods for testing have become more sensitive, and especially with the development of NGS for broad testing of both mutations, fusions and amplifications, performing liquid biopsies are recommended as a supplement to traditional tissue sampling at primary diagnosis (but not sufficient for diagnosis to be made) and can be considered as first approach in the setting of resistance to targeted therapy (35, 41). However, in the case of a negative liquid biopsy, a tissue biopsy should be pursued because of the possibility of false negatives. Furthermore, clearance of ctDNA, i.e., a fall in the amount of ctDNA detected, on therapy, have been demonstrated to correlate with response to treatment and prognosis and has thus been proposed as a predictive tool (36, 42)

1.6 Treatment

There are five main categories of lung cancer treatment, namely surgery, radiotherapy, chemotherapy, immunotherapy and targeted therapies. Most lung cancer patients in Norway are treated according to national guidelines which are updated regularly (28). Patients who are candidates for curative treatment should be discussed in a multidisciplinary team before treatment decisions are made. In the case of metastatic disease, several different aspects should be considered, as described below.

1.6.1 Localized disease

Patients in stage I-II and selected patients with stage III disease are treated with curative intent. They should be considered for surgical resection of the tumour, which usually include either removal of one or two lobes of the lung (lobectomy) or an entire lung (pulmectomy). To be eligible for surgery, the patients are required to have sufficient lung function to tolerate loss of part of or the whole lung. The 5-year survival after surgery is around 65% for all stages combined (43). For patients not fit for major surgery, stereotactic body radiotherapy (SBRT) in which large radiation doses are delivered to the tumour, may be an alternative. Local control rate after SBRT for stage I disease is around 80% at 5 years and the treatment is well tolerated (44).

Adjuvant treatment with chemotherapy after complete resection of NSCLC has been investigated in several studies. A meta-analysis concluded with an absolute survival benefit of cisplatin-based chemotherapy compared with no treatment of 5.4% after 5 years (45). Some studies have demonstrated that stage I-tumours do not benefit from adjuvant treatment (46). Hence, administration of four courses of platinum-doublet chemotherapy after completely resected stage II-III tumours has been implemented as standard of care. Recently, the IMPOWER 010-study was published, in which 12 months treatment of the PDL1-inhibitor atezolizumab was added to adjuvant chemotherapy. In patients with PDL1≥1% there was a significantly increased disease-free survival in the atezolizumab-treated group compared with best supportive care with a hazard ratio of 0.66 (95% confidence interval 0.50-0.88). However, this treatment is at present only approved in the USA (47). Furthermore, adjuvant treatment with the EGFR-TKI osimertinib for resected *EGFR*-mutant lung cancer has recently been approved, this is elaborated in the section on EGFR tyrosine kinase inhibitors.

1.6.2 Locally advanced disease

Stage III disease might be amenable for curative treatment if negative prognostic factors like decreased performance status and significant weight loss are absent. Concurrent treatment with high dose radiotherapy, usually 2 Gy x 30-33, and platinum-based chemotherapy has yielded 5-year survival rates of approximately 15% (48). Recently, a large randomized study in which non-progressing patients were given the immune check point inhibitor durvalumab after concomitant radiochemotherapy, demonstrated that adjuvant immunotherapy increased median progression free survival from 5.6 months to 16.8 months compared with placebo (49). Furthermore, immunotherapy significantly increased survival at two years from 56% in the placebo-group to 66%

in the durvalumab-group (50). Based on these results, one year treatment with durvalumab after radiochemotherapy is standard of care for patients with PDL1≥1%.

1.6.3 Advanced disease

Patients for whom curative treatment is not possible, either due to metastatic disease (stage IV) or locally advanced disease (stage III) with negative prognostic factors, comprise a large and heterogenous group. Several aspects in addition to tumour properties should be taken into consideration when making treatment decisions for these patients. For some patients in poor performance status, with significant comorbidities or elderly frail patients with short life expectancy, best supportive care might be the best option rather than potentially toxic anticancer treatment. Goals for non-curative anti-cancer treatment is to prolong life, maintain control of the disease for as long as possible and to alleviate cancer related symptoms. Palliative care with symptom relief as the main focus should be given to all patients regardless of cancer treatment.

Tumours without driver mutations

For tumours without actionable driver mutations, treatment is chosen based on histology and PDL1-expression. Historically, different combinations of chemotherapy containing either cisplatin or carboplatin in pair with a second agent were the only systemic treatment options, yielding response rates of 20%, median overall survival of 8 months and 2-year survival of only 11% (51). With the advent of immunotherapy however, the prognosis for patients with advanced disease is much improved. Immune checkpoint inhibitors act as inhibitors of receptors involved in regulation of the immune response. In the treatment of lung cancer, PD1- and PDL1-inhibitors are the two most commonly used classes of drugs, either as monotherapy or in combination with chemotherapy. Furthermore, the CTLA4-antibody ipilimumab is also approved. Several of these agents have been shown to confer improved progression-free survival (PFS) and overall survival (OS) in patients with high PDL1-expression (≥50%) compared to platinum-based chemotherapy when given as first line therapy for both adenocarcinomas and squamous cell carcinomas (52-55). Currently, pembrolizumab as monotherapy is the recommended choice for these patients according to the Norwegian treatment guidelines (28). In the phase III-study which lead to approval of this regimen, the median overall survival was 26.3 months in the pembrolizumab-group vs 13.4 months in the chemotherapy-group and the five-year survival rate was reported to be 31.9% for the patients who received pembrolizumab (56). For patients with PDL1-expression below 50%, studies have shown that immune checkpoint inhibitors alone have less effect (57). In the KEYNOTE-189 study, the

combination of platinum-based chemotherapy and pembrolizumab versus chemotherapy alone was evaluated. The combination-arm yielded a superior PFS (median 8.8 months versus 4.9 months) and OS (median 22 months versus 10.7 months) (58) and, importantly, patients with no PDL1-expression also benefited with a hazard ratio of 0.52 (95% confidence interval 0.36 to 0.74) and median OS 17.2 months versus 10.2 months, respectively (59). Similar results have been demonstrated for squamous cell carcinomas (60) and based on these studies, combination therapy is the recommended treatment for patients with PDL1-expression under 50% (28, 30).

Second line treatment

A study published in 2000, in which 204 patients were randomized to docetaxel or best supportive care after treatment with platinum-based chemotherapy, found a response rate of 7.1% and median overall survival of 7.0 months versus 4.6 months in favour of docetaxel (61). Based on this, docetaxel is used as second line treatment not only after progression on chemotherapy, but also after progression on combination treatment with chemotherapy and immunotherapy, although it has not been studied in the combination setting. For platinum-naïve patients who have progression after monotherapy with an immune checkpoint inhibitor, it is regarded reasonable to treat with a platinum doublet in second line, given that the patient is eligible for such treatment (28). This strategy is however also not studied in randomized trials. Several of the immune checkpoint inhibitors were first studied in pre-treated patients progressing on chemotherapy and proved to be more effective than docetaxel (62-65). As such, pembrolizumab, nivolumab and atezolizumab are approved as second line treatment for PDL1-positive patients who have been treated with chemotherapy alone in first line.

Oncogenic driven tumours

Targeted therapies are the primary treatment for patients in whom an actionable target is identified, and the choice of drug depends on the molecular alteration in question. In general, this type of therapy aims to block signalling pathways which are aberrantly activated. The most common class of drugs for this purpose is tyrosine kinase inhibitors (TKIs); small molecular drugs which are administered per mouth. The response rates for treatment with selective TKIs are typically high (above 50%) and many of them have demonstrated a prolonged PFS compared to standard chemotherapy.

EGFR tyrosine kinase inhibitors (EGFR-TKIs) were the first targeted drugs developed for the treatment of lung cancer, and as part of the main topic of this thesis, they will be discussed in greater detail later.

TKIs targeting *ALK* are now regarded standard of care as first line treatment for tumours harbouring an *ALK*-translocation. In 2013, the first randomized phase III-study was published, demonstrating that the first generation TKI crizotinib conferred improved PFS compared to chemotherapy, with 10.9 months versus 7.0 months ($p<0.001$) (66). The study failed to demonstrate a significant improvement in OS, but of note, the majority (84.2%) of the patients in the chemotherapy-arm received subsequent crizotinib upon progression (67). Nevertheless, the median OS in the crizotinib-arm was not reached versus 45 months in the chemotherapy-arm, which was impressive in a NSCLC patient population (67). In the recent years, second and third generation ALK-TKIs have proven to be superior to crizotinib and are now the preferred treatment choices. Alectinib was compared to crizotinib in the ALEX-trial (68) and long-term follow up demonstrated a median PFS 34.8 with alectinib versus 10.9 months with crizotinib. Furthermore, the 5-year OS rate was 62.5% with alectinib, i.e., comparable to early stage resected patients (69). Another second generation TKI, brigatinib, has demonstrated similar results as alectinib when compared to crizotinib (70, 71). Importantly, common for both these agents and typical for newer TKIs, is a superior effect in the CNS. In the alectinib-study, the response rate within the CNS was 59% versus 26% in the alectinib and crizotinib group, respectively. Furthermore, 12% of patients treated with alectinib experienced CNS-progression and 45% in the crizotinib-group (68). Correspondingly, the intracranial response rate was 67% versus 17% with brigatinib versus crizotinib, respectively (70). Among patients treated with brigatinib, 9% had progression in the brain as their first site of progression, compared to 19% in the crizotinib-group. Alectinib and brigatinib are according to the Norwegian National Guidelines regarded equal in terms of choice of first line treatment for ALK-positive patients (28).

Effect of selective protein kinase inhibitors for several other mutations or translocations such as *ROS1*, *BRAF*, *RET*, *MET* and *NTRK* has also been demonstrated. However, these alterations are rare with few patients harbouring each of them, and as such, the documented effect is largely through non-randomized trials with a limited number of patients. Crizotinib has received approval for the use in *ROS1*-translocated patients based on a study with 53 patients where the overall response rate was 72%, median PFS 19.2 months and median overall survival 51.4 months (72, 73). When given in previously treated or untreated patients with a $BRAF^{V600E}$-mutation, two single-arm studies with 57

and 36 patients, demonstrated that dual inhibition with the MEK-inhibitor trametinib and the BRAF-inhibitor dabrafenib gave response rates of 63.2% and 64% and median PFS of 9.7 months and 10.9 months, respectively (74, 75). Similarly, in the case of *RET*-fusions, two different TKIs have been shown to be active. In a phase I-II study, 105 patients treated with selpercatinib had a response rate of 64% and median PFS 16.5 months (76). Furthermore, with the caveat that there were few patients, selpercatinib had promising intracranial activity; in 22 patients with measurable brain metastases, 82% achieved an intracranial response (77). Likewise, another RET-inihibitor, pralsetinib, demonstrated a response rate of 53% in 53 previously treated and 70% in 27 previously untreated patients. The previously untreated patients were patients not eligible for platinum chemotherapy. Median PFS in the two groups were 17.1 and 9.1 months, respectively. Also in this study there was a signal of effect on brain metastases for nine patients with measurable disease intracranially (78). *NTRK*-fusions are gene rearrangements which are rare but can be present in many different cancers. At present, the NTRK-inhibitors entrectinib and larotrectinib have regulatory approval for *NTRK*-positive cancers of any kind including NSCLC, which is an example of tumour agnostic approval, i.e., treatment based solely on a molecular target rather than a specific cancer diagnosis (79, 80). Studies leading to this approval have included patients with a range of different cancers and have demonstrated efficacy of the drugs in *NTRK*-positive patients across cancer types. In one study of larotrectinib, 75% of 12 patients with NSCLC had an objective response (81) and correspondingly, in a study with entrectinib, 8/10 patients had an objective response. The median PFS was 11.2 months for all patients regardless of tumour type for entrectinib (82). Worth noting, entrectinib has inhibitory effect also in *ROS1*-translocated tumour cells. Although entrectinib has not been compared head-to-head with crizotinib, it is recommended as first choice for *ROS1*-positive lung cancer, based on similar results with regards to objective response rate (77% in 53 patients) and PFS (median 19.0 months) and also, the benefit of better CNS-penetrance than crizotinib (83). Among other rare oncogenic drivers, TKIs for *MET*-alterations have demonstrated activity in early phase-studies (84, 85).

As demonstrated above, TKIs are by far the most common class of drugs used in targeted therapies. However, other principles for personalized treatment are developed or in development. Examples of this are inhibitors of KRAS and HER2. In contrast to the above-mentioned rare alterations, *KRAS* is a common driver mutation found in 25-30% of lung adenocarcinomas (25, 86). Although prevalent, it has proven difficult to develop effective drugs against *KRAS*-mutated tumours. Recently, however, a drug directed towards a subtype, *KRAS G12C*, have shown activity in early phase-studies in pre-treated patients (87). Other similar compounds are in development. Different strategies for targeting

the HER2-receptor have been employed especially in the treatment of HER2-positive breast cancer, including antibodies and TKIs. A recent phase II-study demonstrated activity of an antibody-drug conjugate, trastuzumab deruxtecan, in *HER2*-mutated NSCLC (88). This drug consists of a chemotherapeutic molecule coupled to a HER2-directed antibody. The antibody facilitates uptake in the cancerous cells in which the chemotherapeutic exerts its action (88).

1.7 Brain metastases in non-small cell lung cancer

The brain is a common site of metastases in lung cancer and when symptomatic, a possible cause of severe morbidity and impact on quality of life. The true prevalence of brain metastases at diagnosis of lung cancer is uncertain, in part due to lack of screening for asymptomatic brain metastases both in routine and in clinical trials, and in part due to use of less sensitive methods like CT scanning instead of MRI scanning (89). Nevertheless, some epidemiological studies report that 10-20% of all lung cancer patients have brain metastases at diagnosis, and around 25-30% of patients who present with stage IV disease at the time of diagnosis (90, 91). In these studies, adenocarcinomas are estimated to have a higher propensity than squamous cell carcinomas to metastasize to the brain. Furthermore, patients with *EGFR*-mutations and *ALK*-translocations have a high risk of brain metastases. Again, the exact frequency in these groups is unknown, but studies of *EGFR*-mutated lung cancer report that 20-45% present with brain metastases at diagnosis (92-95) and there are indications that more than 50% develop brain metastases at some point during their disease course (92).

The prognosis for patients with brain metastases has historically been poor with median survival of around 6 months, and indeed it is still so if negative prognostic factors are present (91, 96). Fortunately, outcomes have improved in some groups, in particular for *EGFR*- or *ALK*-altered tumours (96).

Treatment of brain metastases consist of either locally applied treatment including surgery, stereotactic radiosurgery (SRS) or whole brain radiotherapy (WBRT) or systemic treatment. There has been a shift towards less use of WBRT as such treatment is associated with neurocognitive toxicity and uncertainty of effect on both survival and quality of life (97). Surgery and SRS are recommended if there are few brain metastases, although exact cut-off regarding number of metastases is not defined for the use of SRS (98). Systemic therapy like chemotherapy have limited

use in the treatment of brain metastases with only modest intracranial response rates due to restricted penetration of the blood-brain barrier (99, 100). Treatment of patients with *EGFR*-mutated lung cancer and brain metastases will be discussed later.

2 Epidermal Growth Factor Receptor and Tumorigenesis

Receptor tyrosine kinases (RTKs) comprise a class of transmembrane receptors that are widely involved in cell signalling and are recognized as important factors in carcinogenesis. Upon binding of their specific ligands (growth factors), they confer a signal through the cell membrane via tyrosine kinase activity and thus activates downstream signalling pathways involved in basal cellular processes like proliferation, survival and apoptosis. Altered RTKs act as oncogenes through dysregulated signalling and hence result in uncontrolled cell proliferation which is one of the six original hallmarks of cancer as proposed by Hanahan and Weinberg (101).

During the 20th century, great efforts were done by the scientific community to understand how cancer arises. One important step on the way was a seminal discovery made by biologist Stanley Cohen in 1962. He observed that a substance in salivary gland extract induced opening of the eyelids and tooth eruption in new-born mice, then isolated the protein responsible for this effect and identified it as the epidermal growth factor (EGF) (102). More than 10 years later, his group identified the receptor to which the growth factor binds, the epidermal growth factor receptor (EGFR) (103). EGFR was later identified as an RTK. Cohen and his colleague Rita Levi-Montalcini received the Nobel Prize in Physiology or Medicine in 1986 for their discovery of growth factors, including EGF and nerve growth factor.

EGFR is one of four members of a family of human epidermal growth factor receptors (EGFR/HER1/ ErbB1, HER2/ErbB2, HER3/ErbB3 and HER4/ErbB4) (Figure 6). They consist of an extracellular domain to which their ligands bind, a transmembrane part and an intracellular part which constitutes the tyrosine kinase domain (104). Several ligands are known to activate EGFR, among which are EGF, transforming growth factor-α (TGF-α) and amphiregulin. Upon binding a specific ligand, the receptors come together to form homodimers (i.e., an EGFR-EGFR-complex) or heterodimers (i.e., a complex of two different receptors like EGFR-HER2). The dimerization activates the receptors and leads to phosphorylation of the tyrosine kinase via binding of adenosine triphosphate (ATP) to a pocket on the intracellular part of the receptor, which then ignites the downstream signalling

cascade. The two main pathways activated by EGFR are the RAS/RAF/MEK/ERK and the phosphatidylinositol 3-kinase (PI3K)/Akt pathways, however, the different pathways involved in signal transduction induced by the ErbB-family constitute a network in which they are connected and influence each other (105, 106).

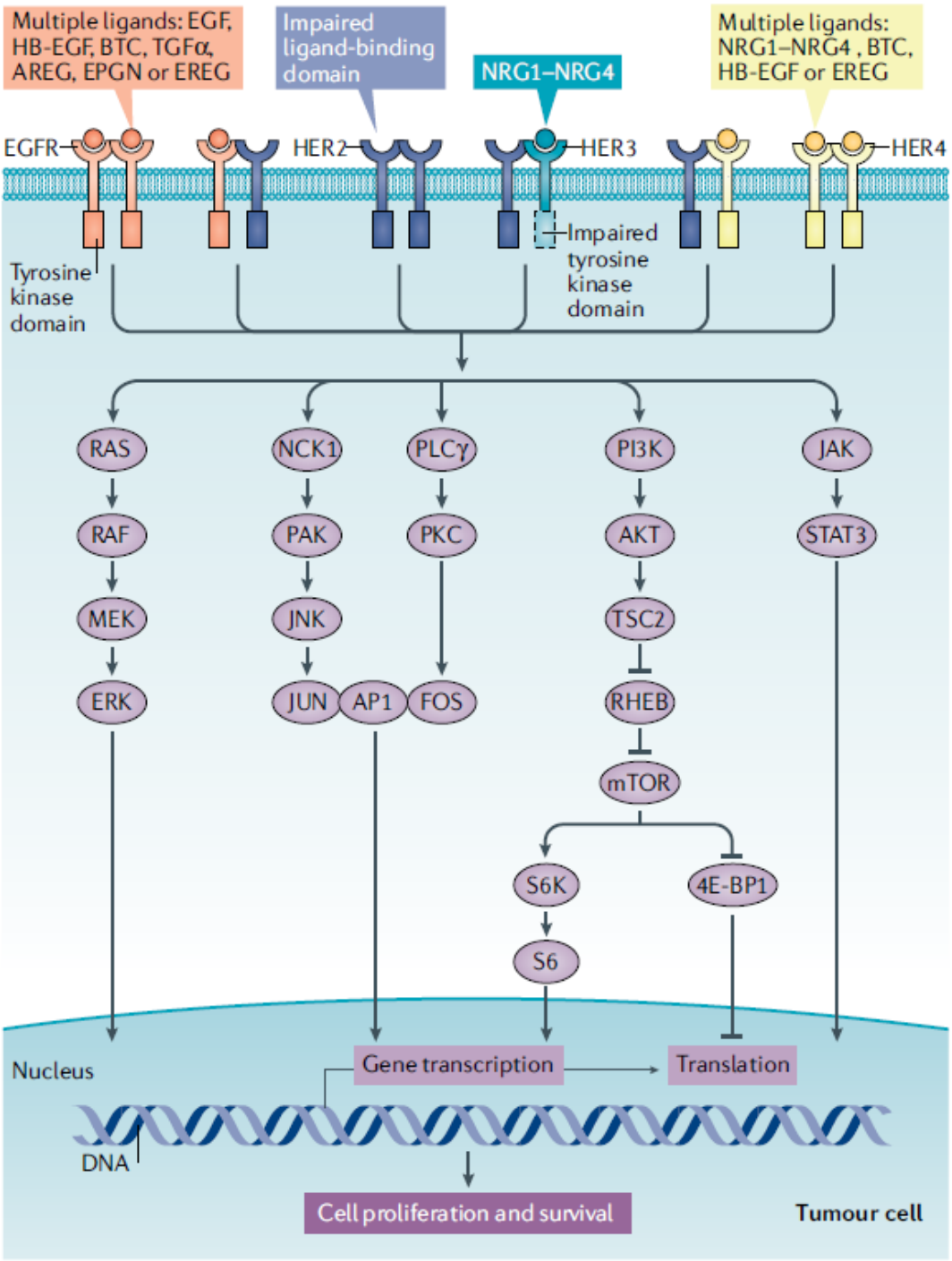


Figure 6. The ErbB-family of receptors and their signalling pathways. From Kumagai, Koyama and Nishikawa. Antitumour immunity regulated by aberrant ERBB family signalling. Nat Rev Cancer 2021 (106). With permission.

2.1 *EGFR*-mutations

Activating mutations in the region of the *EGFR*-gene encoding the tyrosine kinase domain renders the receptor constitutively active independent of the presence of its ligands. The continuously active receptors lead to signalling which escape the normally fine-tuned regulation of cell survival and death, resulting in tumorigenesis. Genomic studies have implicated that *EGFR*-mutations are early events in tumour evolution and hence thought to be important in initiating the development of cancer (107, 108).

The *EGFR*-mutations considered oncogenic mutations are located in exon 18-21 of the gene. The most prevalent sensitizing mutations, often referred to as "common mutations", are deletions in exon 19 (del19) and the point mutation L858R in exon 21. They comprise roughly 80-85% of all *EGFR*-mutations, with del19 being more prevalent than L858R (approximately 45% vs 35-40%, respectively) (Figure 7) (109, 110). The activity of the mutated receptors can be blocked with EGFR-TKIs. The remaining 15-20% of the *EGFR*-mutations (so-called "rare" or "uncommon mutations") comprise a heterogenous group of different mutations of which the exon 18 point mutations G719X (X representing A, C or S), exon 20 S768I and exon 21 L861Q in addition to insertions in exon 20 (ex20ins) are the most studied. Ex20ins have usually been considered to be resistant to EGFR-TKIs whereas the other uncommon mutations confer some degree of sensitivity to at least some of the targeted treatment available as will be described in greater detail later (111, 112).

