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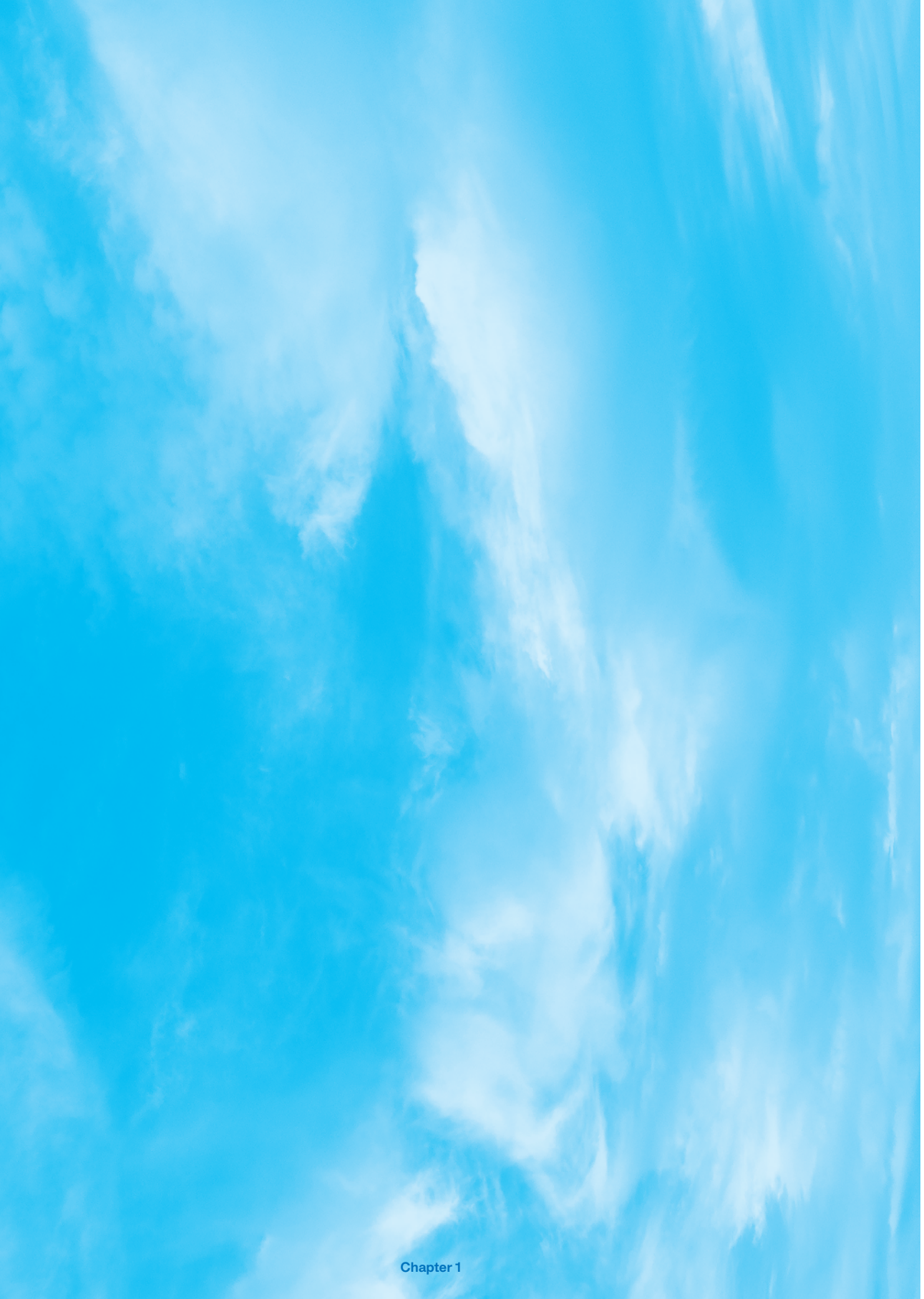

Chapter 1

General introduction and outline of this thesis

General aspects of lung cancer

In 2020, lung cancer remained the leading cause of cancer death worldwide with an estimated 1.80 million deaths (1). Also in the Netherlands, lung cancer has a high mortality rate; although survival rates are still improving, only 21% of the patients are alive 5 years after diagnosis (based on the period 2011 – 2018) (2). In 2019, more than 14.000 people were diagnosed with lung cancer in the Netherlands. Approximately 50% of them has stage IV disease at diagnosis, this means the cancer has spread to distant organs and is in general incurable (2). Lung cancer is mostly a disease of the elderly, around 70% of patients with stage IV disease is 65 years or older (2).

Lung cancer can be divided into two subgroups, non-small cell lung cancer (NSCLC) and small cell lung cancer. Eighty five % of lung cancer patients are classified as NSCLC, a collective name for different subtypes: including adenocarcinoma, squamous cell carcinoma and other less common subtypes. Most lung carcinomas are adenocarcinomas.

Tobacco smoking remains the leading cause of lung cancer (3). The number of lung cancer patients who have never smoked varies widely, from more than 50% in women in Southeast Asia, to approximately 2-6% in men in Western countries (4). Lung cancers that occur in never smokers, defined as less than 100 cigarettes over lifetime, are considered a distinct entity (4, 5). Causative factors in this population are still poorly understood. The demographics of non-smokers with lung cancer are different compared with smokers, with more women and individuals of younger age. The vast majority are adenocarcinomas. In addition, never-smoker lung cancers show a high prevalence of targetable oncogenic driver alterations in nearly 80% of patients of whom approximately 50% harbors an epidermal growth factor receptor (*EGFR*) mutation (6, 7).

The majority of NSCLC patients are diagnosed with advanced disease on first presentation and are unsuitable for curative treatment. Until 2004, the standard treatment for advanced stage NSCLC was platinum based chemotherapy, with modest effects on life expectancy (8).

Over the past two decades, the therapeutic landscape of lung cancer has changed tremendously. The discovery of small-molecule receptor tyrosine kinase inhibitors (TKIs) was regarded as a landmark finding and has brought impressive clinical activity in specific subgroups with oncogenic drivers activated by mutation, translocation or fusion. Molecular analyses to detect oncogenic drivers have become standard in patients with advanced stage disease. The Dutch guideline on NSCLC recommends to test every patient with stage IV adenocarcinoma or never-smokers, regardless their histology, for the following driver alterations: *KRAS*, *EGFR, BRAF, HER2, ALK, MET, ROS1, RET, NTRK1-3* and *NRG1* (9). In the Netherlands, 50% of patients harbors a molecular alteration, mostly KRAS (37.9%), followed by *EGFR* (10.5%) and *BRAF* (4.1%) (10). Unfortunately, only a minority of lung cancer patients harbor a targetable genetic alteration; therefore, most NSCLC patients are not eligible for targeted treatment. This thesis focusses on patients with metastatic NSCLC harboring an *EGFR* mutation.

EGFR mutations

The EGF receptor is a member of the ErbB family, which consists of four related receptor tyrosine kinases: EGFR or HER1 (ErbB-1), HER2neu (ErbB-2), HER3 (ERbB-3) and HER4 (ErbB-4). It is a transmembrane protein receptor activated by binding its specific ligands, including EGF and transforming growth factor-α (11). Binding of EGFR to its ligands results in receptor dimerization followed by autophosphorylation, with leads to downstream activation of the Ras-Raf mitogen-activated protein kinase and the phosphatidyl inositol 3' kinase and Akt pathways. These pathways regulate cell proliferation, angiogenesis and inhibition of apoptosis (12). The physiological activity of the EGF receptor is tightly regulated, otherwise it can lead to uncontrolled cell growth and cancer formation (13).

The epidermal growth factor receptor is frequently overexpressed in common solid tumors and in 50% of NSCLCs (13). This observation made EGFR an interesting target for the development of targeted agents. In May 2003, the EGFR inhibitor gefitinib was approved as third-line therapy for non-small cell lung cancer (14, 15). In general, response rates were low in approximately 10% of patients. However, a small subset of patients showed dramatic, long-lasting responses, independent of EGFR overexpression levels (16, 17). Thereafter, Lynch et al. sequenced the entire coding region of EGFR in tumour samples from patients with a response and found different somatic gain-of-function mutations within the tyrosine kinase domain of EGFR (18). This was the starting point of the discovery of targeted therapy in lung cancer.

The *EGFR* gene is located on chromosome 7 short arm q22 and consists of four domains: an extracellular domain (EGF binding), transmembrane domain and two intracellular domains, the tyrosine kinase and autophosphorylation regions (19). The tyrosine kinase domain can be divided into an N-lobe and a C-lobe, with an ATP-binding site located between the two lobes (19). *EGFR* mutations in NSCLC are almost exclusively clustered within the tyrosine kinase domain of EGFR, and more specifically between exon 18 and 21, around the ATP-binding pocket.

EGFR in-frame deletions in exon 19 are the most prevalent *EGFR* mutations, accounting for 45% of all *EGFR* mutations, followed by *EGFR* L858R missense mutation in exon 21, which is found in 40% of *EGFR* kinase domain mutations. Exon 20 insertions (ex20ins) comprise 4-12% and are the third most common category of *EGFR* mutations found in NSCLC. The remaining *EGFR* mutations consist of a small group of nucleotide substitutions in exon 18 and 20 (13, 20, 21).

Classic *EGFR* mutations

Approximately 85% of *EGFR* mutations comprise the two classic activating *EGFR* mutations: in-frame deletions in exon 19 and L858R substitution mutations in exon 21. Activation of wild-type EGFR is primarily driven by ligand-binding-induced dimerization. Classic *EGFR* mutations hyperactivate the kinase activity of EGFR by stabilizing ligand-independent dimerization with ERBB family receptors (19). Of all *EGFR* mutations, *EGFR* exon 19 deletions exhibit the highest catalytic activity, followed by L858R mutations (22-24). These mutations increase the relative affinity for EGFR TKIs over ATP compared with wild-type EGFR.

Treatment

Two classes of EGFR directed therapies have been developed since 1984. First anti-EGFR monoclonal antibodies (mAb), and in 2003 early generation reversible EGFR TKIs were introduced (25, 26).

The role of anti-EGFR mAb's in the treatment of *EGFR* -mutated NSCLC is limited as they are ineffective as a single agent (27). Anti-EGFR mAb's bind to the extracellular domain of EGFR and block ligand-induced EGFR tyrosine kinase activation by occluding the ligand-binding region (25, 26). Cetuximab was the first anti-EGFR human-to-murine chimeric mAb tested in patients with EGFR overexpression determined by immunohistochemical analysis. Treatment with cetuximab alone was well tolerated. The most frequent adverse events were fever and chills, asthenia, transaminase elevation, nausea, skin toxicities (flushing, dermatitis and acneiform rashes) and allergic reactions (28).

Small-molecule EGFR TKIs like gefitinib and erlotinib, compete reversibly with ATP to bind to the receptor's kinase pocket, which inhibit EGFR autophosphorylation and prevents downstream signaling (26, 29). Gefitinib and erlotinib, two first-generation EGFR TKIs, have shown an improvement of objective response rate (ORR) and progression free survival (PFS) compared to platinum-based chemotherapy and became the standard of care as first-line treatment for advanced *EGFR* mutated NSCLC (30-32). Dose-dependent and reversible diarrhea and acneiform rashes are the most frequent side effects. Thereafter, irreversible second-generation EGFR TKIs (afatinib and dacomitinib) did show a slight clinical improvement compared to first-generation TKIs, however they were associated with higher toxicity rates (33-35). Until the discovery of third generation EGFR TKIs, first- or second generation EGFR TKIs were both used without a clear preference.

Despite significant clinical and durable benefit after treatment with early-generation EGFR TKI's, patients inevitably develop disease progression, mostly driven by acquisition of an exon 20 T790M resistance mutation. This mutation, also referred to as 'gatekeeper' mutation, sterically hinders the binding of early-generation EGFR TKIs to the ATP-binding site of EFGR. Osimertinib, a third generation EGFR TKI, was developed to selectively inhibit both EGFR-TKI-sensitizing and *EGFR* exon 20 T790M resistance mutations while sparing wild-type EGFR (36). As first-line treatment, it also demonstrated a significantly PFS and overall survival (OS) benefit relative to first-generation EGFR TKIs. In addition, osimertinib showed superior activity against brain metastases and has a favorable toxicity profile (37-40). With a median overall survival of more than three years, first-line osimertinib is the preferred option for *EGFR*-positive NSCLC patients at this time (41).

Immune checkpoint inhibitors has revolutionized the treatment of NSCLC, however the efficacy of immunotherapy in *EGFR* -mutated NSCLC appears to be quite limited (42). In addition, the use of immune checkpoint inhibitors prior to or concurrent with osimertinib increases the risk of pneumonitis (43). This underlines the importance of molecular testing at diagnosis to enable personalized treatment.

EGFR exon 20 insertion mutations

EGFR ex20ins mutations comprise approximately 1 to 2% of all NSCLC cases and 4 – 12% of all *EGFR* mutated lung cancers, representing the third most common type of *EGFR* mutations (20, 21, 44, 45).

Similar to the classic *EGFR* mutations, *EGFR* ex20ins are more common among never-smokers and females. The increased incidence among Asian patients is less pronounced. In addition, the ex20ins mutation was more common in older patients compared to *EGFR* exon 19 deletions and L858R mutations (20, 46). The prognosis of patients harboring an *EGFR* ex20ins mutation is worse, compared to patients with common or other uncommon *EGFR* mutations. The median OS ranged from 4.8 – 20 months, similar or slightly better than the *EGFR* wild-type population (46-48).

EGFR ex20ins mutations are a heterogeneous group of activating mutations within the tyrosine kinase domain of EGFR. They represent a combination of in-frame insertions and/or duplications, ranging from three to 21 base pairs, clustered between amino acid positions 762 and 774 of exon 20 (20). This region of exon 20 contains two important regions: the regulatory C-helix (762 – 766) and the adjacent loop that follows it (767-774) (20, 21, 45, 49). To date, more than 60 unique variants are identified, mostly located in the loop following the C-helix (45).

PD-L1 status is negative in approximately 75% of patients with an *EGFR* ex20ins mutation and median tumor mutational burden is low (48, 50). Although little data is available, they seem to derive less benefit from immunotherapy (50).

Treatment

EGFR ex20ins are generally associated with de-novo resistance to early generation EGFR TKIs, except for variant A763_Y764insFQEA (20, 46, 51, 52). Classic *EGFR* mutations directly affect the structure of the ATP-binding pocket and increase the relative affinity for EGFR TKIs over ATP compared with wild-type EGFR. This leads to a large therapeutic window for EGFR TKIs. Targeting *EGFR* ex20ins is challenging because they activate EGFR without diminishing ATP affinity, which makes it difficult to selectively target the ex20ins mutant over wild-type EGFR. In addition, *EGFR* ex20ins mutations induce steric hindrance of the drug-binding pocket by pushing the C-helix into its inward (active state) position, which prevents binding of EGFR TKIs (53). Insertions before residue 764, like the A763_Y764insFQEA variant, do not reveal this stabilized active formation and do not induce drug resistance (21). The role of osimertinib had not yet been established. 3D modeling of the crystal structures of *EGFR* ex20ins showed changes within the drug-binding pocket, similar to T790M (53). Given these similarities, osimertinib could be a treatment option. However, in contrast with T790M mutation, *EGFR* ex20ins mutations are characterized by changes of the a-C helix in an inward, activated position. In addition, 3D modeling showed a shift of the P-loop into the drug-binding pocket, resulting in steric hindrance of the drug-binding pocket from different directions (53). Osimertinib has a large inflexible terminal 1-methylindole group that reduces the binding ability to *EGFR* ex20ins. So based on preclinical data, the role of osimertinib for this patient category was unclear. Overall, platinum-based chemotherapy is to date the most efficacious first-line treatment for patients with an *EGFR* ex20ins mutation (48, 54).

Outline of this thesis

The primary goals of this thesis were to gain insights in primary and secondary resistance mechanisms in patients with *EGFR* mutated NSCLC and to develop new treatment strategies.

The first part of this thesis focuses on a specific subgroup of *EGFR* mutated lung cancer patients, namely patients harboring an *EGFR* ex20ins mutation. We retrospectively and prospectively analyzed the activity of two different treatment regimens for this specific mutation subtype.

The second part focusses on genomic profiling of resistance mechanisms after treatment with a third generation EGFR TKI. In addition, we investigated the activity of combination treatment after cMET driven resistance.

PART I. Targeted treatment of *EGFR* exon 20 insertion mutations

This part of the thesis focuses on finding new targeted treatment options for patients with advanced stage NSCLC harboring an *EGFR* ex20ins mutation. Although the clinical activity of osimertinib for classical *EGFR* mutations is impressive, data about effectiveness of third generation EGFR TKIs for patients harboring an *EGFR* ex20ins mutation were conflicting in pre-clinical studies. Limited clinical data were available. Firstly, we retrospectively collected clinical and molecular data of 21 patients harboring an *EGFR* ex20ins mutation who were treated with osimertinib to gain insight into effectiveness. In **chapter 2**, we described the results of this case series. Almost all patients were treated with osimertinib 80 mg once daily with limited efficacy. Preclinical data showed that the IC50 values of osimertinib for *EGFR* ex20ins mutations were 10 to 100 fold higher compared to classical *EGFR* mutations. We hypothesized that a higher dose of osimertinib might be necessary to effectively treat those patients. In **chapter 3**, we present the results of the POSITION-20 trial, a prospective trial in which 25 patients harboring an *EGFR* exon 20 mutation were treated with high-dose osimertinib in first or second line.

A different strategy to target *EGFR* ex20ins positive NSCLC could be to intensify EGFR blockade by concurrent TKI and EGFR monoclonal antibody treatment. Afatinib is an interesting drug to combine with other EGFR directed therapies like anti-EGFR mAbs, given its broad and irreversible inhibitory profile and low potential for drug interactions (55). First-line afatinib plus cetuximab already showed efficacy in heavily pretreated *EGFR* -mutant NSCLC patients with acquired resistance to first generation EGFR TKIs (56). Dual EGFR blockade with afatinib and cetuximab may also induce tumor responses in *EGFR* ex20ins mutations, based on the treatment outcomes of four *EGFR* exon 20 insertion positive NSCLC patients treated with this combination, presented in **chapter 4**. We tried to validate these results in a dedicated clinical trial. In **chapter 5**, we present the results of a single-arm, phase II trial, where 37 advanced *EGFR* ex20ins positive treatment-naïve or pretreated NSCLC patients were treated with afatinib in combination with cetuximab.

In recent years, several TKIs directed against *EGFR* ex20ins mutations have been developed. However, *EGFR* ex20ins mutated NSCLC cannot be seen as a single disease entity, due to marked heterogeneity in this subgroup. There is a need to expand the knowledge on the structure-function relationship of different *EGFR* ex20ins mutants on drug sensitivity. **Chapter 6** provides a comprehensive assessment of the available literature of pre-clinical and clinical drug sensitivity to currently available TKIs across different *EGFR* ex20ins mutations.

PART II. Analysis an atment of resistance mechanisms after third generation EGFR TKI treatment.

Despite robust activity of osimertinib in first- and second-line settings, eventually acquired resistance occurs. In **chapter 7**, we retrospectively analyzed pre- and post-treatment biopsies in patients who received a third-generation EGFR TKI in second line to gain more insights in innate and acquired resistance mechanisms. Bypass-track mechanisms after third-generation EGFR TKI treatment are most commonly MET amplifications. Little was known about the efficacy of cMET inhibitors in the acquired resistance setting. Finally, in **chapter 8** we retrospectively analyzed the treatment results of patients with acquired cMET amplification after EGFR-directed therapy, who are treated with crizotinib.

References

1. https://www.who.int/news-room/fact-sheets/detail/cancer
2. https://iknl.nl/kankersoorten/longkanker/registratie/overleving
3. Alberg AJ, Brock MV, Ford JG, Samet JM, Spivack SD. Epidemiology of lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2013;143(5 Suppl):e1S-e29S.
4. Couraud S, Zalcman G, Milleron B, Morin F, Souquet PJ. Lung cancer in never smokers--a review. Eur J Cancer. 2012;48(9):1299-311.
5. Subramanian J, Govindan R. Lung cancer in never smokers: a review. J Clin Oncol. 2007;25(5):561-70.
6. Devarakonda S, Li Y, Martins Rodrigues F, Sankararaman S, Kadara H, Goparaju C, et al. Genomic Profiling of Lung Adenocarcinoma in Never-Smokers. J Clin Oncol. 2021;39(33):3747-58.
7. Couraud S, Souquet PJ, Paris C, Dô P, Doubre H, Pichon E, et al. BioCAST/IFCT-1002: epidemiological and molecular features of lung cancer in never-smokers. Eur Respir J. 2015;45(5):1403-14.
8. Chemotherapy in addition to supportive care improves survival in advanced non-small-cell lung cancer: a systematic review and meta-analysis of individual patient data from 16 randomized controlled trials. J Clin Oncol. 2008;26(28):4617-25.
9. Specialists DFoM. Non-Small Cell Lung Cancer - General. https://richtlijnendatabase.nl/richtlijn/niet_kleincellig_longcarcinoom/algemeen.html
10. https://iknl.nl/kankersoorten/longkanker/registratie/incidentie
11. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol. 2001;2(2):127-37.
12. Herbst RS. Review of epidermal growth factor receptor biology. Int J Radiat Oncol Biol Phys. 2004;59(2 Suppl):21-6.
13. Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer. 2007;7(3):169-81.
14. Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected]. J Clin Oncol. 2003;21(12):2237-46.
15. Kris MG, Natale RB, Herbst RS, Lynch TJ, Jr., Prager D, Belani CP, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. Jama. 2003;290(16):2149-58.
16. Giaccone G, Herbst RS, Manegold C, Scagliotti G, Rosell R, Miller V, et al. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial--INTACT 1. J Clin Oncol. 2004;22(5):777-84.
17. Herbst RS, Giaccone G, Schiller JH, Natale RB, Miller V, Manegold C, et al. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial--INTACT 2. J Clin Oncol. 2004;22(5):785-94.
18. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med. 2004;350(21):2129-39.
19. Davies RL, Grosse VA, Kucherlapati R, Bothwell M. Genetic analysis of epidermal growth factor action: assignment of human epidermal growth factor receptor gene to chromosome 7. Proc Natl Acad Sci U S A. 1980;77(7):4188-92.
20. Arcila ME, Nafa K, Chaft JE, Rekhtman N, Lau C, Reva BA, et al. EGFR exon 20 insertion mutations in lung adenocarcinomas: prevalence, molecular heterogeneity, and clinicopathologic characteristics. Mol Cancer Ther. 2013;12(2):220-9.
21. Yasuda H, Park E, Yun CH, Sng NJ, Lucena-Araujo AR, Yeo WL, et al. Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer. Sci Transl Med. 2013;5(216):216ra177.
22. Galdadas I, Carlino L, Ward RA, Hughes SJ, Haider S, Gervasio FL. Structural basis of the effect of activating mutations on the EGF receptor. Elife. 2021;10.
23. Gilmer TM, Cable L, Alligood K, Rusnak D, Spehar G, Gallagher KT, et al. Impact of common epidermal growth factor receptor and HER2 variants on receptor activity and inhibition by lapatinib. Cancer Res. 2008;68(2):571-9.
24. Yun CH, Boggon TJ, Li Y, Woo MS, Greulich H, Meyerson M, et al. Structures of lung cancer-derived EGFR mutants and inhibitor complexes: mechanism of activation and insights into differential inhibitor sensitivity. Cancer Cell. 2007;11(3):217-27.
25. Masui H, Kawamoto T, Sato JD, Wolf B, Sato G, Mendelsohn J. Growth inhibition of human tumor cells in athymic mice by anti-epidermal growth factor receptor monoclonal antibodies. Cancer Res. 1984;44(3):1002-7.
26. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med. 2008;358(11):1160-74.
27. Rossi A, Maione P, Gridelli C. Cetuximab in advanced non-small cell lung cancer. Crit Rev Oncol Hematol. 2006;59(2):139-49.
28. Baselga J, Pfister D, Cooper MR, Cohen R, Burtness B, Bos M, et al. Phase I studies of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin. J Clin Oncol. 2000;18(4):904-14.
29. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer. 2005;5(5):341-54.
30. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med. 2010;362(25):2380-8.
31. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009;361(10):947-57.
32. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol. 2012;13(3):239-46.
33. Park K, Tan EH, O'Byrne K, Zhang L, Boyer M, Mok T, et al. Afatinib versus gefitinib as first-line treatment of patients with EGFR mutation-positive non-small-cell lung

cancer (LUX-Lung 7): a phase 2B, open-label, randomised controlled trial. Lancet Oncol. 2016;17(5):577-89.
34. Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol. 2013;31(27):3327-34.
35. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. Lancet Oncol. 2014;15(2):213-22.
36. Cross DA, Ashton SE, Ghiorghiu S, Eberlein C, Nebhan CA, Spitzler PJ, et al. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. Cancer Discov. 2014;4(9):1046-61.
37. Mok TS, Wu YL, Ahn MJ, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive Lung Cancer. N Engl J Med. 2017;376(7):629-40.
38. Ramalingam SS, Vansteenkiste J, Planchard D, Cho BC, Gray JE, Ohe Y, et al. Overall Survival with Osimertinib in Untreated, EGFR-Mutated Advanced NSCLC. N Engl J Med. 2020;382(1):41-50.
39. Reungwetwattana T, Nakagawa K, Cho BC, Cobo M, Cho EK, Bertolini A, et al. CNS Response to Osimertinib Versus Standard Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Patients With Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. J Clin Oncol. 2018:Jco2018783118.
40. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. N Engl J Med. 2018;378(2):113-25.
41. Planchard D, Popat S, Kerr K, Novello S, Smit EF, Faivre-Finn C, et al. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2018;29 Suppl 4:iv192-iv237.
42. Addeo A, Passaro A, Malapelle U, Banna GL, Subbiah V, Friedlaender A. Immunotherapy in non-small cell lung cancer harbouring driver mutations. Cancer Treatment Reviews. 2021;96:102179.
43. Schoenfeld AJ, Arbour KC, Rizvi H, Iqbal AN, Gadgeel SM, Girshman J, et al. Severe immune-related adverse events are common with sequential PD-(L)1 blockade and osimertinib. Ann Oncol. 2019;30(5):839-44.
44. Beau-Faller M, Prim N, Ruppert AM, Nanni-Metéllus I, Lacave R, Lacroix L, et al. Rare EGFR exon 18 and exon 20 mutations in non-small-cell lung cancer on 10 117 patients: a multicentre observational study by the French ERMETIC-IFCT network. Ann Oncol. 2014;25(1):126-31.
45. Riess JW, Gandara DR, Frampton GM, Madison R, Peled N, Bufill JA, et al. Diverse EGFR Exon 20 Insertions and Co-Occurring Molecular Alterations Identified by Comprehensive Genomic Profiling of NSCLC. J Thorac Oncol. 2018;13(10):1560-8.
46. Burnett H, Emich H, Carroll C, Stapleton N, Mahadevia P, Li T. Epidemiological and clinical burden of EGFR Exon 20 insertion in advanced non-small cell lung cancer: A systematic literature review. PLoS One. 2021;16(3):e0247620.
47. Lo P, Jackman D, Butaney M, Lindeman N, Johnson B, Jaenne P, et al. Clinical Behavior Of Lung Cancers Harboring EGFR Exon 20 Insertions. Journal of Thoracic Oncology. 2012;7:S206-S7.
48. Choudhury NJ, Schoenfeld AJ, Flynn J, Falcon CJ, Rizvi H, Rudin CM, et al. Response to Standard Therapies and Comprehensive Genomic Analysis for Patients with Lung Adenocarcinoma with EGFR Exon 20 Insertions. Clin Cancer Res. 2021;27(10):2920-7.
49. Meador CB, Sequist LV, Piotrowska Z. Targeting EGFR Exon 20 Insertions in Non-Small Cell Lung Cancer: Recent Advances and Clinical Updates. Cancer Discov. 2021;11(9):2145-57.
50. Geng D, Guo Q, Huang S, Zhang H, Guo S, Li X. Clinical and molecular characteristics of epidermal growth factor receptor exon 20 insertion mutations in non-small-cell lung cancer. Clin Transl Oncol. 2022;24(2):379-87.
51. Naidoo J, Sima CS, Rodriguez K, Busby N, Nafa K, Ladanyi M, et al. Epidermal growth factor receptor exon 20 insertions in advanced lung adenocarcinomas: Clinical outcomes and response to erlotinib. Cancer. 2015;121(18):3212-20.
52. Yang JC, Sequist LV, Geater SL, Tsai CM, Mok TS, Schuler M, et al. Clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations: a combined post-hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. Lancet Oncol. 2015;16(7):830-8.
53. Robichaux JP, Elamin YY, Tan Z, Carter BW, Zhang S, Liu S, et al. Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer. Nat Med. 2018;24(5):638-46.
54. Yang G, Li J, Xu H, Yang Y, Yang L, Xu F, et al. EGFR exon 20 insertion mutations in Chinese advanced non-small cell lung cancer patients: Molecular heterogeneity and treatment outcome from nationwide real-world study. Lung Cancer. 2020;145:186-94.
55. Solca F, Dahl G, Zoephel A, Bader G, Sanderson M, Klein C, et al. Target binding properties and cellular activity of afatinib (BIBW 2992), an irreversible ErbB family blocker. J Pharmacol Exp Ther. 2012;343(2):342-50.
56. Janjigian YY, Smit EF, Groen HJ, Horn L, Gettinger S, Camidge DR, et al. Dual inhibition of EGFR with afatinib and cetuximab in kinase inhibitor-resistant EGFR-mutant lung cancer with and without T790M mutations. Cancer Discov. 2014;4(9):1036-45.

Part I

Targeted treatment of *EGFR* exon 20 insertion mutations

Chapter 2

Osimertinib treatment for patients with *EGFR* exon 20 mutation positive non-small cell lung cancer

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ABSTRACT

Objectives: **Epidermal growth factor receptor (*EGFR*) exon 20 insertions comprise 4-10 % of *EGFR* mutations in non-small cell lung cancer (NSCLC) and are associated with primary resistance to first and second generation *EGFR* tyrosine kinase inhibitors (TKIs). *In vitro* and preclinical animal studies have shown that osimertinib exerts antitumor activity against *EGFR* exon 20 mutation positive NSCLC. We report on a cohort of advanced stage NSCLC patients who harbor an *EGFR* exon 20 mutation and received osimertinib treatment.**

Material and methods: **Twenty-one patients were treated with osimertinib 80 or 160 mg once daily from April 2016 to June 2018, in four institutions in the Netherlands. Data were obtained retrospectively. Progression free survival (PFS), disease control rate (DCR), overall survival (OS) and objective response rate (ORR) were assessed using RECIST v1.1.**

Results: **Thirteen patients received prior platinum-based chemotherapy, and three patients a first - or second generation *EGFR* TKI. We observed 1 partial response, 17 patients with stable disease and 3 with progressive disease as best response to osimertinib (ORR 5 %). Median PFS was 3.6 (95 % CI, 2.6-4.5) months. PFS did not differ for patients with co-occurring TP53 mutations (p = 0. 937). The DCR at three months was 71 %. Median OS was 8.7 (95 % CI, 1.1-16.4) months.**

Conclusion: **Osimertinib has limited antitumor activity in patients with *EGFR* exon 20 mutated NSCLC, with an ORR of 5 %.**

1. Introduction

Epidermal growth factor receptor *(EGFR)* exon 20 insertion mutations are identified as a subset (4–12 %) of *EGFR* mutation-positive non-small cell lung cancer (NSCLC) and are the third most common category of *EGFR* activating mutations [1-3]. *EGFR* exon 20 insertion mutations are heterogeneous at the molecular level but can be characterized as in frame duplications (dup) or insertions (ins), or deletion/insertion (delins) mutations. *EGFR* exon 20 insertions are *EGFR* driver mutations that exhibit intrinsic resistance to first and second generation *EGFR* tyrosine kinase inhibitors (TKIs) with overall response rates of only 3-8 % [1,3]. In contrast to the common *EGFR* exon-19 deletion and L858R point mutations, *EGFR* exon 20 insertion mutations alter the a-C helix resulting in steric hindrance of the drug-binding pocket, that prevents binding of first and second generation EGFR TKIs [3,4]. Given the limited activity of first and second generation EGFR TKIs, new treatment options are needed to overcome primary resistance of *EGFR* directed treatment for tumors harboring an *EGFR* exon 20 insertion. Data about effectiveness of third generation EGFR TKIs are conflicting in preclinical studies. *In vivo, in vitro* and 3D modeling by Robichaux et al. showed that osimertinib lacks activity against cell lines harboring an *EGFR* exon 20 insertion, due to steric hindrance as a result of significant changes within the drug-binding pocket [4]. In contrast, a study by Floc'h et al. described anti-tumor activity of osimertinib across xenograft models, representing a variety of *EGFR* exon 20 insertions [5], in line with a previous report presenting *in vitro* activity of osimertinib in exon 20 insertion mutant cell lines [6]. Limited clinical data are available. Two cases with a clinical response to osimertinib have been published [7,8]. Recently, Fang et al. treated six Chinese patients with an *EGFR* exon 20 insertion mutation with osimertinib. This resulted in a median progression free survival (PFS) of 6.2 months [9]. Here, we report on a cohort of advanced stage *EGFR* exon 20 mutation positive NSCLC patients that were treated with osimertinib.

2. Material and methods

Twenty-one patients with advanced stage NSCLC harboring an *EGFR* exon 20 mutation were treated with osimertinib in four institutions in the Netherlands (Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital, University Medical Center Groningen, Amsterdam UMC and Erasmus Medical Center). Data were obtained retrospectively. We started a search by identifying all patients treated with osimertinib from April 2016 to June 2018 in the four institutions. Then we selected the patients with an *EGFR* exon 20 mutation. Patients data were collected by searching electronic medical record databases. Histological or cytological tumor samples from all patients were tested for at least *EGFR* and KRAS mutation status. For 8 patients high resolution melting followed by Sanger sequencing was used to test for *EGFR* and KRAS mutations, while next-generation sequencing (NGS) with different panels was used to test for at the following mutations: BRAF, *EGFR*, HER2, KRAS, MET, PIK3CA. Patients were treated with commercially available osimertinib 80 mg or 160 mg once daily on an off-label basis. Response evaluation was performed every six to eight weeks, according to local practice. PFS, overall survival (OS), disease control rate (DCR) and objective response rate (ORR) were retrospectively assessed by the study team using Response Criteria in Solid Tumors (RECIST) version 1.1. PFS was defined as the interval between start of osimertinib treatment and radiological progression or death, whichever came first. PFS and OS data were calculated using Kaplan Meier survival plots together with the median PFS or OS time and corresponding 95 % confidence intervals (CIs). P values for subgroups were calculated using the log rank test.

Table 1
Patient and tumor characteristics.

	Number	Percentage (%)
Age (years)	63	Range 38-82
Women	14	67
Histology		
Adenocarcinoma	20	95
Large cell neuroendocrine carcinoma	1	5
Tumor samples		
Histology	19	90
Cytology	2	10
Mutation type		
EGFR exon 20 insertion/duplication	17	81
EGFR exon 20 (bi/tri) nucleotide substitutions	4	19
Number of lines for advanced disease		
0	6	28.5
1	7	33.5
2	6	28.5
3	2	9.5
Prior platinum based chemotherapy	13	62
Prior TKI		
First generation	1	5
Second generation	2	10
Brain metastases at baseline	11	52

EGFR: epidermal growth factor receptor; TKI: tyrosine kinase inhibitor.

Table 2
Response to treatment and co-occurring mutations.

Patient	Mutation type	Best response	Best change from baseline according RECIST (%)	PFS (months)	Co-mutations
1	p.(His773_Val774delinsLeuMet)	PR	−38	8.3	TP53
2	p.(His773delinsTyrAsnProTyr)	SD	−26	3.6	TP53; PTEN
3	p.(Asn771delinsThrHis)	PD	−22	1.2	None
4	p.(His773_Val774insAlaHis)	SD	−21	6.5	N/A
5	p.(Gly779Phe)	SD	−19	2.7	TP53; EGFR amplification
6	p.(Ala767_Val769dup)	SD	−17	8.3	N/A
7	p.(Asn771_His773dup)	SD	−16	17.3	TP53
8	p.(Ser768_Asp770dup)	SD	−12	3.8	N/A
9	p.(Asn771_Pro772insHis)	SD	−11	11.4	TP53
10	p.(Asn771_Pro772insArgHis)	SD	−9	9.3	None
11	p.(Asn771delinsGlyTyr)	SD	−7	12.6	N/A
12	p.(Asn771_His773dup)	SD	−5	7.9	N/A
13	p.(Ser768_Val769delinsIleLeu)	SD	−5	2.6	N/A
14	p.(Ala767_Val769dup)	SD	−4	4.0	EGFR amplification
15	p.(His773_Val774delinsLeuMet)	SD	0	3.2	N/A
16	p.(His773_Val774insAlaHis)	SD	0	1.7	TP53
17	p.(Val769_Asp770insGlyGly)	SD	+6	3.7	Notch1
18	p.(Asn771delinsGlyHis)	SD	+12	3.0	None
19	p.(Val769_Asp770insSerPheLeu)	PD	+17	1.7	N/A
20	p.(Ala767_Val769dup)	PD	NE	3.1	TP53; CDKN2A
21	p.(Ala767_Val769dup)	PD	NE	0.7	TP53

NE: non-evaluable; N/A: not applicable; PD: progressive disease; PFS: progression free survival; PR: partial response; RECIST: Response Evaluation Criteria in Solid Tumors; SD: stable disease.

3. Results

3.1 Patient and tumor characteristics

Patient and tumor characteristics are summarized in Table 1. Median age was 63 years (range 38-82), 67 % of patients were female and median number of prior systemic treatments was 1 (range 0-3). Thirteen patients (62 %) received prior platinum-based chemotherapy. Three patients were treated with a first or second generation EGFR TKI, erlotinib (n = 1) or afatinib (n = 2); one patient experienced stable disease (SD) for 11 months after having received afatinib, while the other two patients had progression as best response. One patient was treated with luminespib (HSP-90 inhibitor) for nineteen months. Median time on the previous line of systemic treatment prior to osimertinib treatment was

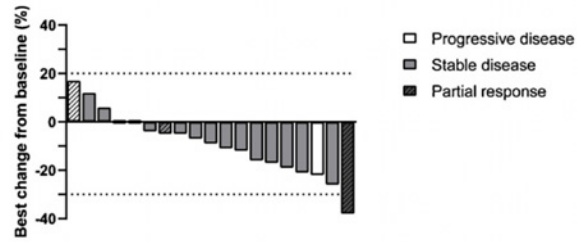


Fig. 1. Waterfall plot of best percentage change in tumor size during osimertinib treatment.
Two patients were non-evaluable according to RECIST version 1.1.
The patients who received a prior TKI are shaded with stripes.

4 months (95 % confidence interval [CI], 3.0-12.0). Eleven (52 %) of the treated patients had brain metastases at baseline. Cranial irradiation (whole brain radiotherapy (n = 1) or stereotactic radiotherapy (n = 2) was performed in three patients prior to starting osimertinib.

Response to osimertinib treatment and mutation findings are summarized in Table 2. All exon 20 mutations were clustered between Ala767 and Gly779. The most common mutation was p. Ala767_ Val769dup (n = 4). No patients with the known sensitizing *EGFR* exon 20 insertion variant A763_Y764insFQEA were included in this cohort. Data about co-occurring genetic alterations and copy number variations were available for 13 patients (62 %). Tumor protein p53 *(TP53)* mutations were the most common type of co-occurring mutations detected (n = 8; 62 %), followed by other less common alterations in cyclin dependent kinase inhibitor 2A *(CDKN2A),* phosphatase and tensin homolog *(PTEN),* notch 1 gene *(NOTCHI)* and erb-b2 receptor tyrosine kinase 4 *(ERBB4).* In two patients (15 %) *EGFR* amplification was detected.

3.2. Response to osimertinib treatment

Patients were treated with osimertinib 80 mg (n = 20) or 160 mg (n = 1) once daily. Osimertinib treatment was well tolerated with none of the patients requiring dose modification. Two patients experienced grade I nausea and three patients reported grade I skin toxicity. We observed one (5 %) partial response (PR), sixteen (76 %) patients with SD and four (19 %) with progressive disease (PD) as best response (Fig. 1). Median PFS was 3.6 (95 % CI, 2.6-4.5) months. The DCR at three months was 71 % (n = 15) (Fig. 2). After five months, eight patients (38 %) were still on osimertinib treatment. In 14 patients (67 %), osimertinib treatment resulted in tumor regression (range: -4 to -38 percent from baseline according to RECIST v1.1 [Fig. 1]). Two patients were not evaluable due to the absence of measurable lesions. Median OS was 8.7 (95 % CI, 1.1-16.4) months. Patients with co-occurring *TP53* mutations did not appear to fare better or worse than those without in terms of PFS (p = 0.937) or OS (p = 0.107). Three patients had brain metastases that showed a decrease in lesional size, including the patient with a partial response. There were no differences in PFS between patients with or without brain metastases; 3.4 and 3.6 months (95 % CI, 2.6-4.5; p = 0.740), respectively.

One patient, who progressed after 9.3 months osimertinib treatment, subsequently received carboplatin gemcitabine. After progression, a higher dose of osimertinib (160 mg once daily) was re-introduced, with stable disease after six weeks and progression after three months of treatment.

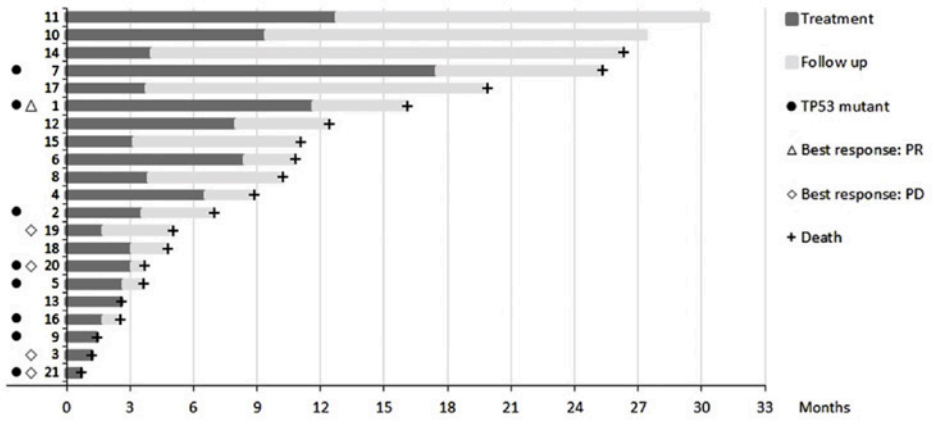


Fig. 2. Swimmerplot of progression-free survival and overall survival after osimertinib treatment.

A post-progression biopsy was available for the patient with a PR after osimertinib treatment. Besides the activating *EGFR* exon 20 mutation this biopsy revealed high *cMET* amplification, a known resistance mechanism to osimertinib treatment, with 34 copies of *cMET,* as assessed by whole genome sequencing. NGS at baseline, prior to platinum based chemotherapy and (unsuccessful) erlotinb treatment, did not show an increased *cMET* copy number.

4. Discussion

EGFR exon 20 insertions represent a heterogeneous group of genomic aberrations with an unmet clinical need in precision oncology. *EGFR* exon 20 insertions after residue 764 are resistant to first and second generation EGFR TKIs [1]. Data about effectiveness of third generation EGFR TKIs are limited. Our series describes the results of osimertinib treatment, a third generation EGFR TKI, in 21 patients with advanced stage NSCLC harboring an *EGFR* exon 20 mutation. Despite the low response rate of 5 %, 14 of 21 patients (67 %) experienced a decrease in tumor size (Fig. 1). The DCR at three months was 71 %. In addition, a subgroup of patients (38 %) derived durable disease stabilization of five months and beyond. However, the median PFS of the treatment line prior to osimertinib was 4 months, so it is questionable if this represents osimertinib efficacy or natural evolution of the disease. Recently, a series of six Chinese patients with an *EGFR* exon 20 insertion mutation, treated with osimertinib 80 mg once daily was published. Four patients achieved a PR (including one patient with a known sensitizing variant A763_Y764insFQEA in combination with a *T790M* mutation after gefitinib treatment) and the other two patients showed stable disease as best response [9]. One patient with a PR harbored the same *EGFR* exon 20 mutation (p.A767_V769dup) as a patient in our cohort with SD as best response.

Together, the results of these two case series show that osimertinib has limited efficacy in patients with *EGFR* exon 20 insertion mutations with sized based tumor responses in the majority of patients. However, the results of osimertinib treatment do not even approach those that can be obtained for patients with *EGFR* exon 19 deletion and exon 21 L858R mutations.

Our results show that patients with exactly the same insertion mutation can have different treatment outcomes. The potential role of co-occurring aberrations like *TP53* during EGFR TKI treatment is not well characterized [10]. In this small series, there was no association between reduction in efficacy of osimertinib treatment and co-occurring *TP53* mutations. Analysis of the post-progression biopsy of the only patient with a PR after osimertinib treatment, revealed *cMET* amplification, suggestive of *cMET* bypass track resistance, a known resistance mechanism to EGFR TKIs.

The dose of osimertinib might be a relevant factor in the treatment of patients with an *EGFR* exon 20 insertion mutation. Pharmacokinetic analysis revealed that geometric mean plasma concentrations for osimertinib doses of 80 mg and 160 mg once daily are around 500 nM and 1000 nM, respectively [11]. Hirano et al. used *in vitro* models to identify the therapeutic window of osimertinib for *EGFR* exon 20 alterations. The IC50 values of osimertinib for the tested *EGFR* exon 20 insertion mutations were 10-100 fold higher compared to classic *EGFR* mutations. Thus, a higher dose of osimertinib greater than an 80 mg oral dose may be necessary to effectively treat patients with *EGFR* exon 20 mutations [6]. In our experience, one patient received osimertinib 160 mg after having progressed on osimertinib 80 mg and another patient, included in this cohort, started with osimertinib 160 mg. Neither of the two patients showed a radiological response.

Currently, two phase 2 trials are active to investigate the efficacy of this higher dose (NCT03191149 and ESR-16-12212). Also, the results of a prospective phase II study with 80 mg osimertinib are awaited (NCT03414814).