2.1.1 Clinical characteristics

EGFR-mutations are found in around 10-15% of lung adenocarcinomas in Western populations and in 40-50% of cases in Asians (113-115). In addition to ethnicity, some clinical features are particularly associated with *EGFR* mutant lung cancer; it is more common in females, younger aged patients and never- or light-smokers (115, 116). In fact, these clinical traits were observed to increase the probability of effect of EGFR-TKIs even before the link between activating mutations and response to these drugs was revealed (117).

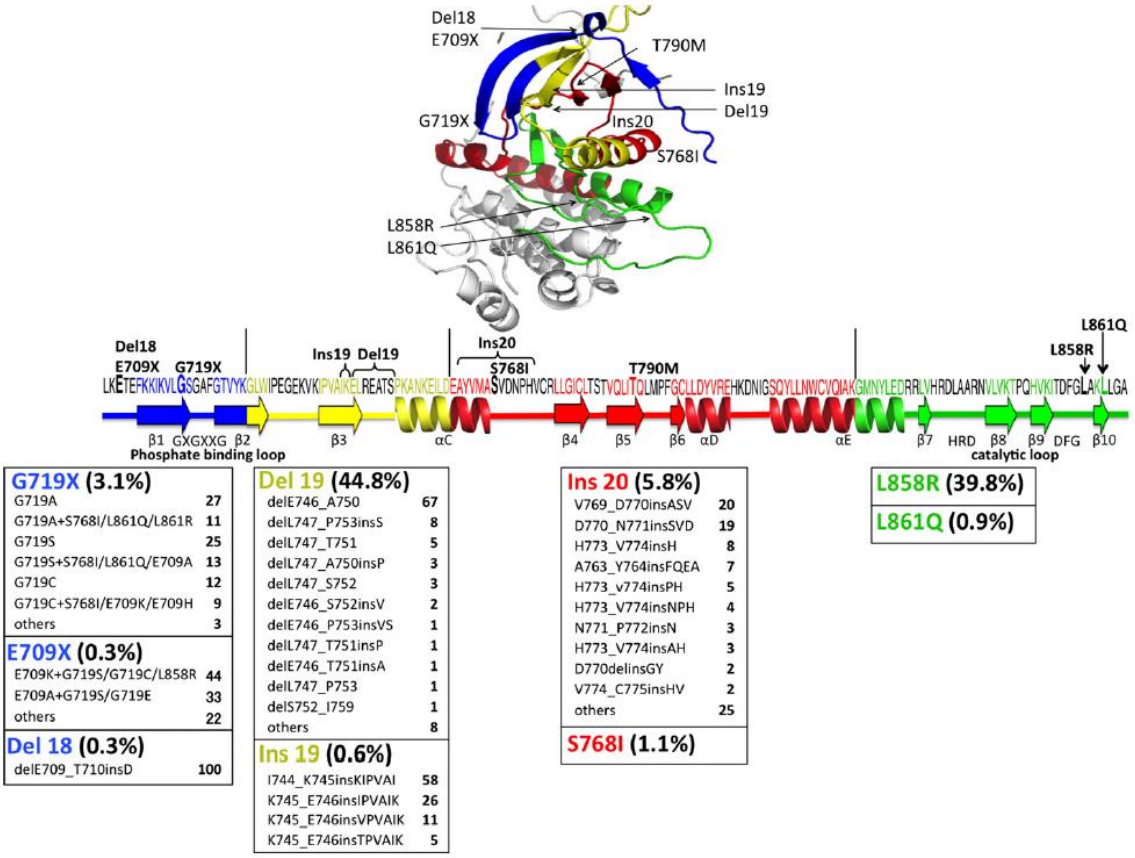


Figure 7. Structure of the EGFR-protein and localization and frequencies of the different *EGFR*-mutations. From Kobayashi et al., Not all epidermal growth factor receptor mutations in lung cancer are created equal: Perspectives for individualized treatment strategy, Cancer Sci, 2016 (109). With permission.

2.2 EGFR tyrosine kinase inhibitors

2.2.1 First- and second-generation drugs

EGFR-TKIs are small-molecular drugs targeting the tyrosine kinase domain of the EGF-receptor. The first-generation drugs gefitinib and erlotinib reversibly block the ATP-binding pocket on the tyrosine kinase, thus hindering the phosphorylation necessary for activation of the downstream signalling pathways. These drugs were developed before the discovery of *EGFR*-mutations and originally designed to target the wild-type (i.e., non-mutated) EGFR (118). In 2004, however, landmark studies from two independent groups were published almost simultaneously, describing distinct mutations in the gene encoding the EGFR in patients who had had clinical effect of gefitinib (119, 120). In the non-responders, they failed to detect such mutations. Shortly after, a third paper with similar results was published (121), confirming these findings. The identification of specific mutations predicting

response to corresponding tyrosine kinase inhibitors, was the first example of the now rapidly growing field of precision medicine in lung cancer.

Several randomized trials have demonstrated high response rates (62.1%-83%) and superior PFS for gefitinib and erlotinib compared to chemotherapy when given as first-line treatment for metastatic *EGFR*-mutated lung cancer, with median PFS in the range of 9.2-13.1 months for patients treated with TKIs versus 4.6-6.3 months in chemotherapy-treated patients (122-127). However, none of these studies found a significant difference in overall survival, most likely because of a high rate of cross-over to EGFR-TKI at progression for the patients in the chemotherapy arm of the studies. A meta-analysis of the studies in ref. 122-127 with individual patient data excluding those without known *EGFR*-mutations, confirmed these results and reported a median OS of 25.8 months vs 26.0 months for EGFR-TKI treated patients and chemotherapy treated (bearing in mind that many of them received EGFR-TKI at progression on chemotherapy), respectively (p=0.84) (128). Nonetheless, this was a substantial improvement in OS compared to historical data on OS in patients treated with chemotherapy only (51).

The second-generation drugs afatinib and dacomitinb are, in contrast to the first-generation drugs, irreversible inhibitors of the tyrosine kinase. In addition, they are pan-HER inhibitors and originally designed to overcome some of the most common resistance mechanisms to the first generation TKIs. Although they failed to prove effective as second line drugs in clinical trials, trials have demonstrated results comparable or superior to gefitinib/erlotinib when given as upfront treatment (129-132).

The first- and second-generation TKIs are approved for first line treatment of metastatic *EGFR*-mutant lung cancer. Head-to-head comparisons have confirmed similar efficacy across the different drugs (133-135), with the exception of dacomitinib which had a statistical significantly longer PFS than gefitinib in one study, and also an improved overall survival (132, 136).

2.2.2 Mechanisms of resistance to first- and second-generation EGFR-TKIs

Although the majority of patients respond to targeted therapies, development of resistance is virtually inevitable. Resistance might be due to selection of pre-existing resistant clones which survive when the drug-sensitive cells in the tumour are eliminated with therapy. The emerging

resistance alteration will then be identical to the one detected before therapy. Another, probably more common mechanism, is the presence of so-called drug-tolerant cells which survive for a longer period of time despite therapy, and thus have time to incidentally acquire different types of alterations capable of circumvent the action of the drug, resulting in resistance (137-139) (Figure 8).

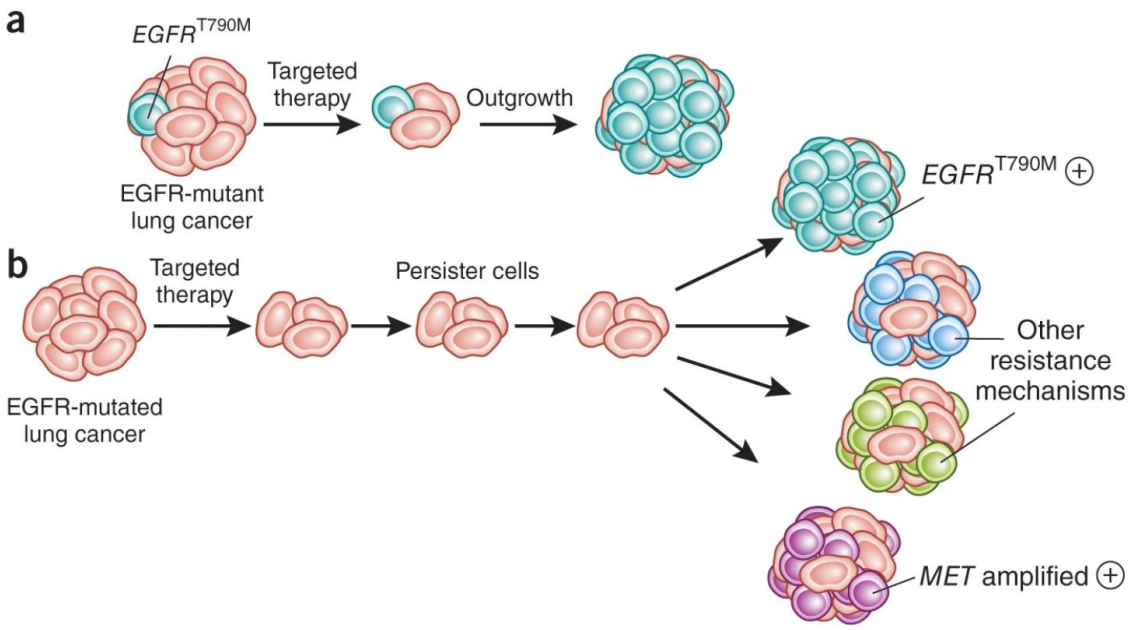


Figure 8. Different paths to resistance to targeted therapy. From Oxnard, The cellular origins of drug resistance in cancer. Nature Medicine 2016 (139). With permission.

Several different mechanisms mediating resistance to first- and second-generation EGFR-TKIs have been identified, of which the majority are *EGFR*-dependent and others *EGFR*-independent (140, 141) (Figure 9). The most common *EGFR*-dependent resistance mutation after treatment with first- or second-generations drugs is the emergence of a second point mutation, T790M, in exon 20 of the gene in which threonine is substituted with methionine. T790M is found in approximately 50-70% of patients after progression on first- or second-generation drugs (140-142). One study reported a prevalence of T790M as high as 73.1% after treatment with afatinib and based on liquid biopsies (142). A T790M mutation increases the affinity of ATP to its binding site and thereby hinders the reversible EGFR-TKIs which normally competes with ATP (143), resulting in resistance to these drugs.

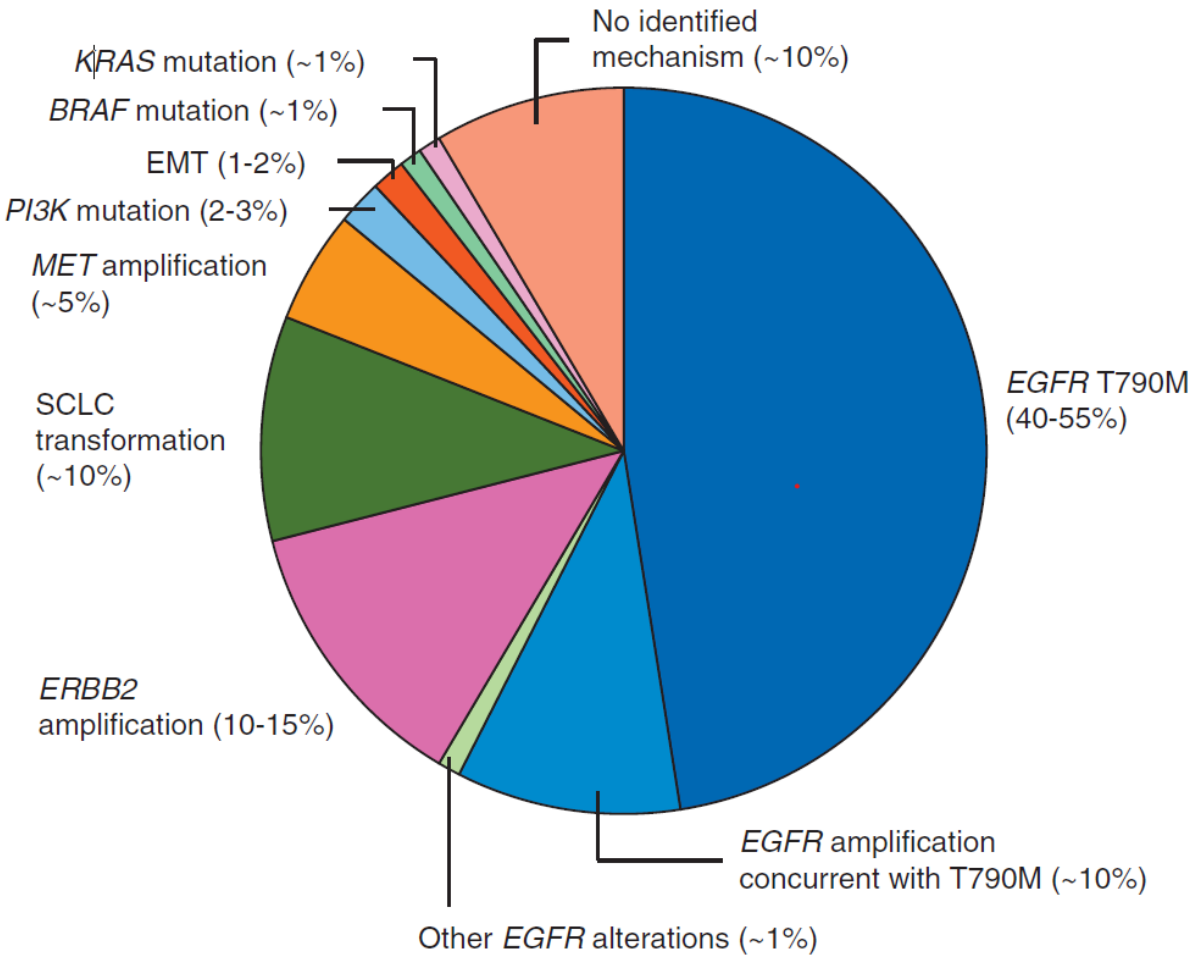


Figure 8. Mechansims of resistance to first- and second-generation EGFR-TKIs with approximate prevalence. From Westover et al., Mechansims of resistance to first- and second-generation EGFR tyrosine kinase inhibitors. Ann Oncol 2018 (146). With permission.

Among non-*EGFR* alterations are amplifications of *MET* or *HER2*, mutations in *BRAF*, *PI3K* and *KRAS* (140, 141, 144, 145). Many of these bypass EGFR by activating some of the same downstream pathways as EGFR. In addition, histological transformation to small-cell lung cancer has been described (140, 141). Also, epithelial to mesenchymal transition (EMT) occurs in a few cases.

2.2.3 Third-generation EGFR-TKIs

The recognition of T790M as a prevalent resistance mutation to EGFR-TKIs led to the development of third-generation drugs. Osimertinib was designed to target T790M in addition to the sensitizing mutations del19 and L858R. It is an irreversible inhibitor and binds covalently to the Cysteine 797 (Cys797) residue in the ATP-pocket of the tyrosine kinase, irrespective of increased ATP-affinity. Also, it displays a selectivity for mutant EGFR, i.e., it has less activity against the wild-type receptor than the older generation drugs (147). Preclinical studies have also indicated that osimertinib has a greater capability of crossing the blood-brain-barrier than the earlier generation TKIs (148). Thus far, osimertinib is the only third-generation drug which has reached regulatory approvement by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), whereas the third-generation TKIs aumolertinib and furmonertinib are approved for T790M-positive in China based on studies conducted in Chinese centres only (149, 150).

2.2.4 Osimertinib in second or later lines

The first clinical studies evaluating the effect of osimertinib, included patients previously treated with at least one EGFR-TKI and who had developed the T790M-mutation. The phase I-study even included 62 patients without detectable T790M (151). However, based on an objective response rate (ORR) of 28% and median PFS 2.8 for the T790M negative patients in this study, the subsequent studies and eventually the regulatory approval only included T790M-positive patients. In the randomized phase III AURA3-study, osimertinib was compared with standard platinum-based chemotherapy in T790M-positive patients. Both the response rate and median PFS were in favour of osimertinib (ORR 71% vs 31% and median PFS 10.1 vs 4.1 months, respectively) (152). However, the OS did not differ significantly between the groups (median 26.8 vs 22.5 months, respectively, $p = 0.277$) (153). Of note, 60% of the patients in the chemotherapy group crossed over to receive osimertinib at progression, which is probably contributing to the fact that the improved PFS did not translate into a survival benefit. Similar results for osimertinib were also achieved in a pooled analysis of two phase II studies with T790M positive patients (though without a control arm), with median PFS 9.9 months and median OS 26.8 months (154).

2.2.5 Osimertinib in first line

In the phase III FLAURA-study, 556 patients with metastatic *EGFR*-mutated lung cancer were randomized to either osimertinib or a first-generation TKI (erlotinib or gefitinib) as first treatment. Only patients with del19 or L858R were included. The response rate was similar for the two arms (80% vs 76%, p = 0.24). However, the median PFS was significantly longer in the osimertinib-arm with 18.9 months vs 10.2 months in the comparator TKI-arm and the hazard ratio for PFS was 0.46 (p < 0.001) (155). Furthermore, the FLAURA-study also demonstrated a survival benefit for the osimertinib-treated patients with a median OS of 38.6 months vs 31.8 months in the control group, p = 0.046 (156). In this study, 31% of the patients who received a first-generation drug as their first treatment, crossed over to receive osimertinib at progression.

Osimertinib was first approved by the FDA for treatment of T790M-positive patients in November 2015 and in Europe shortly thereafter (157, 158). In 2018 it also received approval for treatment naïve patients with del19 or L858R based on the results from the FLAURA-trial. It is now recommended as first choice for metastatic *EGFR*-mutated NSCLC in both national and international guidelines (28, 30, 41).

2.2.6 Resistance to osimertinib

Resistance to osimertinib consists of a complex landscape of multiple different molecular alterations, many of which are overlapping with regard to whether osimertinib is given as first- or second-line treatment. Whereas *EGFR*-dependent mechanisms of resistance are most common after first- and second-generation TKIs, only around 10-26% of resistant cases are due to new *EGFR*-mutations or -amplification after osimertinib treatment. There are several emerging *EGFR*-mutations described, of which C797S in exon 20 is the most commonly occurring (159, 160). When osimertinib is given as second-line treatment for T790M-positive disease, loss of T790M is described in around half of the cases at resistance and is associated with the emergence of non-*EGFR*-alterations (160). Of non-*EGFR*-alterations, *MET*-amplifications are the most frequent and lead to bypass signalling (161). Among other non-*EGFR*-alterations are *HER2*-amplifications, fusions in *RET, ALK, BRAF* and other oncogenes, cell cycle gene alterations and histological transformation to small-cell lung cancer or squamous cell carcinoma (161). Different mechanisms of resistance and their frequencies as summarized by Leonetti at al. (162), are shown in figure 9.

Figure 9. Resistance mechanisms after treatment with osimertinib. From Leonetti et al., Resistance mechanisms to osimertinib in *EGFR*-mutated non-small cell lung cancer, Br J Cancer 2019 (162). Under licence of the Creative Commons CC-BY.

2.3 EGFR-TKIs as adjuvant treatment for early-stage disease

The idea of giving targeted adjuvant treatment with EGFR-TKIs for patients undergoing surgery for early-stage disease harbouring *EGFR*-mutations, could be attractive. A few clinicals trials randomizing patients to either first-generation TKIs or standard chemotherapy have been conducted. Of two studies including patients with both stage II and III-disease, one demonstrated a prolonged time to relapse with TKI (163). However, a recent study did not find a benefit in disease free survival (DFS) (164) and none of them have been able to show an improved overall survival.

A phase III-study of osimertinib in the adjuvant setting (the ADAURA trial), demonstrated an impressive DFS with a hazard ratio 0.17 ($p < 0.001$) in favour of osimertinib versus placebo (165). In contrast to previous studies, patients could receive chemotherapy prior to osimertinib according to the treating physician's choice. Patients received osimertinib for three years. Although promising results, there are still many questions to be answered regarding the true value of adjuvant osimertinib among which the question of whether such treatment really offers a higher rate of cure or simply delays disease recurrence as data on overall survival is yet to be published. Furthermore,

the role of EGFR-directed therapy after relapse on adjuvant osimertinib is unclear. In fact, *EGFR*-mutated patients with relapse after surgery (without adjuvant osimertinib), might have a longer post-relapse survival than non-mutated patients, thus diluting the potential effect of adjuvant TKI treatment (166). As such, it is still a matter of debate whether adjuvant treatment with osimertinib should be endorsed as standard of care (167, 168).

2.4 Side effects of EGFR-directed therapy

Side effects of EGFR-TKIs are mainly caused by blockage of wild-type EGFR in non-cancerous cells. Normal EGFRs are abundant in skin and in the mucosa of the gastrointestinal tract (169). Almost all patients treated with first- or second-generation drugs experience some side effects, mainly of mild or moderate character, but more serious toxicities also occur. The most prevalent dermatologic event (affecting approximately 60-80% of patients) is a characteristic acneiform rash located in the face and chest, or in more severe cases even extends across large parts of the body surface (126, 133). In addition, pruritus, dry skin, paronychia and change of hair texture are other effects on the body surface. Furthermore, diarrhoea is frequently occurring. Some patients also experience nausea and loss of appetite. A proportion of patients (10-20%) will have treatment-related elevated liver aminotransferases (126, 133, 134). A potentially serious (but rare) toxicity includes interstitial lung disease. The irreversible inhibitors afatinib and dacomitinib are associated with more pronounced side effects than the reversible inhibitors, especially skin rash and diarrhoea which more often lead to dose reductions or even discontinuation of the drug (132, 134).

Osimertinib have much the same toxicity profile as the first- or second-generation drugs (155, 170, 171), but of even milder character due to the affinity of the drug to mutated receptors and sparing of wild-type EGFR (147). In head-to-head comparison with first-generation TKIs, patients receiving osimertinib reported less rash (58% versus 78%) most of which were grade 1 (155). QT-prolongation occurs in 3-10% of the patients, this is also mainly of mild or moderate grade. Thrombocytopenia of mild degree is also reported in some patients.

2.5 EGFR-TKIs in combination with other drugs

In the pursuit of maximizing treatment effect, combinations with EGFR-TKIs and different drugs have been studied in several clinical trials. The rationale would be to find a means of targeting the different subclones in heterogenous tumours or targeting other tumour promoting factors like

angiogenesis alongside blockage of EGFR-mediated signalling. In the primary treatment setting, the combination of EGFR-TKIs and anti-vascular endothelial growth factor (VEGF) agents or chemotherapy, respectively, have demonstrated clinical benefit relative to EGFR-TKI monotherapy. Early studies of combinatory treatment with chemotherapy and gefitinib or erlotinib, included patients with unknown *EGFR*-mutational status. In some of the studies PFS was prolonged for the combination compared to chemotherapy alone, but none could demonstrate a survival benefit (172-174). More recently however, two phase III-studies with *EGFR*-mutated patients investigated chemotherapy plus gefitinib versus gefitinib alone and both studies demonstrated a clinically relevant and statistically significant increase in both PFS (median 16 vs 8 months and 20.9 vs 11.9 months, respectively) and OS (median not reached vs 17 months, HR 0.45, and 50.9 vs 38.8 months, HR 0.72, respectively) (175, 176). The rate of grade 3 or higher toxicities was approximately doubled in the combination arms than that in the TKI-arm in both studies. The use of second line osimertinib in these studies was low, only 15% and 22% received osimertinib upon progression where one could expect around 50% to be T790M positive. As osimertinib is now more widely available with results in first line resembling the results reported in these two trials (155), and also given the increased toxicity associated with the combination, the utility of the chemotherapy plus first-generation TKI-regimen remains uncertain. Of note, there are ongoing trials studying osimertinib in combination with chemotherapy (i.e., NCT04035486 and NCT04410796).