In addition, multiple clinical trials are pending with EGFR TKIs that were specifically designed to target *EGFR* and *HER2* exon 20 insertion mutations. The clinical activity of poziotinib was evaluated in a single arm phase 2 trial with an ORR of 43 %. Poziotinib is a small and flexible molecule that can circumvent the steric changes of *EGFR* exon 20 insertions. However, toxicity was substantial with 60 % grade ≥3 toxicity, mainly skin toxicity and diarrhea. Despite the high response rate, median PFS was 5.5 months [12]. A phase I/II trial with another EGFR TKI, TAK-788, showed a 43 % confirmed ORR and 7.3 months median PFS. Toxicity profiling showed 40 % treatment related grade ≥3 toxicity, mainly diarrhea and nausea [13]. The combination of afatinib and cetuximab resulted in a partial response in 3 out of 4 patients and a median PFS of 5.4 months in a retrospective case series [14]. This combination treatment is being prospectively evaluated in a single arm phase two trial (NCT03727724).

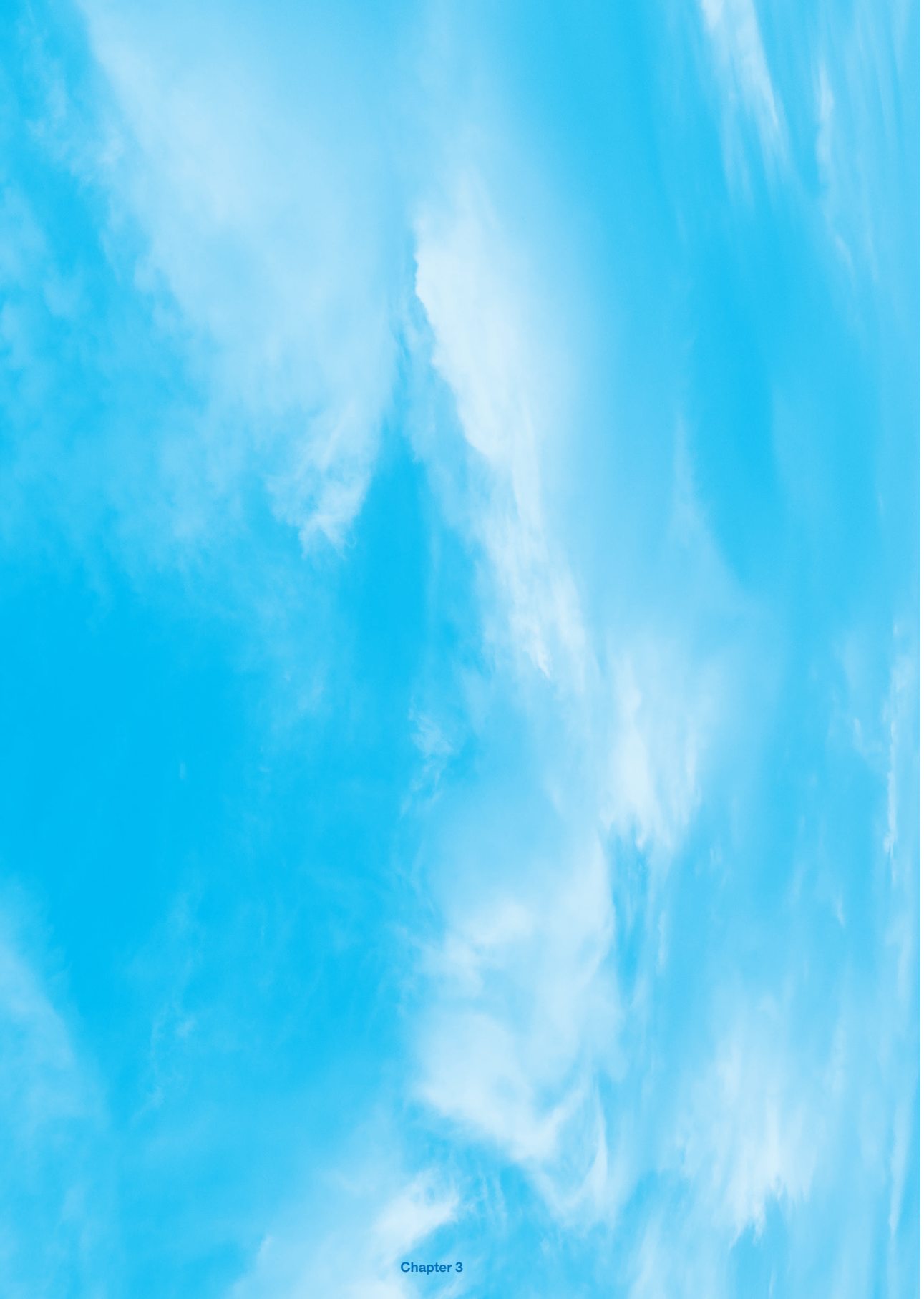
Our study has several limitations. At first hand, because of the retrospective nature of this study. Data about co-occurring genetic alterations were available for only 62 % of patients. Also, the sample size of our cohort is relatively small. However, to our knowledge this is the largest case series of patients with *EGFR* exon 20 mutation positive NSCLC, treated with osimertinib.

5. Conclusion

In conclusion, our study shows that osimertinib 80 mg once daily has limited antitumor activity in patients with *EGFR* exon 20 mutated NSCLC, with an ORR of 5 %.

References

1. H. Yasuda, S. Kobayashi, D.B. Costa, EGFR exon 20 insertion mutations in non small-cell lung cancer: preclinical data and clinical implications, Lancet Oncol. 13 (1) (2012) e23-31.
2. J.W. Riess, D.R. Gandara, G.M. Frampton, R. Madison, N. Peled, J.A. Bufill, et al., Diverse EGFR Exon 20 Insertions and Co-Occurring Molecular Alterations Identified by Comprehensive Genomic Profiling of NSCLC, J. Thorac. Oncol. 13 (10) (2018) 1560-1568.
3. H. Yasuda, E. Park, C.H. Yun, N.J. Sng, A.R. Lucena-Araujo, W.L. Yeo, et al., Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer, Sci. Trans!. Med. 5 (216) (2013) 216ra177.
4. J.P. Robichaux, Y.Y. Elamin, Z. Tan, B.W. Carter, S. Zhang, S. Liu, et al., Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer, Nat. Med. 24 (5) (2018) 638–646.
5. N. Floc'h, M.J. Martin, J.W. Riess, J.P. Orme, A.D. Staniszewska, L. Menard, et al., Antiturnor activity of Osimertinib, an irreversible mutant-selective EGFR tyrosine kinase inhibitor, in NSCLC harboring EGFR exon 20 insertions, Mo!. Cancer Ther. 17 (5) (2018) 885-896.
6. T. Hirano, H. Yasuda, T. Tani, J. Hamamoto, A. Oashi, K. Ishioka, et al., In vitro modeling to determine mutation specificity of EGFR tyrosine kinase inhibitors against clinically relevant EGFR mutants in non-small-cell lung cancer, Oncotarget. 6 (36) (2015) 38789-38803.
7. Z. Piotrowska, F.J. Fintelmann, L V. Sequist, B. Jahagirdar, Response to Osimertinib in an EGFR exon 20 insertion-positive lung adenocarcinoma, J. Thorac. Oncol. 13 (10) (2018) e204---e206.
8. S. Byeon, Y. Kim, S.W. Lim, J.H. Cho, S. Park, J. Lee, et al., Clinical outcomes of EGFR exon 20 insertion mutations in advanced non-small cell lung Cancer in Korea, Cancer Res. Treat. 51 (2) (2019) 623-631.
9. W. Fang, Y. Huang, S. Hong, Z. Zhang, M. Wang, J. Gan, et al., EGFR exon 20 insertion mutations and response to osimertinib in non-small-cell lung cancer, BMC Cancer 19 (1) (2019) 595.
10. M. Canale, E. Petracci, A. Delmonte, E. Chiadini, C. Dazzi, M. Papi, et al., Impact of TP53 mutations on outcome in EGFR-Mutated patients treated with first-line tyrosine kinase inhibitors, Oin. Cancer Res. 23 (9) (2017) 2195-2202.
11. D. Planchard, K.H. Brown, D.W. Kim, S.W. Kim, Y. Ohe, E. Felip, et al., Osimertinib Western and Asian clinical pharmacokinetics in patients and healthy volunteers: implications for formulation, dose, and dosing frequency in pivotal clinical studies, Cancer Chemother. Pharmacol. 77 (4) (2016) 767-776.
12. J. Heymach, M. Negrao, J. Robichaux, et al., OA02.06 A Phase II Trial of Poziotinib in EGFR and HER2 exon 20 Mutant Non-Small Cell Lung Cancer (NSCLC), J. Thorac. Oneal. 13 (2018) S323-S324.
13. P.A. Janne, J.W. Neal, D.R. Camidge, A.I. Spira, Z. Piotrowska, L. Hom, et al., Antitumor activity of TAK-788 in NSCLC with EGFR exon 20 insertions, J. Clin. Oneal. 37 (15_suppl) (2019) 9007.
14. B. van Veggel, A.J. de Langen, S.M.S. Hashemi, K. Monkhorst, D.A.M. Heideman, E. Thunnissen, et al., Afatinib and Cetuximab in Four Patients With EGFR Exon 20 Insertion-Positive Advanced NSCLC, J. Thorac. Oncol. 13 (8) (2018) 1222-1226



Chapter 3

High dose osimertinib in patients with advanced stage *EGFR* exon 20 mutation-positive NSCLC: Results from the phase 2 multicenter POSITION20 trial

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ABSTRACT

Introduction: **Patients with life-threatening advanced non-small cell lung cancer (NSCLC) who harbor an exon 20 deletion and/or insertion mutation (EGFRex20 +) have limited effective treatment options. The high dose 3rd generation tyrosine kinase inhibitor (TKI) osimertinib shows promising *in vitro* activity in EGFRex20 + NSCLC tumors.**

Methods: **The POSITION20 is a single arm phase II, multicenter study investigating 160 mg osimertinib in patients with EGFRex20+, T790M negative NSCLC. We allowed patients to be treatment naïve and to have asymptomatic brain metastases. The primary endpoint was overall response rate (ORR). Secondary outcomes were duration of response (DoR), progression free survival (PFS), overall survival (OS), and treatment related adverse events (trAEs).**

Results: **From June 2018 to October 2021, 25 patients were enrolled across five centers in the Netherlands. The median age was 70 years (range, 47–87), 20 patients (80%) were women, and the median number of previous lines of therapy was 1 (range, 0–3). The exon 20 mutations were clustered between A763 and L777. The most common exon 20 mutations were p.(N771_H773dup) (n = 3) and p.(A767_V769dup) (n = 3). The ORR was 28% (95% CI, 12–49%), including seven partial responses, with a median DoR of 5.3 months (range, 2.7–27.6). The median PFS was 6.8 months (95% CI, 4.6–9.1) and the median OS was 15.2 months (95% CI, 14.3–16.0). The most common trAEs were diarrhea (72%), dry skin (44%), and fatigue (44%). The primary reason for discontinuation was progressive disease in 14 patients (56%).**

Conclusion: **The POSITION20 study showed modest antitumor activity in patients with EGFRex20 + NSCLC treated with 160 mg osimertinib, with a confirmed ORR of 28% and acceptable toxicity.**

1. Introduction

The treatment landscape of metastatic non-small cell lung cancer (NSCLC) changed radically in recent years, due to the identification of activating mutations within the tyrosine kinase domain of the epidermal growth factor receptor (*EGFR*) [1,2]. Patients with NSCLC with common *EGFR* mutations (exon 19 and 21, accountable for approximately 90% of all detected *EGFR* mutations) benefit from treatment with EGFR tyrosine kinase inhibitors (EGFR-TKIs) [3–5]. The third most common subset (4–12%) of *EGFR* activating mutations in patients with NSCLC are exon 20 deletion and/or insertion (EGFRex20 +) mutations [6,7]. Patients with NSCLC harboring an EGFRex20 + mutation lack clinical benefit to current 1st and 2nd generation EGFR-TKIs, with the general exception of the sensitive insertions in the αC-helix (for example, A763insFQEA) [8–10].

The inability to clinical benefit is most likely caused by the three-dimensional structure of the current EGFR-TKIs and the influence of the EGFRex20 + mutation on the kinetics of drug and ATP binding, which determines the resistance or sensitivity to *EGFR* inhibitors [11–13]. Therefore, new treatment options are needed to overcome the primary resistance of the EGFRex20 + mutation. Osimertinib is an oral, third-generation, irreversible EGFR-TKI approved for both classical- and *EGFR* T790M resistant mutations [14–18]. To date, the evidence regarding efficacy of osimertinib 80 mg daily in patients with EGFRex20 + NSCLC is disappointing [19–23,49] (see Supplementary Table 1, showing the efficacy of osimertinib in several trials). The results of the different studies showed that patients with EGFRex20 + NSCLC are heterogenous in their response to osimertinib 80 mg, in which patients harboring the A763_Y764insFQEA variant in the αC-helix showed better response in comparison to other near- and far loop EGFRex20 + mutations [24].

Higher dosage of osimertinib could be beneficial, as *in vitro* data hints [13,25]. A pharmacokinetic analysis revealed that geometric mean plasma concentrations for multiple dosing of osimertinib with daily 80 mg and 160 mg were around 500 nM and 1000 nM [25]. Thus, a higher dose of osimertinib will be necessary to effectively treat patients with EGFRex20 + NSCLC. In addition to preclinical research, the phase II ECOG-ACRIN 5162 trial assessed osimertinib 160 mg once daily in 20 patients with EGFRex20 + NSCLC who previously received at least one line of treatment and had Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–1. The study showed an objective response rate (ORR) of 24% and a disease control rate (DCR) of 85% [26], in which the responders all harbored an EGFRex20 + mutation in the loop following C-helix (see Supplementary Table 2, showing the efficacy of osimertinib 160 mg for 21 patients with NSCLC EGFRex20 + within the study of Piotrowska et al., 2020). However, the ECOG-ACRIN 5162 trial does not provide evidence spanning the entire spectrum of exon 20 mutations (excluding the αC-helix), and patients were always pre-treated. In addition, we describe the results of the POSITION20 trial assessing the antitumor activity of osimertinib 160 mg in patients with EGFRex20 + NSCLC as first or second line treatment.

2. Material and methods

2.1. Study design and patients

The POSITION20 study was a single arm, phase II, multicenter study conducted in five institutes in the Netherlands. The study was approved by all medical research ethics committees at each site and informed consent was obtained from all patients according to the Declaration of Helsinki, prior to the study [27]. The study was listed in the Netherlands

trial register (trialregister.nl), NL6705. AstraZeneca provided funding for and access to the study medication osimertinib. The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding- and first author had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

General inclusion criteria included ≥ 18 years of age, ECOG PS 0–2 and being diagnosed with advanced NSCLC, harboring an EGFRex20 + mutation (deletion and/or insertion), T790M negative. Histological or cytological tumor samples from all patients were locally tested using next-generation sequencing (NGS) (e.g. single-molecule molecular inversion probes [smMIP] and Ion Torrent). We allowed patients to be pre-treated (chemo- or immunotherapy, multiple lines of treatment were allowed) and to have asymptomatic brain metastases. Patients pre-treated with other generation (EGFR-)TKIs or EGFR-MET bispecific antibody were excluded.

2.2. Procedure

Patients were treated with a double dosage of osimertinib 160 mg orally once daily until progression or unacceptable toxicity. The treatment started within 28 days of screening enrolment. Follow-up was done after 2 and 6 weeks, and thereafter every 6 weeks. Patients were monitored for ECG machine-derived QTc value changes at baseline, after 2 and 6 weeks, and further on indication in case of an abnormal QTc value.

The primary endpoint of this study was efficacy of osimertinib defined as ORR by investigator assessment using RECIST 1.1 [28]. Disease assessment using computed tomography (CT) and/or magnetic resonance imaging (MRI) was performed at baseline and every 6 weeks, until week 24. Thereafter, during the treatment phase every 12 weeks, until disease progression, discontinuation or withdrawal from the study. Brain imaging was not mandated at baseline, and only (subsequently) performed during the study at the discretion of the investigator when clinically indicated. Palliative radiotherapy to control symptoms (including gamma knife technique), e.g. to control brain disease, was permitted. Treatment beyond progression was allowed by investigators judgment in case of persistent clinical benefit. Secondary outcomes were treatment efficacy observed by duration of response (DoR), progression free survival (PFS), overall survival (OS) [28], clinical benefit rate (CBR), and safety endpoints defined as treatment related adverse events (trAEs). The CBR was defined as the proportion of patients with a complete or partial response (PR) or with durable stable disease (SD) ≥ week 24. Adverse events were reported and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.0 (CTCAE) [29].

2.3. Statistical analysis

Descriptive statistics – median (IQR), n (%) – were used to describe patient and disease baseline characteristics. The association between characteristics and survival outcome was univariately assessed using a stratified Cox regression model. If possible, variables with a p-value ≤ 0.15 in the univariate analyses were analyzed in a multivariable stratified Cox regression model. The median PFS and median OS were estimated using the Kaplan–Meier method.

The trial aimed to show a confirmed ORR of 30%, adopting Simon's optimal two-stage design with a predefined one-sided alpha of 5% and a power of 80%. Twenty-five patients were planned to be recruited in total, if at least one patient showed a response in the first fifteen treated patients. Else, the study would be terminated. The ORR was estimated according to Clopper-Pearson. The Barnard's test was used to compare different ORR proportions. The efficacy data cutoff for the analyses was October 1, 2021.

3. Results

3.1. Patient characteristics

From August 2018 to August 2021, 29 patients were screened of whom 25 patients were enrolled and treated with 160 mg osimertinib once daily. The median follow-up time was 11.5 months (range, 0.8–32.0). The median age was 70 years (range, 47–87), 20 patients (80%) were woman and four patients (6%) never smoked (Table 1). Three patients

Table 1
Patient characteristics at baseline.

Characteristic	Efficacy population (N = 25)
Gender	
Male	5 (20%)
Female	20 (80%)
Age (median, range)	70 (47 – 87)
Ethnicity	
White	25 (100%)
Histology	
Adenocarcinoma	25 (100%)
Tumor samples	
Histology	20 (80%)
Cytology	5 (20%)
Smoking status	
Never	4 (6%)
Former	16 (64%)
Current	5 (20%)
ECOG performance status	
0	9 (36%)
1	15 (60%)
2	1 (4%)
Baseline brain metastases (asymptomatic)	3 (14%)
Prior lines of therapy (median, range)	1 (0 – 3)
Prior therapy	
Chemotherapy	10 (40%)
Chemo-immunotherapy	2 (8%)
Chemotherapy – TKI	1 (4%)
None	12 (48%)
EGFR exon20 mutation variants	p.(A767_V769dup) (12%)
(most common >1 are listed)	p.(N771_H773dup) (12%)
See Supplementary Table 3 for all EGFRex20 + mutation variants and co-occurring alterations in this study.	p.(S768_D770dup) (8%)
	p.(D770_N771insG) (8%)
	p.(N771_P772insH) (8%)
	p.(P772_H773dup) (8%)

Abbreviations: ECOG = Eastern Cooperative Oncology Group, TKI = tyrosine kinase inhibitor.

(12%) had asymptomatic brain metastases at baseline, in which one patient (4%) had a history of treated brain lesions. Thirteen patients (52%) had undergone previous treatment. The median number of previous lines of therapy was 1 (range, 0–3). Two patients (8%) had received chemo-immunotherapy and 11 patients (44%) platinum-based chemotherapy.

Despite per-protocol exclusion criteria, one patient (4%) was treated with an EGFR-TKI in combination with an anti-EGFR monoclonal antibody (afatinib/cetuximab, partial response as best overall response [BOR]). Histological or cytological tumor samples from all patients were tested for at least BRAF, EGFR, HER2, HRAS, KIT, KRAS, MET, NRAS, and PIK3CA. The observed EGFRex20 + mutations were clustered between amino acids A763 and L777. The most common EGFRex20 + mutation variants were p.(N771_H773dup) (n = 3) and p. (A767_V769dup) (n = 3). Data regarding co-occurring alterations were available for 14 patients (56%). The tumor protein p53 (TP53) mutation was the most common type of co-occurring alteration detected (n = 5; 36%), (see Supplementary Table 3, for all EGFRex20 + mutation variants and co-occurring alterations in this study).

3.2. Clinical efficacy

Among the 25 patients, seven patients had a confirmed PR (ORR: 28%; 95% confidence interval [CI], 12–49). No complete responses were observed. Thirteen patients (52%) had SD, four patients (16%) were inevaluable (NE) for response due to symptomatic deterioration before the first radiological assessment, and one patient (4%) had progressive disease (PD) as BOR. The CBR was 60% (95% CI, 39–79). The median DoR was 5.3 months (range, 2.7–27.6), with five patients remaining on treatment after the data cutoff (Fig. 1). Eighty percent of the patients showed radiographically tumor shrinkage of any quantity (Fig. 2). When considering the treatment line (first-line vs. later line setting), there was no significant difference (p = 0.625) between the time to progression (see Supplementary Fig. 1). The ORR was 31% (95% CI 9–61) and 25% (95% CI 6–57) in patients receiving osimertinib 160 mg as first line or in later line setting (p = 0.464).

All three patients with brain metastases showed a decrease in brain lesion size during osimertinib treatment. Two patients had brain metastasis as target lesions with a brain

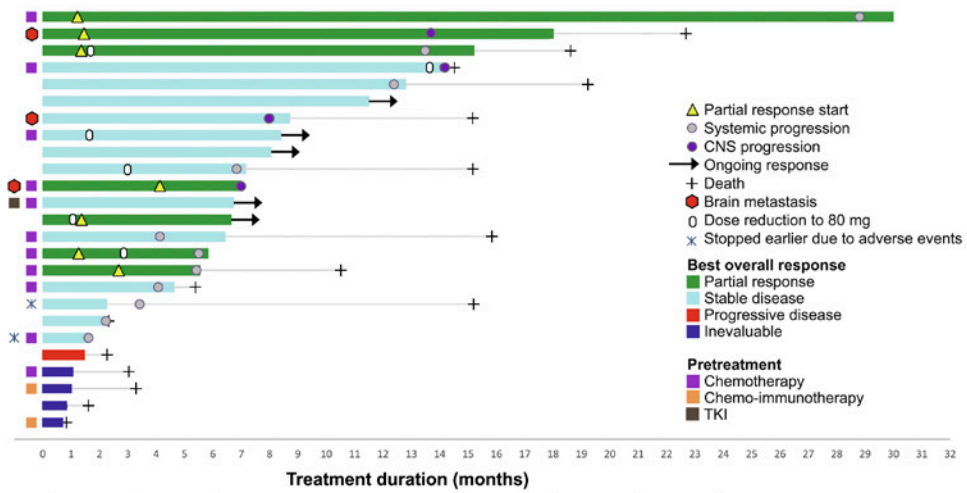

Fig. 1. Swimmers plot showing the duration of osimertinib treatment and responses for all evaluable patients. The lower four patients are inevaluable for response due to symptomatic deterioration before the first radiological assessment. Abbreviations TKI = tyrosine kinase inhibitor, CNS = central nervous system.