Different anti-VEGF agents like the antibodies bevacizumab and ramucirumab and the TKI lapatinib in combination with first generation EGFR-TKIs have all demonstrated PFS benefits in the range of 13.7-19.4 months compared to EGFR-TKI alone (177-180). In contrast, a phase II study which evaluated osimertinib plus bevacizumab in previously TKI-treated T790M-positive patients failed to demonstrate a PFS-benefit (181). In fact, median PFS for the combination was numerically shorter than for osimertinib as single agent treatment (9.4 vs 13.5 months). Likewise, a recent randomized phase II study with osimertinib and bevacizumab given as first line treatment was negative (PFS 22.1 vs 20.2 months for the combination and osimertinib alone, respectively) (182). Like for other combinations, toxicity is increased with the addition of anti-VEGF drugs to EGFR-TKIs as compared to single agent therapy and the role of these combinations in the current treatment landscape is still unclear.

2.6 Immunotherapy in *EGFR*-mutated lung cancer

Data on immunotherapy in *EGFR*-mutated patients are limited by small early phase-studies, retrospective studies or subgroup analyses of phase III-studies, and unfortunately with less encouraging results than for non-oncogenic driven lung cancer. *EGFR*-mutated tumours have a lower tumour mutational burden and the correlation between PDL1-expression and effect of immunotherapy is less convincing than for non-mutated cancers, which may explain at least some of the lack of efficacy of the PD1-/PDL1-agents. In a meta-analysis of studies with immune checkpoint inhibitors versus docetaxel, OS was not improved for *EGFR*-mutated patients (183). In one study of pembrolizumab given to previously untreated patients, the ORR was 0% in 10 *EGFR*-mutated patients (184). Also, increased toxicity when given concurrently or in sequence with EGFR-TKIs has also been a limiting factor. Noteworthy, a phase IB-study of osimertinib plus durvalumab was terminated because of a higher-than-expected rate of pneumonitis (185). In a retrospective analysis, patients treated with osimertinib subsequently to a PD1-/PDL1-inhibitor had a high risk (15%) of developing severe immune-related toxicities (but not when given in the opposite order) (186).

In a randomized phase III-study (IMpower150) evaluating the four-drug regimen of a platinum doublet plus atezolizumab and bevacizumab, a subgroup analysis of a total of 79 patients with an *EGFR*-mutation demonstrated an HR of 0.61 for overall survival in favour of the four-drug combination relative to chemotherapy plus bevacizumab, although not statistically significant (187). Among 50 patients who previously had received at least one EGFR-TKI the corresponding HR was 0.30. Based on this subgroup analysis and of the above discussed lack of evidence of efficacy of immune checkpoint blockade in monotherapy, the quadruple treatment is now the recommended treatment after progression on osimertinib as per the Norwegian national guidelines (28).

2.7 EGFR-directed therapy for uncommon *EGFR*-mutations

With a few exceptions, all phase III-studies on EGFR-TKIs excluded patients with other mutations than the common sensitizing mutations del19 and L858R. Hence, the evidence of efficacy of TKIs for treatment of cancer with uncommon mutations is limited and comprises mainly studies of retrospective or observational character and with small patient numbers.

In a post-hoc analysis, 38 patients harbouring either L861Q, S768I or G719X mutations and treated with afatinib were pooled from three trials (one single arm and two randomized trials). The objective

response rate was 71.1% and PFS was 10.7 months, which led to FDA-approval of afatinib for these specific mutations (in addition to the common mutations) (111). In the same analysis, the group consisting of 23 patients with exon 20 insertions the objective response rate to afatinib was only 8.7% and PFS 2.7 months (111). Similar results were reported from a database of patients included in different clinical trials and expanded-access programs (127 patients with uncommon mutations and 77 with exon 20 insertions) (188).

The data on activity of first-generation EGFR-TKIs for uncommon mutations demonstrate a somewhat modest efficacy compared to historical data on TKIs for common mutations. For example, patients with tumours harbouring L861Q or G719X was analysed in a post-hoc manner from the NEJ002-study in which patients was randomized to either chemotherapy or gefitinib. Patients with uncommon mutations in the gefitinib-arm had significantly shorter PFS than patients with common mutations (2.2 months vs 11.4 months) and OS (12 months vs 28.4 months), respectively (189). However, there were only 5 patients with uncommon mutations in each arm in this study. In a larger Taiwanese retrospective study, 161 patients with G719X, S768I or L861Q treated with gefitinib or erlotinib were compared with a control group of patients with common mutations. The ORR was 41.6% in the uncommon group vs 66.5% among patients with common mutations ($p<0.001$). Furthermore, both median PFS (7.7 months vs 11.4 months for uncommon and common, respectively) and median OS (17.2 vs 27.8 months) were significantly shorter in the uncommon group (190).

Osimertinib has been evaluated in patients with uncommon mutations in a prospective Korean single-arm study. Of 36 patients, 50% had an objective response and the median PFS was 8.2 months (191). The median OS was not reached at the time of the analysis. Whether there was a difference between the patients with compound and single mutations was not addressed in this study.

2.8 Treatment of brain metastases in *EGFR*-mutated NSCLC

First- and second-generation EGFR-TKIs have limited penetration through the blood-brain barrier (192) and radiotherapy has in practice been the mainstay in the treatment of brain metastases also for *EGFR*-mutated lung cancer. However, there are some clinical data indicating effect of first- and second-generation TKIs on brain metastases, mainly from small and/or retrospective studies (193-195). Furthermore, data regarding the timing of brain radiotherapy when treating with first- or

second-generation drugs is unclear as there is a lack of randomized trials, although some studies indicate that deferred radiotherapy is inferior to radiotherapy applied before commencing TKI-treatment (196-198).

However, there are preclinical data suggesting a more efficient penetrance into the central nervous system for osimertinib than the earlier generation of drugs (148, 192). Evaluation of efficacy of osimertinib in the subgroups with brain metastases was conducted both in the phase II and phase III-AURA-trials of T790M-positive patients treated in second- or later lines, and in the first-line FLAURA-study. In the single-arm phase II study, 50 T790M-positive patients had measurable disease in the brain, and they displayed an intracranial ORR of 54% and median intracranial PFS was not reached (199). In the randomized AURA3-study comparing osimertinib to chemotherapy as second-line treatment for patients with T790M, the intracranial ORR was 70% in 30 patients with measurable brain metastases in the osimertinib arm versus 31% (n=16) in the chemotherapy arm. Median intracranial PFS was 11.7 vs 5.6 months, respectively (200). In the FLAURA-trial in which osimertinib or first-generation TKI was given upfront, the intracranial ORR was 91% (n=22) for osimertinib and 68% (n=19) for the comparator TKI. Median PFS in the brain was not reached in the osimertinib-arm and 13.9 in the control arm (HR 0.48, p=0.014) (201). Hence, these clinical data support the use of osimertinib in the presence of intracerebral metastases. However, whether radiotherapy should be given upfront or deferred to progression on osimertinib is less clear. To date, there are no prospective studies investigating this, but in a recent retrospective analysis, there was no difference in time to neither CNS-progressive disease nor overall progressive disease between patients treated with osimertinib alone or radiation plus osimertinib, though with the caveat of some methodological issues in the retrospective nature of the study and the fact that they defined progression based on radiological reports and not standardized criteria like RECIST v1.1 (202). Furthermore, as T790M-negative patients were excluded from the AURA-studies after the phase I-study, there is a lack of knowledge as to whether osimertinib has a role in treatment of brain metastases in the T790M-negative resistance setting.

3 Clinical development of new medicines and approval processes

After preclinical studies of a new pharmaceutical compound including in vitro studies and often animal studies, the drug enters a series of clinical evaluations before regulatory approval can be applied for. The clinical part of the development consists of three phases and the drug moves from one phase to the next if the endpoints of the previous phase are met. Sometimes even a fourth phase of studies is conducted (203).

In phase I-studies, the main focus is safety, pharmacokinetics and pharmacodynamics. They usually include a small number of patients or sometimes even healthy subjects who are treated with the drug in question and closely monitored for safety. Increasing doses might be given to find the maximum tolerated dose and to determine which dose to be used in the subsequent studies (203, 204).

Phase II-studies are conducted to investigate both safety and efficacy of the drug. They typically include 100 or more participants and can be either of single-arm design or randomized trials (203, 204).

Phase III-studies are confirmatory trials with a large number of patients where the new treatment is compared to either standard-of-care treatment or placebo in a randomized manner to determine the efficacy and also provide further data on safety (203, 204).

Phase IV-studies are trials investigating drugs which have approval and are used in routine practice, with the aim of generating even more data on different aspects of the treatment in question (203, 204).

When there is sufficient clinical evidence of efficacy and safety of a medicine, the drug owner (usually a pharmaceutical company) can apply for approval of the drug to be sold on the market. Approval of a drug is usually based on the results from phase III-trials, although both the FDA and EMA have “fast-track” programs to grant approval before larger studies have been conducted. This is meant for drugs that are promising for serious diseases to be able to reach the patients faster, and

thus some oncology drugs receive such “accelerated approval”. In such cases, the approval from both EMA and FDA is conditional on the drug company conducting confirmatory studies after the drug have been released on the market (205, 206). When a drug has received marketing authorisation in Europe (i.e., through EMA), the approval is also valid in Norway. However, to be reimbursed, drugs which are used in the specialist health service in Norway (for instance oncological drugs) also need to be approved through “The National System for Managed Introduction of New Health Technologies within the Specialist Health Service in Norway” (in Norwegian “Nye Metoder”) (207).

4 Aims

- The overarching aim of this thesis was to evaluate the efficacy of osimertinib in a Northern European population of patients with advanced or metastatic *EGFR*-mutant lung cancer

- Furthermore, evaluate the efficacy of osimertinib in patients resistant to first- or second-generation EGFR-TKIs with or without the presence of a T790M-mutation

- Investigate the efficacy of osimertinib in patients with brain metastases in both T790M-positive and -negative patients

- Assess the activity of osimertinib in patients with uncommon *EGFR*-mutations treated in either first line or in later lines

5 Material and Methods

This thesis is predominantly based on data from one clinical study, TREM. Paper III also includes a subset of patients from a similar trial, FIOL (Figure 10). Both studies evaluated osimertinib in *EGFR*-mutant non-small cell lung cancer patients.

5.1 The TREM-study

5.1.1 Overview

The TREM-study was a phase II-study of single-arm design with the aim of investigating the efficacy and safety of osimertinib in second or later lines of treatment. The study was an academic investigator-initiated study with 14 participating centres in Norway, Sweden, Denmark, Finland and Lithuania, with Oslo University Hospital as sponsor. A total of 199 patients were included during a 2.5-years period from 2015 to 2017. All patients had advanced *EGFR*-mutated NSCLC and had progressed on or after at least one EGFR-TKI prior to inclusion. A rebiopsy before inclusion was performed to determine mutational status at resistance to treatment with first- or second-generation TKIs, including the presence or absence of T790M. However, patients were enrolled regardless of their T790M-status. The complete list of inclusion- and exclusion criteria is shown in Frame 1 and an overview of the study in Figure 11.

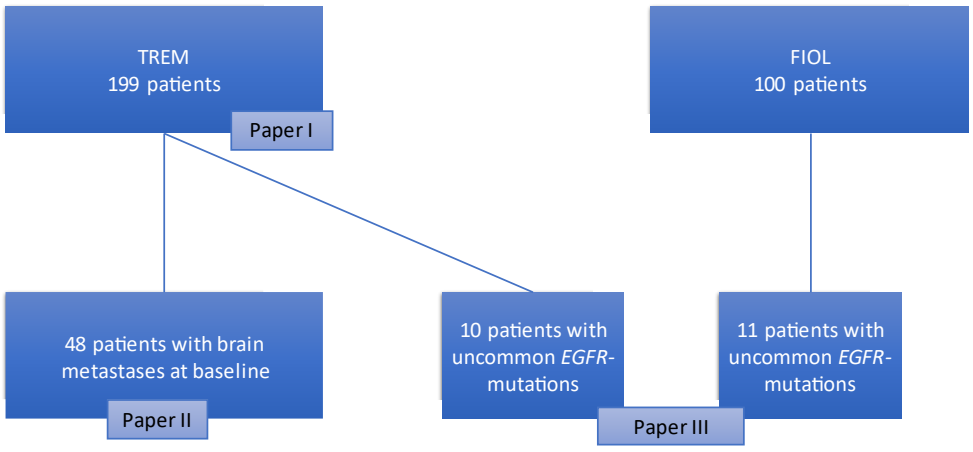


Figure 10. Patient disposition.

Inclusion criteria:

1. Provision of signed and dated, written informed consent.
2. Age > 18 years.
3. Histologically or cytologically documented locally advanced or metastatic NSCLC not amenable to curative surgery or radiotherapy.
4. Radiological disease progression following at least one prior EGFR-TKI.
5. Documented EGFR mutation known to be associated with EGFR-TKI sensitivity (also including T790M).
6. ECOG status 0-2 and a minimum life expectancy of 12 weeks.
7. At least one lesion, not previously irradiated and not chosen for biopsy during the study screening period, that can be accurately measured at baseline according to RECIST 1.1.
8. Females should be using adequate contraceptive measures, should not be breast feeding and must have a negative pregnancy test prior to start of dosing if of child-bearing potential or must have evidence of non-child-bearing potential by fulfilling one of the following criteria at screening:
    - Post-menopausal defined as aged more than 50 years and amenorrhoeic for at least 12 months following cessation of all exogenous hormonal treatments
    - Women under 50 years old would be considered postmenopausal if they have been amenorrheic for 12 months or more following cessation of exogenous hormonal treatments and with Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) levels in the post-menopausal range for the institution
    - Documentation of irreversible surgical sterilisation by hysterectomy, bilateral oophorectomy or bilateral salpingectomy but not tubal ligation.
9. Male subjects must be willing to use barrier contraception.

Exclusion criteria:

1. Treatment with an EGFR-TKI within 8 days or approximately 5x half-life, whichever is the longer, of the first dose of study treatment.
2. Treatment with cytotoxic chemotherapy, investigational agents or other anticancer drugs from a previous treatment regimen or clinical study within 14 days or approximately 5x half-life, whichever is the longer, of the first dose of study treatment.
3. Previous treatment with AZD9291, or another EGFR-TKI with similar profile, e.g., CO-1686
4. Major surgery within 4 weeks of inclusion
5. Radiotherapy treatment to more than 30% of the bone marrow or with a wide field of radiation within 4 weeks of inclusion
6. Subjects currently receiving (or unable to stop using) potent inhibitors or inducers of CYP3A4
7. Any unresolved toxicities from prior therapy greater than CTCAE grade 1 (with the exception of alopecia grade 2) at the time of starting study treatment.
8. Spinal cord compression or brain metastases unless asymptomatic and on stable steroid dosage for at least 2 weeks prior to start of study treatment.
9. Any evidence of severe or uncontrolled systemic diseases which in the investigator's opinion makes it undesirable for the subject to participate in the trial or which would jeopardise compliance with the protocol, or active infection including hepatitis B, hepatitis C and human immunodeficiency virus (HIV). Screening for chronic conditions is not required.
10. Gastrointestinal conditions incompatible with swallowing or precluding absorption of AZD9291.
11. Exclude based on any of the following cardiac criteria:
    - Mean resting corrected QT interval (QTc using Fredericia's formula) > 470 msec
    - Any clinically important abnormalities in rhythm, conduction or morphology of resting ECG (e.g., complete left bundle branch block, third degree heart block, second degree heart block)
    - Any factors that increase the risk of QTc prolongation or risk of arrhythmic events such as heart failure, hypokalemia, congenital long QT syndrome, family history of long QT syndrome or unexplained sudden death under 40 years of age in first degree relatives or any concomitant medication known to prolong the QT interval
12. Current or previous significant interstitial lung disease or radiation pneumonitis
13. Absolute neutrophil count < 1.5 x 109/L
14. Platelet count < 100 x 109/L
15. Haemoglobin < 80 g/L
16. Alanine aminotransferase (ALT) > 2.5 times the upper limit of normal (ULN) if no demonstrable liver metastases or > 5 times ULN in the presence of liver metastases
17. Aspartate aminotransferase (AST) > 2.5 times ULN if no demonstrable liver metastases or > 5 times ULN in the presence of liver metastases
18. Total bilirubin > 1.5 times ULN if no liver metastases or > 3 times ULN in the presence of documented Gilbert's Syndrome (unconjugated hyperbilirubinaemia) or liver metastases
19. Creatinine >1.5 times ULN concurrent with creatinine clearance < 50 ml/min (measured or calculated by Cockcroft and Gault equation),
20. History of hypersensitivity of AZD9291 (or drugs with a similar chemical structure or class.
21. Women who are pregnant or breast-feeding, or have a positive (urine or serum) pregnancy test prior to study entry
22. Judgment by the investigator that the subject should not participate in the study if the subject is unlikely to comply with study procedures, restrictions and requirements.

Frame 1. Complete list of inclusion- and exclusion criteria in the TREM-study.

5.1.2 Study procedures

5.1.2.1 Histopathological and molecular pathological assessment

Data on *EGFR*-mutations present at primary diagnosis was captured in the electronic case report form (eCRF). Archival tissue from the time of primary diagnosis, before first line EGFR-TKI treatment, was collected for research purposes (data not included in this thesis).

At inclusion in the study, a new tumour biopsy or a cytology specimen was required to determine mutational status upon commencing osimertinib. Molecular analysis was done per local routine at the different centres, which at the time of study conduction was predominantly PCR-based. Only a few cases were examined with NGS. Although a tissue or cytology specimen was required per protocol, some patients were included based on liquid biopsies if tissue/cytology was not feasible and an *EGFR*-mutation was confirmed in a plasma sample. In feasible cases, a new biopsy or cytology specimen was collected at the time of progression on osimertinib. At each visit, blood for translational research was drawn and plasma was frozen and stored in a biobank (data published, not included in this thesis (208-210)).

5.1.2.2 Determination of T790M-status

Patients with a tissue biopsy (or cytology) in which an activating *EGFR*-mutation was present, but no T790M-mutation detected, were defined as T790M-negative (n = 52). In 27 patients, T790M-status was not possible to determine due to either insufficient amount of tumour cells in the biopsy to do mutational analysis or a biopsy was not technically possible or safe to perform. Two of these cases included patients with only liquid biopsies available without any detectable *EGFR*-mutations and hence these were also classified as unknown. The remaining 120 patients had T790M confirmed in either tissue or plasma and were grouped as T790M-positive cases.

5.1.2.3 Radiological assessments

Radiological imaging consisted of CT scans of thorax and abdomen and in case of known or suspected brain metastases, MRI or CT of the brain was done. Imaging was performed at baseline and subsequently every 8 weeks the first year and every 12 weeks thereafter until progression. Tumour response at each time point was determined by the local investigators according to the RECIST v1.1. criteria (211) and registered in the eCRF. For the analysis of brain metastases (paper II),

brain CT/MRI from all time points were collected from all patients with baseline brain metastases and reviewed by an independent radiologist who were blinded for clinical data.

5.1.2.4 Treatment

All patients received treatment with osimertinib 80 mg taken orally once daily. Dose reduction to 40 mg in case of toxicity was allowed. Treatment continued until radiological progression, although treatment could also be sustained after progression if the treating physician judged it beneficial for the patient. Other reasons for discontinuation could be unacceptable toxicity, non-compliance or patient's wish.

5.1.2.5 Other assessments

The first clinical visit was done after two weeks of treatment, mainly as a toxicity visit. Thereafter the patients were followed with clinical visits at the time points corresponding to tumour imaging. Each visit included blood samples, ECG recording and registration of adverse events. Adverse events were assessed using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Four weeks after discontinuation of study treatment, a follow-up visit for toxicity was done. Patients were followed for survival after exit from the study.

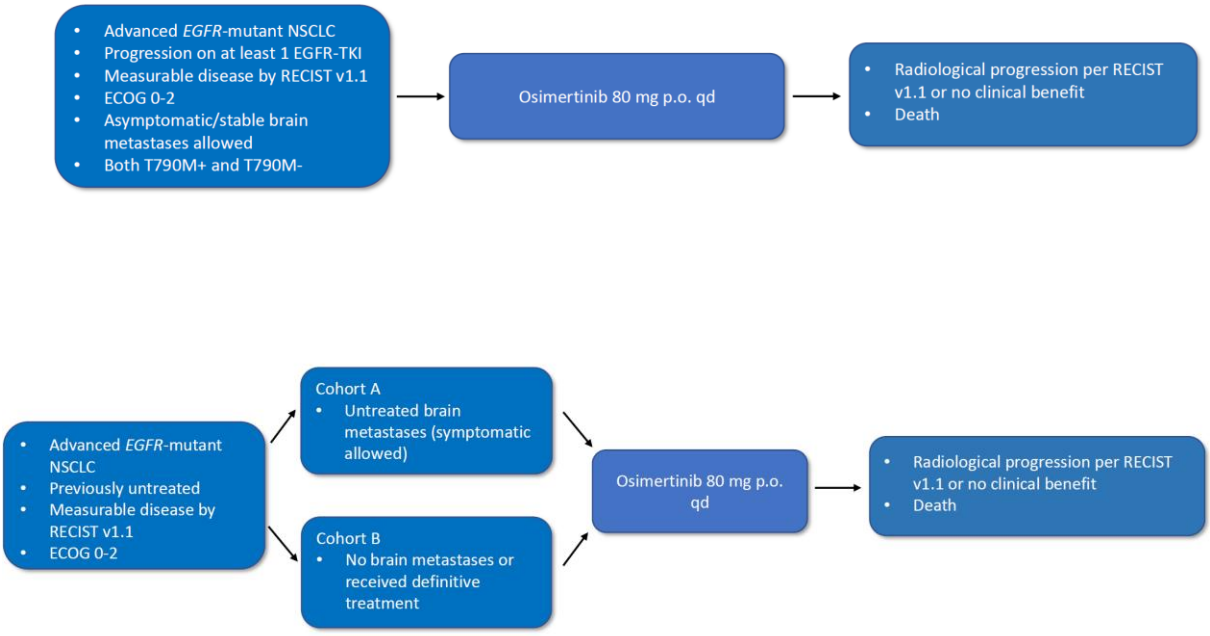


Figure 11. Study overview of the two trials. TREM in top panel, FIOL in bottom panel.

5.2 The FIOL-study

The FIOL-study had a similar design as TREM, i.e., a single-arm phase II design (Figure 11). As in TREM, patients had advanced non-small cell lung cancer with a confirmed sensitizing *EGFR*-mutation. In FIOL, however, patients were treatment-naïve and osimertinib was given as first-line treatment. Also, in contrast to the TREM-study, patients with untreated or symptomatic brain metastases were allowed to be enrolled. Furthermore, all patients in FIOL were screened for brain metastases at baseline using MRI and all patients were evaluated with MRI of the brain at each tumour assessment, regardless of the presence of baseline brain metastases.

To ensure safe follow-up of the patients with potentially unstable brain metastases, such patients were allocated to a separate cohort from the rest of the study population and had extra weekly clinical follow-up visits the first month of treatment and an early tumour evaluation with MRI of the brain after 4 weeks of treatment to reveal early progression in need of local intervention. Patients without baseline brain metastases followed the same schema as described for the TREM-study.

One-hundred patients were included in FIOL from December 2018 through June 2021. The patients were treated with osimertinib 80 mg daily. Other study assessments were as described above for the TREM-study.

5.2.1 Analysis of ctDNA in FIOL

In the FIOL-study, blood for ctDNA was collected at all visits and analysed with CAPP-Seq (cancer personalized profiling by deep sequencing) which is an NGS-method developed specifically to analyse ctDNA (212). In paper III, we report results from ctDNA-analyses at baseline and after two weeks of treatment. With NGS it is possible to sequence either targeted gene regions of interest, exomes or whole genomes. It is a stepwise procedure where DNA is extracted from the material in question (usually tissue or plasma). Then the DNA is divided into fragments and adapters are added to the ends of the fragments to create a so-called library. Then the library is loaded onto the sequencing platform and amplified after which the fragments are read. Finally, biostatistical software is applied to analyse the data.

In FIOL, blood was drawn and collected in Streck tubes (Streck Cell-Free DNA BCT©, Streck, La Vista, NE) and then centrifugated at 1400 g for 15 minutes to separate plasma from the red blood cells and the buffy coat (i.e., the layer of white blood cells and platelets), which was then stored in separate aliquots at -80°C. Cell-free DNA was isolated from plasma with the AVENIO cfDNA Isolation Kit (Roche) using the recommended 4 mL of plasma when available. The libraries were prepared with AVENIO ctDNA Surveillance Kit (Roche, Basel, Switzerland). The sequencing was done on NextSeq 500 High Output Lane (Illumina, San Diego, CA) with the 198 kb AVENIO ctDNA Surveillance panel (Roche) covering 197 genes related to lung and colorectal cancer. The 197 genes are shown in reference 213. The data was processed using the AVENIO Oncology Analysis Software v.2.0.0 (Roche).

5.3 End points

The primary end point in both TREM and FIOL was objective response rate (ORR). Secondary end points included disease control rate (DCR) and time-to-event end points like progression-free survival (PFS), overall survival (OS) and duration of response (DoR) as well as safety.

5.3.1 Definition of end points

The Recist-guideline defines a partial response (PR) as at least 30% reduction in the sum of diameter of target lesions from baseline. A complete response (CR) is disappearance of all target lesions as well as non-target lesions. In these studies, responses had to be confirmed, i.e., with a subsequent scan done a minimum of 4 weeks after the response was first assessed. Progressive disease (PD) is defined as either an increase in sum of diameter of 20% (from the smallest sum registered on study), appearance of new lesions or an "unequivocal progression of non-target lesions". Stable disease (SD) is assigned in cases where neither response nor progressive disease is observed.

ORR was defined as the proportion of patients who achieved CR or PR as per Recist v1.1 (PR + CR/all patients with radiologically evaluable disease). To be regarded evaluable, there had to be measurable target lesions at baseline as defined by the Recist-guideline, i.e., at least one lesion of 10 mm diameter or more which is not previously irradiated. Lymph nodes had to have a short axis of at least 15 mm to be regarded measurable. Non-measurable lesions were registered as non-target lesions and followed for response or progression.

DCR was the proportion of patients with either CR, PR or SD (CR + PR + SD/all evaluable patients).

Duration of response was defined as the time from the first time point response was observed and until progression or death whichever happened first. Patients with ongoing response at data cut-off was censored at the time of their latest tumour assessment.

PFS was defined as the time from start of treatment and until progressive disease, or in cases where the patient died before progression, the time of death. Patients were censored if they were still alive and progression-free at cut-off. If lost to follow up, they were censored at the latest time they were known to be progression-free.

OS was the time from start of treatment and until death. Patients alive at data cut-off were censored for OS.

To evaluate the efficacy of osimertinib in CNS metastases we assessed the above-mentioned end points in the brain in the subset of patients who had CNS lesions at baseline. Intracranial ORR (iORR) was the proportion of patients displaying a PR or CR of brain lesions and correspondingly intracranial DCR (iDCR) was the proportion of patients with at least stable disease in the brain. By definition, PR or SD is not possible to assess in the cases where only non-measurable lesions are present at baseline. Hence, in these cases, in absence of CR or PD, the response was designated as non-CR/non-PD. Intracranial PFS (iPFS) was the time from start of treatment until progression of brain disease regardless of status extra-cranially. Patients with no sign of CNS progression were censored at the time of the latest radiological assessment of the brain.

5.4 Statistical analysis

The statistical analyses were performed with either IBM SPSS Statistics for Windows, Versions 25.0-27.0 (Armonk, NY, USA: BMI Corp.) or R version 3.6.1. A two-sided p-value of less than 0.05 was regarded as significant in all analyses.

Groups of baseline characteristics were compared with the chi-square test or Fisher's exact test for categorical data and continuous data were compared with the Student's *t*-test. In paper III, differences in concentration of mutations in ctDNA between groups were analysed with a two-way ANOVA and adjusted for multiple comparisons with Šidák correction.

Confidence intervals for proportions (i.e., ORR and DCR) were calculated with the normal approximation method in paper I. Due to the small sample size in paper III, normal distribution of the data could not be readily assumed and therefore we chose to apply the exact method for confidence intervals in paper III. In paper II we refrained from calculating confidence intervals of the ORR and DCR due to the very limited sample size and few events, hence these results were regarded descriptive. In paper I, univariate comparison of subgroups with regard to ORR were analysed with the chi-square-test and logistic regression modelling was applied for multivariate analysis of response rates.

Time-to-event endpoints (PFS, OS, DoR, iPFS) were analysed with the Kaplan-Meier method and visualized in Kaplan-Meier plots. We used the log rank test to examine whether there were

significant differences between groups in univariate analyses. In paper I, we used the Cox proportional hazards model to calculate hazard ratios and perform multivariate analyses of subgroups with regards to PFS. Calculation of follow-up times were done with the reverse Kaplan-Meier method as described by Schemper et al (214).

In paper II, a competing risk analysis was performed to assess the risk of progression in the brain (215). Competing events were extra-cranial progression or death. Patients were censored if they had not experienced any of the events at cut-off. Cumulative incidences for the primary event (CNS-progression) and competing events were calculated and visualized in a cumulative-incidence plot.

6 Summary of results

Paper I

Osimertinib in T790M-positive and -negative patients with EGFR-mutated advanced non-small cell lung cancer

In paper I, we presented the main efficacy data from the TREM-study. In total, 199 patients were included in the trial over a period of approximately 2.5 years and treated with osimertinib 80 mg daily until disease progression, death or unacceptable toxicity. All patients had received at least one previous line of EGFR-TKI treatment and 56% had received 2 or more lines of prior treatment. As patients were included regardless of their T790M-status, 60% (n=120) had detectable T790M, 26% (n=52) were T790M-negative and 14% (n=27) had unknown T790M-status. Fifteen percent of the patients presented in reduced performance status (ECOG 2), and 24% had CNS metastases at baseline. Around half of the patients were never-smokers and 70% were female, reflecting a typical *EGFR*-mutated population. We compared the baseline characteristics between the T790M-positive and the T790M-negative patients, and the only statistically significant difference was the distribution of L858R which was more common in the T790M-negative group (23% vs 44%, p=0.006).

The primary endpoint ORR was 48% among 191 patients who were evaluable for response. Moreover, the ORR was significantly higher in T790M-positive patients vs -negative (60% vs 28%, respectively, p<0.001). A significant difference was also found between patients with del19 (61%) vs L858R (32%), p=0.001. Contrary, there was no statistical difference in ORR for patients with or without brain metastases (55% vs 46%, respectively, p=0.471). This was also true when looking at the patients with brain metastases within the T790M-positive group (66% vs 58%) and the T790M-negative group (33% vs 26%), respectively. In multivariate analysis, positive T790M-status and del19-mutation were variables significantly correlated with response.

There was a statistically significant difference in median PFS between T790M-positive and -negative patients (10.8 months vs 5.1 months, respectively, p=0.007). Moreover, the difference in median PFS between patients with del19 (11.3 months) and L858R (7.3 months) was also significant with a p-value of 0.001. Furthermore, the median PFS for T790M-positive patients with or without CNS-metastases did not differ (10.6 vs 11.4 months, respectively, p=0.819). However, for T790M-negative patients with CNS-disease, the median PFS was significantly shorter than for T790M-negative patients without CNS-disease (1.6 vs 5.6 months, respectively, p=0.009).

Results for overall survival had a similar pattern as PFS, with an overall median OS of 17.9 months and longer OS for T790M-positive than -negative patients (22.5 months vs 13.4 months, p=0.002). As for PFS, there was no difference in OS for T790M-positive patients with or without brain metastases, whereas T790M-negative patients with brain metastases had a poorer survival than patients without (7.5 months vs 17.0 months, p=0.002).

Adverse events were as expected for osimertinib.

To summarize, in this paper we found that in this Northern European patient population, osimertinib had comparable efficacy for T790M-positive patients as in the previously published randomized AURA3-trial (152). Some activity of the drug was also seen in the T790M-negative patients which to date have limited treatment options in the second- or later line setting. In line with the known CNS-penetrance of osimertinib, patients with brain metastases also seemed to benefit from the treatment.

Paper II

Intracranial effect of osimertinib in T790M-positive and -negative non-small cell lung cancer patients

In paper II, we investigated the intracranial efficacy of osimertinib in the TREM-study and examined differences in T790M-positive and -negative patients. We collected MRI or CT brain scans from the patients known to have baseline brain metastases and an independent radiologist reviewed all the scans. Forty-eight patients were identified to have brain metastases prior to or at inclusion in TREM. Patients with brain metastases were significantly younger than patients without brain metastases (median 61.5 vs 68 years, p=0.037) and with more patients with poor performance status (ECOG 2), otherwise similar to the non-CNS cohort including the prevalence of T790M. Most of the patients had received local treatment to the brain before inclusion, including 73% with prior whole brain radiotherapy. Brain scans for radiological evaluation were available from 42 patients. The overall intracranial ORR (iORR) was 10%, and similar for T790M-positive and -negative patients. However, few patients had measurable disease according to the Recist-criteria due to the extensive use of prior whole brain radiotherapy. In the T790M-positive group, the rate of intracranial disease control

(iDCR) was 89% whereas 55% of the T790M-negative patients achieved at least stable disease in the brain (p=0.031). The median intracranial PFS (iPFS) was 39.7 months vs 3.5 months (p<0.001) for T790M-positive and -negative, respectively. Patients with T790M were less prone to experience progressive disease in the brain (6% at 12 months) than those without T790M (17% at 12 months), regardless of whether they had brain metastases at baseline.

In conclusion, we demonstrated that in this cohort of patients with mainly previously irradiated brain metastases, T790M-positive patients had durable intracranial control and a low risk of CNS progressive disease when treated with osimertinib whereas the opposite was observed for the T790M-negative patients indicating that caution is warranted with respect to choose osimertinib as treatment for such patients.

Paper III

Osimertinib in non-small cell lung cancer with uncommon EGFR-mutations: a post-hoc subgroup analysis with pooled data from two phase II clinical trials

Most studies on EGFR-TKIs have only included patients with the common sensitizing mutations del19 and L858R. Thus, knowledge of the efficacy of these drugs for the 10-15% of *EGFR*-mutations other than del19/L858R is limited. In both the TREM- and FIOL-study, inclusion of patients was not restricted to the common mutations. Hence, in paper III, we did a post-hoc analysis of patients with uncommon mutations from the two studies. There were 10 patients in TREM and 11 in FIOL with non-del19/L858R mutations. We analysed the 21 patients together and in two groups based on treatment line in which they received osimertinib (first line or pre-treated) and we also looked at patients with compound mutations with G719X vs other mutations.

The ORR was 47.6% for all patients, numerically higher in the first line cohort vs the pre-treated cohort (63.6% vs 30.0%, respectively), as well as in the G719X compound group vs the other mutations group (62.5% vs 38.5%, respectively). The median PFS was equal (5.5 months) between the first line group and the pre-treated group, however there was a significantly longer median PFS in the G719X compound group vs the other mutations group (13.7 months vs 3.5 months, p=0.003). Overall survival reflected the PFS-results with no significant difference regarding treatment line, but a significant difference between the mutational groups (29.3 months vs 7.5 months, p=0.001, for

compound mutations vs other mutations, respectively). Among 11 patients with baseline brain metastases, intracranial responses were observed across all groups, the overall iORR being 36.4%. No patients had progressive disease as their best intracranial response.

ctDNA from patients in the first line cohort was analysed. Of the 11 patients, 9 had a reduction in the amount of ctDNA after two weeks of treatment, indicating an ongoing clearance.

In summary, we observed clinical meaningful activity of osimertinib in this group of patients with uncommon *EGFR*-mutations. The effect was more pronounced in patients with G719X compound mutations, suggesting that such mutations render the kinase more sensitive to osimertinib.

7 Ethical considerations

There are several ethical aspects to conducting clinical studies involving human beings. Many of these are addressed in the Declaration of Helsinki (DOH) which is regarded as the most important guideline to ensure high ethical standards in research. The overarching principle in the DOH is the well-being of and respect for the individual (216).

Some examples of dilemmas in research involving new interventions could be that new experimental treatments might come with unknown harms and might even prove to be inefficacious. Moreover, study related procedures like extra biopsies represent a risk of procedure related complications for the patient. These procedures might not be of benefit for the individual, but rather contribute to increasing knowledge for a group of patients or also on a more general basis, although the risk inevitably is on the individual patient. On the other hand, clinical trials can offer patients treatment options not available in clinical routine practice including early access to new drugs, possibly with effects leading to less symptoms or even prolongation of life. Furthermore, participating in clinical trials will often secure the patient more standardized and closer follow-up than in routine practice. Taking these conflicting considerations into account, the process of informed consent is central to the conduct of trials and is described in detail in the DOH. The patient must receive thorough and balanced information on potential benefits and potential risks of all aspects of the study to be able to make an informed decision on whether to partake in the study. Furthermore, as stated in the DOH, simply giving the information is not sufficient, it is also important ascertain that the patient has understood the information (216, Paragraph 26). Moreover, physicians who include patients in clinical trials should be aware of their role as a treating physician versus the interest of including patients in their trials, and as such, always have the individual patient's best interest in mind when considering whether to recommend inclusion in a trial or treatment according to standard practice. In both trials included in this thesis, all patients signed written informed consent upon inclusion in the trials, and an additional written consent was provided before performing a re-biopsy at the time of progression on osimertinib, which was done in feasible cases.

Another important guideline concerning clinical trials is the ICH-guideline of Good Clinical Practice (ICH-GCP). It describes standards for all the stages in the conduct of clinical studies, from planning through execution to closure and reporting of results, and also of monitoring and ensuring data

quality (217). All clinical personnel involved in the conduct of such studies are obliged to be trained in GCP.

The conduct of clinical trials including trials involving medical drugs is also tightly regulated by law, including the obligation to adhere to the DOH and ICH-GCP. Among the most important laws regulating human research in Norway are “Helseforskningsloven” and “Legemiddelloven” (218, 219).

Furthermore, also regulated by law, is the principal of ethical approval of projects on beforehand. As such, approvals from the regional ethics committees and regulatory authorities in each country involved in TREM and FIOL were secured before commencement of the studies (in Norway, REK ref. no.: TREM 2015/181, FIOL 2018/1028). Another important principle is that of trial registration in public registers to secure transparency and prevent publication bias. Moreover, registration will make the trials more accessible both for researchers, referring physicians and the public. Many scientific journals require registration to publish clinical trial results. Both our studies were registered with ClinicalTrials.gov (NCT02504346 and NCT03804580 for TREM and FIOL, respectively) and the European Union Drug Regulating Authorities Clinical Trials Database (EudraCT) with reference numbers 2015-000307-10 (TREM) and 2018-001863-21 (FIOL).

8 Discussion

8.1 Methodological considerations

8.1.1 Study design

Both TREM and FIOL were single-arm phase II-studies. Moreover, the sample size in TREM was not determined as a result of a formal power calculation. To be able to draw firm conclusions on efficacy of a study drug, the gold standard is to perform a randomized controlled trial, and with a target sample size (i.e., number of patients in each treatment group) based on a calculation of which power and which statistical uncertainty is accepted in the trial. Without a control group in TREM and FIOL, the efficacy end points must be interpreted cautiously and regarded as *signals* of efficacy rather than *evidence* for efficacy. Although results can be compared with historical data, bias with respect to which patients were included in the present trials versus historical trials might be present. Also, determining whether some subgroups perform better than others is due to a drug effect, inherent biological features characterizing the subgroup in question or other factors is impossible to discern and such analyses should be regarded as hypothesis generating only. However, the decision to conduct TREM as a single-arm study was made at a time point where only phase I-data on osimertinib was published and hence it was natural to choose a phase II-design. Furthermore, as an academic investigator-led study with somewhat limited resources, both financially and regarding available study personnel, and conducted within a low-prevalence area of the world, the sample size was chosen based on estimated feasibility of conducting the study within the existing budget and a reasonable time frame. Indeed, the TREM-study was fully included in 2.5 years. It was also of worth to generate enough biological material to be able to perform translational analyses, possibly coupled to clinical data. Hence, despite the limitations that follow the study design, this study can contribute with valuable knowledge in different ways.

First, in terms of characterising a Nordic population of *EGFR*-mutated NSCLC patients which has not been done previously. Because of the higher prevalence of *EGFR*-mutations in Asia, the main bulk of evidence of this disease including treatment is based on studies with a majority of Asian patients. Due to differences in metabolism and pharmacokinetics of a drug between ethnic groups, it is not given that neither the efficacy nor safety profile is the same within different populations (220).

Second, the Northern European TREM- and FIOL-studies give a notion of the efficacy of osimertinib in this particular patient group. In both studies, the inclusion was less strict than in the industry-led

trials, allowing patients in ECOG performance status 2 to participate. Moreover, the randomized trials of osimertinib in second line, have not included T790M-negative patients, and thus the TREM-trial contributes to describe this special subgroup, although in a descriptive manner rather than definitive. Also, patients with uncommon mutations were included in our trials whereas in the majority of studies of EGFR-directed therapies, only patients with the common del19 and L858R are included. Thus, we were able to describe this small but not negligible subgroup through the two trials, providing some more data clinicians may take into account when choosing between treatment options for these patients. Lastly, a positive consequence of the TREM-study, is the informal formation of a Nordic network of lung cancer researchers and clinicians which already has yielded several other clinical studies and translational projects with both immediate and possible long-term benefits for lung cancer patients.

8.1.2 Choice of end points/RECIST-evaluation

The primary end point of the TREM-study was ORR which is a well-known measure of tumour response commonly used as a primary end point in non-comparative studies. It has been demonstrated to correlate with other perhaps more clinically relevant end points like PFS and in some cases also OS (221). Hence, it was a natural choice as primary end point in both TREM and FIOL due to the single-arm design of the studies, and further, to regard PFS and OS (among others) as secondary end points.

The application of the RECIST-criteria to evaluate tumour response and define the time of progression which ultimately is necessary to calculate the time-to-event end points PFS and duration of response, is a means to secure a standardized and independent measure of these end points. Evaluation by RECIST v.1.1 has become a standard method in clinical trials, and hence chosen as the method of assessment of radiological evaluations also in the TREM- and FIOL-trials. The RECIST-guideline was first published in 2000 with the intent to provide a reproducible way of measuring tumour response in clinical trials and especially in phase II-trials in which tumour shrinkage was considered a sufficient measurement for screening of new drugs before entering phase III-studies (222). A revision to its present form came in 2009 with some additional clarifications in 2016 (211, 223) and provides a standardized set of “rules” on how to radiologically assess tumour response. In particular, in the setting of multi-centre trials like TREM and FIOL with multiple investigators involved, using a guideline like RECIST v.1.1. is crucial to obtain objective results and contributes to reducing bias in evaluations. The RECIST-criteria were, however, developed to assess response to

chemotherapy. As newer modalities like molecular targeted treatments with inhibitors of signalling pathways have gained widespread use, the question as to whether RECIST v1.1 apply to these treatments with other mechanisms of action than cytotoxic treatment has been addressed, and indeed been shown to have similar results for targeted agents as for chemotherapy (224). In the TREM-study, we monitored the data entrance on radiological measurements and the corresponding response assessment through the eCRF to ensure preciseness of this important end point.

There are some potential pitfalls with the RECIST-criteria, and especially when it comes to the evaluation of brain metastases. For instance, the disease might respond differently to treatment in the CNS versus extra-CNS and if there are targets lesions both in the brain and extracranially, interpretation of overall response might be difficult if responses differ in the different sites. In paper II, we reviewed CT- or MRI-scans of all the patients with known brain metastases at baseline and all their subsequent scans until progression and applied the RECIST-criteria separately on the brain scans. With this isolated brain analysis, the problem of interpreting differential responses extra- and intracranially were avoided. There is another guideline developed by the Response Assessment in Neuro-Oncology Group (RANO) for the evaluation of brain metastases which, to some extent, have been adopted in clinical trials, RANO-BM (225). The main difference from RECIST is the incorporation of clinical information like use of corticoids and clinical status (worse/stable/improved) whereas the radiological assessment is similar to RECIST. Since our radiological analysis of brain metastases was done in a post-hoc manner, the CRF did not specifically ask for these clinical data and hence we chose the RECIST-criteria as the preferred method. Another methodological challenge in paper II, was that the majority of patients had received radiotherapy to the brain before inclusion. The RECIST-criteria allows irradiated lesions to be included as target lesions only if they have displayed progression after radiotherapy as a sign of active tumours. Irradiated stable lesions were thus included as non-target lesions, and it is in many cases not possible to conclude whether these lesions were actually active tumours responding to treatment (if stable) or merely fibrous tissue after definitive irradiation. In the latter case, the presence of scar tissue included as non-target lesions will preclude complete responses to be registered and hence the overall response rate will be underestimated.