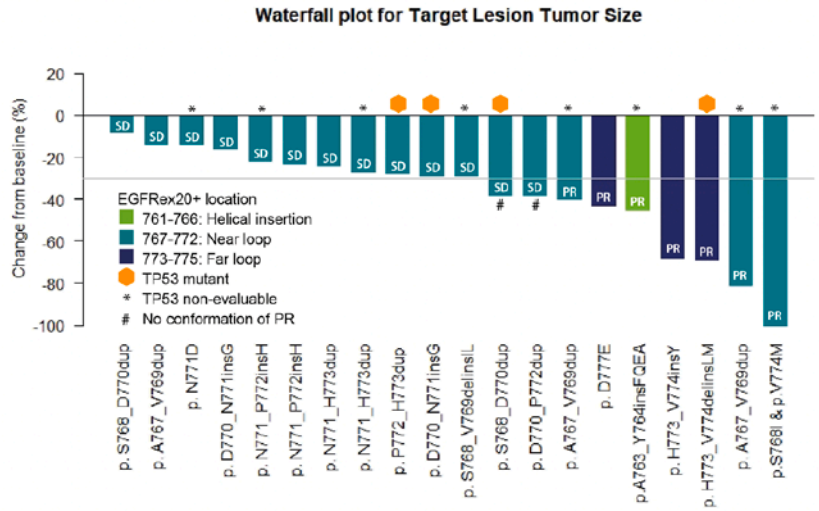

Fig. 2. Waterfall plot showing best percent change from baseline in sum of target lesion diameters by location of EGFRex20 + mutations for all evaluable patients. In addition, the best overall response for each patients is shown. Four patients were radiological non-evaluable, due to symptomatic deterioration before the first radiological assessment, and are not shown in the waterfall plot. The patient with PD as best response, showed pleural effusion on CT which was non measurable, and is therefore not shown in the waterfall plot. Two patients showed a percent change of more than 30% decrease in sum of target lesion diameters, but the decrease was not confirmed on the next time point. Therefore, the best responses of these patients is SD. The patients whose TP53 status is unknown are indicated with an asterisk. Abbreviations: SD = stable disease, PR = partial response, PD = progressive disease, EGFRex20+ = exon 20 deletion and/or insertion mutation.

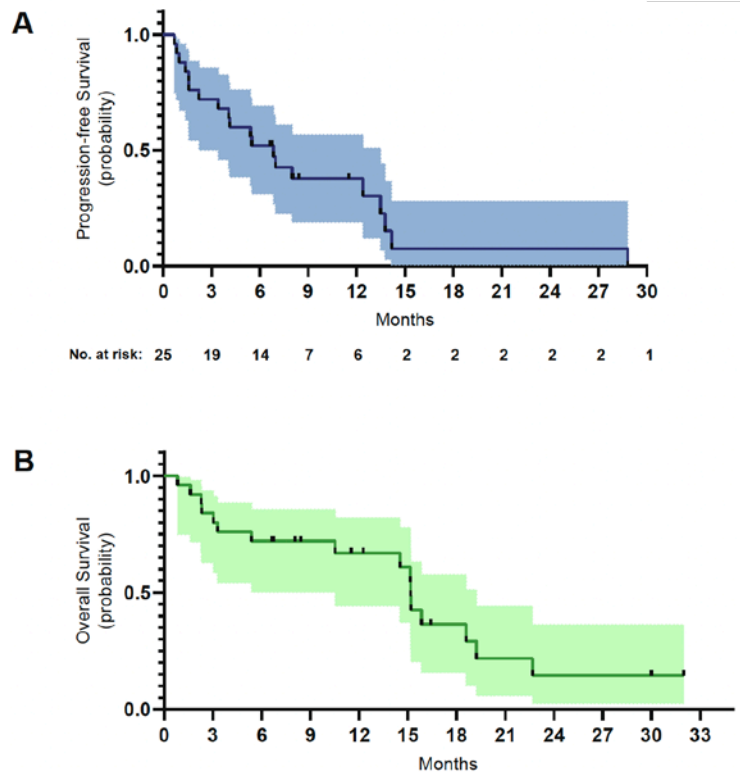

Fig. 3. Kaplan-Meier curve with 95% confidence interval showing progression-free survival (A) and overall survival (B).

lesion size decrease of 4% (SD as BOR) and 40% (PR as BOR). The patient with a decrease of 40% was pre-treated with radiotherapy. The third patient (PR as BOR) had non-target lesions as brain metastasis, which were at one point non-measurable. However, this patient experienced oligoprogression in the brain for which whole brain radiotherapy (WBRT) was indicated during osimertinib treatment. Hereafter, the patient remained stable on 160 mg osimertinib treatment for 5.3 months. The median duration of central nervous system (CNS) disease response was 6.6 (range, 2.8–7) months.

The primary reasons for discontinuation were PD in 14 patients (56%), symptomatic deterioration due to malignant disease in four patients (16%), and grade 3 trAEs in two patients (8%). Within the patients who experienced PD, 10 patients (71%) had systemic progression and four patients (29%) had CNS disease progression (Fig. 1). At data cutoff, progression or death due to PD occurred in 20 patients (80%). The median PFS was 6.8 months (95% CI, 4.6–9.1) and the median OS was 15.2 months (95% CI, 14.3–16.0) (Fig. 3). We found no significant correlation between the time to progression and the different baseline characteristics (see Supplementary Fig. 1). There was no significant difference in antitumor responses (p = 0.90) between the helical region (known sensible insertion regions) or loop region (less sensible insertion regions) of the EGFRex20 + mutations. To distinguish further between the different mutations and co-occurring TP53 mutations, we compared the best percent change from baseline in sum of target lesion diameters to location of insertion regions and presence of TP53 shown in Fig. 2. The patient with the longest PFS (16.4 months) had a known sensible *EGFR* exon 20 insertion variant p.(A763_Y764insFQEA) (helical region) (Fig. 2).

3.3. Safety

Among the 25 patients that were included in the safety analysis, adverse events of any cause were reported in all patients during the treatment (see Supplementary Table 4, showing adverse events regardless of causality in ≥ 1 patient). TrAEs of osimertinib of any grade were reported in 92% of the patients and are summarized in Table 2. The most common trAEs were diarrhea (72% of patients), dry skin (grouped term: 44% of patients), fatigue (44% of patients) and rash or acne (grouped term: 40% of patients). Five patients (20%) experienced grade 3 or worse trAEs. Serious adverse events (SAEs) were reported in nine patients (36%) (see Supplementary Table 5), in which three cases (12%) were considered treatment-related. These three SAEs included pneumonitis, dehydration, and hypokalemia caused by diarrhea. The patient who experienced pneumonitis did not receive immunotherapy in prior lines of treatment. Two patients discontinued treatment prior to progression due to SAE pneumonitis (n = 1) after 1.6 months and due to grade 3 trAE left ventricular systolic dysfunction (n = 1) after 2.3 months.

A dose interruption and/or reduction at any time during the treatment of osimertinib from 160 mg to 80 mg was required in eight patients (32%) (Fig. 1). Two patients required a dose interruption for only 2 weeks due to diarrhea or increased creatine kinase (CK) and continued thereafter with 160 mg. Two patients had to interrupt the regimen due to grade 3 trAEs hepatotoxicity and increased CK, and continued after the interruption without any sequels at a reduced dose of 80 mg. Four patients had a dose reduction to 80 mg (after 41-, 86-, 88- and 417 days) without interruption because of grade 2 fatigue, QTc prolongation, skin problems or nausea. No patient required a dose reduction to 40 mg. There were no new significant safety findings regarding treatment-related adverse events to osimertinib.

Table 2
Treatment-related adverse events (safety population).

Treatment-related adverse events	Safety population (N = 25) *Number of patients (percent)*				
	Grade 1	Grade 2	Grade 3	Grade 4	All grades
Any adverse event	22 (88)	11 (48)	5 (20)	0	23 (92)
Diarrhea	14 (56)	3 (12)	1 (4)	0	18 (72)
Dry skin*	10 (40)	1 (4)	0	0	11 (44)
Fatigue	9 (36)	2 (8)	0	0	11 (44)
Rash or acne*	8 (32)	1 (4)	1 (4)	0	10 (40)
Dyspnea	8 (32)	1 (4)	0	0	9 (36)
Paronychia	9 (36)	0	0	0	9 (36)
Anemia	6 (24)	1 (4)	1 (4)	0	8 (32)
Coughing	7 (28)	0	0	0	7 (28)
Myalgia	4 (16)	2 (8)	1 (4)	0	7 (28)
Anorexia	3 (12)	3 (12)	0	0	6 (24)
CPK increased	3 (12)	1 (4)	2 (8)	0	6 (24)
Back pain	4 (16)	1 (4)	0	0	5 (20)
Dry eyes	5 (20)	0	0	0	5 (20)
Mucositis oral	4 (16)	1 (4)	0	0	5 (20)
Nausea	5 (20)	0	0	0	5 (20)
Platelets decreased	4 (16)	1 (4)	0	0	5 (20)
Dry mouth	3 (12)	0	0	0	3 (12)
Pruritus	0	3 (12)	0	0	3 (12)
Fissures	3 (12)	0	0	0	3 (12)

The adverse events that were considered by the investigators to be related to osimertinib treatment observed in at least 10% of the patients are recorded.
* This category is a grouped term.

4. Discussion

Patients with life-threatening advanced NSCLC who harbor a rare EGFRex20 + mutation (4–12%) have limited effective treatment options. This study aimed to investigate whether the increased dosage of osimertinib 160 mg once daily would induce a stronger anti-tumor response and prolong survival. Our confirmed ORR was 28% (95% CI 12–49). Whilst this study did not reach the ORR of 30% we assumed in our power calculation, the treatment did partially substantiate clinical benefit. We found that 20 (80%) patients experienced a decrease in tumor size. In addition, the confirmed CBR of our study is 60% (15 of 25 patients) at 6 months. Within the 15 patients who experienced CBR, 10 patients received osimertinib 160 mg as first line treatment suggesting a more effective strategy in first compared to second line.

Currently, this is the largest phase II study which investigated osimertinib 160 mg in patients with EGFRex20 + NSCLC. Similar studies with osimertinib in this subset of patients are being conducted (ClinicalT rials.gov, Identifier: NCT04974879) and are summarized in Supplementary Table 1. The evidence reviewed in our study suggests benefit for osimertinib 160 mg over 80 mg, with a confirmed ORR of 28% and a CBR of 60%. This study has found responses across the entire spectrum of the EGFRex20 + mutation (see Supplementary Table 3), including the helical region and point mutations. Comparison of our findings considering trAEs with studies evaluating osimertinib 80 mg daily, confirms that treatment with osimertinib 160 mg daily result in higher rates of diarrhea (72% any grade), fatigue (44% any grade), and acneiform rash (40% any grade). However, the occurred trAEs are tolerable and comparable with other new therapy strategies for EGFRex20 + NSCLC patients [30,31]. In comparison to the ECOG-ACRIN 5162, our study provides additional evidence that osimertinib 160 mg once daily shows a similar confirmed ORR in first-line setting. This combination of findings further supports the idea that osimertinib 160 mg is a relevant first-line treatment option for patients with EGFRex20 + NSCLC.

New directed treatment strategies, including poziotinib and mobocertinib, attempt to overcome steric hindrance at the active site of the EGFRex20 + mutation, while minimizing toxicity by preserving selectivity against the wild type *EGFR* [30,31]. Poziotinib showed an average potency 100 times greater than osimertinib in EGFRex20 + *in vitro* [31], but an ORR of 14.8% among 115 patients with EGFRex20 + NSCLC [32]. Another group of 114 patients with EGFRex20 + NSCLC, progressing on platinum-based chemotherapy, were treated with mobocertinib and showed a confirmed ORR of 28% (95% CI, 20–37) by IRC assessment and 35% (95% CI, 26–45) by investigator assessment, with a DoR of 17.5 months (95% CI, 7.4–20.3) [33]. Due to the encouraging duration of the responses, mobocertinib was granted FDA accelerated approval in September 2021 [34]. Unfortunately, mobocertinib could not preserve selectivity against the wild type *EGFR* and provoked grade ≥ _3 trAEs in 46% of the patients and diarrhea in 90% (21% trAEs grade 3–4). Taken together, treatment with osimertinib 160 mg shows no improvement regarding ORR by investigator assessment, but less clinical burden for patients in terms of less severe trAEs compared to poziotinib and mobocertinib.

The exploratory CHRYSALIS single arm phase II study evaluated the intravenous treatment amivantamab in 81 patients who were pre-treated with chemotherapy [35]. Park et al. (2021) reported an ORR of 40% with a median DoR of 11.1 months, a median PFS of 8.3 months, and an expected safety profile associated with dual inhibition of *EGFR* and MET with grade 3–4 trAEs reported in 16% of all patients. Based on these results, amivantamab was granted FDA accelerated approval in May 2021 and EMA approval in December 2021 for patients with EGFRex20 + NSCLC with disease progression on or after platinum-based chemotherapy [36,37]. However, the treatment failed to address yet the clinical activity in CNS disease since it was an exclusion criteria [35]. Our study showed a decrease in brain lesion size in all patients with CNS disease, suggesting benefit of osimertinib for this subgroup. Another disadvantage of amivantamab is the possible adverse clinical burden due to more hospital visits because of the intravenous treatment, with associated infusion-related reactions (IRRs). Therefore, the disadvantages of amivantamab treatment raises the possibility that osimertinib 160 mg oral can be a good alternative for patients with EGFRex20 + NSCLC with CNS disease and deteriorated ECOG performance status.

Recently, more exciting new treatment options are being established for patients with EGFRex20 + NSCLC. Clinical data from a phase I study (ClinicalTrials.gov, Identifier:NCT04036682) evaluating CLN-081 (TAS6417) reported 13 confirmed PR and 8 unconfirmed PR among the 42 evaluable patients [41]. The findings from the phase II portion indicated 54% of patients experienced a PR (six confirmed, one unconfirmed) following treatment with a 100 mg twice daily dose of CLN- 081, granting a breakthrough therapy designation from the FDA in January 2022 [42,43]. Another promising breakthrough treatment option is the selective, irreversible *EGFR* inhibitor DZD9008 [44]. In a cohort of 97 patients treated at the recommended phase 2 dose of 300 mg once daily, the ORR was 48.4%, and the disease control rate was 90.3% [45]. DZD9008 is currently being evaluated in phase II clinical development (ClinicalTrials.gov, Identifier:NCT03974022) for the treatment of patients with EGFRex20 + NSCLC and HER2-mutated NSCLC.

The effect of co-occurring alterations, for example TP53, PTEN or STK11 mutations, or the different EGFRex20 + mutation variants is not well identified [24,46]. In this study, four out of five patients, showed at least 25% of tumor shrinkage while harboring the TP53 co-occurring alteration. With a small sample size, caution must be applied. Furthermore, there was no significant difference in antitumor responses between the EGFRex20 + mutation variants. However, the uncommon *EGFR* mutation is characterized as heterogenous showing different sensitivity among the mutational spectrum [47,48]. A further larger study and review with more focus on the co-occurring alterations and the EGFRex20 + mutation variants is therefore suggested and will help define unmet needs for future therapeutic advances.

Our study has several limitations. Firstly, it should be acknowledged that the distribution of EGFRex20 + mutation variants in this cohort is skewed towards near-loop mutations, with only three far-loop insertions. The responses almost all occur in EGFRex20 + mutation variants reported as sensitive to osimertinib in preclinical models, including the sensitizing variant p.(A763_Y764insFQEA). This knowledge should be taken into account while interpreting the survival data and therefore the different EGFRex20 + mutation variants were extensive elaborated. Secondly, a potential source of bias for the study is the influence the researchers had upon the response review, since the trial's lack of central review. Thirdly, the sample size of our cohort is relatively small, exists of only White patients with EGFRex20 + NSCLC, and are all being treated in a single country. In spite of its limitations, this prospective phase 2 POSITION20 study evaluates a relatively large case series of patients with EGFRex20 + NSCLC treated with 160 mg osimertinib, providing valuable insights. [42].

It is important to test and characterize the different EGFRex20 + mutation variants, co-occurring alterations, and evaluate the performance status of the patient to target the EGFRex20 + tumor effectively, and achieve clinical benefit. Osimertinib cannot be routinely recommended at either 80 or 160 mg daily for patients with EGFRex20 + NSCLC, but further development of higher dosing schemes is warranted. Approved and/or newer drugs in clinical trials should be considered first. Osimertinib 160 mg once daily can only be considered in patients who cannot access clinical trials and do not have other standard-of-care options available, particularly if CNS disease is present.

5. Conclusion

In conclusion, osimertinib 160 mg showed modest antitumor activity in patients with EGFRex20 + NSCLC, with a confirmed ORR of 28% and acceptable toxicity.