8.1.3 Statistical considerations

Although not the method for assessing the primary end point, survival analysis (also known as time-to-event analysis), has been a large part of this work. There are some specific challenges to such

data which require the application of dedicated statistical methods. This is because not all observations are complete due to censoring, meaning that all individuals will not have experienced the event in interest at the time of analysis and hence some of the survival times will be unknown. Therefore, ordinary statistical methods based on calculation of a mean cannot be used. There are different ways of handling analysis of survival times, of which the Kaplan-Meier method and Cox proportional hazard regression are the most used, and also applied in our work. The Kaplan-Meier method estimates the probability of being event-free at any specific time point and serves as a basis for the survival curves which is a nice way of visualizing the survival probabilities and differences between groups.

There are three main reasons for censoring: an individual has not reached the end point at the time of analysis/end of study, individuals might be lost to follow-up or there might be competing risks (i.e., an individual experiences an event which precludes the outcome in interest from happening). An important assumption for the abovementioned methods, is that the censoring is independent. End-of-study censoring is assumed to be done independently, but loss-to-follow-up or the presence of competing risks could be a source of non-independent censoring. However, in our studies, only a negligible number of patients were lost to follow-up or experienced competing risks and hence we could assume independent censoring.

Because of the study design without a control group, we considered it relevant to examine the impact of T790M on progression-free survival (PFS) adjusted for potential confounding baseline factors that could affect prognosis or effect of treatment. Hence, in paper I, multivariate analysis with Cox regression was performed in which three variables (T790M-status, del19 and longer duration of treatment prior to inclusion in the study) were significantly correlated with PFS. An underlying assumption to this method is that of proportional hazards. In retrospect, we found that the T790M-variable violated this assumption with respect to PFS. There are different methods to deal with non-proportional hazards which we could have applied and reported in the paper. For instance, we could have divided the time into periods and analysed the data within these periods to investigate how the impact of the variable changes over time. Indeed, when repeating the multivariate Cox regression analysis for periods of 6 months from start of treatment, we can show that T790M-positive status has a very small and highly statistically significant hazard ratio (HR) the first 6 months of treatment, indicating a large difference in effect of this variable on PFS (Table 1).

Thereafter, the HR increases and after 6 months, the 95% confidence intervals for the HRs crosses 1, hence the HRs for T790M are not statistically significant anymore.

Period – months from start of treatment	0-6	6-12	12-18
T790M HR (95% CI)	0.08 (0.03 – 0.23)	0.95 (0.38 – 2.38)	0.59 (0.18 – 1.94)

Table 1. Hazard ratios for T790M-status in multivariate Cox regression for PFS in different time periods.

These findings correspond well to the shape of the Kaplan-Meier curve for PFS for T790M-positive versus -negative (Figure 3B in paper I) where there is a steeper fall for T790M-negative patients early on, after which the curves for the two groups follow a more parallel course. This feature can be explained by the fact that fewer patients without T790M respond to treatment with osimertinib than the T790M-positives. Thus, as the ones with no effect of treatment experience a PFS-event early, there is a larger difference in hazard rates between the groups in the first period. The non-significant effect of T790M later on corresponds to the finding that T790M-negative patients who do respond, have similar duration of response as the T790M-postive patients (median duration of response was 10.7 versus 11.8 months, p = 0.229).

Having in mind that the HR for T790M in the multivariate analysis of PFS, when differences in time were not taken into account (and as reported in paper I), was 0.49 with a 95% confidence interval of 0.33-0.73, it is apparent that the early strong effect of T790M was “diluted” and the power of the analysis in fact reduced. Still, the association between positive T790M-status and treatment effect of osimertinib is so strong that there was a clear statistically significant p-value and a convincing hazard ratio despite the non-proportional hazards.

Other approaches to deal with non-proportional hazards are stratifying the analysis on the variable with non-proportional hazards, but as T790M was the variable with greatest interest, a stratification

on this variable would not be appropriate. A more advanced method could be to perform an Aalen additive regression-method which takes differences over time into account (226), and would probably have revealed the early effect of T790M as discussed above.

In paper II, we estimated the intracranial progression-free survival with a Kaplan-Meier estimate based on results from the brain scans alone. In this analysis, patients were censored at the date of the latest brain scan available if they were progression-free within the brain. This time point often coincided with the time of extra-cranial progression because the systematic follow-up then ceased. This might be a possible bias leading to overestimation of the iPFS. In addition, we were interested in exploring the risk of progression in the brain as the first event. This was identified as a situation with competing risks, as extra-cranial progression or death would prevent CNS-progression to happen first. Therefore, we performed a competing risk analysis in which CNS-progression was the event of interest and compared the T790M-positive and -negative groups. In such analysis, cumulative incidence of the event in interest is calculated with the competing events taken into account (227). As such, this analysis is also a supplement to the iPFS analysis to better get an impression on the CNS-effect of the drug in this setting.

8.2 Discussion of results

In the three papers included in this thesis, we evaluated the efficacy and safety of osimertinib in patients who had progressed on at least one previous EGFR-TKI, regardless of the presence of the resistance mutation T790M, in patients with or without brain metastases and with common and uncommon sensitizing *EGFR*-mutations. In paper III, a subset of the patients was treated in first line.

Although osimertinib is currently approved only for T790M-positive disease in patients refractory to first- or second-generation EGFR-TKIs, the TREM-study also included patients without detectable T790M. The rationale for this was results from a phase I-study published in 2015 (in the same period as the initiation of TREM) which included both T790M-positive and -negative patients and where patients without detectable T790M had a response rate of 21% and a median PFS of 2.8 months (151). This was interpreted as a sign of efficacy also in patients without T790M. In the TREM-study, the T790M-negative patients had a response rate of 28% and a median PFS of 5.1 months which in this setting, where there are no clear treatment options other than chemotherapy-containing regimens with considerable toxicity, is arguably clinically meaningful. Indeed, in the chemotherapy-arm of the AURA III-study published in 2016, the response rate was 31% and median PFS 4.4 months

(152), hence comparable to the results in the T790M-negative treated with osimertinib in TREM. A subgroup analysis of *EGFR*-mutated patients from the IMpower150 trial, demonstrated a median PFS of 10.2 months for the 34 patients treated with the quadruple regimen of platinum doublet-chemotherapy, atezolizumab and bevacizumab (187). However, this was a small subgroup of selected patients, and the treatment regimen probably suitable only for fit patients.

The definition of T790M-negative status in TREM was absence of T790M in tissue biopsy. It is well known that tumour heterogeneity might lead to false negatives in small tissue samples, hence some of the responding patients in this group might still be T790M-positive. To come closer to a true mutational status, a plasma ctDNA analysis of tissue negative-patients could have been performed and might have revealed some additional T790M-positive patients. However, it is only recently that ctDNA-analysis is becoming more widely available for clinical use, and as there was no central lab involved in the conduct of TREM, the molecular analyses were done locally in clinical labs and with the available methods which was mainly tissue PCR. Thus, although molecular pathological methods have evolved since the initiation of TREM, in real-world practice, a re-biopsy at progression is not always feasible to obtain or plasma analysis for ctDNA might be false negative, hence there will still be patients with false negative or unknown T790M-status in the clinic. Hence, clinical data on possible treatment options for such patients are of value.

The results for the T790M-positive group of patients in TREM were in line with what is observed in other studies, both in terms of ORR and PFS/OS. Although cross-study comparisons should be done with great caution, this can be interpreted as a sign of similar efficacy in patients treated with osimertinib in our part of the world as in the larger phase II AURAII and phase III AURA III-studies in which more than 60% of the participants were of Asian origin (152, 170). Furthermore, in the TREM-study 15% of the patients were in ECOG-status 2 indicating poorer performance status than in the AURA-studies and with more than half of the patients receiving osimertinib in later than second line. However, there are studies suggesting that T790M-positive disease might have a more indolent course than T790M-negative, and due to the single-arm design of our study, one cannot with certainty rule out that at least some of the duration of progression-free time is due to the biology in the disease itself (228). In line with this, we found that longer duration of previous treatment before osimertinib, was associated with PFS only for the T790M-positive patients which could possibly be explained by a less aggressive course of disease rather than sole treatment effect of prior treatments. Nevertheless, being the only study in the later line setting evaluating osimertinib in a

Nordic population of patients, TREM is an important addition to data from the existing randomized trials.

In paper I, we demonstrated that PFS and OS on osimertinib did not differ between patients with or without brain metastases in the cohort with T790M-positive disease. In contrast to this, the T790M-negative group performed significantly worse in the presence of brain metastases. We investigated this further in paper II in which we found that T790M-negative patients had worse outcome also in the intracranial endpoints. However, T790M-positive patients had durable intracranial disease control. There are some caveats to these analyses which mean the interpretation of the results must be done with caution. First, this was an exploratory analysis of a small subgroup from TREM, and as such, only hypothesis generating conclusions can be made. Second, the majority of the patients had received prior radiotherapy to the brain, and in the absence of progression of the brain lesions, it is not possible to discern whether the stable lesions were in fact scar tissue from "dead" lesions rather than active brain metastases responding to the drug. Third, the radiological modality varied between CT scans and MRI scans with the latter being more sensitive, and thus details might be lost in the patients with CT scans. However, a strength was that the review of all scans was done by one designated radiologist securing consistent evaluations of the radiological material at hand.

The evidence on treatment and prognosis of T790M-negative brain metastatic disease is scarce and conflicting. There are a few small or retrospective studies evaluating osimertinib in this setting which, in contrast to our results, indicate similar outcome for T790M-positive and -negative patients (229-231). On the other hand, one study demonstrates worse prognosis for brain metastatic patients without T790M after progression on EGFR-TKIs (232), supporting the theory that the poor outcome for the T790M-negative group in our study, might be due to a combination of poor prognosis and the overall limited efficacy of osimertinib patients without T790M.

The optimal treatment strategy for patients harbouring *EGFR*-mutations other than del19/L858R is not clear as most of the large, randomized phase III-studies on the different EGFR-TKIs excluded such patients. To date, afatinib is the only TKI approved for G719X/S768I/L861Q-mutations based on an analysis of a small subset of patients from the LUX-LUNG studies (111). In our analysis of uncommon mutations from TREM and FIOL (paper III), we found an ORR and median PFS consistent with what was found in a prospective single-arm Korean study of 36 patients with uncommon mutations

treated with osimertinib upfront (191). A multi-national retrospective analysis of 60 patients have recently confirmed these results (233). Keeping in mind that we cannot draw any firm conclusions from our study of post-hoc nature and with few patients, we did some intriguing findings which if feasible, could warrant further studies and perhaps help guiding treatment choices; the subgroup of patients with G719X-mutations in combination with one of the other uncommon mutations seemed to have a higher probability of response and longer duration of clinical benefit of osimertinib than patients with single mutations. There are indicatives that this might be the case for patients treated with first generation TKIs (190), but to our knowledge has not previously been investigated in the setting of osimertinib-treatment. The TREM-cohort of pre-treated patients was heterogenous, with three patients harbouring T790M while the rest of the patients were T790M-negative. As such, the results from this cohort should be regarded as descriptive only. However, to our knowledge, the existing data on osimertinib in uncommon mutations thus far is limited to EGFR-TKI naïve patients.

With analysis of ctDNA from patients with uncommon mutations in the FIOL-cohort we were able to demonstrate that most patients had detectable ctDNA in plasma at treatment initiation and most of them had a drop in mutant molecules after only two weeks of treatment. We interpreted this as a signal of activity of osimertinib in the patients displaying a clearing of ctDNA and observed this across the different mutations. However, whether this translates into a clinical benefit in terms of PFS or OS, is unclear and is currently under investigation in our material.

Most patients treated with osimertinib will experience side effects, as was also the case in the TREM-study. However, the rate of severe side effects is low, and the drug is generally well tolerated as demonstrated across studies. A limitation to our study is that we did not include patient-related outcome measures including quality of life-reporting which could have contributed to highlight the patients' perspective as an addition to the investigators' adverse events-reporting.

8.3 Concluding remarks and future perspectives

In the recent years, osimertinib has been moved into first line with the longest PFS demonstrated to date, and with an OS benefit compared to upfront first generation TKIs and hence, is now standard of care for most newly diagnosed patients with metastatic *EGFR*-mutated lung cancer. Therefore, it is plausible to raise the question of whether the role of osimertinib in second or later lines is still relevant. However, there are still some patients receiving first- or second-generation EGFR-TKIs who

have yet to progress and who potentially later will benefit from osimertinib. Secondly, osimertinib is still not widely available in first line in all parts of the world, thus data on efficacy and use in later lines is important. Thus, the TREM-study is a contribution to the existing pool of data and with patients perhaps more similar to real-world patients than in the industry-led studies. Thirdly, the concept of sequencing of treatment in the setting of *EGFR*-mutant NSCLC is not definitely settled. There is a lack of studies properly designed and powered to answer whether giving osimertinib first, regarded as the most effective TKI to date, or sequencing (i.e., a first- or second-generation drug upfront and osimertinib for T790M-positive disease in second line) would be better for all or at least a subset of patients. A challenge with the sequencing approach is the fact that despite all progress in diagnostics and treatment the recent years, a relevant proportion of patients (20-40% in randomized trials) treated with EGFR-TKIs of any generation upfront never proceed to second line treatment. Furthermore, around 50% of the patients do not have T790M at progression. Taken together, with less than half of patients being candidates for osimertinib second line, this is an argument for giving the most efficacious treatment first. Efforts to maximize first line-treatment effects are made. Some recent studies have demonstrated a PFS benefit for patients treated with first generation TKIs in combination with chemotherapy compared to TKI alone (175, 176). However, these regimens are not compared to osimertinib and their role in the treatment landscape remain somewhat undecided. Studies of osimertinib in combination with other drugs, including a randomized trial comparing first line osimertinib plus chemotherapy with osimertinib alone is ongoing (NCT04035486).

The choice of second line treatment depends on different factors, among which is what treatment was given upfront and, if possible to decide, which mechanism of resistance is present. Furthermore, the pattern of progression is of relevance. If there is only one or a few sites progressing, (oligoprogression), local ablative approaches with either radiotherapy or surgery followed by continuation of the EGFR-TKI is a possible strategy as indicated in some small series (234-236). Selected patients, probably in the case of slowly progressing disease, could benefit from continuation of the first TKI beyond progression, delaying the time to need of new therapy (237, 238). The addition of chemotherapy to EGFR-TKI could be a compelling approach based on the rationale that the chemotherapy would be cytotoxic to the resistant clones, whereas continued EGFR-inhibition would keep the still sensitive clones under control. However, this approach has failed to prove efficacious in the setting of first-generation TKIs (239). If treated with a first-generation drug, the emergence of T790M would inform the use of osimertinib in second line, as

discussed in this thesis. However, when a non-T790M resistance mechanism is present, or none identified, the choice might be less clear.

There is a large heterogeneity in resistance mechanisms to osimertinib regardless of which treatment line it is being used in, making continued targeted treatment with single drugs challenging in the setting of osimertinib-resistance. Chemotherapy in combination with immunotherapy and the anti-angiogenic agent bevacizumab is therefore an option if no druggable targets are identified (187). Results of multiple ongoing studies on drugs targeting different resistance mechanisms alone or in combinations are awaited.

The biomarker-guided treatment approach assumes repeated testing for these markers during or between treatment lines. With the possibility of detecting mutations and translocations in plasma, surveillance of the evolving mutational landscape of the disease has become more accessible. However, not all mechanisms of resistance are captured in plasma samples. Up to 15% of patients are reported to undergo histological transformation to either squamous cell carcinoma or small-cell carcinoma (140, 141, 240), which both mandates different chemotherapy-regimens. To reveal histological transformation, tissue re-biopsy remains an important method although in many cases not feasible at the time of progression. Hence, despite improved molecular pathological methods, there will probably always be some patients where resistance mechanisms are unknown and precision medicine is out of reach.

Metastatic spread to the CNS remains an important clinical problem, and an advantage of osimertinib is its superior efficacy on brain metastases compared to earlier generation TKIs (200, 201). However, the role of local therapy in this setting is still not determined. Even in the years before the implementation of osimertinib, when the less CNS-penetrant EGFR-TKIs were widely used, the evidence for radiation therapy or surgery in combination with or in sequence with systemic treatment where conflicting and based on retrospective studies or small series of patients. Thus, there is a need for further studies examining the use of local therapies in the era of newer, better CNS-penetrant drugs and perhaps sparing patients for the toxicity of brain radiation therapy. Furthermore, as newer targeted agents are more effective intracerebrally, clinical studies should prospectively screen patients for brain metastases at baseline and regularly at follow-up to gain true insights to the prevalence and treatment effect on brain metastases.

There are many remaining questions regarding how to handle the heterogenous group of uncommon *EGFR*-mutations as data are scarce. The main challenge is the rarity and with increasing use of NGS, diversity, of these mutations. As is the case also for other rare cancers, randomized trials powered to establish efficacy of treatment for each of these mutations or even for groups of them, might not be feasible. Robichaux et al proposed a new classification of *EGFR*-mutations by identifying four groups of structural changes in the tyrosine kinase domain of the EGFR which is induced by different mutations. These conformational changes affect the drug-binding site of the molecule and hence might be indicative of sensitivity of different classes of TKIs (241). Perhaps such structure-function based classification could help inform treatment or inclusion in clinical trials in the future and is a step towards even more personalized approaches to patients with rare mutations.

Both the TREM- and FIOL-study have limitations as discussed in the sections above, perhaps most importantly the inherent limitations in the single-arm study design. Moreover, paper II and III are analyses of subgroups of exploratory nature and with small sample sizes. Still, such independent studies with wider inclusion criteria than studies designed within drug development programs, provide valuable insights. We were able to produce clinical data on treatment of patients possibly more like real-world patients and in a population in our part of the world. An immediate value for the individual patients, was the opportunity to gain access to an additional treatment line of EGFR-TKIs which was not yet reimbursed at the time of study conduct. Furthermore, studies like TREM and FIOL are important sources of material for biomarker- and translational research which hopefully will help the research community to gain insights in the biology of the disease, predictive and prognostic markers and resistance mechanisms to treatment, and which eventually might be a contribution to improved care of patients.

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Osimertinib in T790M-positive and -negative patients with *EGFR*-mutated advanced non-small cell lung cancer (the TREM-study)

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ABSTRACT

Objectives: In non-small cell lung cancer patients with acquired resistance to first- or second-generation EGFR-TKIs, osimertinib is approved in the presence of the T790 M resistance mutation. We assessed the efficacy of osimertinib in both T790M-positive and T790M-negative patients.

Materials and methods: The TREM-study is an investigator-initiated, multi-centre, single-arm, phase 2 clinical trial conducted in five Northern European countries. Patients with progression on at least one previous EGFR-TKI were assigned to treatment with 80 mg of osimertinib daily until radiological progression or death. Patients were included regardless of the presence of T790 M. The primary endpoint was objective response rate (ORR).

Results: Of 199 included patients, 120 (60 %) were T790M-positive, 52 (26 %) were T790M-negative and 27 (14 %) had unknown T790M-status. 24 % had brain metastases and 15 % had an ECOG performance status of 2. Overall ORR was 48 % (95 % CI, 41 %–55 %), 60 % (51 %–69 %) for T790M-positive patients and 28 % (15 %–41 %) for T790M-negative patients, $p < 0.001$. ORR for patients with co-occurring del19 vs L858R was 61 % vs 32 %, $p = 0.001$. Duration of response was similar between the T790M-positive and –negative groups (11.8 vs 10.7 months, $p = 0.229$). Overall median progression-free survival (PFS) was 8.9 months (95 % CI, 7.4–10.5), and 10.8 vs 5.1 months for T790M-positive vs –negative patients (HR 0.62, $p = 0.007$). Median overall survival (OS) was 17.9 months (95 % CI, 14.4–21.3). For T790M-positive vs –negative median OS was 22.5 vs 13.4 months, (HR 0.55, $p = 0.002$).

Conclusions: This study confirms the efficacy of osimertinib for T790M-positive patients. There was also clinically significant activity of osimertinib in a proportion of T790M-negative patients.

Clinical trial registration: This trial is registered with ClinicalTrials.gov (NCT02504346).

1. Introduction

Lung cancer is one of the most common cancers worldwide, and by far the most fatal with 1.8 million cancer-related deaths yearly [1]. The majority of patients have advanced disease at the time of diagnosis and hence a poor prognosis. Mutations in the tyrosine kinase domain of the epidermal growth factor receptor (*EGFR*) gene act as oncogenic drivers and are present in about 10 % of patients with non-squamous non-small cell lung cancer (NSCLC) in Western countries [2–6]. *EGFR*-mutations are predictive of response to first- and second-generation tyrosine

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kinase inhibitors (EGFR-TKIs) such as erlotinib, gefitinib, afatinib or dacomitinib, with response rates of around 70 %. Unfortunately, all patients inevitably develop resistance to these drugs within a median of 9–15 months [7–10]. The most frequent resistance mechanism is the point mutation T790M on exon 20, which is detectable in about 60 % of patients at the time of progression [11].

Osimertinib is a third generation, irreversible EGFR-TKI, targeting both the sensitizing mutations and the T790M resistance mutation [12]. In patients harbouring the T790M-mutation after progressing on a first- or second-generation EGFR-TKI, osimertinib was superior to platinum doublet chemotherapy with a higher response rate (71 % vs 31 %) and longer progression free survival (10.1 vs 4.4 months, $p < 0.001$) [13]. The median overall survival was 26.8 vs 22.5 months, $p = 0.277$ [14]. In a phase 1 study which, in addition to T790M-positive patients, also included EGFR-TKI pre-treated patients without the T790M-mutation, the latter group demonstrated an overall response rate of 21 % and a median PFS of 2.8 months, indicating some activity of osimertinib despite the absence of T790M [15]. In the phase 3 FLAURA-study, osimertinib achieved both a longer median PFS and OS than first-generation EGFR-TKIs in the first-line setting (median PFS 18.9 vs 10.2 months, $p < 0.001$ and median OS 38.6 vs 31.8 months, $p = 0.0462$), thus establishing osimertinib as an option not only at the time of resistance, but also as the primary treatment of advanced *EGFR*-mutated NSCLC [16,17].

We conducted this single-arm prospective clinical study to evaluate the efficacy of osimertinib in patients progressing on standard EGFR-TKI treatment regardless of T790M-status. We hypothesized that osimertinib would have similar activity in a Northern European cohort of patients as previously shown in studies with a high proportion of Asian patients [13], and that some patients without detectable T790M-mutation would benefit.

2. Material and methods

2.1. Trial design

The TREM-study is an investigator-initiated, multi-institutional, single-arm, phase 2 clinical trial conducted in 14 centres in five Northern European countries (Norway, Sweden, Denmark, Finland and Lithuania).

2.2. Patients

Eligible patients were over 18 years old with advanced (stage IIIB or IV) histologically or cytologically confirmed non-small cell lung cancer with a documented sensitizing *EGFR* mutation. They had radiologically assessed disease progression on or after at least one previous EGFR-TKI. There had to be measurable disease according to Response Evaluation Criteria in Solid Tumours (RECIST) guidelines version 1.1 at baseline, an Eastern Cooperative Oncology Group (ECOG) status of 0–2 and a life expectancy of minimum 12 weeks. Patients with asymptomatic brain metastases on stable steroid dosage the last two weeks before start of study treatment could be enrolled. Patients could have had more than one line of TKI-treatment or other systemic anticancer therapies prior to study entry. Patients also had to have adequate liver, kidney and bone marrow function.