References

1. M.G. Kris, B.E. Johnson, L.D. Berry, D.J. Kwiatkowski, A.J. Iafrate, I.I. Wistuba, M. Varella-Garcia, W.A. Franklin, S.L. Aronson, P.-F. Su, Y.u. Shyr, D.R. Camidge, L.V. Sequist, B.S. Glisson, F.R. Khuri, E.B. Garon, W. Pao, C. Rudin, J. Schiller, E.B. Haura, M. Socinski, K. Shirai, H. Chen, G. Giaccone, M. Ladanyi, K. Kugler, J.D. Minna, P.A. Bunn, Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs, JAMA - J. Am. Med. Assoc. 311 (19) (2014) 1998.
2. N. Normanno, The role of EGF-related peptides in tumor growth, Front. Biosci. 6 (1) (2001) d685.
3. T. Kosaka, Y. Yatabe, H. Endoh, H. Kuwano, T. Takahashi, T. Mitsudomi, Mutations of the Epidermal Growth Factor Receptor Gene in Lung Cancer, Biol. Clin. Implic. (2004).
4. T.J. Lynch, D.W. Bell, R. Sordella, S. Gurubhagavatula, R.A. Okimoto, B.W. Brannigan, P.L. Harris, S.M. Haserlat, J.G. Supko, F.G. Haluska, D.N. Louis, D.C. Christiani, J. Settleman, D.A. Haber, Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non–Small-Cell Lung Cancer to Gefitinib, N. Engl. J. Med. 350 (21) (2004) 2129–2139.
5. S.S. Ramalingam, J. Vansteenkiste, D. Planchard, B.C. Cho, J.E. Gray, Y. Ohe, C. Zhou, T. Reungwetwattana, Y. Cheng, B. Chewaskulyong, R. Shah, M. Cobo, K.H. Lee, P. Cheema, M. Tiseo, T. John, M.-C. Lin, F. Imamura, T. Kurata, A. Todd, R. Hodge, M. Saggese, Y. Rukazenkov, J.-C. Soria, Overall Survival with Osimertinib in Untreated, EGFR -Mutated Advanced NSCLC, New Engl. J. Med. 382 (2020) 41–50, https://doi.org/10.1056/nejmoa1913662.
6. M.E. Arcila, K. Nafa, J.E. Chaft, N. Rekhtman, C. Lau, B.A. Reva, M.F. Zakowski, M.G. Kris, M. Ladanyi, EGFR exon 20 insertion mutations in lung adenocarcinomas: Prevalence, molecular heterogeneity, and clinicopathologic characteristics, Mol. Cancer Ther. 12 (2013) 220–229, https://doi.org/10.1158/1535-7163.MCT-12-0620.
7. J.P. Robichaux, X. Le, R.S.K. Vijayan, J.K. Hicks, S. Heeke, Y.Y. Elamin, H.Y. Lin, H. Udagawa, F. Skoulidis, H. Tran, S. Varghese, J. He, F. Zhang, M.B. Nilsson, L. Hu, Poteete, W. Rinsurongkawong, X. Zhang, C. Ren, X. Liu, L. Hong, J. Zhang, L. Diao, R. Madison, A.B. Schrock, J. Saam, V. Raymond, B. Fang, J. Wang, M.J. Ha, J. Cross, J.E. Gray, J. v. Heymach, Structure-based classification predicts drug response in EGFR-mutant NSCLC, Nature. 597 (2021) 732–737. doi:10.1038/ s41586-021-03898-1.
8. G.R. Oxnard, P.C. Lo, M. Nishino, S.E. Dahlberg, N.I. Lindeman, M. Butaney, D.M. Jackman, B.E. Johnson, P.A. J¨anne, Natural history and molecular characteristics of lung cancers harboring egfr exon 20 insertions, J. Thoracic Oncol. 8 (2013) 179–184, https://doi.org/10.1097/ JTO.0b013e3182779d18.
9. A.M. Li, A. Boichard, E. Felip, R. Kurzrock, New therapeutic approaches to overcoming resistant EGFR exon 20 alterations, Crit. Rev. Oncol./Hematol. 151 (2020), https:// doi.org/10.1016/j.critrevonc.2020.102990.
10. P.E.N.S. Vasconcelos, C. Gergis, H. Viray, A. Varkaris, M. Fujii, D. Rangachari, P.A. VanderLaan, I.S. Kobayashi, S.S. Kobayashi, D.B. Costa, EGFR-A763_ Y764insFQEA Is a Unique Exon 20 Insertion Mutation That Displays Sensitivity to Approved and In-Development Lung Cancer EGFR Tyrosine Kinase Inhibitors, JTO Clin. Res. Rep. 1 (2020), 100051, https://doi.org/10.1016/j.jtocrr.2020.100051.
11. H. Yasuda, E. Park, C.H. Yun, N.J. Sng, A.R. Lucena-Araujo, W.L. Yeo, M.S. Huberman, D.W. Cohen, S. Nakayama, K. Ishioka, N. Yamaguchi, M. Hanna, G.R. Oxnard, C.S. Lathan, T. Moran, L. v. Sequist, J.E. Chaft, G.J. Riely, M.E. Arcila, R.A. Soo, M. Meyerson, M.J. Eck, S.S. Kobayashi, D.B. Costa, Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer, Science Translational Medicine. 5 (2013). doi: 10.1126/scitranslmed.3007205.
12. T. Jiang, C. Su, S. Ren, F. Cappuzzo, G. Rocco, J.D. Palmer, N. van Zandwijk, F. Blackhall, X. Le, N.A. Pennell, C. Zhou, A consensus on the role of osimertinib in non-small cell lung cancer from the AME Lung Cancer Collaborative Group, J. Thorac. Dis. 10 (7) (2018) 3909–3921.
13. T. Hirano, H. Yasuda, T. Tani, J. Hamamoto, A. Oashi, K. Ishioka, D. Arai, S. Nukaga, M. Miyawaki, I. Kawada, K. Naoki, D.B. Costa, S.S. Kobayashi, T. Betsuyaku, K. Soejima, In vitro modeling to determine mutation specificity of EGFR tyrosine kinase inhibitors against clinically relevant EGFR mutants in non-small- cell lung cancer, Oncotarget. 6 (2015). doi:10.18632/oncotarget.5887.
14. J.C.H. Yang, M.J. Ahn, D.W. Kim, S.S. Ramalingam, L. v. Sequist, W.C. Su, S.W. Kim, J.H. Kim, D. Planchard, E. Felip, F. Blackhall, D. Haggstrom, K. Yoh, S. Novello, K. Gold, T. Hirashima, C.C. Lin, H. Mann, M. Cantarini, S. Ghiorghiu, P.A. Jänne, Osimertinib in pretreated T790M-positive advanced non-small-cell lung cancer: AURA study phase II extension component, Journal of Clinical Oncology. 35 (2017) 1288–1296. doi:10.1200/JCO.2016.70.3223.
15. G. Goss, C.M. Tsai, F.A. Shepherd, L. Bazhenova, J.S. Lee, G.C. Chang, L. Crino, M. Satouchi, Q. Chu, T. Hida, J.Y. Han, O. Juan, F. Dunphy, M. Nishio, J.H. Kang, M. Majem, H. Mann, M. Cantarini, S. Ghiorghiu, T. Mitsudomi, Osimertinib for pretreated EGFR Thr790Met-positive advanced non-small-cell lung cancer (AURA2): a multicentre, open-label, single-arm, phase 2 study, Lancet Oncol. 17 (2016) 1643–1652, https://doi.org/10.1016/S1470-2045(16)30508-3.
16. T.S. Mok, Y.-L. Wu, M.-J. Ahn, M.C. Garassino, H.R. Kim, S.S. Ramalingam, F.A. Shepherd, Y. He, H. Akamatsu, W.S.M.E. Theelen, C.K. Lee, M. Sebastian, A. Templeton, H. Mann, M. Marotti, S. Ghiorghiu, V.A. Papadimitrakopoulou, Osimertinib or Platinum-Pemetrexed in EGFR T790M–Positive Lung Cancer, N. Engl. J. Med. 376 (2017) 629–640, https://doi.org/10.1056/nejmoa1612674.
17. V. Gregorc, C. Lazzari, N. Karachaliou, R. Rosell, M. Santarpia, Osimertinib in untreated epidermal growth factor receptor (EGFR)-mutated advanced non-small cell lung cancer, Translational Lung Cancer Research. 7 (2018) S165–S170. doi: 10.21037/tlcr.2018.03.19.
18. G. Goss, C.M. Tsai, F.A. Shepherd, M.J. Ahn, L. Bazhenova, L. Crino`, F. de Marinis, E. Felip, A. Morabito, R. Hodge, M. Cantarini, M. Johnson, T. Mitsudomi, P.A. Jänne, J.C.H. Yang, CNS response to osimertinib in patients with T790M- positive advanced NSCLC: Pooled data from two phase II trials, Ann. Oncol. 29 (2018) 687–693, https://doi.org/10.1093/annonc/mdx820.
19. T.M. Kim, C.-Y. Ock, M. Kim, S.H. Kim, B. Keam, Y.J. Kim, D.-W. Kim, J.-S. Lee, D.S. Heo, 1529P - Phase II study of osimertinib in NSCLC patients with EGFR exon 20 insertion mutation: A multicenter trial of the Korean Cancer Study Group (LU17- 19), Ann. Oncol. 30 (2019), v628, https://doi.org/10.1093/annonc/mdz260.051.
20. G.J. Yang, J. Li, H.Y. Xu, Y. Sun, L. Liu, H.S. Li, L. Yang, Y. Zhang, G.H. Li, Y. Wang, Osimertinib for Chinese advanced non-small cell lung cancer patients harboring diverse EGFR exon 20 insertion mutations, Lung Cancer. 152 (2021) 39–48, https://doi.org/10.1016/j.lungcan.2020.11.027.
21. W. Fang, Y. Huang, S. Hong, Z. Zhang, M. Wang, J. Gan, W. Wang, H. Guo, K. Wang, L. Zhang, EGFR exon 20 insertion mutations and response to osimertinib in non-small-cell lung cancer, BMC Cancer. 19 (2019), https://doi.org/10.1186/ s12885-019-5820-0.
22. H. Yasuda, E. Ichihara, J. Sakakibara-Konishi, Y. Zenke, S. Takeuchi, M. Morise, K. Hotta, M. Sato, S. Matsumoto, A. Tanimoto, R. Matsuzawa, K. Kiura, Y. Takashima, S. Yano, J. Koyama, T. Fukushima, J. Hamamoto, H. Terai, S. Ikemura, R. Takemura, K. Goto, K. Soejima, A phase I/II study of osimertinib in EGFR exon 20 insertion mutation-positive non-small cell lung cancer, Lung Cancer. 162 (2021) 140–146, https://doi.org/10.1016/j.lungcan.2021.10.006.
23. B. van Veggel, J.F.V. Madeira R Santos, S.M.S. Hashemi, M.S. Paats, K. Monkhorst, D.A.M. Heideman, M. Groves, T. Radonic, E.F. Smit, E. Schuuring, A.J. van der Wekken, A.J. de Langen, van der Wekken, A.J. de Langen, Osimertinib treatment for patients with EGFR exon 20 mutation positive non-small cell lung cancer, Lung Cancer. 141 (2020) 9–13.
24. G.-J. Yang, J. Li, H.-Y. Xu, Y. Sun, L. Liu, H.-S. Li, L. Yang, Y. Zhang, G.-H. Li, Y. Wang, Osimertinib for Chinese advanced non-small cell lung cancer patients harboring diverse EGFR exon 20 insertion mutations, Lung Cancer. 152 (2021) 39–48, https://doi.org/10.1016/j.lungcan.2020.11.027.
25. D. Planchard, K.H. Brown, D.W. Kim, S.W. Kim, Y. Ohe, E. Felip, P. Leese, M. Cantarini, K. Vishwanathan, P.A. Ja¨nne, M. Ranson, P.A. Dickinson, Osimertinib Western and Asian clinical pharmacokinetics in patients and healthy volunteers: Implications for formulation, dose, and dosing frequency in pivotal clinical studies, Cancer Chemother. Pharmacol. 77 (2016) 767–776, https://doi.org/10.1007/ s00280-016-2992-z.
26. Z. Piotrowska, Y. Wang, L. v Sequist, S.S. Ramalingam, ECOG-ACRIN 5162: A phase II study of osimertinib 160 mg in NSCLC with EGFR exon 20 insertions., Journal of Clinical Oncology. 38 (2020) 9513. doi:10.1200/JCO.2020.38.15_ suppl.9513.
27. World Medical Association declaration of Helsinki: Ethical principles for medical research involving human subjects, JAMA - Journal of the American Medical Association. 310 (2013) 2191–2194. doi:10.1001/jama.2013.281053.
28. E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij, New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1), Eur. J. Cancer 45 (2009) 228–247, https://doi.org/10.1016/j.ejca.2008.10.026.
29. Common Terminology Criteria for Adverse Events (CTCAE), n.d. http://www. meddramsso.com.
30. G.J. Riely, J.W. Neal, D.R. Camidge, A.I. Spira, Z. Piotrowska, D.B. Costa, A.S. Tsao, J.D. Patel, S.M. Gadgeel, L. Bazhenova, V.W. Zhu, H.L. West, T. Mekhail, R.D. Gentzler, D. Nguyen, S. Vincent, S. Zhang, J. Lin, V. Bunn, S. Jin, S. Li, P.A. Ja¨nne, Activity and safety of mobocertinib (Tak-788) in previously treated non–small cell lung cancer with egfr exon 20 insertion mutations from a phase i/ii trial, Cancer Discovery. 11 (2021) 1688–1699. doi:10.1158/2159-8290. CD-20-1598.
31. J.P. Robichaux, Y.Y. Elamin, Z. Tan, B.W. Carter, S. Zhang, S. Liu, S. Li, T. Chen, A. Poteete, A. Estrada-Bernal, A.T. Le, A. Truini, M.B. Nilsson, H. Sun, E. Roarty, S.B. Goldberg, J.R. Brahmer, M. Altan, C. Lu, V. Papadimitrakopoulou, K. Politi, R.C. Doebele, K.K. Wong, J. v. Heymach, Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer, Nature Medicine. 24 (2018) 638–646. doi:10.1038/s41591-018-0007-9.
32. X. Le, J.W. Goldman, J.M. Clarke, N. Tchekmedyian, Z. Piotrowska, D. Chu, G. Bhat, F.M. Lebel, M.A. Socinski, Poziotinib shows activity and durability of responses in subgroups of previously treated EGFR exon 20 NSCLC patients, J. Clin. Oncol. 38 (2020) 9514, https://doi.org/10.1200/JCO.2020.38.15_suppl.9514.
33. S.S. Ramalingam, C. Zhou, T.M. Kim, S.-W. Kim, J.-C.-H. Yang, G.J. Riely, T. Mekhail, D. Nguyen, M.R. García Campelo, E. Felip, S. Vincent, S. Jin, V. Bunn, J. Lin, H.M. Lin, M. Mehta, P.A. Janne, Mobocertinib (TAK-788) in EGFR exon 20 insertion (ex20ins)+ metastatic NSCLC (mNSCLC): Additional results from platinum-pretreated patients (pts) and EXCLAIM cohort of phase ½ study, J. Clin. Oncol. 39 (2021) 9014, https://doi.org/10.1200/JCO.2021.39.15_ suppl.9014.
34. FDA Approves Mobocertinib for EGFR Exon 20 Insertion+ NSCLC, U.S. Food & Drug Administration. (2021).
35. K. Park, E.B. Haura, N.B. Leighl, P. Mitchell, C.A. Shu, N. Girard, ; Santiago Viteri, J.-Y. Han, ; Sang-We Kim, ; Chee, K. Lee, J.K. Sabari, ; Alexander, I. Spira, ; Tsung- Ying Yang, ; Dong-Wan Kim, ; Ki, H. Lee, R.E. Sanborn, J. Joś, J. Trigo, ; Koichi Goto, J.-S. Lee, ; James, C.-H. Yang, ; Ramaswamy Govindan, J.M. Bauml, ; Pilar Garrido, ; Matthew, G. Krebs, K.L. Reckamp, ; John Xie, J.C. Curtin, ; Nahor Haddish-Berhane, A. Roshak, D. Millington, P. Lorenzini, ; Meena Thayu, ; Roland, E. Knoblauch, B.C. Cho, Amivantamab in EGFR Exon 20 Insertion-Mutated Non- Small-Cell Lung Cancer Progressing on Platinum Chemotherapy: Initial

Results From the CHRYSALIS Phase I Study, J Clin Oncol. 39 (2021) 3391–3402. doi: 10.1200/JCO.21.
36. Committee for Medicinal Products for Human Use (CHMP): 8-11 November 2021, (2021). https://www.ema.europa.eu/en/events/committee-medicinal-products-human-use-chmp-8-11-november-2021 (accessed December 24, 2021).
37. FDA grants accelerated approval to amivantamab-vmjw for metastatic non-small cell lung cancer, U.S. Food & Drug Administration. (2021). https://www.fda.gov/ drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-a mivantamab-vmjw-metastatic-non-small-cell-lung-cancer (accessed December 24, 2021).
38. H. Udagawa, S. Hasako, A. Ohashi, R. Fujioka, Y. Hakozaki, M. Shibuya, N. Abe, T. Komori, T. Haruma, M. Terasaka, R. Fujita, A. Hashimoto, K. Funabashi, H. Yasuda, K. Miyadera, K. Goto, D.B. Costa, S.S. Kobayashi, TAS6417/CLN-081 is a pan- mutation-selective EGFR tyrosine kinase inhibitor with a broad spectrum of preclinical activity against clinically relevant EGFR mutations, Molecular Cancer Research. 17 (2019) 2233–2243. doi:10.1158/1541-7786.MCR-19-0419.
39. Z. Piotrowska, H.A. Yu, J.-C.-H. Yang, M. Koczywas, E.F. Smit, D.-S.-W. Tan, V.-H.- F. Lee, R.A. Soo, J.M. Wrangle, A.I. Spira, V. Velcheti, M.A. Socinski, A. Page, D. Witter, L. Zawel, J.M. Wigginton, M.S. Clancy, D. Nguyen, Safety and activity of CLN-081 (TAS6417) in NSCLC with EGFR Exon 20 insertion mutations (Ins20), J. Clin. Oncol. 39 (2021) 9077, https://doi.org/10.1200/JCO.2021.39.15_ suppl.9077.
40. FDA Grants Breakthrough Therapy Designation to CLN-081 for Locally Advanced/ Metastatic EGFR Exon 20–Mutant NSCLC, U.S. Food & Drug Administration. (2022).
41. Dizal Pharmaceutical Co. Ltd., FDA grants breakthrough therapy designation for dizal pharmaceutical's DZD9008 in patients with locally advanced or metastatic non-small cell lung cancer harboring EGFR exon20 insertion., (2022).
42. J.-C.-H. Yang, M. Wang, P. Mitchell, J. Fang, W. Nian, C.-H. Chiu, J. Zhou, Y. Zhao, W.-C. Su, D.R. Camidge, T.-Y. Yang, V.W. Zhu, M. Millward, Y. Fan, W.T. Huang, Y. Cheng, L. Jiang, L. Zheng, X. Ye, P.A. Janne, Preliminary safety and efficacy results from phase 1 studies of DZD9008 in NSCLC patients with EGFR Exon20 insertion mutations, J. Clin. Oncol. 39 (2021) 9008, https://doi.org/10.1200/JCO.2021.39.15_suppl.9008.
43. J.W. Riess, D.R. Gandara, G.M. Frampton, R. Madison, N. Peled, J.A. Bufill, G.K. Dy, S.-H.-I. Ou, P.J. Stephens, J.D. McPherson, P.N. Lara Jr, R.A. Burich, J.S. Ross, V.A. Miller, S.M. Ali, P.C. Mack, A.B. Schrock, Diverse EGFR Exon 20 Insertions and Co-Occurring Molecular Alterations Identified by Comprehensive Genomic Profiling of NSCLC, J. Thorac. Oncol. 13 (2018) 1560–1568, https://doi.org/10.1016/j.jtho.2018.06.019.
44. I.S. Kobayashi, H. Viray, D. Rangachari, S.S. Kobayashi, D.B. Costa, Egfr-d770>gy and other rare egfr exon 20 insertion mutations with a g770 equivalence are sensitive to dacomitinib or afatinib and responsive to egfr exon 20 insertion mutant-active inhibitors in preclinical models and clinical scenarios, Cells. 10 (2021), https://doi.org/10.3390/cells10123561.
45. K. Sehgal, D. Rangachari, P.A. VanderLaan, S.S. Kobayashi, D.B. Costa, Clinical Benefit of Tyrosine Kinase Inhibitors in Advanced Lung Cancer with EGFR-G719A and Other Uncommon EGFR Mutations, Oncologist 26 (2021) 281–287, https:// doi.org/10.1002/onco.13537.
46. T.M. Kim, C.-Y. Ock, M. Kim, S.H. Kim, B. Keam, Y.J. Kim, D.-W. Kim, J.-S. Lee, D.S. Heo, Phase II study of osimertinib in NSCLC patients with EGFR exon 20 insertion mutation: A multicenter trial of the Korean Cancer Study Group (LU17-19), Ann. Oncol. 30 (2019), v628, https://doi.org/10.1093/annonc/mdz260.051.

Supplementary information

Supplementary Table 1.
Efficacy of osimertinib 80 mg or 160 mg daily for patients with EGFRex20+ NSCLC in several trials.

Clinical trial	Patient (No.)	ORR (%)	DCR (%)	PFS (months)	Reference
Retrospective	80 mg/d: 6	67.7	100.0	6.2	[21]
NCT03414814	80 mg/d: 15	0	46.7	3.5	[49]
UMIN000031929	80 mg/d: 12	0	58.3	3.8	[22]
Retrospective	80 mg/d: 20	5.0	71.0	3.6	[23]
	160 mg/d: 1				
Retrospective	80 mg/d: 53	6.5	53.2	2.3	[20]
	160 mg/d: 9				
ECOG-ACRIN 5162 trial	160 mg/d: 20	25.0	85.0	9.7	[26]

Supplementary Table 2.
Efficacy of osimertinib 160 mg for 21 patients with NSCLC EGFRex20+ within the study of Piotrowska et al., 2020[26].

	Best response				
***EGFR* exon 20 variant (No.)**	**CR**	**PR**	**SD**	**PD**	**Location**
A767_V769dup (5)	1	0	4	0	Near loop
V769_D770insASV (1)	0	0	1	0	Near loop
D770_N771insG (2)	0	1	0	1	Near loop
D770_N771insNPH (1)	0	1	0	0	Near loop
D770_N771insSVD (1)	0	0	1	0	Near loop
N771_P772insH (1)	0	0	1	0	Near loop
N771_H773dup (1)	0	0	1	0	Near loop
P772_H773ARG (1)	0	1	0	0	Near loop
P772_H773insF (1)	0	0	1	0	Near loop
H773_V774insAH (1)	0	0	1	0	Far loop
H773_V774insPH (1)	0	1	0	0	Far loop
Unknown (5)	0	0	3	3	n.a.
TOTAL (21)	**1**	**4**	**13**	**4**	

Supplementary Table 3.
The EGFRex20+ mutation variants and co-occurring alterations harbored by patients with NSCLC in the POSITION20 study and the response to treatment.

EGFR exon 20 variants (No.)	Best response	Location	Co-occurring alterations
A763_Y764insFQEA	PR	Helical region	N/A
A767_V769dup	SD	Near loop	None
A767_V769dup	PR	Near loop	N/A
A767_V769dup	PR	Near loop	N/A
S768_D770dup	SD	Near loop	TP53; RB1
S768_D770dup	PR	Near loop	None
S768_V769delinsIL	SD	Near loop	N/A
S768I & p.V774M	PR	Near loop – point mutations	N/A
D770_N771insG	SD	Near loop	TP53
D770_N771insG	SD	Near loop	None
D770_P772dup	SD	Near loop	None
N771_P772insH	SD	Near loop	N/A
N771_P772insH	SD	Near loop	None
N771_P772insV	NE	Near loop	N/A
N771_H773dup	SD	Near loop	None
N771_H773dup	NE	Near loop	N/A
N771_H773dup	SD	Near loop	N/A
N771D	SD	Near loop – point mutation	N/A
P772_H773dup	PD	Near loop	None
P772_H773dup	SD	Near loop	TP53
P772_H773insR	NE	Near loop	TP53
P772_H773insQPNP	NE	Near loop	N/A
H773_V774delinsLM	PR	Far loop	TP53
H773_V774insY	PR	Far loop	None
D777E	SD	Far loop – point mutation	None

PR = partial response, N/A = not applicable, SD = stable disease, NE = non-evaluable, PD = progressive disease.

Supplementary Table 5.
Serious adverse events (safety population)

Serious adverse events (SAE) *SAEs occurring in ≥1 patient*	**Number of patients (percent)**	**Relation to treatment**
Seizures	1 (4)	Unrelated
Pneumonitis	1 (4)	Probably
Haematuria	1 (4)	Unrelated
Fractures (fall stairs)	1 (4)	Unrelated
Hyponatraemia	1 (4)	Unrelated
Hypokalaemia due to diarrhoea	1 (4)	Possible
Thoracic pain	1 (4)	Unrelated
Dyspnoea	1 (4)	Unrelated
Dehydration	1 (4)	Possible

All the SAEs occurring in ≥1 patient are recorded. Unrelated: no relation between the treatment of osimertinib 16mg once daily and the SAE. Probably: relation between the treatment of osimertinib 160mg once daily and the SAE not completely excludable. Possible: known relation between the treatment of osimertinib 160mg once daily and the SAE.

Supplementary Table 4.
Adverse events regardless of causality in ≥1 patient in the safety population

Adverse events	**Safety population (N = 25)** *Number of patients (percent)*				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**	**All grades**
Any adverse event	25 (100)	15 (60)	8 (32)	0	25 (100)
Diarrhoea	14 (56)	3 (12)	1 (4)	0	18 (72)
Dry skin*	10 (40)	1 (4)	0	0	11 (44)
Fatigue	9 (36)	2 (8)	0	0	11 (44)
Rash or acne*	8 (32)	1 (4)	1 (4)	0	10 (40)
Dyspnoea	8 (32)	1 (4)	0	0	9 (36)
Paronychia	9 (36)	0	0	0	9 (36)
Anaemia	6 (24)	1 (4)	1 (4)	0	8 (32)
Coughing	7 (28)	0	0	0	7 (28)
Myalgia	4 (16)	2 (8)	1 (4)	0	7 (28)
Anorexia	3 (12)	3 (12)	0	0	6 (24)
CPK increased	3 (12)	1 (4)	2 (8)	0	6 (24)
Back pain	4 (16)	1 (4)	0	0	5 (20)
Dry eyes	5 (20)	0	0	0	5 (20)
Mucositis oral	4 (16)	1 (4)	0	0	5 (20)
Nausea	5 (20)	0	0	0	5 (20)
Platelets decreased	4 (16)	1 (4)	0	0	5 (20)
Constipation	2 (8)	1 (4)	0	0	3 (12)
Dry mouth	3 (12)	0	0	0	3 (12)
Pruritus	0	3 (12)	0	0	3 (12)
Fissures	3 (12)	0	0	0	3 (12)
Pain joint	3 (12)	0	0	0	3 (12)
Urinary tract infection	3 (12)	0	0	0	3 (12)
Pain in extremity	2 (8)	0	0	0	2 (8)
Localized edema	1 (4)	1 (4)	0	0	2 (8)
Alopecia	2 (8)	0	0	0	2 (8)
Dizziness	2 (8)	0	0	0	2 (8)
Conjunctivitis	2 (8)	0	0	0	2 (8)
Upper respiratory infection	0	2 (8)	0	0	2 (8)
Vomiting	2 (8)	0	0	0	2 (8)
Alanine aminotransferase increased	1 (4)	1 (4)	0	0	2 (8)
Creatinine increased	1 (4)	1 (4)	0	0	2 (8)
Headache	2 (8)	0	0	0	2 (8)
Skin induration	1 (4)	0	0	0	1 (4)
Fever	1 (4)	0	0	0	1 (4)
Palpitations	1 (4)	0	0	0	1 (4)
Gastritis	1 (4)	0	0	0	1 (4)
Malaise	0	1 (4)	0	0	1 (4)
Dysgeusia	1 (4)	0	0	0	1 (4)
Hypertension	0	0	1 (4)	0	1 (4)
Red face	1 (4)	0	0	0	1 (4)
Amnesia	1 (4)	0	0	0	1 (4)
Embolism	0	1 (4)	0	0	1 (4)
Lumbago	1 (4)	0	0	0	1 (4)
ECG QT corrected interval prolonged	0	1 (4)	0	0	1 (4)
Stomach pain	0	1 (4)	0	0	1 (4)
Dyspepsia	1 (4)	0	0	0	1 (4)
Thrush	0	1 (4)	0	0	1 (4)
Anxiety	1 (4)	0	0	0	1 (4)
Vascular access complication	0	0	1 (4)	0	1 (4)
Joint range of motion decreased	1 (4)	0	0	0	1 (4)
Aspartate aminotransferase increased	1 (4)	0	0	0	1 (4)
Leukopenia	1 (4)	0	0	0	1 (4)
Hepatotoxicity	0	0	1 (4)	0	1 (4)
Left ventricular systolic dysfunction	0	0	1 (4)	0	1 (4)
Hyponatraemia	0	1 (4)	0	0	1 (4)
Hypokalaemia	1 (4)	0	0	0	1 (4)
Hypomagnesaemia	1 (4)	0	0	0	1 (4)

All the adverse events (including adverse events that were considered by the investigators to be related to osimertinib treatment) that were considered and observed by the investigators that were observed in at least ≥1 patient are recorded.
* This category is a grouped term

Chapter 4

Afatinib and cetuximab in four patients with *EGFR* exon 20 insertion-positive advanced NSCLC

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ABSTRACT

Introduction: **Epidermal growth factor receptor (EGFR) exon 20 insertions comprise 4-9% of EGFR mutated non-small-cell lung cancer (NSCLC). Despite being an oncogenic driver, they are associated with primary resistance to EGFR tyrosine kinase inhibitors (TKIs). We hypothesized that dual EGFR blockade with afatinib, an irreversible EGFR TKI, and cetuximab, a monoclonal antibody against EGFR, could induce tumor responses.**

Methods: **Four patients with EGFR exon 20 insertion positive NSCLC were treated with afatinib 40 mg once daily and cetuximab 250-500 mg/m2 every two weeks.**

Results: **All patients had stage IV adenocarcinoma of the lung harboring an EGFR exon 20 insertion mutation. Previous lines of treatment consisted of platinum doublet chemotherapy (n=4) and EGFR TKI (n=2). Three out of four patients showed a partial response according to RECIST 1.1. Median progression-free survival was 5.4 months (95% confidence interval 0.0 – 14.2 months; range 2.7 – 17.6 months). Toxicity was manageable with appropriate skin management and dose reduction being required in two patients.**

Conclusions: **Dual EGFR blockade with afatinib and cetuximab may induce tumor responses in patients with EGFR exon 20 insertion positive NSCLC.**

Introduction

Epidermal growth factor receptor (*EGFR*) exon 20 insertion/duplication mutations (further collectively referred to as 'exon 20 insertions') comprise 4-9% of *EGFR* mutated non-small-cell lung cancer (NSCLC).[1-3] They represent a combination of in-frame insertions and duplications of 3 to 21 nucleotide base pairs, predominantly clustered between codons 767 and 774. Similar to the more common 'classic' exon 19 deletion and exon 21 L858R *EGFR* point mutation, *EGFR* exon 20 insertions result in sustained *EGFR* signaling and are oncogenic drivers.[4] However, in contrast to the classic *EGFR* mutations, insertion mutants in exon 20, with the exception of insertion mutation A763_Y764insFQEA, are associated with resistance to *EGFR* tyrosine kinase inhibitors (TKIs), including first and second generation *EGFR* TKIs and the third generation *EGFR* TKI rociletinib.[5-7] Although two other third generation *EGFR* TKIs, osimertinib and EGF816, demonstrated preclinical antitumor activity in *EGFR* exon 20 insertion models,[8, 9] it is uncertain whether these results will translate into clinical benefit.

A different strategy could be to intensify *EGFR* blockade by concurrent TKI and monoclonal antibody (mAb) treatment. A study with the second generation *EGFR* TKI afatinib and the *EGFR* mAb cetuximab in patients with advanced *EGFR* mutation positive NSCLC and acquired resistance to first generation *EGFR* TKIs, showed that combination treatment is able to overcome *EGFR* TKI resistance in patients with classic *EGFR* mutations, irrespective of T790M status.[10] A study with afatinib and the *EGFR* mAb nimotuzumab showed comparable results.[11] These studies suggest that dual *EGFR* blockade with the irreversible *EGFR* TKI afatinib and a mAb is able to induce tumor responses in patients with a tumor that is driven by *EGFR*, but resistant to first and second generation *EGFR* TKIs. Based on these results, the rationale of *EGFR* exon 20 insertion being an oncogenic driver and that *EGFR* TKI resistance can be overcome by dual *EGFR* inhibition, we treated four patients with *EGFR* exon 20 insertion positive NSCLC with afatinib and cetuximab.

Material and methods

Four patients with stage IV NSCLC harboring an *EGFR* exon 20 insertion were treated with afatinib and cetuximab in two institutions (VU University Medical Center and The Netherlands Cancer Institute) in the Netherlands. Data were obtained retrospectively by medical record review. *EGFR* mutation status was assessed by next-generation sequencing (NGS) or high resolution melting (HRM) followed by Sanger sequencing. *EGFR* amplification status was determined by using chromogenic in situ hybridization (ISH). Mutation and copy number variation analysis by NGS for KRAS, HER2, EGFR, BRAF, MET and fusion analysis by immunohistochemistry or ISH for ALK, ROS1 and RET were performed on pre-treatment biopsy samples and, if available, on post-progression biopsy samples as well.

The first three patients were treated with afatinib (40 mg once daily) and cetuximab (500 mg/m² intravenously (i.v.) every 2 weeks) until disease progression or unacceptable toxicity. The fourth patient was treated with afatinib 40 mg once daily and cetuximab 250 mg/m² i.v. every 2 weeks. Premedication consisted of metoclopramide 10 mg orally, clemastin 2 mg i.v. and dexamethasone 8 mg i.v. Supportive medication consisted of minocycline 100 mg once daily, skin cream and loperamide 2 mg as needed. Follow-up was performed biweekly with physical examination and blood analysis. Radiological follow-up was performed every six weeks with CT of the thorax and upper abdomen. Response was assessed according to Response Criteria In Solid Tumors (RECIST) 1.1.[12] Progression free survival (PFS) was defined as the interval between initiation of afatinib plus cetuximab and the date of radiological progression.

Table 1. Patient characteristics and outcome to afatinib and cetuximab treatment

Patient	Mutation type[1]	EGFR CISH (gene copy number)	Lines systemic therapy	Prior Platinum based therapy	Prior EGFR therapy	Best RECIST response	Progression free survival
1	p.(Ser768_Asp770dup)	3-7	1	Yes	None	PR	17.6 months
2	p.(Asn771_His773dup)	1-2	3	Yes	None	PR	4.4 months
3	p.(His773dup)	1-2	4	Yes	Gefitinib	SD	2.7 months
4	p.(Ala767_Val769dup)	1-2	6	Yes	Osimertinib	PR	6.4 months (treatment ongoing)

[1]predicted consequence, i.e. experimental evidence at DNA sequence.
EGFR, epidermal growth factor receptor; CISH, chromogenic in situ hybridization; RECIST: Response Evaluation Criteria in Solid Tumors; PR, partial response; SD, stable disease.

Results

All patients had stage IV adenocarcinoma of the lung harboring an exon 20 insertion. Patient and mutation characteristics are depicted in Table 1. Additional molecular analysis, including mutations and fusions, did not detect any other common oncogenic drivers. *EGFR* copy number gain was only present in the tumor biopsy of the first patient.
All patients received prior platinum doublet chemotherapy and two patients were treated with an *EGFR* TKI as well: patient 3 with a first generation *EGFR* TKI and patient 4 with a third generation *EGFR* TKI, both without a radiological response. Of the four patients treated with afatinib plus cetuximab, three experienced an objective partial response (PR) according to the RECIST 1.1 (Figure 1). The median PFS was 5.4 months (95% confidence interval 0.0 – 14.2 months; range 2.7 – 17.6 months). Patient 1 showed a PFS of 17.6 months. After 10.5 months of treatment this patient requested for temporary interruption of treatment because of a planned holiday. After 8 weeks a new CT scan showed clear progression. Upon re-introduction of afatinib and cetuximab, the tumor responded again for 6.5 months.

Toxicity was acceptable and in line with the known toxicity profile of afatinib-cetuximab combination therapy. Patient 1 experienced grade II skin toxicity and paronychia. After 15 months of treatment, this led to a dose reduction of afatinib to 30 mg once daily. The afatinib dose was reduced to 30 mg as well in patient 2 due to grade III skin toxicity and grade II diarrhea. This was followed by a dose reduction of cetuximab (250 mg/m2) because of ongoing toxicity. Patient 3 had grade I skin toxicity and grade I diarrhea. Due to progression, treatment was discontinued after 2.7 months. Patient 4 was treated with a lower dose of cetuximab (250 mg/m2) in combination with afatinib 40 mg once daily because of WHO performance status of 3. She experienced grade I skin toxicity and paronychia and grade II diarrhea that were managed with supportive medication. She experienced a PR and her WHO performance status improved to 1.

Post-progression biopsy samples were available for both patients that experienced a PR, while patient 4 is still on treatment with an ongoing response. Both biopsy samples were analyzed for mutations with NGS, as well as copy number changes for EGFR, MET and HER2 with ISH. Both samples were positive for the known exon 20 insertion and negative for T790M point mutation. In the paired biopsy samples of patient 1 polysomy of the MET proto-oncogene was present with 4-5 copies/nucleus compared to 3 copies before treatment, suggestive of bypass track resistance.

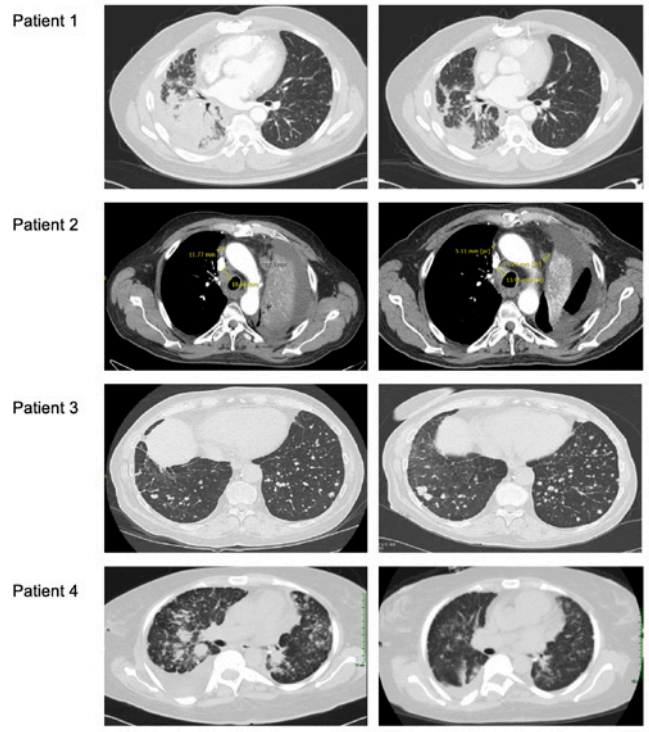


Figure 1. CT scans of the thorax performed prior (baseline) and after treatment onset (response).

Discussion

EGFR exon 20 insertions are associated with primary resistance to *EGFR* TKI treatment. These mutations activate *EGFR* without reducing the affinity for ATP or increasing the affinity for *EGFR* TKIs like sensitive *EGFR* mutants do, possibly by rearrangement of the C-helix.[4] At present, treatment options for these patients are limited. The treatment of choice in the first-line setting is platinum based chemotherapy and responses are consistent with the results seen with treatment of advanced lung adenocarcinoma in general.[7]

In this series, we have treated four patients with previously described *EGFR* exon 20 insertion variants, which are thought to confer resistance to *EGFR* TKIs.[1, 3, 4] Two out of four patients were treated with an *EGFR* TKI, including one patient with the third generation *EGFR* TKI osimertinib, without a radiological response. All patients received afatinib plus cetuximab, a combination of an irreversible ErbB family blocker that selectively blocks signaling from all ErbB family receptors and a chimeric, human-murine anti-EGFR mAb that binds with high affinity to the extracellular domain of *EGFR*. Three of the four patients had a PR and the median PFS was 5.4 months. In all cases, toxicity was manageable.

The clinical activity of afatinib plus cetuximab supports the hypothesis that dual *EGFR* inhibition can be effective in patients with *EGFR* exon 20 insertion positive NSCLC. Because these tumors are dependent on *EGFR* signaling, we hypothesized that intensification of *EGFR* blockade could induce tumor responses, as was also shown for patients with *EGFR* TKI sensitive *EGFR* mutations and secondary resistance to *EGFR* TKIs.[10] Wheler et al.[13]

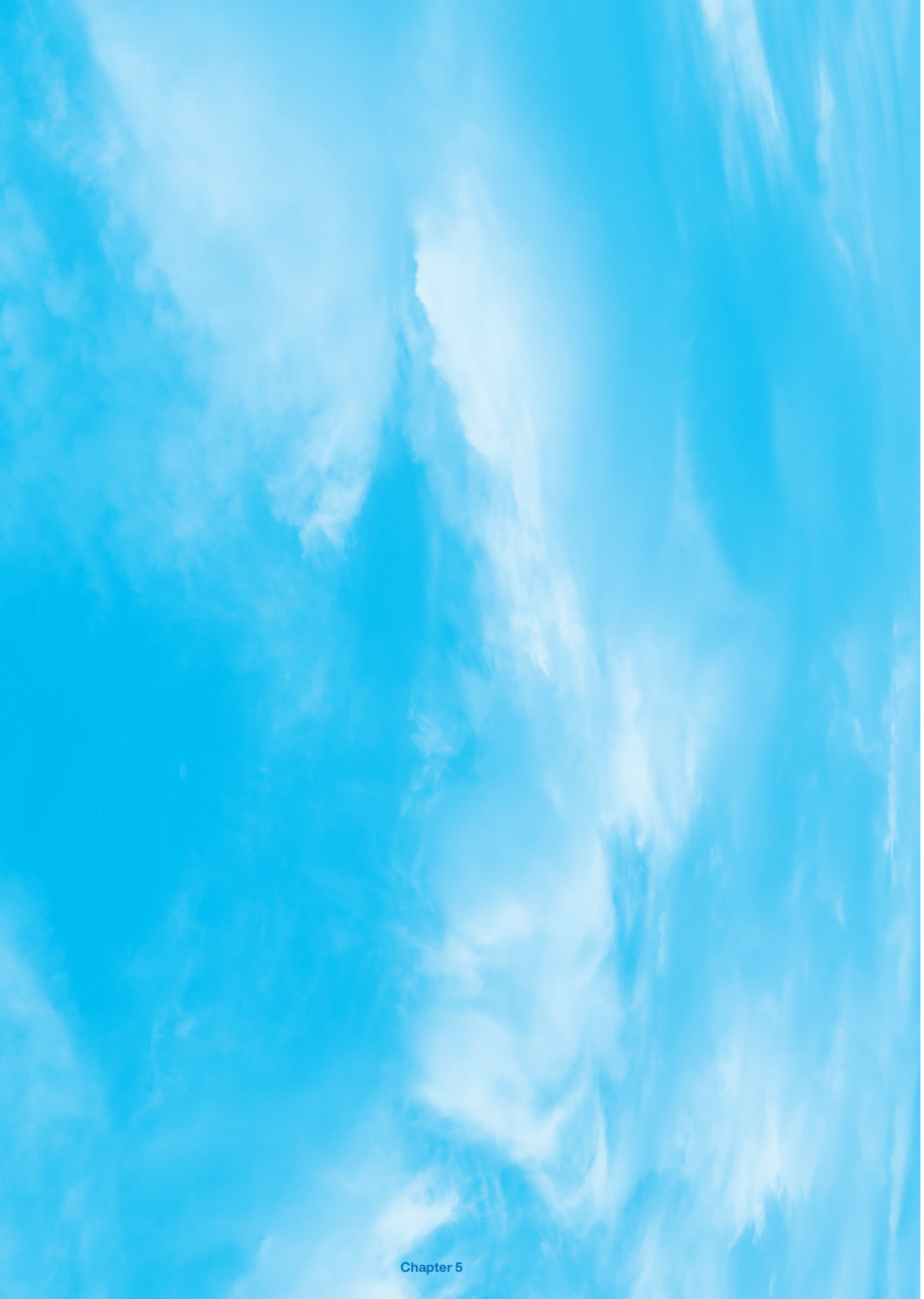
treated 20 NSCLC patients with erlotinib and cetuximab. One patient with an *EGFR* exon 20 insertion was included in this trial and this patient experienced a PR. Vanderbilt et al.[14] reported on two patients with *EGFR* exon 20 insertions that showed a PR on different cetuximab containing regimens (one patient was treated with afatinib and cetuximab and one with carboplatin and gemcitabine in combination with cetuximab).

Analysis of post-progression samples revealed an increase in the MET gene copy number in one patient, suggestive of MET bypass track resistance, a known resistance mechanism to *EGFR* TKIs.[15, 16] *EGFR* gene amplification has been postulated as a possible biomarker for *EGFR* mAb treatment benefit. A recent study observed a trend for greater necitumumab benefit in *EGFR* FISH positive squamous NSCLC patients.[17] However, in our case series pre-treatment *EGFR* gene copy numbers were low for two of the three responding patients.

In conclusion, this case series suggests that afatinib and cetuximab combination treatment is able to overcome primary *EGFR* TKI resistance in patients with *EGFR* exon 20 insertion positive NSCLC. Treatment with the combination resulted in a partial response in 3 out of 4 patients and a median PFS of 5.4 months. These results will be validated in a dedicated clinical trial.

References

1. Arcila ME, Nafa K, Chaft JE, et al. EGFR exon 20 insertion mutations in lung adenocarcinomas: prevalence, molecular heterogeneity, and clinicopathologic characteristics. Molecular cancer therapeutics 2013;12:220-229.
2. Mitsudomi T, Yatabe Y. Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. The FEBS journal 2010;277:301-308.
3. Oxnard GR, Lo PC, Nishino M, et al. Natural history and molecular characteristics of lung cancers harboring EGFR exon 20 insertions. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer 2013;8:179-184.
4. Yasuda H, Park E, Yun CH, et al. Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer. Science translational medicine 2013;5:216ra177.
5. Yasuda H, Kobayashi S, Costa DB. EGFR exon 20 insertion mutations in non-small-cell lung cancer: preclinical data and clinical implications. The Lancet Oncology 2012;13:e23-31.
6. Walter AO, Sjin RT, Haringsma HJ, et al. Discovery of a mutant-selective covalent inhibitor of EGFR that overcomes T790M-mediated resistance in NSCLC. Cancer discovery 2013;3:1404-1415.
7. Naidoo J, Sima CS, Rodriguez K, et al. Epidermal growth factor receptor exon 20 insertions in advanced lung adenocarcinomas: Clinical outcomes and response to erlotinib. Cancer 2015;121:3212-3220.
8. Hirano T, Yasuda H, Tani T, et al. In vitro modeling to determine mutation specificity of EGFR tyrosine kinase inhibitors against clinically relevant EGFR mutants in non-small-cell lung cancer. Oncotarget 2015;6:38789-38803.
9. Jia Y, Juarez J, Li J, et al. EGF816 Exerts Anticancer Effects in Non-Small Cell Lung Cancer by Irreversibly and Selectively Targeting Primary and Acquired Activating Mutations in the EGF Receptor. Cancer research 2016;76:1591-1602.
10. Janjigian YY, Smit EF, Groen HJ, et al. Dual inhibition of EGFR with afatinib and cetuximab in kinase inhibitor-resistant EGFR-mutant lung cancer with and without T790M mutations. Cancer discovery 2014;4:1036-1045.
11. Lee JY, Sun JM, Lim SH, et al. A Phase Ib/II Study of Afatinib in Combination with Nimotuzumab in Non-Small Cell Lung Cancer Patients with Acquired Resistance to Gefitinib or Erlotinib. Clinical cancer research : an official journal of the American Association for Cancer Research 2016;22:2139-2145.
12. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). European journal of cancer (Oxford, England : 1990) 2009;45:228-247.
13. Wheler JJ, Tsimberidou AM, Falchook GS, et al. Combining erlotinib and cetuximab is associated with activity in patients with non-small cell lung cancer (including squamous cell carcinomas) and wild-type EGFR or resistant mutations. Molecular cancer therapeutics 2013;12:2167-2175.
14. Vanderbilt CM. Two cases of NSCLC with EGFR Exon 20 Insertions with Major Clinical Response to Cetiximab-containing Therapies. 2015.
15. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039-1043.
16. Bean J, Brennan C, Shih JY, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proceedings of the National Academy of Sciences of the United States of America 2007;104:20932-20937.
17. Hirsch FR BT, Thatcher N, et al. EGFR IHC and FISH correlative analyses (SQUIRE trial): Necitumumab + gemcitabine-cisplatin vs gemcitabine-cisplatin in 1st-line squamous NSCLC. 16th World Conference on Lung Cancer. Abstract ORAL32.05. Presented September 9, 2015.



Chapter 5

A phase II trial combining afatinib with cetuximab in patients with *EGFR* exon 20 insertion positive non-small cell lung cancer

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Abstract

Introduction: **Epidermal growth factor receptor (*EGFR*) exon 20 insertion (ex20ins) mutations are the third most common *EGFR* mutations in patients with non-small cell lung cancer (NSCLC) and are associated with primary resistance to EGFR tyrosine kinase inhibitors (TKIs). There is evidence of activity of combining EGFR TKIs with monoclonal antibodies. Here we report on the efficacy and safety of afatinib in combination with cetuximab.**

Methods: **In this single-arm phase II trial, patients with advanced NSCLC harboring an EGFR ex20ins mutation were treated with afatinib 40 mg once daily, in combination with cetuximab 500 mg/m2, every two weeks. The primary endpoint was disease control rate (DCR) after 18 weeks of treatment.**

Results: **Thirty-seven patients started treatment: median age 65 years (range 40 – 80 years), 78% female, 35 (95%) Caucasian. The study achieved its primary endpoint, with a DCR of 54% at 18 weeks, an overall response rate (ORR) of 43% and a 32% confirmed ORR. Best responses were partial (n=16), stable (n=16), progressive disease (n=2) or not evaluable (n=3). Median progression free survival was 5.5 months (95% CI: 3.7 – 8.3 months) and median overall survival was 16.8 months (95% CI: 10.7 – 25.8 months). Most common treatment related adverse events (TRAEs) were diarrhea (70%), rash (65%), dry skin (59%), paronychia (54%) and erythema (43%). Grade III TRAEs were reported in 54% of all patients.**

Conclusions: **Combination treatment with afatinib and cetuximab demonstrated antitumor activity with a DCR of 54% at 18 weeks and a 32% confirmed ORR. Toxicity was significant, however manageable after dose reduction.**

Introduction

Epidermal growth factor receptor (*EGFR*) exon 20 insertion mutations (ex20ins) comprise 4% to 12% of *EGFR* mutated non-small cell lung cancer (NSCLC) and approximately 2% of all NSCLC cases [1-4]. In contrast to the more common in-frame deletions in exon 19 and the L858R point mutation in exon 21, *EGFR* ex20ins are generally resistant to EGFR tyrosine kinase inhibitors (TKIs), except for the *EGFR* A763_Y764insFQEA variant [2, 5-8]. Targeting *EGFR* ex20ins is challenging because these mutations activate EGFR without diminishing ATP affinity, resulting in a small therapeutic window for EGFR TKIs [9]. Although there is progress in developing novel targeted therapies for these patients, currently platinum-based chemotherapy remains the first line treatment of choice [10-12].

EGFR ex20ins are unresponsive to EGFR TKIs in general, including afatinib, a second generation irreversible EGFR TKI, with overall response rates (ORR) below 10% [8]. In addition, a pooled analysis of 70 patients treated with afatinib monotherapy demonstrated modest activity with an ORR of 24.3% [13]. There is some evidence of increased anti-tumor activity when adding cetuximab to an EGFR TKI. Cetuximab, an anti-EGFR monoclonal antibody (mAb), binds with high affinity to the extracellular domain of the EGFR receptor, partially blocks the ligand-binding domain and sterically hinders EGFR dimer formation [14]. Cetuximab can also target EGFR by diminishing EGFR phosphorylation [15]. Consequently, in combination with an EGFR TKI, mAbs induce a more potent inhibitory effect *in vitro*, *in vivo* and in xenografts than either therapy alone [16]. In 2017, we described tumor responses in three of four *EGFR* ex20ins positive NSCLC patients after afatinib and cetuximab combination treatment [17]. In conclusion, there is evidence of activity of combining EGFR TKIs with EGFR monoclonal antibodies. However, the safety profile of this combination is of special interest, since treatment with afatinib and cetuximab led to high rates of EGFR-related toxicity, especially dermatological side effects and diarrhea [18, 19].

We previously presented interim-data of the first 17 patients with *EGFR* ex20ins positive metastatic NSCLC who received afatinib in combination with cetuximab in our single arm, phase II trial (AFACET). The antitumor activity of this combination was demonstrated by a disease control rate (DCR) at 18 weeks of 59%, an ORR of 47% and median progression free survival (PFS) of 5.5 months. Almost 60% of patients experienced grade ≥3 toxicity [20]. Here, we report the final results of this trial.