Exclusion criteria included current or previous interstitial lung disease or radiation pneumonitis, prolonged QTc-interval or treatment with osimertinib or other EGFR-TKIs with similar profile prior to inclusion. Any remaining toxicity from previous treatment had to be less than Common Terminology Criteria for Adverse Events (CTCAE v4.0) grade 2 (except alopecia).

2.3. Ethics

All patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki and the ICH-guidelines of Good Clinical Practice, and according to regulatory requirements and individual Ethics Committees approval in the countries of each participating site. The trial is registered with ClinicalTrials.gov (NCT02504346).

2.4. Roles of the sponsor, authors and the funding sources

This was an academic study designed by the principal investigators and Oslo University Hospital was sponsor. Neither the sponsor nor the participating sites had any financial benefit from the study. The funding sources did not have access to data, nor did they take part in analyses, interpretation of the results or writing of the manuscript.

2.5. Assessments and procedures

Eligible patients received osimertinib with a starting dose of 80 mg orally once daily until radiological progression by RECIST v 1.1 or death, whichever occurred first, or unacceptable toxicity. Patients could continue treatment beyond radiological progression if they had clinical benefit, as judged by the investigator. Archival tumour material from the time of diagnosis was collected, and a rebiopsy to determine mutational status was required prior to the first dose of osimertinib. If a biopsy was not possible to obtain, a plasma sample for mutational analysis could be collected if methods for analysing plasma were available at the study centre in question. T790M-negative status was defined as absence of T790M in the presence of an activating *EGFR*-mutation in tissue. In the case of a negative plasma sample and no tissue sample available, the mutational status was regarded as unknown. Analysis for *EGFR*-status in tissue or blood at inclusion was done per local practice at the different centres, and methods included mainly quantitative PCR and in a few cases next generation sequencing. Patients received osimertinib regardless of T790M-status.

Adverse events were graded according to the CTCAE version 4.0. A first visit for toxicity assessment was done two weeks after commencing osimertinib. Tumour assessments were done with CT-scans of the thorax and abdomen every 8 weeks the first 48 weeks of treatment, thereafter every 12 weeks. MRI of the brain was done at baseline in patients with known or suspected brain metastases, and repeated in the same intervals as the CT-scans if there were brain involvement. Patients who discontinued treatment for other reasons than progression or death, continued assessments until disease progression. Biochemistry, tumour markers, electrocardiogram-recordings and toxicity assessment were done at each visit. At every visit including baseline, blood was collected and stored for analyses of liquid biopsies and other translational research purposes (not reported here). At progression on osimertinib, the patients were asked to undergo a new biopsy sampling for molecular profiling and exploratory research.

2.6. Outcomes

The primary endpoint was objective response rate (ORR) defined as the percentage of patients with partial or complete response according to RECIST v 1.1, assessed by the investigators. All responses were confirmed with a subsequent scan at least 4 weeks after the response was first assessed. Secondary endpoints were progression-free survival (PFS), duration of response (DoR), disease control rate (DCR), overall survival (OS) and safety. Progression-free survival was defined as the time from start of treatment until progression or death in absence of progression, whichever occurred first. Duration of response was defined as the time from a response was first assessed (and later confirmed) until progression. Disease control rate was the proportion of patients who achieved at least stable disease as best overall response (stable disease, partial response or complete response). Overall survival was defined as the time from start of treatment until death of any cause.

2.7. Statistical methods

All time-to-event endpoints were calculated with the Kaplan-Meier method. Univariate comparisons were done with the log-rank test. The Cox proportional hazards model were used to calculate hazard ratios and to perform multivariate analyses. Categorical data were analysed with the chi-square test or the Fisher's exact test. The Student's *t*-test was used for continuous data. Logistic regression modelling was done to evaluate subgroups of response rates. For all analyses a two-sided p-value less than 0.05 was considered statistically significant. No formal power calculation was done, but a sample size of 200 patients was considered adequate to establish evidence on efficacy and to provide robust material for translational research. All statistical analyses were performed with IBM SPSS Statistics for Windows, Version 25.0 (Armonk, NY: IBM Corp.).

3. Results

3.1. Patients

199 patients were included from July 2015 to November 2017. Baseline characteristics are summarized in Table 1. The median age was 66 years, 70 % were female and 52 % never-smokers. 15 % had an ECOG status of 2. 24 % of the patients had brain metastases including one patient with leptomeningeal disease. 25 % had had more than one line of EGFR-TKI and 44 % also had had at least one line of other systemic cancer therapy, mainly chemotherapy, prior to study entry.

All patients had a documented *EGFR*-mutation before treatment prior to the study. *EGFR*-mutational status was assessable in rebiopsies in 172 of the 199 patients (in tissue from 157 patients and in plasma from 15 patients) at inclusion. 120 (60 %) patients were T790M-positive and 52 (26 %) T790M-negative. Reasons for unknown mutational status (27 patients, 14 %) included not enough biopsy material available, biopsy considered not feasible for technical or safety reasons, or only liquid biopsy with no mutation detected. In more than 95 % of the patients, the activating mutation found at diagnosis was retained at inclusion.

The most common sensitizing mutations at baseline were deletions in exon 19 (del19) (53 %) and the L858R point mutation in exon 21 (26 %). There was a statistically significant lower prevalence of the L858R-mutation in the T790M-positive group vs the T790M-negative group (23 % vs 44 %, p = 0.006). The other baseline characteristics were equally distributed between T790M-positive and -negative patients.

3.2. Response rates and duration of response

Data cut-off was January 7, 2019. The median follow-up was 27.0 months and the median duration of treatment was 11.8 months (range 0–40.6+ months). 191 patients were evaluable for response, defined as patients with measurable disease at baseline. The overall response rate was 48 % (95 % CI, 41–55 %) (Table 2). Among the T790M-positive patients, 60 % (95 % CI, 51–69 %) achieved an objective response vs 28 % (95 % CI, 15–41 %) in the T790M-negative patients (p < 0.001). There was a statistically significant difference between the ORR in patients with del19 and L858R, 61 % vs 32 % respectively, p = 0.001 (Fig. 1A). Within the T790M-positive group, the ORR for patients with del19 and L858R was 70 % vs 44 % (p = 0.017) and in the T790M-negative group 33 % vs 15 % (p = 0.162). The ORR for patients with baseline brain metastases was 55 % vs 46 % for patients without brain metastases, p = 0.296 (Fig. 1B). The T790M-positive patients with and without brain metastases had an ORR of 66 % vs 58 %, respectively, p = 0.471. In the T790M-negative group the ORR was 33 % for patients with brain metastases vs 26 % for patients without, p = 0.718. There were no statistically significant differences in ORR between the other subgroups.

The disease control rate was 83 % (95 % CI, 77–88 %) overall, 91 % (95 % CI, 85–96 %) for T790M-positive patients and 64 % (95 % CI, 50–78 %) for T790M-negative patients, p < 0.001 (Table 2).

A logistic regression model including T790M-status, gender, smoking history, age, ECOG status, del19/L858R, baseline brain metastases, 1 vs 2 or more previous lines of therapy and previously only TKI or TKI and chemotherapy was fitted to identify independent predictors of response. T790M-positive status (OR 4.0, p = 0.001) and del19 (OR 3.2, p = 0.002) were the only variables significantly associated with ORR.

The overall median duration of response was 10.7 months (95 % CI, 8.5–12.9). There was no statistically significant difference between the T790M-positive and -negative groups with a DoR of 11.8 vs 10.7 months, respectively, p = 0.229 (Fig. 2). However, DoR differed between patients with del19 and L858R (12.0 vs 8.9 months, p = 0.042) (data not shown).

3.3. Progression-free survival

The median progression-free survival for all patients (n = 199) was 8.9 months (95 % CI, 7.4–10.5) (Fig. 3A). Median PFS for T790M-positive patients was 10.8 months vs 5.1 months for T790M-negative, HR 0.62 (95 % CI, 0.43-0.88), p = 0.007 (Fig. 3B). For patients with del19 mutation, the median PFS was 11.3 vs 7.3 months for patients with L858R, HR 0.55 (95 % CI, 0.38-0.80), p = 0.001. In the T790M-positive group, the median PFS for patients with del19 vs L858R was 12.6 vs 10.6 months, HR 0.61 (95 % CI, 0.38-0.99), p = 0.044, whereas in the T790M-negative group it was 5.7 vs 1.7 months, HR 0.61 (95 % CI, 0.32–1.13), p = 0.112 (Fig. 3C–D). For patients with brain metastases at baseline, the median PFS was 7.3 vs 9.1 months for patients without brain metastases, HR 1.28 (95 % CI, 0.90–1.82), p = 0.165, regardless of T790M-status. There was also no significant difference in PFS for T790M-positive patients with or without brain metastases, but in the T790M-negative group the median PFS was significantly shorter for patients with brain involvement than without (1.6 vs 5.6 months, HR 2.46 (95 % CI, 1.23–4.93), p = 0.009) (Fig. 3E–F). We performed a multivariate analysis including T790M-status, age, gender, smoking-status, ECOG-status, CNS-metastases, del19 or L858R, one or more prior lines of treatment and duration of previous treatment. The variables that were significantly associated with PFS were T790M-positive status (HR 0.49 (95 % CI, 0.33–0.73), p < 0.001), del19 (HR 0.52 (95 % CI, 0.35–0.78), p = 0.002) and longer duration of previous treatment (HR 0.52 (95 % CI, 0.34–0.80), p = 0.003). We also performed multivariate analyses for the T790M-negative and -positive groups separately. The only statistically significant variable in the T790M-negative group was presence of CNS metastases (HR 2.95 (95 % CI, 1.37–6.33)), p = 0.006. In the T790M-positive group, the statistically significant variables were longer duration of previous treatment (HR 0.51 (95 % CI, 0.30-0.86)), p = 0.011, more than one previous line of treatment (HR 1.98 (CI 95 %, 1.17–3.35), p = 0.011 and del19-mutation (HR 0.50 (95 % CI, 0.30–0.84), p = 0.008.

3.4. Overall survival

At the data cut-off, 127 of 199 (64 %) patients had died. The median overall survival for the whole study cohort was 17.9 months (95 % CI, 14.4–21.3) (Fig. 4A). The survival rates at 12 and 24 months were 67 % and 39 %, respectively. The median OS for T790M-positive vs -negative patients was 22.5 vs 13.4 months, HR 0.55 (95 % CI, 0.37–0.81), p = 0.002 (Fig. 4B).

For patients with del19 or L858R mutations, the median overall survival was 21.8 vs 15.2 months, respectively, HR 0.65 (95 % CI, 0.43–1.00), p = 0.046. However, no such difference in median OS was seen with regards to sensitizing mutations within the T790M-positive or the T790M-negative groups (Fig. 4C–D).

There was no statistical difference in median OS for patients with or without brain metastases overall, 15.2 months vs 20.2 months, HR 1.33

Table 1
Baseline characteristics.

Characteristic	Overall n = 199	T790M-positive n = 120	T790M-negative n = 52	P-value (T790M pos vs neg)
Median age (range) – years	66 (33–90)	65 (38–86)	69 (33–90)	
Sex				
Male	60 (30 %)	35 (29 %)	17 (33 %)	0.644
Female	139 (70 %)	85 (71 %)	35 (67 %)	
Smoking history				
Never-smoker	104 (52 %)	64 (53 %)	26 (50 %)	0.787
Former smoker[a]	81 (41 %)	49 (41 %)	24 (46 %)	
Current smoker[b]	14 (7 %)	7 (6 %)	2 (4 %)	
ECOG status				
ECOG 0	64 (32 %)	40 (33 %)	14 (27 %)	0.513
ECOG 1	102 (51 %)	58 (48 %)	30 (58 %)	
ECOG 2	30 (15 %)	20 (17 %)	7 (14 %)	
Missing data	3 (2 %)	2 (2 %)	1 (2 %)	
Histology				
Adenocarcinoma[c]	197 (99 %)	120 (100 %)	51 (98 %)	0.302
Squamous cell carcinoma	1 (< 1 %)	0	1 (2 %)	
Other[d]	1 (< 1 %)	0	0	
EGFR mutation at inclusion				
Exon 18	5 (3 %)	3 (3 %)	2 (4 %)	0.639
Exon 19	105 (53 %)	79 (66 %)	26 (50 %)	0.168
Exon 20 excl T790M	4 (2 %)	1 (1 %)	3 (6 %)	0.083
Exon 21[e]	51 (26 %)	28 (23 %)	23 (44 %)	0.006
T790M	120 (60 %)	120 (100 %)	0	
Unknown	27 (14 %)			
Disease classification				
Stage III	2 (1.0 %)	0	2 (4 %)	0.090
Stage IV	197 (99 %)	120 (100 %)	50 (96 %)	
Extent of disease				
CNS[f]	47 (24 %)	29 (24 %)	13 (25 %)	0.907
Intrathoracic[g]	196 (99 %)	119 (99 %)	52 (100 %)	1.000
Extrathoracic visceral	68 (34 %)	45 (38 %)	19 (37 %)	0.905
Bone	96 (48 %)	60 (50 %)	21 (40 %)	0.246
Previous EGFR-TKI therapy				
First line				
Erlotinib	127 (64 %)	84 (70 %)	30 (58 %)	
Gefitinib	57 (29 %)	27 (23 %)	18 (35 %)	
Afatinib	15 (8 %)	9 (8 %)	4 (8 %)	
Second line				
Erlotinib	19 (10 %)	11 (9 %)	2 (4 %)	
Gefitinib	7 (4 %)	5 (4 %)	2 (4 %)	
Afatinib	24 (12 %)	13 (11 %)	6 (12 %)	
Third line				
Erlotinib	4 (2 %)	2 (2 %)	1 (2 %)	
Gefitinib	0	0	0	
Afatinib	2 (1 %)	1 (1 %)	1 (2 %)	
Total no. of systemic anti-cancer treatments (TKIs, chemotherapy and other)				
1	89 (45 %)	55 (46 %)	25 (48 %)	0.962
2	67 (34 %)	39 (33 %)	17 (31 %)	
≥3	43 (22 %)	26 (22 %)	11 (21 %)	

[a] Stopped smoking at least one year before inclusion.
[b] Included stopped smoking the last year before inclusion.
[c] One patient initally diagnosed with bronchoalveolar carcinoma, later reclassified to adenocarcinoma.
[d] One patient with adenosquamous carcinoma.
[e] L858R, except four patients with L861Q.
[f] 46 patients with brain metastases, one with leptomeningeal disease.
[g] Lung, pleura or mediastinum.

(95 % CI 0.89–1.98), p = 0.162, but for patients with T790M-negative status and brain metastases the median OS was substantially worse than for T790M-negatives without brain metastases (7.5 vs 17.0 months, HR 3.08 (95 % CI 1.46–6.51), p = 0.002) (Fig. 4F). For the T790M-positives the median OS was similar regardless of brain involvement, 21.8 vs 22.5 months, HR 0.87 (95 % CI, 0.50–1.51), p = 0.611 (Fig. 4E).

There was a markedly shorter median overall survival for patients in poor performance status, 20.8 months (ECOG 0–1) vs 5.6 months (ECOG 2), p = 0.001 (data not shown). This difference was also seen in the T790M-positive and -negative groups with a median OS of 24.2 vs 9.4 and 13.7 vs 2.0 months, respectively, although the difference was not statistically significant in the T790M-negative group.

There were no statistically significant differences in median OS across other subgroups.

3.5. Safety

196 of 199 (98.5 %) patients experienced an adverse event, most of which were of grade 1-2. The most commonly reported adverse events were fatigue (67 %), decreased appetite (45 %), dyspnoea (44 %), rash (43 %), paronychia (42 %) and diarrhoea (42 %). 29 % of the patients had an adverse event of grade 3 or higher. 10 patients (5 %) needed a permanent dose reduction and only five patients (2.5 %) discontinued treatment due to adverse events (three with pneumonitis, one with

Table 2
Response rates. CR – complete response, PR – partial response, SD – stable disease, PD – progressive disease, ORR – overall response rate, DCR – disease control rate.

Type of response	Overall (n = 191) n (%)	T790M+ (n = 117) n (%)	T790M- (n = 50) n (%)
CR	2 (1)	1 (< 1)	1 (2)
PR	89 (47)	69 (59)	13 (26)
SD	67 (35)	36 (31)	18 (36)
PD	26 (14)	8 (7)	15 (30)
ORR (PR + CR)	91 (48)	70 (60)	14 (28)
95 % CI	41-55	51-69	15-41
DCR (PR + CR + SD)	158 (83)	106 (91)	32 (64)
95 % CI	77-88	85-96	50-78

ventricular tachycardia and one with cerebral ischemia). There were 14 cases of QTc-prolongation, all grade 1 except one grade 2 event, and 8 cases of pneumonitis, one grade 3 and the rest of lower grades. There were no treatment-related deaths.

4. Discussion

Osimertinib has emerged as a new standard of care in patients with advanced *EGFR*-mutated NSCLC, both in the first-line setting and after acquired resistance against first- and second-generation EGFR-TKIs in patients with the resistance mutation T790 M [13,16]. In the present study, we demonstrated the efficacy of osimertinib in a Northern European population of patients with advanced *EGFR*-mutated NSCLC with acquired resistance to first- or second-generation EGFR-TKIs, regardless of the presence of the T790M-mutation.

The patients in the current cohort were heavily pre-treated. In contrast, 96 % of the patients in AURA3 had received only one line of prior treatment. Furthermore, 15 % of the patients in our trial had an ECOG performance status of 2 while in the AURA-studies only patients in good performance status (PS 0–1) were included. The median age (66 years) was also higher than in the AURA-trials (60–63 years). Despite this, the ORR for T790M-positive patients in our study (60 %) is only slightly lower than in the AURA3-study (71 %) and comparable to the ORR in AURA1 (61 %) [13,15]. The DCR of 91 % is similar to the DCR in AURA3 (93 %). The median PFS of 10.8 months for the T790M-positive patients is in line with the median PFS observed in the AURA-studies, and the median OS of 22.5 months mirrors the OS in AURA3 (26.8 months) [14]. Thus, the study population in our trial is a less selected group, which better represents real world-patients. Still, the efficacy of osimertinib in T790M-positive patients is in line with previous reports, and is consistent with population-based observational studies [18,19].

Interestingly, osimertinib also showed clinically relevant activity in the T790M-negative group. To date, there are few approved treatment options for patients without the T790M-mutation who are refractory to

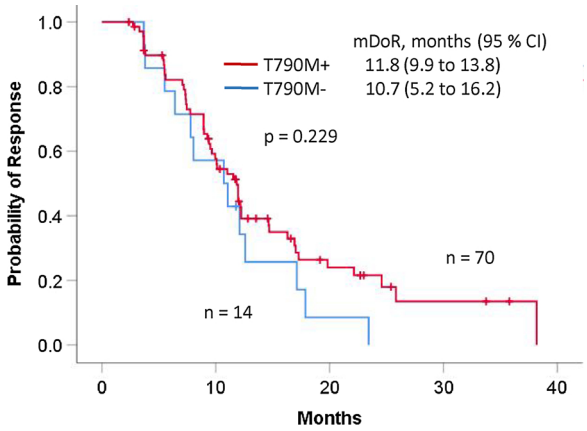

Fig. 2. Duration of response for T790M-positive and -negative patients. *mDoR – median duration of response.*

EGFR-TKIs, except chemotherapy or, in some countries, a combination of chemotherapy and immunotherapy [20] for fit patients. Immunotherapy alone seems less effective in this population [21]. In our material, the T790M-negative cohort had a response rate (28 %) and PFS (5.1 months) comparable to that of the chemotherapy-arm in the randomized AURA3-study. Furthermore, the duration of response in the present study was similar for T790M-positive and -negative patients, suggesting that despite the lower likelihood of response for T790M-negative patients, those who achieve a response, have a similar benefit as T790M-positive patients. Our trial is the second study to evaluate the effect of osimertinib in T790M-negative patients. In the phase 1 AURA1-trial, 61 T790M-negative patients achieved an ORR of 21 % and a median PFS of 2.8 months [15]. However, this trial was a dose expansion trial, and 20 of the patients received daily doses lower than the recommended 80 mg, which might explain the lower efficacy compared to our study.

The reason for this observed activity of osimertinib in patients without the T790M-mutation remains unclear, but might at least in part be due to false negative biopsies because of tumour heterogeneity. To minimize the number of false negatives, a negative tissue biopsy could be followed by mutation testing in plasma [22]. However, the prevalence of the T790M-mutation in our material is similar to what has previously been reported [11], and the testing was done with methods available in routine practice at the different centres. Thus, we have no reason to believe that the rate of false negatives should be higher in our cohort than in a clinical setting. Taking into consideration that osimertinib is a less toxic treatment than combination chemotherapy, and appears to have similar efficacy, osimertinib might represent an attractive treatment option for selected T790M-negative patients. Still, there is a need for additional approaches to identify those who remain EGFR-dependent and therefore are most likely to respond to continued EGFR-inhibition.

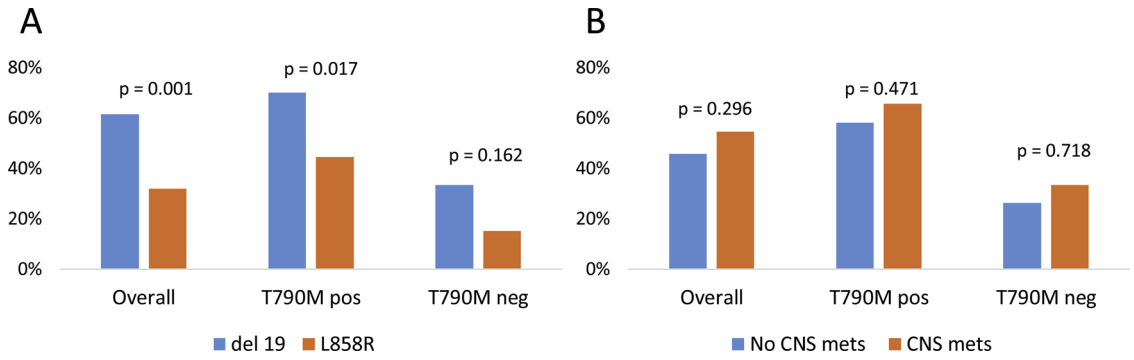

Fig. 1. (A) Response rates for patients with del19 or L858R. (B) Response rates for patients with or without CNS-metastases.