Material and methods

Study Design and Patients

This single-arm, open-label, investigator-initiated phase II trial was conducted in five academic institutions in the Netherlands. Eligible patients had advanced NSCLC with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of ≤ 2 and had measurable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [21]. *EGFR* mutation status was locally tested using an amplicon based next-generation sequencing (NGS) hotspot panel that was validated for detection of *EGFR* ex20ins. Previous systemic therapy was allowed, but not mandatory. Key exclusion criteria were prior treatment with EGFR targeting antibodies (prior treatment with EGFR TKIs was allowed) and symptomatic brain metastases. Untreated asymptomatic brain metastases were allowed.

The protocol was approved by the medical research ethics committee. All patients provided written informed consent prior to study procedures and the study was done in accordance with the Declaration of Helsinki [22]. This study is registered with ClinicalTrials.gov, NCT03727724.

Treatment and Study Assessments

Patients received afatinib 40 mg orally once daily, in combination with cetuximab 500 mg/m^2 intravenously every two weeks, until disease progression or unacceptable toxicity. Patients who discontinued treatment for reasons other than disease progression continued with tumor assessments until disease progression. Supportive medication consisted of minocycline 100 mg once daily, loperamide as needed and emollient skin creams two-four times per day. Two dose reductions were allowed: firstly, a dose reduction of afatinib to 30 mg in combination with 400 mg of cetuximab, followed by a second dose reduction of afatinib 30 mg once daily and cetuximab 250 mg. If unacceptable toxicity recurred following two dose modifications, study treatment was permanently discontinued. Tumor assessments according to RECIST v1.1 were locally performed by an independent thoracic radiologist using computed tomography (CT) scans. CT scans were performed at baseline and every six weeks thereafter until radiographic progression. Magnetic resonance imaging (MRI) of the brain was performed at baseline, and thereafter every six weeks only in case brain metastases were present at screening. Selected patients were permitted to continue treatment beyond disease progression in case of ongoing clinical benefit.

Outcomes

The primary objective was to determine the DCR after 18 weeks of afatinib and cetuximab treatment. Secondary endpoints included investigator-assessed ORR, duration of response (DoR), PFS, overall survival (OS) and safety. Adverse events were graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.03.

Table 1: Baseline patient and tumor characteristics (n = 37)

Characteristics	Number of patients (%)
Median age, years (range)	65 (40 – 80)
Female	29 (78)
Histology	
Adenocarcinoma	36 (97)
Squamous cell carcinoma	1 (3)
Smoking status	
Never	13 (35)
Former	22 (59)
Current	2 (5)
Ethnicity	
Caucasian	35 (95)
African Descent	1 (3)
East/Southeast Asian	1 (3)
ECOG performance status	
0	11 (30)
1	23 (62)
2	3 (8)
Brain metastases at baseline	14 (38)
Local radiotherapy at baseline	2 (14)
Untreated asymptomatic brain metastases at baseline	12 (86)
Most common *EGFR* ex20ins mutations (>10%)	
S768_D770dup	7 (19)
A767_V769dup	4 (11)
D770_N771insG	4 (11)
Prior treatment	
Platinum based chemotherapy	10 (27)
Chemo-immunotherapy	6 (16)
Osimertinib	7 (19)
Median prior lines of therapy (range)	0 (0 - 5)
0	19 (51)
1	12 (32)
2	5 (14)
5	1 (3)

Abbreviations: ECOG: Eastern Cooperative Oncology Group; *EGFR* ex20ins: epidermal growth factor receptor exon 20 insertion.

Statistical Analysis

A Simon's two-stage optimal design was used for sample size determination. The null hypothesis was tested against a one-sided alternative, with α: 0.10, power of 90. The trial aimed to show a DCR of ≥ 40% at 18 weeks after treatment initiation. Seventeen patients were planned for inclusion in the first stage. If at least four patients experienced disease control after 18 weeks of treatment, the trial expanded to a total sample size to 37 patients. The study treatment was accepted for further development if at least 11 of 37 patients experienced disease control at 18 weeks. A uniform minimum variance unbiased estimator for the response rate was used on that matter proposed by Jung [23], and as a 95% confidence interval (CI) the Koyama et al [24] method was used on that purpose. The clinfun package, version 1.1.0, in R programming was used for that calculation, and specifically the twostage.inference function.

Overall response and disease control were estimated, including their two-sided 95% confidence intervals (CIs). DCR was defined as percentage of patients who had achieved complete response, partial response or stable disease at eighteen weeks of treatment. Time-to-event endpoints (PFS, DoR and OS) were analyzed using the Kaplan-Meier method. PFS was defined as the interval between initiation of study treatment and the date of radiological progression or death from any cause, whatever occured first. The data cutoff for the analyses was December 18, 2022. P values for subgroups were calculated using the log rank test. Statistical software program R was used for all the analyses.

Results

Patients

Between Jan 2019 and Dec 2021, 40 patients were screened. Three patients did not meet all inclusion criteria and were registered as screen failure. Thirty-seven patients were eligible and started study treatment. All patients had stage IV NSCLC harboring an *EGFR* ex20ins mutation based on NGS results of histological or cytological tumor samples. Baseline characteristics are shown in Table 1. The majority of patients (78%) were female and 35% had never smoked. Among fourteen patients (38%) with brain metastases at baseline, two were treated previously with local radiotherapy. The most common *EGFR* ex20ins mutation was S768_D770dup (19%, n = 7), followed by A767_V769dup (11%, n = 4) and D770_N771insG (11%, n = 4). No patients with the known sensitizing *EGFR* ex20ins variant A763_Y764insFQEA were included in this cohort. Eighteen patients (49%) received previous treatment with chemotherapy, chemo-immunotherapy and/or osimertinib. None of the patients received another (new-generation) EGFR TKI prior to enrollment in the study. The median number of previous lines of therapy was zero (range, 0 – 5).

Efficacy

The primary endpoint was met as disease control was achieved in 54% (95% CI: 25 – 63, n = 20) after 18 weeks of treatment. Best responses were partial (n = 16), stable (n = 16) or progressive disease (PD) (n = 2) (Figure 1). Three patients were not evaluable (NE) for response due to a treatment-related adverse event (TRAE) or symptomatic deterioration before the first radiological assessment. The ORR was 43%. Among the 16 patients with a PR, 12 were confirmed at subsequent imaging resulting in a confirmed ORR rate of 32% (95% CI, 20 – 49). Median DoR was 4.7 months (range 0.3 – 16.6 months), median PFS 5.5

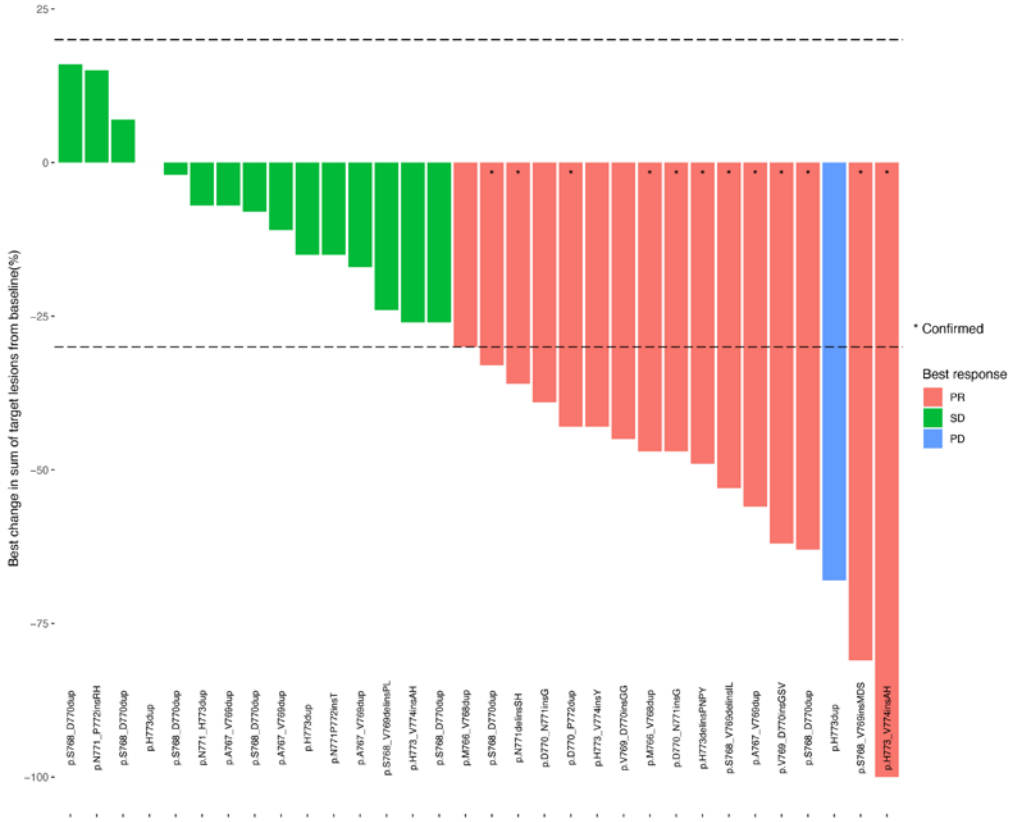


Figure 1. Tumor response to afatinib and cetuximab. Waterfall plot of the best percent change from baseline in the sum of target lesion diameters by locations of EGFR ex20ins mutations based on investigator assessment in patients with evaluable disease. The dashed lines at 20% and -30% indicate the thresholds for progressive disease and partial response, respectively, for RECIST response. Confirmed response rates are indicated with an asterisk. EGFR indicates epidermal growth factor receptor; ex20ins, exon 20 insertion; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.

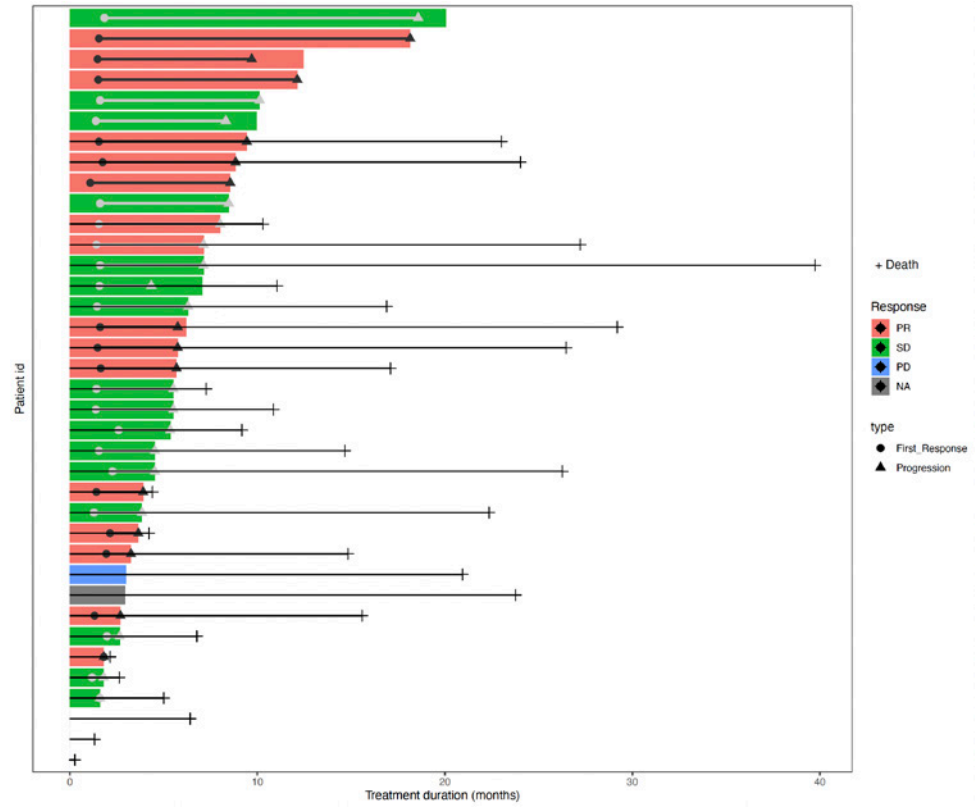


Figure 2. Duration of treatment. Duration of afatinib and cetuximab treatment in all treated patients (N = 37). Each bar represents one subject in the study. NA indicates nonapplicable; PD, progressive disease; PR, partial response; SD, stable disease.

months (95% CI: 3.7 – 8.3 months) and median OS was 16.8 months (95% CI: 10.7 – 25.8 months). At data cutoff (December 2022) all patients were off study treatment (Figure 2). The primary reasons for discontinuation were PD (73%) or adverse event(s) (22%). Two patients refused further treatment (5%).

In the subset of patients with brain metastases at baseline, the median PFS was similar to those without brain metastases (p-value = 0.87). Three patients continued study treatment beyond cerebral disease progression for a median of 7 weeks (range 6 – 14 weeks). One patient received whole brain radiotherapy during study treatment. Seven weeks later, treatment was permanently discontinued due to further progression. Of the patients with brain metastases at baseline, all patients (except two patients not evaluable for response) had cerebral progression as first site of progression.

Although one partial response was observed in the subgroup of patients pretreated with osimertinib (n = 9), patients pretreated with osimertinib did fare worse than those without in terms of PFS (p = 0.00055; Log-rank test).

When considering the treatment line, patients receiving study treatment as first line treatment showed a statistically longer PFS compared to later lines (p = 0.0001; Log-rank test).

Table 2: Mutation type and response to treatment

Insertion region	Mutation type	Best response	Best change from baseline according RECIST (%)	PFS (months)
Helical region n = 2 ORR 100%	p.M766_V768dup	PR	-30	5.5
	p.M766_V768dup	PR	-47	3.2
Near loop region n = 28 ORR 39%	p.A767_V769dup	SD	-11	19.6
	p.A767_V769dup	SD	-17	5.5
	p.A767_V769dup	PR	-56	8.5
	p.A767_V769dup	SD	-7	6.2
	p.S768_V769insMDS	PR	-81	5.5
	p.S768_D770dup	PR	-63	6.9
	p.S768_D770dup	SD	-8	3.7
	p.S768_D770dup	SD	7	9.7
	p.S768_D770dup	SD	-2	1.6
	p.S768_D770dup	SD	16	1.4
	p.S768_D770dup	SD	-26	5.5
	p.S768_D770dup	PR	-33	9.7
	p.S768_V769delinsIL	NE	NE	0
	p.S768_V769delinsIL	PR	-53	8.8
	p.S768_V769delinsPL	SD	-24	8.3
	p.V769_D770insGSV	PR	-62	18.0
	p.V769_D770insGG	PR	-45	1.6
	p.D770_N771insG	PD	NE	0
	p.D770_N771insG	PR	-47	12.0
	p.D770_N771insG	SD	NE*	12.7
	p.D770_N771insG	PR	-39	2.8
	p.D770_P772dup	NE	NE	3.0
	p.D770_P772dup	PR	-43	7.8
	p.N771delinsGY	NE	NE	1.0
	p.N771_P772insRH	SD	15	3.7
	p.N771_P772insT	SD	-15	3.9
	p.N771delinsSH	PR	-36	9.7
	p.N771_H773dup	SD	-7	2.1
Far loop region n = 7 ORR 43%	p.H773dup	PD	-68	1.4
	p.H773dup	SD	0	6.9
	p.H773dup	SD	-15	4.1
	p.H773delinsPNPY	PR	-49	9.2
	p.H773_V774insY	PR	-43	2.1
	p.H773_V774insAH	SD	-26	4.6
	p.H773_V774insAH	PR	-100	3.7

*There was no measurable lesion conforming to RECIST at baseline

NE: non-evaluable; ORR: overall response rate; PD: progressive disease; PFS: progression free survival; PR: partial response; RECIST: Response Evaluation Criteria in Solid Tumors; SD: stable disease

Table 3: Summary of TRAEs of any grade reported in 10% or more of patients or grade 3 or higher.

	Patients, No. (%)			
TRAE ≥ 10%	**Total**	**Grade 1**	**Grade 2**	**Grade ≥ 3**
Diarrhea	26 (70)	19 (51)	2 (5)	5 (14)
Rash*	24 (65)	8 (22)	11 (30)	5 (14)
Dry skin	22 (59)	10 (27)	7 (19)	5 (14)
Paronychia	20 (54)	9 (24)	10 (27)	1 (3)
Erythema multiforme	16 (43)	8 (22)	6 (16)	2 (5)
Fatigue	14 (38)	9 (24)	5 (14)	0 (0)
Hypertrichosis	13 (35)	11 (30)	2 (5)	0 (0)
Nausea	13 (35)	6 (16)	6 (16)	1 (3)
Anorexia	9 (24)	5 (14)	4 (11)	0 (0)
Mucositis	9 (24)	2 (5)	6 (16)	1 (3)
Dysgeusia	8 (22)	7 (19)	1 (3)	0 (0)
Pruritus	7 (19)	4 (11)	2 (5)	1 (3)
Dry mouth	6 (16)	6 (16)	0 (0)	0 (0)
Chills	5 (14)	4 (11)	1 (3)	0 (0)
Dry eye	5 (14)	4 (11)	1 (3)	0 (0)
Infusion related reaction	5 (14)	0 (0)	5 (14)	0 (0)
Headache	4 (11)	2 (5)	2 (5)	0 (0)

Abbreviations: TRAE: treatment-related adverse event
*Rash is defined by rash papulopustular, rash, maculo-papular, dermatitis and rash acneiform.

Responses were observed across the entire spectrum of *EGFR* ex20ins mutations (Table 2). There were no differences in ORR within the near-loop and far-loop regions of exon 20. Only two patients harbored an EGFR ex20ins within the helical region with an ORR of 100%.

Safety

Most common TRAEs were diarrhea (70%), rash (65%), dry skin (59%), paronychia (54%) and erythema (43%). Grade III TRAEs were reported in 54% of patients. Grade III TRAEs ≥ 10% included diarrhea (n=5; 14%), rash (n=5; 14%) and dry skin (n=5; 14%). All TRAEs of any grade reported in 10% or more or grade three or higher TRAEs are listed in Table 3. No grade IV treatment-related toxicity was observed. One patient died due to respiratory failure after the first infusion of study medication, probably related to disease progression, possibly treatment related. Two other patients died during study treatment due to non-treatment related events, respectively COVID-19 infection and cardiac arrest. Twenty-five (68%) patients required a dose reduction, including five patients (14%) who had two dose reductions. Rate of treatment discontinuation due to TRAEs was 16% (n=6), including one grade III allergic reaction after the first infusion of cetuximab.

Discussion

In this phase II trial, combination treatment with afatinib and cetuximab was effective for patients with *EGFR* ex20ins mutated NSCLC, resulting in a DCR of 54% after 18 weeks and a confirmed ORR of 32%. The median PFS was 5.5 months. Twenty-eight (76%) patients experienced a decrease in tumor size.

Multiple other TKIs, specifically designed to target *EGFR* ex20ins, have recently been tested in clinical trials. This led to the FDA approval of mobocertinib, for patients harboring an *EGFR* ex20ins mutation, who progressed on or after prior platinum-based chemotherapy. The ORR was 28% with a median PFS of 7.3 months [25, 26]. Poziotinib, another EGFR ex20ins-directed TKI demonstrated a 14.8% ORR [27]. In addition, the EGFR-MET bispecific antibody amivantamab was active in pretreated patients with an ORR of 40% and median PFS of 8.3 months [28]. Although progress has been made in the treatment of previously considered untargetable *EGFR* ex20ins, the effectiveness of these new agents is not on the same level as the treatment options for classical *EGFR* mutations and toxicity remains a major concern.

EGFR ex20ins mutations lack the therapeutic advantage of increased affinity for TKI versus ATP. Therefore, these mutations have a small therapeutic window for EGFR TKIs, resulting in high rates of typical EGFR related toxicity. In our study, grade III or higher TRAEs were reported in 54% of all patients, mostly consisting of diarrhea and skin toxicity. As a result, 68% of patients required a dose reduction and 16% percent discontinued treatment due to adverse events. With close monitoring, dose reductions and timely referral to a dermatologist, skin-related toxicity was generally manageable. However, this also clearly shows that adding an anti-EGFR mAb to afatinib enhances on-target side effects associated with inhibition of EGFR. Previous studies with TKI-antibody combinations already showed an increase in TRAEs, resulting in more than 50% grade ≥ III toxicity [29, 30].

In addition, other TKIs like poziotinib and mobocertinib, specifically designed to target *EGFR* ex20ins mutations, could not preserve selectivity against wild type EGFR. During poziotinib treatment, 28% and 26% of patients had grade ≥ 3 rash and diarrhea TRAEs, respectively [27]. Results from the expanded access program showed 66% grade ≥ 3 TRAEs[31]. These high toxicity rates led to a new trial to evaluate a lower dose and twice daily dosing. Mobocertinib

resulted in 46% grade ≥ 3 TRAEs [26]. Toxicity of these new agents seems comparable to our study. Possibly, a lower starting dose of afatinib 30 mg daily will lead to fewer serious adverse events, while maintaining effectiveness [32].

Most of the clinical trials regarding new *EGFR* ex20ins directed therapies excluded patients with active or untreated brain metastases. Therefore, the intracranial activity of these agents is largely unknown. Regarding intracranial activity in our study, the majority of patients with brain metastases at baseline were untreated (n=12). Although most patients experienced some decrease of tumor shrinkage at the first tumor evaluation, all of these patients had first progression in the brain, suggesting brain radiotherapy is required before start treatment with afatinib plus cetuximab.