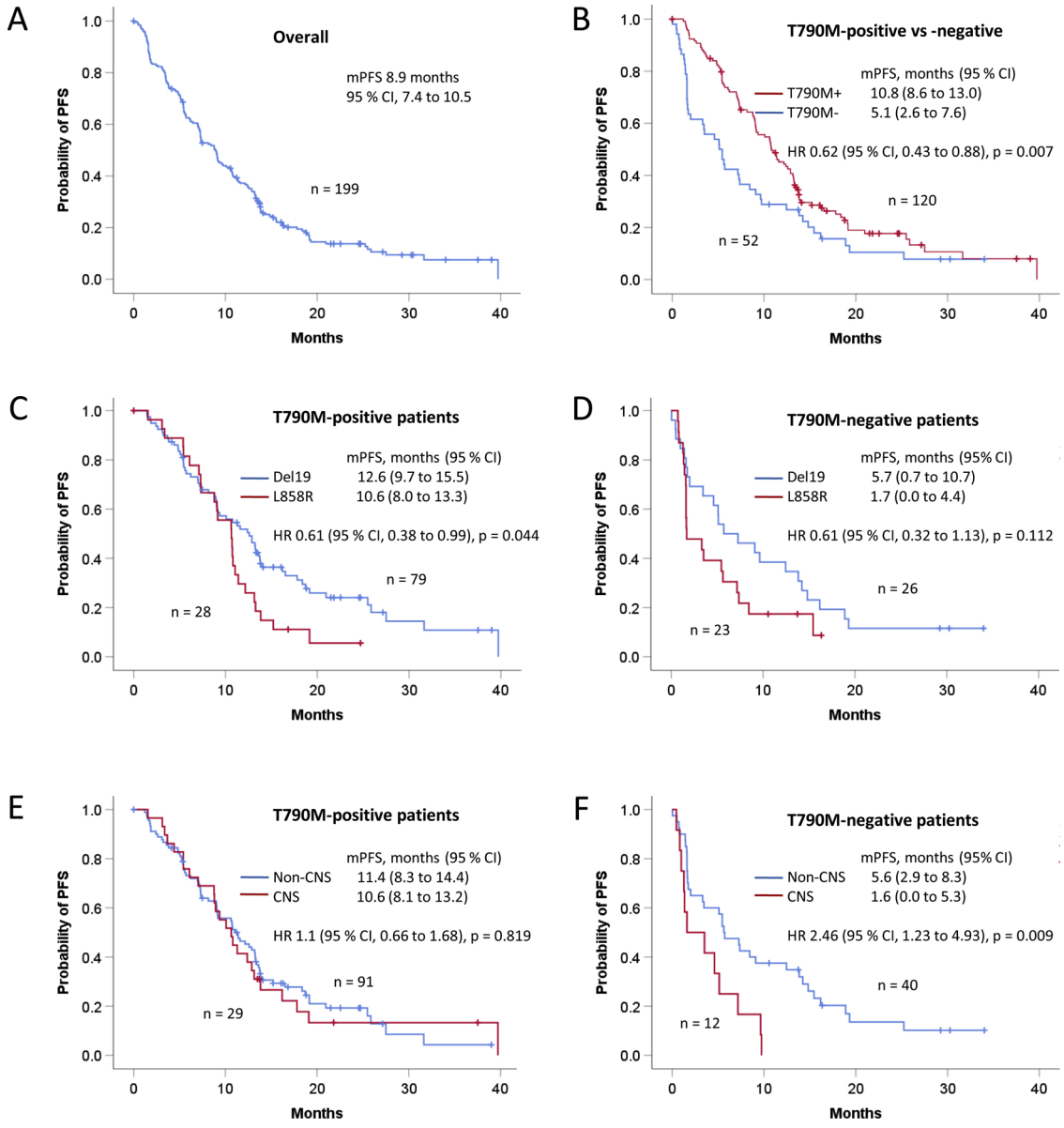

Fig. 3. (A) Progression-free survival (PFS) in the overall study population. (B) PFS in the T790M-positive vs -negative cohort. (C) PFS in patients with del19 vs L858R in the T790M-positive cohort. (D) PFS in patients with del19 vs L858R in the T790M-negative cohort. (E) PFS in patients with or without CNS-metastases in the T790M-positive cohort. (F) PFS in patients with or without CNS-metastases in the T790M-negative cohort.

In the present study, the response rate for patients harbouring a sensitizing deletion in exon 19 was higher than for patients with L858R, both overall and within the T790M-positive and -negative cohorts. The association with a better response rate remained significant when adjusted for other factors. The tendency of a more favourable outcome for patients with del19-mutation compared to patients with L858R was also seen across other efficacy endpoints such as PFS and OS. Existing data suggest that patients with del19-mutations have longer PFS when treated with first- or second-generation TKIs in the first line setting [23–25]. Moreover, in a pooled analysis of two trials comparing the second generation TKI afatinib with chemotherapy, there was a statistically significant survival benefit for patients with del19 treated with afatinib, but not for patients with L858R [26]. Similarly, in the setting of acquired resistance to first-line EGFR-TKIs and presence of T790M, patients with co-occurring del19-mutation treated with osimertinib tended to have a higher response rate, longer PFS and OS [18,27,28]. In our material, the prevalence of T790M is higher in patients with del19 than with L858R, consistent with previously reported data, indicating that the T790M mutation is more likely to emerge in the context of a del19-mutation [29,30]. Thus, our results add to the growing body of evidence that del19 and L858R are distinct subtypes of *EGFR*-mutated NSCLC with different prognosis and response to treatment with EGFR-TKIs.

Both preclinical and clinical data have demonstrated that osimertinib is effective in the CNS [31,32]. Consistent with this, there were no differences in response rate, PFS or OS for patients with or without brain metastases at study entry. This was also true within the T790M-positive cohort, but for patients without the T790M-mutation, both PFS and OS were worse for patients with brain involvement. Furthermore, the presence of brain metastases was the only variable statistically significantly associated with the outcome in multivariate analysis. This might reflect the lower probability of overall response in the T790M-negative cohort, combined with the in general worse prognosis for patients with brain metastases.

The adverse events reported in this trial were mainly of mild character and in line with that observed previously. Some of the most

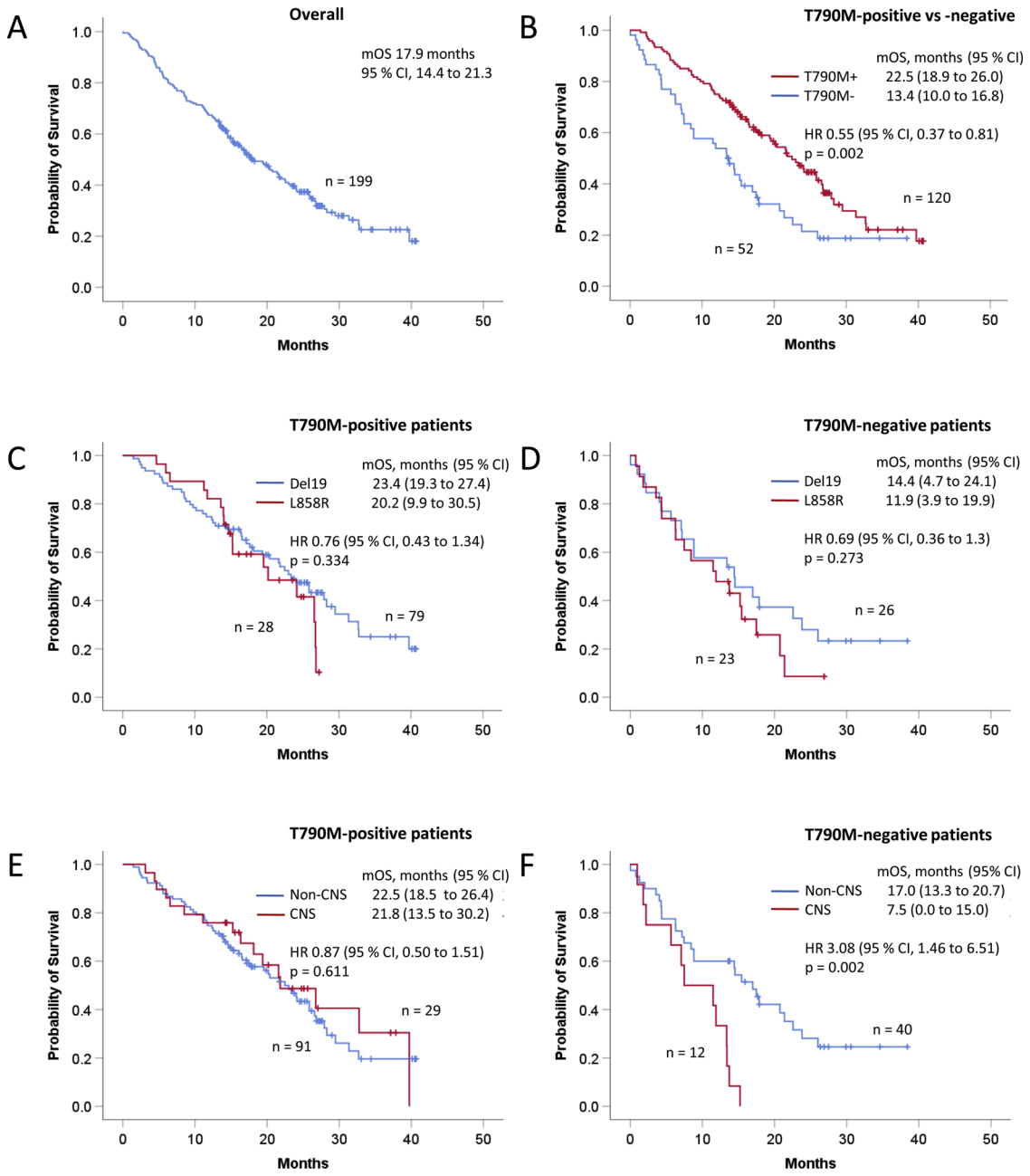

Fig. 4. (A) Overall survival (OS) in the overall study population. (B) OS in the T790M-positive vs -negative cohort. (C) OS in patients with del19 vs L858R in the T790M-positive cohort. (D) OS in patients with del19 vs L858R in the T790M-negative cohort. (E) OS in patients with or without CNS-metastases in the T790M-positive cohort. (F) OS in patients with or without CNS-metastases in the T790M-negative cohort.

frequent adverse events like fatigue and dyspnoea could be related to symptoms from the disease itself rather than being a side effect of the drug. Overall, there were no new safety signals.

In conclusion, this study confirms the efficacy and tolerability of osimertinib as second or later line treatment in patients with advanced *EGFR*-mutated NSCLC in a Northern European cohort. For T790M-positive patients, the results are consistent with the existing evidence, from both clinical trials and real-world data, and show similar efficacy in patients with and without brain metastases. Osimertinib also exhibits activity in the T790M-negative cohort, and might be a treatment option for selected patients in whom EGFR-TKI resistance is not due to T790M-mutation.The ongoing translational analyses based on this study might contribute to elucidate this.

Authors contribution statement

Design of the study: OTB, ÅH, SE, AM, JK, BHG. Data collection: All authors. Data analysis and interpretation: IJZE, OTB. Manuscript draft: IJZE. Editing and revision of manuscript and approval of final version: All authors.

Transparency document

The Transparency document associated with this article can be found in the online version.

Declaration of Competing Interest

IJZE, ÅH, SE and SC have nothing to disclose. AM has received

grants from AstraZeneca. KHH has received honoraria for lectures or advisory boards for Takeda, Roche, MSD, AstraZeneca, Pierre Fabre and BMS. JK has received honoraria for lectures or advisory boards from AstraZeneca, BMS, Boehringer-Ingelheim, MSD and Roche. BHG has received honoraria for lectures and advisory boards for AstraZeneca. OTB has received grants from Roche and Pfizer.

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II

III

Original Article

Osimertinib in non-small cell lung cancer with uncommon *EGFR*-mutations: a *post-hoc* subgroup analysis with pooled data from two phase II clinical trials

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Background: Osimertinib is standard of care for *EGFR*-mutated non-small cell lung cancer (NSCLC) patients. The efficacy of the drug in patients with mutations other than the common deletion in exon 19 and L858R in exon 21 is largely unknown.

Methods: We identified patients with uncommon *EGFR*-mutations from two prospective clinical phase II, single-arm studies for previously treated patients and untreated patients, respectively, and pooled data for this analysis. All patients received treatment with osimertinib 80 mg daily until radiological progression or death. The primary endpoint of both trials was objective response rate (ORR), with progression-free survival (PFS), overall survival (OS) and intracranial efficacy as key secondary endpoints. Circulating tumour DNA (ctDNA) was analysed before and two weeks after treatment initiation in the first line cohort.

Results: Of 299 enrolled patients in the two trials, 21 patients with uncommon mutations were identified; 12 patients had a single mutation (G719X or L861Q), one patient had L861Q and an exon 20 insertion, and 8 patients had compound mutations with G719X and either L861Q or S768I. Three of the 10 pretreated patients had the T790M resistance mutation. ORR was 47.6% and disease control rate (DCR) 85.7%. The median duration of response (DoR) was 7.9 months. Among 11 patients treated with osimertinib in first line, ORR was 63.6% *vs.* 30.0% of 10 previously treated patients. The median PFS was 5.5 months in both groups. Patients with G719X-compound mutations had a higher response rate (62.5% *vs.* 38.5%), a longer median PFS (13.7 *vs.* 3.5 months) and median OS (29.3 *vs.* 7.5 months) than patients with other mutations. Most first line treated patients (81.8%) displayed a reduction in ctDNA after two weeks of treatment.

Conclusions: Osimertinib demonstrates activity in patients with uncommon *EGFR*-mutations, and especially for G719X-compound mutations.

Keywords: Osimertinib; uncommon *EGFR* mutations; circulating tumour DNA (ctDNA); T790M

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Introduction

Treatment with tyrosine kinase inhibitors targeting epidermal growth factor receptor (EGFR-TKIs) is established as standard of care for patients with advanced or metastatic *EGFR*-mutated non-small cell lung cancer (NSCLC). Deletions in exon 19 (del19) and the L858R point mutation in exon 21 constitute around 85% of the *EGFR*-mutations. The remaining 10–15% consist of a variety of mutations in exons 18 through 21, of which insertions in exon 20 (ex20ins) are the most frequent followed by the point mutations G719X (X representing A, C or S) in exon 18 either alone or in combination with others, S768I in exon 20 and L861Q in exon 21, respectively (1-3).

Multiple phase III trials have demonstrated the superiority of first and second generation EGFR-TKIs to chemotherapy for patients harbouring a sensitizing *EGFR*-mutation, with median progression-free survival (PFS) in the range of 9–15 months (4-7). Furthermore, the third generation TKI osimertinib, which is active against the sensitizing mutations and the T790M resistance mutation, had a median PFS of 18.9 months when used as first line treatment and 10.1 months in patients with acquired T790M-mediated resistance to previous EGFR-TKI treatment (8,9). However, most of the landmark studies of these agents only included patients with the common mutations del19 and L858R, and hence there are limited prospective data on the efficacy of these drugs in patients with uncommon *EGFR*-mutations. Whereas ex20ins are regarded as resistant to currently available EGFR-TKIs, retrospective studies have indicated some activity of first generation EGFR-TKIs in tumours harbouring the other uncommon mutations, albeit to a lesser degree than what is commonly reported for del19/L858R mutations (2,10-12). However, in a post-hoc analysis of 38 patients with G719X, L861Q or S768I from three clinical trials the overall response rate to the second generation EGFR-TKI afatinib was 71.1% and the median PFS was 10.7 months (13). Furthermore, the objective response rate (ORR) was 50% and the median PFS 8.2 months in a recent phase II trial with 36 EGFR-TKI naïve patients with uncommon mutations who were treated with osimertinib (14). Of the 36 patients in this study, 22 patients received osimertinib as first line therapy, whereas the remaining 14 had received at least one line of chemotherapy. Still, data on efficacy of these drugs in patients with the uncommon mutations are limited and prospective data are scarce.

We conducted two clinical phase II trials where *EGFR*-mutated patients received osimertinib as frontline treatment, and second or later line treatment, respectively. With the present analysis, we aimed to evaluate the activity of osimertinib in the subgroup of patients harbouring uncommon *EGFR*-mutations with pooled data from these two trials. We present the following article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-21-995/rc).

Methods

We pooled individual data from a subgroup of patients with uncommon *EGFR*-mutations from two prospective clinical trials on osimertinib (NCT02504346 and NCT03804580). Both trials were phase II trials, had a single-arm design and were run in multiple centres in Northern Europe. Patients had advanced or metastatic NSCLC with an activating *EGFR*-mutation. One trial included patients previously treated with at least one EGFR-TKI; details of this trial have been published previously (15). The second trial included untreated patients. Patients in both trials were aged 18 years or older and provided written informed consent. In the previously treated patients, a re-biopsy was done after the last line of therapy and before commencement of the study treatment (osimertinib). For the untreated patients a biopsy done at diagnosis was accepted unless they had received adjuvant systemic cancer therapy after which a new biopsy was required. Testing for mutational status in tissue biopsies was done per local practice and included mainly real-time polymerase chain reaction (PCR) or next generation sequencing (NGS).

Blood samples were collected from each patient in the previously untreated cohort immediately before treatment initiation and after two weeks of treatment. Circulating tumour DNA (ctDNA) was isolated from blood samples and prepared for sequencing using the AVENIO ctDNA Surveillance Kit (Roche, Basel, Switzerland) as previously described (16). The samples were sequenced on NextSeq

500 High Output Lane (Illumina, San Diego, CA, USA).

All patients had an Eastern Cooperative Oncology Group (ECOG) status of 0–2, had adequate liver, renal and bone marrow function and had measurable disease as defined by RECIST v1.1. Imaging of all tumour lesions was done every 8 weeks the first 48 weeks and every 12 weeks thereafter. In the second line trial an MRI or CT scan of the brain was done if the patient had known or suspected brain metastases at baseline and was repeated throughout the study at the times of overall response assessments, whereas in the first line trial all patients were screened with a brain MRI at baseline and on every subsequent tumour assessment even in the absence of baseline brain metastases. All patients were treated with osimertinib 80 mg once daily until progressive disease by RECIST v1.1, or as long as they had clinical benefit as judged by the investigator. Dose reduction to 40 mg daily was allowed in case of significant toxicity. Reasons for discontinuation other than progressive disease were unacceptable toxicity, non-compliance or patient's wish.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the national ethics committees in each participating country (Norway 2018/1028 and 2015/181, Sweden 2016/10-31/1 and 2019-02941, Finland 59/2015, Lithuania P-16-8 and P-18-85, Denmark H-15005843 and S-20180149) and informed consent was taken from all individual participants upon inclusion in the trials. The trials were registered at ClinicalTrials.gov (identifier: NCT02504346, NCT03804580). AstraZeneca and the South-Eastern Norway Regional Health Authority provided funding for the studies. The funding sources did not contribute to data collection, analyses, interpretation of the results or writing of the manuscript.

Outcome

The primary endpoint in the two clinical trials was ORR. Secondary endpoints included PFS, disease control rate (DCR), duration of response (DoR) and overall survival (OS). Other endpoints were intracranial progression-free survival (iPFS) and intracranial objective response rate (iORR). In this post-hoc, pooled analysis we assessed the efficacy endpoints of osimertinib in all patients with uncommon *EGFR*-mutations. Further, we divided the patients into two groups based on treatment line in which they received osimertinib (first line *vs.* pretreated) as this is clinically relevant. We also described efficacy according to the sensitizing mutations, with patients with G719X compound mutations in one group ("G719X compound group"), and patients with single mutations or combinations excluding G719X in the other group ("other mutation group"). As T790M is a resistance mutation rather than a sensitizing mutation, it was not regarded a compound to the other mutation when present. Furthermore, one patient was identified as having a single G719X mutation in tissue biopsy, but plasma NGS later revealed a rare partner mutation (L833V), which is of unknown clinical significance. As such, we categorized this patient in the "other mutation group".

Statistical analysis

All time-to-event endpoints were analysed with the Kaplan-Meier method and subgroups compared with the log rank test. Two-way ANOVA test with Šidák correction was used to compare mutant ctDNA molecule concentration between groups. Two-sided P values of less than 0.05 were considered statistically significant. Confidence intervals were calculated with the exact method. All analyses were performed with IBM SPSS Statistics for Windows, Version 27.0 (Armonk, NY, USA: IBM Corp.).

Results

Patients

A total of 21 patients were included in the analysis, 10/199 patients from the second line study and 11/100 patients from the first line study. Patients were included from July 2015 to November 2018 and from December 2018 to June 2021 in the two trials, respectively. All patients received at least one dose of study medication and no patients were lost to follow up. The median age was 69 (range, 52–90) years, 81% were female, 19% were never-smokers and 24% had an ECOG-status of 2. At baseline, 38% of the patients had a G719X compound mutation with either S768I or L861Q as a partner mutation. L861Q and G719X as single mutations were found in 33% and 24% of the patients, respectively. In the previously treated patients, 30% had a T790M-mutation. For all pretreated patients, the median time on first or second generation EGFR-TKI before commencement of osimertinib was 18.0 months. The three T790M-positive patients had received a prior EGFR-TKI for 2.9, 16.1 and 34.7 months, respectively. Detailed baseline characteristics are presented in *Table 1*.

Table 1 Baseline characteristics

Baseline characteristics	Overall (n=21)	First line cohort (n=11)	Second or later line cohort (n=10)
Median age [range], years	69 [52–90]	75 [52–83]	60.5 [52–90]
Sex			
Male	4 (19.0%)	2 (18.2%)	2 (20.0%)
Female	17 (81.0%)	9 (81.8%)	8 (80.0%)
Smoking history			
Never-smoker	4 (19.0%)	3 (27.3%)	1 (10.0%)
Former smoker[i]	15 (71.4%)	6 (54.5%)	9 (90.0%)
Current smoker[ii]	2 (9.5%)	2 (18.2%)	0
ECOG status			
ECOG 0–1	16 (76.2%)	8 (72.7%)	8 (80.0%)
ECOG 2	5 (23.8%)	3 (27.3%)	2 (20.0%)
Histology			
Adenocarcinoma	21 (100.0%)	11 (100.0%)	10 (100.0%)
EGFR-mutation at start osimertinib			
G719X + S768I	6 (28.6%)	3 (27.3%)	3 (30.0%)[iii]
G719X + L861Q	2 (9.5%)	1 (9.1%)	1 (10.0%)[iv]
G719X	5 (23.8%)	2 (18.2%)	3 (30.0%)[v]
L861Q	7 (33.3%)	5 (45.5%)	2 (20.0%)
L861Q + ex20ins	1 (4.8%)	0	1 (10.0%)
Disease classification			
Stage III	1 (4.8%)	0	1 (10.0%)
Stage IV	20 (95.2%)	11 (100.0%)	9 (90.0%)
Extent of disease			
Lung	21 (100.0%)	11 (100.0%)	10 (100.0%)
Regional lymph nodes	6 (28.6%)	5 (45.5%)	1 (10.0%)
Liver	5 (23.8%)	3 (27.3%)	2 (20.0%)
Adrenal gland	5 (23.8%)	4 (36.4%)	1 (10.0%)
CNS	12 (57.1%)	7 (63.6%)	5 (50.0%)
Bone	12 (57.1%)	6 (54.5%)	6 (60.0%)
No. of previous EGFR-TKI regimens			
1	–	–	9 (90.0%)
2	–	–	1 (10.0%)
Previous EGFR-TKI therapy			
First line	–	–	
Erlotinib	–	–	8 (80.0%)
Gefitinib	–	–	1 (10.0%)
Afatinib	–	–	1 (10.0%)
Second line	–	–	
Erlotinib	–	–	0
Gefitinib	–	–	0
Afatinib	–	–	1 (10.0%)
No. of other previous systemic anti-cancer treatments[vi]			
0	–	–	2 (20.0%)
1	–	–	6 (60.0%)
2	–	–	2 (20.0%)

Data are presented as number (percentage). [i], stopped smoking at least one year ago; [ii], included stopped smoking the last year; [iii], one T790M positive; [iv], one T790M positive; [v], one T790M positive; [vi], systemic anticancer therapy for metastatic disease or adjuvant treatment ≤6 months before metastatic disease. ECOG, Eastern Cooperative Oncology Group; CNS, central nervous system.

Efficacy

Data cut-off was October 29, 2021. The ORR was 47.6% (95% CI: 25.7–70.2%) (*Table 2* and *Figure 1*). In the first line cohort, the ORR was 63.6% (95% CI: 30.8–89.1%) and in the pretreated cohort 30.0% (95% CI: 6.7–65.2%). There were no complete responses. The DCR was 85.7% overall, and 100.0% and 70.0% in the first line and pretreated cohorts, respectively. The median DoR in the first line cohort was 12.1 months (95% CI: 0–29.2) *vs.* 7.8 months (95% CI: 4.2–11.4) in the pretreated cohort, P=0.602. We analysed response rates according to different *EGFR*-mutations (*Table 2*). For patients with a G719X compound mutation (n=8), the ORR was 62.5% (95% CI: 24.5–91.5%) and 38.5% (95% CI: 13.9–68.4%) in the group with other mutations (n=13). The DCR was 100.0% (95% CI: 63.1–100.0%) and 76.9% (95% CI: 46.2–95.0%) for the two groups, respectively. The median DoR was 12.4 months for the G719X-compound group *vs.* 3.8 months

Table 2 Response to osimertinib

Patient groups	Objective response, %, (95% CI)	Disease control, %, (95% CI)	DoR, months, (95% CI)
Overall (n=21)	47.6 (25.7, 70.2)	85.7 (63.7, 97.0)	7.9 (0, 17.0)
1st line cohort (n=11)	63.6 (30.8, 89.1)	100 (71.5, 100)	12.1 (0, 29.2)[§]
Pretreated cohort (n=10)	30.0 (6.7, 65.2)	70.0 (34.8, 93.3)	7.8 (4.2, 11.4)[§]
G719X compound mutations (n=8)	62.5 (24.5, 91.5)	100.0 (63.1, 100)	12.4 (11.9, 12.9)*
G719X + S768I (n=5)			
G719X + S768I + T790M (n=1)			
G719X + L861Q (n=1)			
G719X + L861Q + T790M (n=1)			
Other mutations (n=13)	38.5 (13.9, 68.4)	76.9 (46.2, 95.0)	3.8 (2.5, 4.1)*
G719X (n=4)			
G719X + T790M (n=1)			
L861Q (n=7)			
L861Q + ex20ins (n=1)			

Note that the cohorts with different mutations are overlapping with the line of treatment cohorts. [§], DoR first line *vs.* pretreated (P=0.602); *, DoR combination *vs*. other (P=0.007). DoR, duration of response.

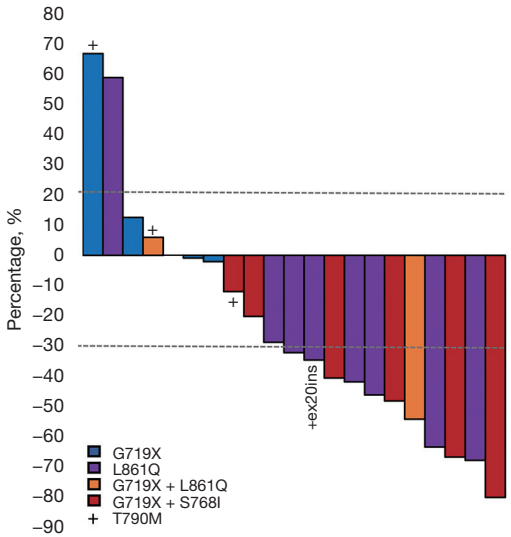

Figure 1 Change in sum of diameters of target lesions from baseline. ex20ins, insertion in exon 20.

for the other mutations group, respectively, P=0.007.

Median PFS was 5.5 months (95% CI: 4.2–6.7) (*Figure 2A*). The median PFS was equal in the first line *vs.* pretreated cohort with 5.5 months in both groups, P=0.682 (*Figure 2B*). However, there was a statistically significant difference in PFS between the G719X compound mutation group and the other mutation group with a median PFS of 13.7 *vs.* 3.5 months, respectively, P=0.003 (*Figure 2C*).

The median OS was 11.9 months (95% CI: 2.1–21.7) (*Figure 2D*). There was no statistically significant difference in OS between the first line and pretreated cohort with a median OS of 17.5 *vs.* 11.9 months, respectively (P=0.882) (*Figure 2E*). The median OS was longer in the compound mutations group with a median of 29.3 *vs.* 7.5 months in the group with other mutations, P=0.001 (*Figure 2F*).

Three of the patients harboured a T790M-mutation in addition to the uncommon mutation (*Figures 1,3*). We analysed the data with and without the T790M positive patients. The median PFS and median OS was identical whether the T790M positive were included or not (5.5 and 11.9 months, respectively).

Of 12 patients with brain metastases at baseline, 11 patients had brain scans available for review. Of these, eight patients had untreated brain metastases, two patients had received prior whole brain radiotherapy and one patient had been treated with stereotactic radiosurgery prior to inclusion. The iORR was 36.4% overall (*Table 3*) and the median iPFS was 6.1 months (95% CI: 1.3–10.8) (data not shown). In the first line cohort, the iORR was 42.3% *vs.* 25.0% in the pretreated cohort. The intracranial DCR was 100.0% in both subgroups. Further, in the G719X

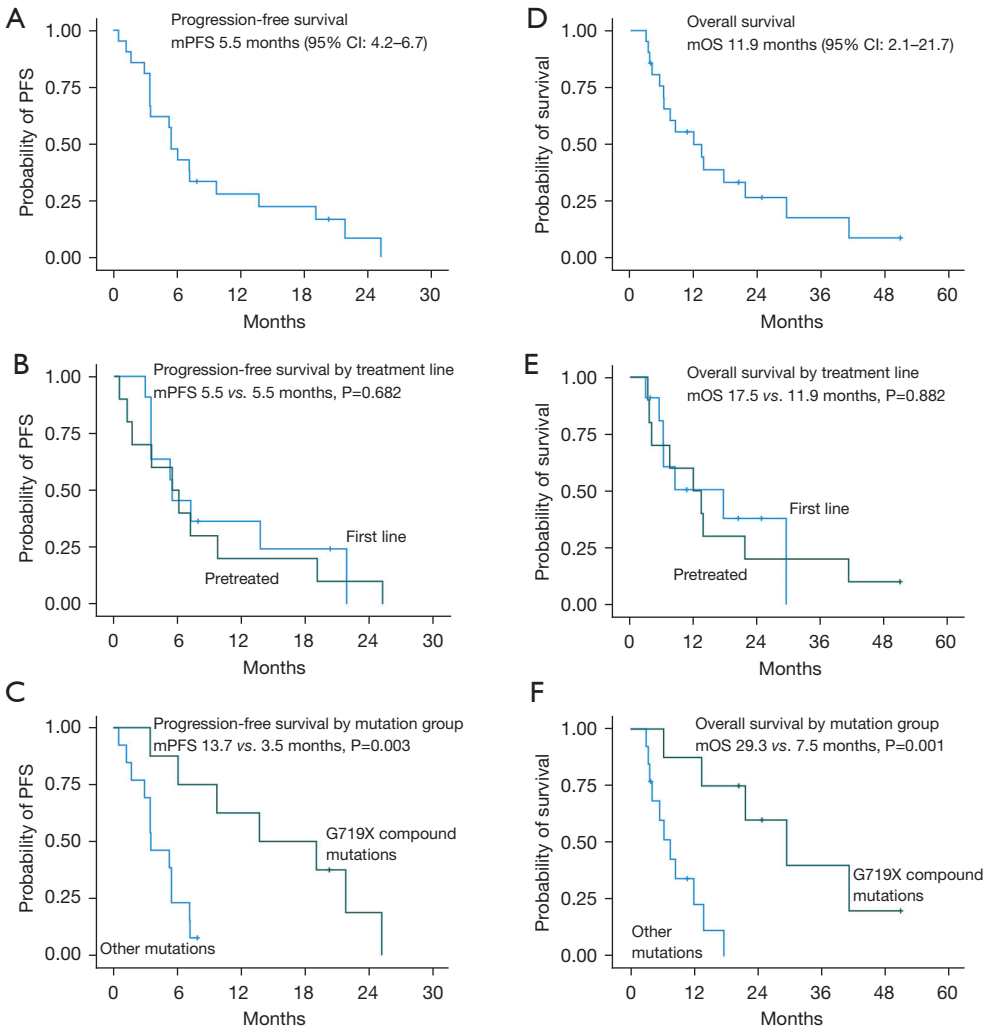

Figure 2 PFS and OS. (A) PFS for all patients. (B) PFS in the first line *vs.* pretreated cohort. (C) PFS in the G719X compound mutation *vs.* other mutations cohort. (D) OS for all patients. (E) OS in the first line *vs.* pretreated cohort. (F) OS in the G719X compound mutation *vs.* other mutations cohort. PFS, progression-free survival; mPFS, median PFS; OS, overall survival; mOS, median OS.

compound group, the iORR was 75.0% *vs.* 14.3% in the other mutation group. We did not calculate median iPFS in the subgroups due to small sample size.

ctDNA analysis

Sequencing of ctDNA from the first-line cohort led to identification of the identical uncommon *EGFR*-mutations in 9 out of the 11 patients that were identified in a tissue biopsy. Furthermore, in one patient with a single G719X mutation detected in tissue biopsy, ctDNA analysis identified an additional uncommon *EGFR*-mutation (L833V). The median number of all mutations identified was 3 mutations at baseline (range, 0–6) and 1 mutation after two weeks of treatment (range, 0–4). The number of mutant molecules per mL plasma was measured for all mutations, and the mean was calculated for each of the patients. All patients had a decrease in the mean number of mutant molecules per mL plasma after two weeks, except one patient with a small amount of ctDNA (8.64 to 13.01 mutant molecules per mL plasma), and one patient where no mutations were detected, neither before nor after treatment initiation. The decrease in ctDNA was detected in both the G719X compound group and in the group with a single uncommon *EGFR*-mutation (*Figure 4*). No significant difference was found between the number of

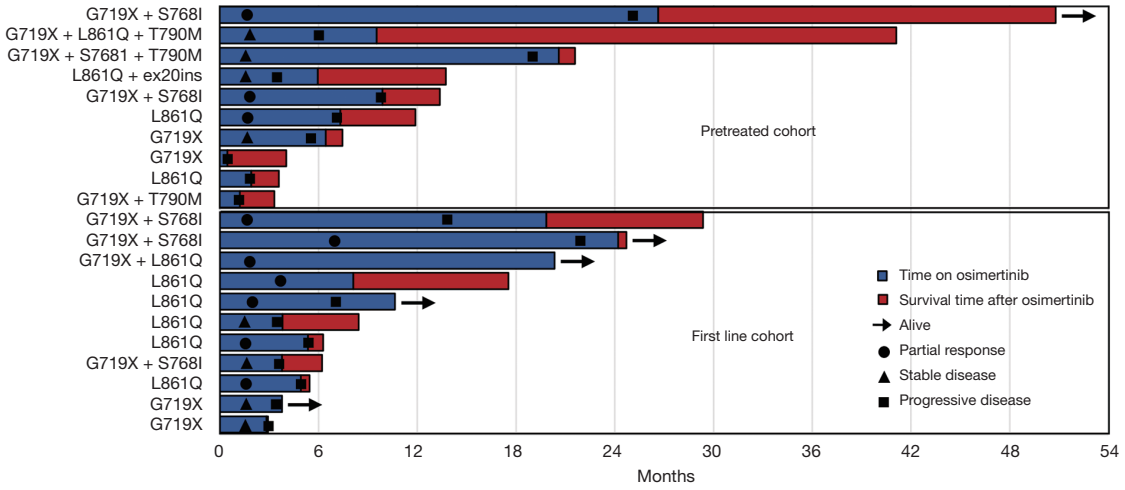

Figure 3 Overview of the clinical course from start of treatment with osimertinib. Each bar represents individual patients. ex20ins, insertion in exon 20.

Table 3 Intracranial response rates

CNS response	Overall* (n=11)	First line cohort (n=7)	Pretreated cohort (n=4)	G719X comb (n=4)	Other (n=7)
CR	2	1	1	2	0
PR	2	2	0	1	1
SD	4	2	2	1	3
Non-CR/non-PD	3	2	1	0	3
PD	0	0	0	0	0
iORR, %	36.4	42.3	25.0	75.0	14.3
iDCR, %	100.0	100.0	100.0	100.0	100.0

*, 6 patients had measurable disease, 5 patients only non-target lesions. CNS, central nervous system; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; iORR, intracranial objective response rate; iDCR, intracranial disease control rate.

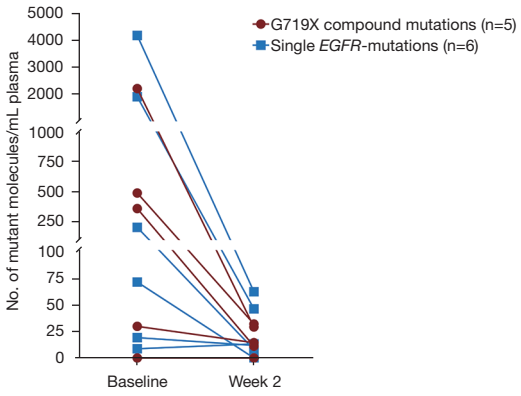

Figure 4 Mean number of mutant molecules per mL plasma for patients in the first line cohort. Each line represents individual patients, stratified into patients with G719X compound mutations (n=5) and patients with a single uncommon *EGFR*-mutation (n=6).

mutant molecules in the plasma of the two groups (P=0.714 at baseline and P>0.999 at week 2) (data not shown). The *EGFR*-mutations in the G719X compounds had a similar allelic frequency (AF) in the baseline samples suggesting that they are in fact compound mutations present in the same cells (*Table 4*).

Safety

All patients reported at least one adverse event, with decreased appetite (13/21), nausea (10/21), rash (9/21) and paronychia (9/21) as the most common. The majority of the adverse events were of grade 1–2. Grade 3 or higher adverse events regardless of relation to study treatment were seen in 11/21 patients. Among these, three cases of pulmonary

Table 4 List of AF for patients with G719X compound mutations

ID	Baseline (AF)		Difference between *EGFR* baseline mutations, %	Week 2 (AF)	
	G719X	Other *EGFR* mutation		G719X	Other *EGFR* mutation
1	22.98	22.01 (S768I)	4.22	0.21	–
2	7.77	8.62 (S768I)	9.86	–	–
3	–	–	–	–	–
4	2.77	2.25, 0.11 (L861Q, ex19del)	18.77	–	0.15 (L861Q)
5	0.53	0.40 (L833V)	24.53	0.22	0.12 (L833V)

AF, allelic frequencies.

embolism, two cases of nausea and one case of erythema multiforme were considered as possibly treatment related (all grade 3). One patient discontinued osimertinib because of an adverse event (erythema multiforme). There were two deaths other than of progressive disease; one patient died of possible treatment-related pneumonitis and one patient died of cholecystitis not related to study treatment.

Discussion

Osimertinib has been shown to be superior to first generation EGFR-TKIs in patients with common *EGFR*-mutations (8,17) and to chemotherapy in patients with the T790M resistance mutation in addition to del19/L858R (9). However, although preclinical data suggest that osimertinib is active also in tumours with uncommon mutations (18), there are limited prospective clinical data to support this. In this post-hoc pooled analysis, we demonstrated that osimertinib has clinical activity in patients with uncommon mutations, with an overall ORR of 48% and DCR of 86%, respectively. Furthermore, the ORR of 64% (95% CI: 31–90%) in the first line cohort was consistent with the ORR (50%, 95% CI: 33–67%) in a Korean phase II-trial of patients treated in the first line setting (14). Although the ORR was lower in the second line cohort (30%), there was a clinically meaningful DCR of 70%. The DoR was similar, and clinically significant in both groups.

Despite the encouraging ORR, the median PFS of 5.5 months was modest and similar to that reported by Cho *et al.* (14) (8.2 months). Interestingly, the median PFS was identical in the first and second line cohorts and close to what we have previously found in the 52 T790M-negative patients in the overall patient population in the second line study (median PFS 5.1 months) (15). However, across all the efficacy parameters, there was a more favourable outcome for patients with G719X compound mutations than for patients with other mutations, which to our knowledge has not been described previously. For instance, there was a large and highly statistically significant difference in OS (median 29.3 *vs.* 7.5 months, P=0.001). In a retrospective observational study of patients with uncommon *EGFR*-mutations treated with gefitinib or erlotinib, Chiu *et al.* (10) demonstrated that the PFS was significantly longer for those with compound mutations than for single mutations (median PFS 11.9 *vs.* 6.5 months, P=0.010, respectively). Similar results were also reported in a Dutch study, indicating that uncommon compound mutations are more responsive to early generation EGFR-TKIs than single uncommon mutations (19). Furthermore, recent data indicate that response to EGFR-TKIs depends on mutational subgroups, and that osimertinib may differ from other EGFR-TKIs with respect to inhibition of the atypical mutation subtypes (20). ctDNA analysis showed that mutations within the G719X compound had a similar AF, which indicates that the two *EGFR*-mutations are in fact compound mutations existing in the same clone. This could possibly explain the favourable PFS and OS, however, the effect of double mutations on the structure-functional characteristics of the *EGFR* mutants is to our knowledge not known.

When looking at the number of ctDNA mutant molecules per mL plasma we found no significant difference between the G719X compound group and the group with a single *EGFR*-mutation. However, we saw a reduction in the mutant molecule concentration within the first two weeks of treatment, independent of mutational group. This ctDNA decrease may be a sign of early response to treatment. A study by Ebert *et al.* (21) showed that clearing of the ctDNA in plasma was correlated with both PFS and OS, independent of the rapidity of the clearing. We suggest that the observed decrease in ctDNA after two weeks of

treatment illustrates ongoing clearing. Our results may indicate that uncommon *EGFR*-mutations, and especially compound mutations involving G719, render the kinase sensitive to osimertinib. Further studies are needed to explore whether these observations are due to inherent biological differences leading to a more indolent course of disease for the compound mutations, or due to a better treatment effect.

Of note, only 30% of the subset of patients with uncommon mutations who had developed resistance to an EGFR-TKI prior to treatment with osimertinib had detectable T790M. Despite the limited sample size in our material, this finding is in line with a recent retrospective study of patients with disease progression on EGFR-TKIs, where there was a significantly lower incidence of T790M in 27.1% of 48 patients with uncommon mutations *vs.* 55.2% and 37.2% in patients with del19 and L858R, respectively (22). Furthermore, studies have demonstrated that a longer duration of exposure to first generation EGFR-TKIs is associated with a higher incidence of T790M (23,24). Uncommon mutations have been reported to be less sensitive to first generation EGFR-TKIs with shorter PFS than the common mutations (2,10-12). However, of the three T790M-positive patients in our material, only one patient had a duration of EGFR-TKI prior to osimertinib that was longer than median (34.7 months, median 18.0 months) and one patient had short time on first generation TKI (2.9 months). Hence, whether fewer cases of T790M-mediated resistance are a consequence of shorter treatment time on first generation drugs remains uncertain.

Osimertinib is central nervous system (CNS)-penetrant and studies have shown a high activity in patients with common mutations and brain metastases (25,26). In the present analysis, the iORR was 36% and the iDCR 100%, demonstrating intracranial effect of osimertinib in the case of uncommon mutations as well. Furthermore, as for the overall efficacy endpoints, the iORR was markedly higher in patients with G719X compound mutations than for the other mutations (75% *vs.* 14%), indicating that the favourable outcome observed for G719X compound mutations also applies in the presence of brain metastases.

There are some limitations to this study. First, the number of patients is small precluding conclusive results based on this study alone. However, given the rarity of these mutations, it is challenging to perform large scale studies and, as such, all patient samples will add to the knowledge on the subject. Also, an ongoing Japanese phase II-study (jRCTs071200002) of osimertinib for previously untreated uncommon *EGFR* mutant NSCLC, which aims to include 40 patients, will be an important contribution to this (27). Second, because the second line trial included patients regardless of T790M-status (15), the pre-treated cohort consists of patients with and without T790M. We have therefore presented individual response data and performed extra analysis of PFS and OS which indicate similar efficacy regardless of T790M status.

In summary, we demonstrated that osimertinib exerts activity in patients with uncommon *EGFR*-mutations, both in treatment naïve patients and after progression on first or second generation EGFR-TKIs. The uncommon mutations are a heterogeneous group of mutations of which compound mutations with G719X seem to be most sensitive to treatment whereas single mutations including G719X alone are less responsive.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-21-995/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-21-995/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-21-995/coif). IJZE has received honoraria for lectures or advisory boards for Novartis and Boehringer-Ingelheim. ÅH has received honoraria for lectures or advisory boards to her institution, from AstraZeneca, Takeda, Pfizer, Bayer, BMS and Roche. SC has received honoraria for lectures for Pfizer, Roche and AstraZeneca. JK has received grants from AstraZeneca and Boehringer-Ingelheim and honoraria for lectures or advisory boards from AstraZeneca, BMS, Boehringer-

Ingelheim, MSD, Amgen, Eli Lilly, Sanofi and Roche. BHG has received honoraria for lectures and advisory boards for AstraZeneca, Pfizer, Takeda, Roche, MSD and Janssen. OTB has received honoraria for lectures or advisory boards from AstraZeneca, Roche, Novartis, Eli Lilly, Pfizer, Sanofi, Bayer, MSD, BMS, Boehringer-Ingelheim and grants from Astra Zeneca. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the national ethics committees in each participating country (Norway 2018/1028 and 2015/181, Sweden 2016/10-31/1 and 2019-02941, Finland 59/2015, Lithuania P-16-8 and P-18-85, Denmark H-15005843 and S-20180149) and informed consent was taken from all individual participants upon inclusion in the trials. The trials were registered at ClinicalTrials.gov (identifier: NCT02504346, NCT03804580).

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