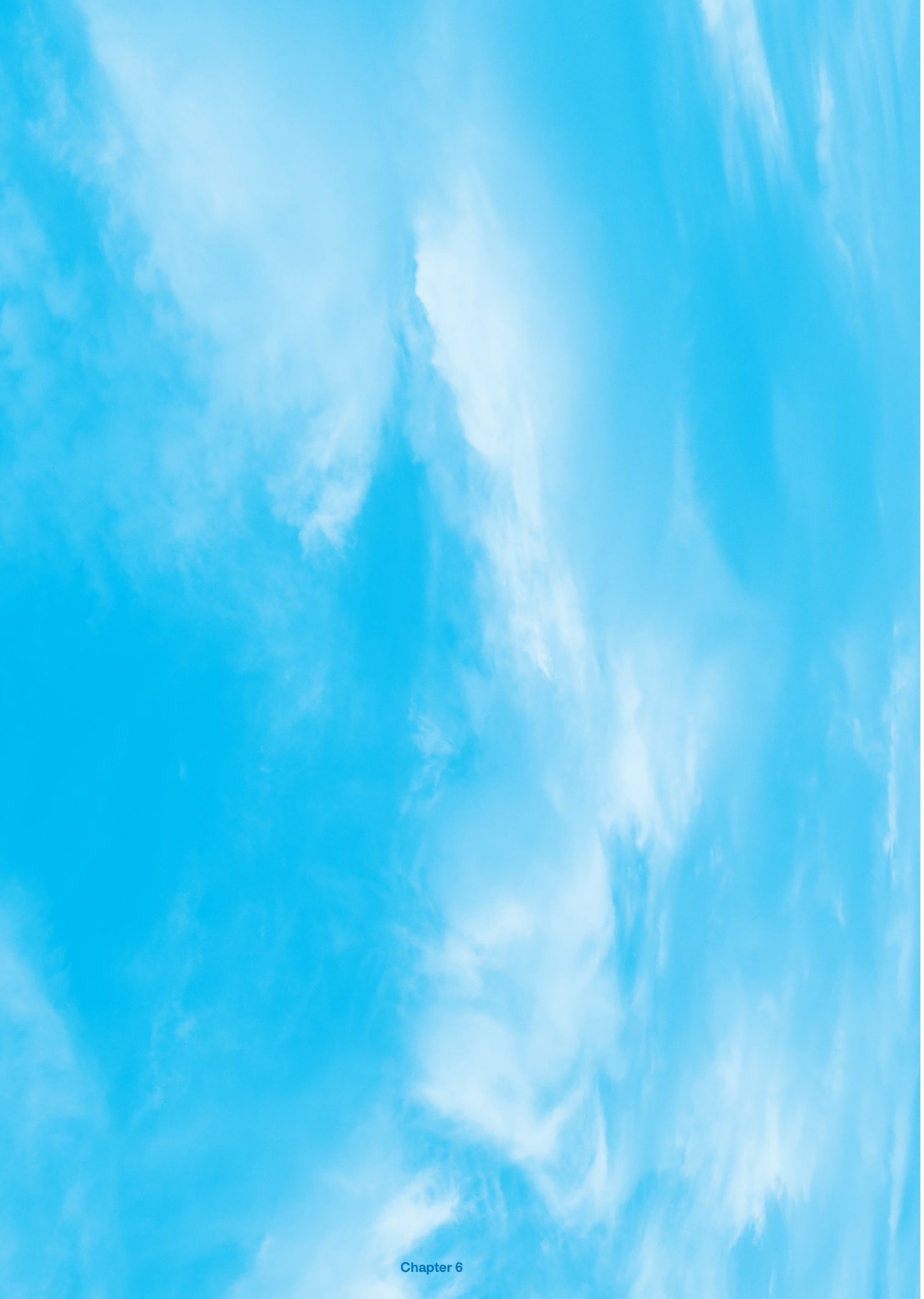
Our study has several limitations, including the lack of a control arm and an independent blinded radiological review. In addition, no previous line of treatment was required. Patients treated with afatinib and cetuximab as first line treatment showed a statistically longer PFS compared to later lines. Therefore, comparison to other predominantly second line studies involving new EGFR ex20ins directed targeted treatment options is difficult.

In conclusion, combination treatment with afatinib and cetuximab demonstrated antitumor activity in patients with *EGFR* ex20ins positive NSCLC, with a DCR of 54% at 18 weeks and a 32% confirmed ORR. EGFR-related toxicity was significant, however manageable after dose reduction.

References

1. Beau-Faller M, Prim N, Ruppert AM, et al. Rare EGFR exon 18 and exon 20 mutations in non-small-cell lung cancer on 10 117 patients: a multicentre observational study by the French ERMETIC-IFCT network. Ann Oncol 2014;25:126-131.
2. Fang W, Huang Y, Hong S, et al. EGFR exon 20 insertion mutations and response to osimertinib in non-small-cell lung cancer. BMC Cancer 2019;19:595.
3. Kosaka T, Tanizaki J, Paranal RM, et al. Response Heterogeneity of EGFR and HER2 Exon 20 Insertions to Covalent EGFR and HER2 Inhibitors. Cancer Res 2017;77:2712-2721.
4. Riess JW, Gandara DR, Frampton GM, et al. Diverse EGFR Exon 20 Insertions and Co-Occurring Molecular Alterations Identified by Comprehensive Genomic Profiling of NSCLC. J Thorac Oncol 2018;13:1560-1568.
5. Naidoo J, Sima CS, Rodriguez K, et al. Epidermal growth factor receptor exon 20 insertions in advanced lung adenocarcinomas: Clinical outcomes and response to erlotinib. Cancer 2015;121:3212-3220.
6. Robichaux JP, Elamin YY, Tan Z, et al. Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer. Nat Med 2018;24:638-646.
7. van Veggel B, Madeira RSJFV, Hashemi SMS, et al. Osimertinib treatment for patients with EGFR exon 20 mutation positive non-small cell lung cancer. Lung Cancer 2020;141:9-13.
8. Yang JC, Sequist LV, Geater SL, et al. Clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations: a combined post-hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. Lancet Oncol 2015;16:830-838.
9. Yasuda H, Park E, Yun CH, et al. Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer. Sci Transl Med 2013;5:216ra177.
10. Choudhury NJ, Schoenfeld AJ, Flynn J, et al. Response to Standard Therapies and Comprehensive Genomic Analysis for Patients with Lung Adenocarcinoma with EGFR Exon 20 Insertions. Clin Cancer Res 2021;27:2920-2927.
11. Shah MP, Aredo JV, Padda SK, et al. EGFR exon 20 Insertion NSCLC and Response to Platinum-Based Chemotherapy. Clin Lung Cancer 2021.
12. Hendriks LE, Kerr KM, Menis J, et al. Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol 2023;34:339-357.
13. Yang JC, Schuler M, Popat S, et al. Afatinib for the Treatment of NSCLC Harboring Uncommon EGFR Mutations: A Database of 693 Cases. J Thorac Oncol 2020;15:803-815.
14. Li S, Schmitz KR, Jeffrey PD, et al. Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. Cancer Cell 2005;7:301-311.
15. Brand TM, Iida M, Wheeler DL. Molecular mechanisms of resistance to the EGFR monoclonal antibody cetuximab. Cancer Biol Ther 2011;11:777-792.
16. Hasegawa H, Yasuda H, Hamamoto J, et al. Efficacy of afatinib or osimertinib plus cetuximab combination therapy for non-small-cell lung cancer with EGFR exon 20 insertion mutations. Lung Cancer 2019;127:146-152.
17. van Veggel B, de Langen AJ, Hashemi SMS, et al. Afatinib and Cetuximab in Four Patients With EGFR Exon 20 Insertion-Positive Advanced NSCLC. J Thorac Oncol 2018;13:1222-1226.
18. Goldberg SB, Redman MW, Lilenbaum R, et al. Randomized Trial of Afatinib Plus Cetuximab Versus Afatinib Alone for First-Line Treatment of EGFR-Mutant Non-Small-Cell Lung Cancer: Final Results From SWOG S1403. J Clin Oncol 2020;38:4076-4085.
19. Janjigian YY, Smit EF, Groen HJ, et al. Dual inhibition of EGFR with afatinib and cetuximab in kinase inhibitor-resistant EGFR-mutant lung cancer with and without T790M mutations. Cancer Discov 2014;4:1036-1045.
20. Veggel Bv, Wekken AJvd, Paats M, et al. Interim results of a phase II single arm trial combining afatinib with cetuximab in patients with EGFRex20ins positive NSCLC. Journal of Clinical Oncology 2021;39:9112-9112.
21. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-247.
22. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. Jama 2013;310:2191-2194.
23. Jung SH, Kim KM. On the estimation of the binomial probability in multistage clinical trials. Stat Med 2004;23:881-896.
24. Koyama T, Chen H. Proper inference from Simon's two-stage designs. Stat Med 2008;27:3145-3154.
25. Duke ES, Stapleford L, Drezner N, et al. FDA Approval Summary: Mobocertinib for Metastatic Non-Small Cell Lung Cancer with EGFR Exon 20 Insertion Mutations. Clin Cancer Res 2023;29:508-512.
26. Zhou C, Ramalingam SS, Kim TM, et al. Treatment Outcomes and Safety of Mobocertinib in Platinum-Pretreated Patients With EGFR Exon 20 Insertion-Positive Metastatic Non-Small Cell Lung Cancer: A Phase ½ Open-label Nonrandomized Clinical Trial. JAMA Oncol 2021;7:e214761.
27. Le X, Goldman JW, Clarke JM, et al. Poziotinib shows activity

and durability of responses in subgroups of previously treated EGFR exon 20 NSCLC patients. Journal of Clinical Oncology 2020;38:9514-9514.
28. Park K, Haura EB, Leighl NB, et al. Amivantamab in EGFR Exon 20 Insertion-Mutated Non-Small-Cell Lung Cancer Progressing on Platinum Chemotherapy: Initial Results From the CHRYSALIS Phase I Study. J Clin Oncol 2021;39:3391-3402.
29. Cortot AB, Madroszyk A, Giroux-Leprieur E, et al. First-Line Afatinib plus Cetuximab for EGFR-Mutant Non-Small Cell Lung Cancer: Results from the Randomized Phase II IFCT-1503 ACE-Lung Study. Clin Cancer Res 2021;27:4168-4176.
30. Goldberg SB, Redman MW, Lilenbaum R, et al. Randomized Trial of Afatinib Plus Cetuximab Versus Afatinib Alone for First-Line Treatment of EGFR-Mutant Non–Small-Cell Lung Cancer: Final Results From SWOG S1403. Journal of Clinical Oncology 2020;38:4076-4085.
31. Prelaj A, Bottiglieri A, Proto C, et al. Poziotinib for EGFR and HER2 exon 20 insertion mutation in advanced NSCLC: Results from the expanded access program. Eur J Cancer 2021;149:235-248.
32. Yang CJ, Tsai MJ, Hung JY, et al. The clinical efficacy of Afatinib 30 mg daily as starting dose may not be inferior to Afatinib 40 mg daily in patients with stage IV lung Adenocarcinoma harboring exon 19 or exon 21 mutations. BMC Pharmacol Toxicol 2017;18:82.



Chapter 6

A comprehensive overview of the heterogeneity of *EGFR* exon 20 variants in NSCLC and (pre) clinical activity to currently available treatments

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Abstract

Activating EGFR mutations are commonly observed in non-small cell lung cancer (NSCLC). About 4-10% of all activating epidermal growth factor receptor (EGFR) mutations are heterogenous in-frame deletion and/or insertion mutations clustering within exon 20 (EGFRex20+). NSCLC patients with EGFRex20+ mutations are treated as a single disease entity, irrespective of the type and location of the mutation. Here, we provide a comprehensive assessment of the literature reporting both in vitro and clinical drug sensitivity across different EGFRex20+ mutations. The activating A763_Y764insFQEA mutation has a better tumor response in comparison with mutations in the near- and far regions directly following the C-helix and should therefore be treated differently. For other EGFRex20+ mutations marked differences in treatment responses have been reported indicating the need for a classification beyond the exon-based classification. A further classification can be achieved using a structure-function modeling approach and experimental data using patient-derived cell lines. The detailed overview of TKI responses for each EGFRex20+ mutation can assist treating physicians to select the most optimal drug for individual NSCLC patients.

Introduction

The epidermal growth factor receptor (EGFR) is a transmembrane protein that influences the pathogenesis in a subset of non-small cell lung cancers (NSCLC) [1]. Activation of EGFR is achieved by somatic mutations that lead to a ligand-independent activation of EGFR and uncontrolled cell growth [2,3]. Over the past decades, multiple tyrosine kinase inhibitors (TKIs) have been developed to inhibit growth of *EGFR*-mutated NSCLC. These drugs affect the intrinsic mitochondrial apoptotic pathway and thereby induce cell death [4]. The most common activating *EGFR* mutations, referred to as classical mutations, are in-frame deletions in exon 19 and a mutation resulting in a leucine to arginine amino acid change at position 858 (L858R) in exon 21. Together, these variants represent about 90% of all activating *EGFR* mutations in NSCLC and tumor cells harboring the classical variants demonstrate high sensitivity to first-, second and third generation EGFR-TKI's [5–7].

About 4-12% of all activating *EGFR* mutations are in-frame deletion and/or insertion mutations clustering within exon 20 of *EGFR* (EGFRex20+) [8,9]. These insertions and duplications are heterogeneous and cluster in a region of 15 amino acids, encompassing residues 761 to 775 [10,11]. The in-frame insertions and duplications vary in size between 1 to 7 amino acids (3 and 21 base pairs). Most EGFRex20+ mutations (90%) affect the loop following the C-helix (amino acids 767 to 775). Only 10% of the EGFRex20+ mutations occur towards the C-terminal end of the C-helix (amino acids 761 to 766) [11,12]. (Figure [Fig.] 1). EGFRex20+ patients are diagnosed at a median age of 60 years. The variant V769_D770insASV is more common in older patients (≥65 years) while the A763_Y764insFQEA variant is more common in younger patients (≤65 years) [13]. Clinical characteristics of NSCLC patients harbouring EGFRex20+ mutations are similar to patients with common *EGFR* mutations, i.e. being more common in woman and non-smokers [14–16].

EGFRex20+ mutation positive NSCLC are resistant to clinically achievable doses of most TKIs (i.e. gefitinib, erlotinib or afatinib), with the exception of the A763_Y764insFQEA mutation which likely leads to a structurally distinct protein compared to the other EGFRex20+mutations [11]. The homology model of the A763_Y764insFQEA mutation, strongly suggest that the inserted amino acids form an additional helical turn within the C-helix (amino acids 761 to 766, Fig. 1). Subsequent *in vitro* experiments indicates that the additional helical turn results in a changed interaction of the C-helix within EGFR. The changed interaction consequently shifts the entire C-helix toward its N-terminus, resulting in a larger distance created between the end of the β3 strand and start of the N-terminus (β3-αC loop; Fig. 1), pulling the C-helix towards the C-in position. No rearrangements in the TKI binding sites were observed. This leads to an increase in the catalytic activity (comparable to the *EGFR* exon 21 L858R mutation at a structural and enzyme kinetic level), which makes it sensitive to TKIs [11,17].

On the contrary, the crystal structure of the insensitive D770_N771insNPG mutation shows an insertion of three amino acids at the C-terminal end of the C-helix, which indicates a reposition of the C-helix. The reposition of the C-helix provides additional steric hindrance for the transition from the active to inactive conformation, strongly promoting the C-helix to remain in its active kinase conformation. Thereby, enzyme kinetic studies of the D770_N771insNPG structure show that the mutation activates EGFR without increasing its affinity for EGFR TKIs, meaning there is no advantage in competition for ATP. Therefore, inhibition of the mutant protein is not possible without inhibition of the wild type (WT) protein which leads to untoward toxicity [11,12].

At this moment, patients with EGFRex20+ mutations are treated as a single disease entity, irrespective of the type of EGFRex20+ mutation. However, a marked degree of heterogeneity in drug sensitivity has been shown across different EGFRex20+ mutations [18,19]. Hence, there is a need to characterize TKI responses of EGFRex20+ mutant NSCLC cases, going beyond the concept of being a homogeneous subgroup. In this study, we reviewed current knowledge on the structure-function relationship of different EGFRex20+ mutations on drug sensitivity based on previously reported *in vitro* and *in vivo* studies. This comprehensive overview can serve as a practical guide for clinicians to select the most optimal drug for individual NSCLC EGFRex20+ patients in clinical practice.

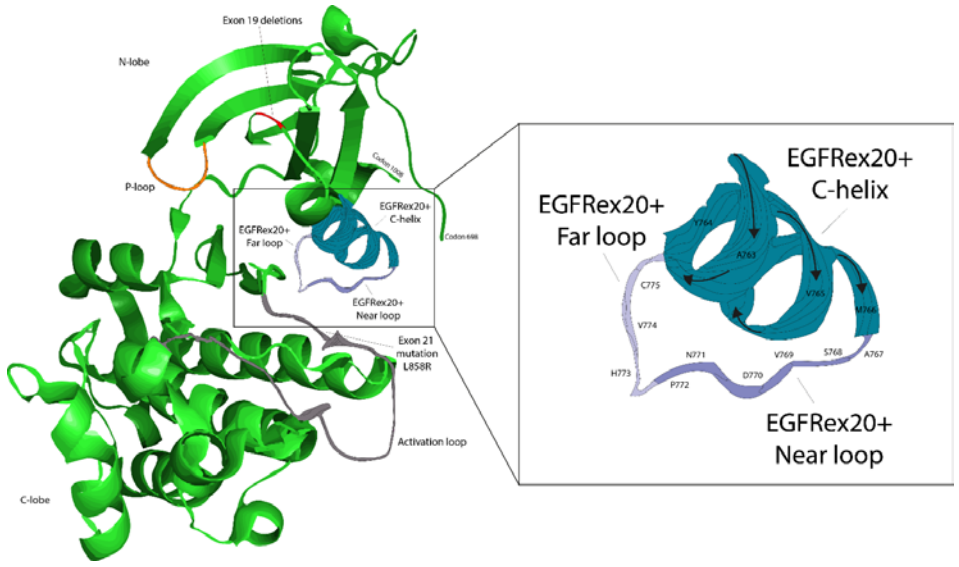


Figure 1. The active conformation of the wild-type tyrosine kinase domain of EGFR (codons 698 to 1008). It consists of a smaller N-lobe and a larger C-lobe. Within the cleft of the two lobes lies the ATP binding site and active site of the kinase. The location of the EGFRex20+ mutations are highlighted. The different regions corresponding to the C-helix, near loop, and far loop are shown in detail with the amino acid positions labelled. For orientation, the P-loop is indicated in orange and the activation loop is indicated in grey. The most common activating EGFR mutations exon 19 and exon 21 L858R are also shown. Image created using PyMOl and PDB ID: 4zau [20]).

Material and methods

Worldwide studies of EGFRex20 mutation-positive NSCLC

A PubMed search using the syntax: "NSCLC" "EGFR" "exon 20" was performed on March 23rd, 2023. Nine-hundred-thirty-eight articles were screened on title and abstract to select clinical studies reporting real-world data and trial data from different countries around the world i.e. North-America, Latin-America, Australia, China, Taiwan, Korea, France, and the Netherlands. We focused on studies that report separate EGFRex20+ mutations to provide a real-world overview (Supplementary [Sup.] Table 1). We excluded studies/cases with non-specified EGFRex20+ mutations and EGFRex20 mutations coexisting with other non-exon 20 *EGFR* mutations. The S768I mutation [a substitution at codon 768 of exon 20 (c.2303G>T, p.S768I)] was also excluded, because it is typically seen in conjunction with other sensitizing EGFR mutations. The EGFRex20+ mutations were converted into the amino acid variant annotation according to the HGVS nomenclature using the NP_005219.2 transcript as a reference (Sup. Table 2).

Literature review: comparing sensitivity of in vitro *models*

A second literature review using the same PubMed search was performed to identify studies reporting the *in vitro* sensitivity towards TKI's of EGFRex20+ mutant patient-derived cell lines which were shown to be dependent on EGFR signaling for growth (tested with EGFR siRNAs or shRNA) (Sup. Table 3). In addition, we included studies with Ba/F3 cells, which is a murine pro-B cell line that is dependent on interleukin-3 (IL-3) for survival and proliferation [21]. By ectopic expression of a driver gene, the Ba/F3 cells become independent of IL-3 [22]. This makes the Ba/F3 cell line a suitable model to study effectivity of TKIs on different EGFRex20+ mutations [23]. The EGFRex20+ mutations were converted into the correct amino acid variant as described above (Sup. Table 2). Studies were included based on reporting IC50 values for individual TKIs. Exclusion criteria were missing TKI concentrations, missing IC50 values for EGFR WT Ba/F3, and studies with a secondary T790M EGFR mutation. To compare the sensitivities, we listed the reported IC_{50} values [unit: nmol/L] of the mutant and WT EGFR Ba/F3 cells (Sup. Table. 4) and calculated the ratio of mutant IC50 to WT IC50. We defined a mutation as sensitive for the tested TKIs when the ratio was below one (<1), meaning that the mutant Ba/F3 cells were more sensitive to the TKI as compared to EGFR WT Ba/F3 cells. A ratio of ≥1 was defined as insensitive, indicating that the mutant Ba/F3 cells were less sensitive to the TKI as compared to EGFR WT Ba/F3 cells. Sensitivity of the patient-derived cell lines for specific TKIs was determined by comparing the IC50 value of, if available, the matching mutated or WT Ba/F3 IC50 to the same TKI (Sup. Table. 5).

Assessment of treatment results of NSCLC patients carrying EGFRex20 mutation-positive

Therapeutic efficacy is different for patients with EGFRex20+ mutations in comparison to common EGFR mutations. We included several studies with recent clinical treatment results in which NSCLC patients harboring different EGFRex20+ mutations were treated with different TKIs (Sup. Table 6). Parameters that were extracted included molecular characteristics of the specific EGFRex20+ mutation, radiological best overall response (BOR) according to RECIST criteria 1.1, progression-free survival (PFS), and duration of response (DoR). For each EGFRex20+ mutation the PFS reported were collected and the total median PFS (mPFS) was calculated and tabulated. Studies describing responses to non-specified EGFRex20+ mutations were excluded.

Results

Distribution and frequency of EGFRex20 mutation-positive NSCLC patients

We reviewed 36 studies to evaluate the distribution and frequency of EGFRex20+ mutations observed in NSCLC (Sup. Table 1, Sup. Fig 1). In Fig. 2, we summarized the distribution and relative frequency of the EGFRex20+ mutations by amino acid position. We found 104 different pathogenic mutations in NSCLC patients with EGFRex20+ mutations.

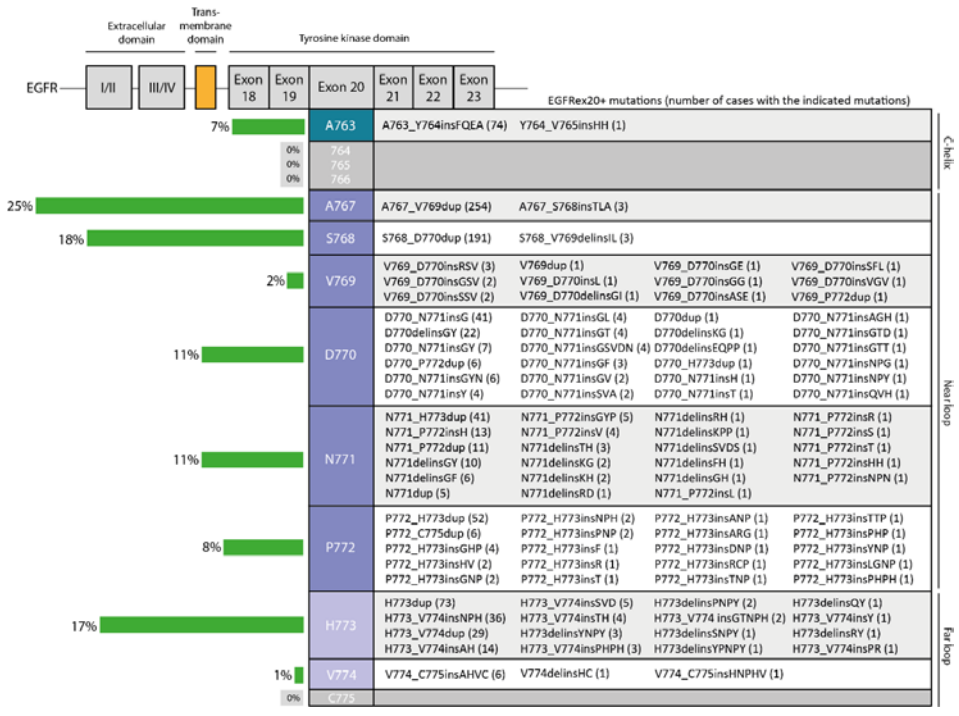


Figure 2. EGFR exon 20 structure with the distribution and relative frequency of 104 different somatic mutations by amino acid position in at least 1059 patients that have been published in several studies. The prevalence of each mutation spectrum by amino acid position are indicated by the green bar with the corresponding relative frequency. Mutations were converted into amino acid variant description according to the HGVS nomenclature (Sup. Table 2).

Seven percent of the mutations occur at the C-terminal end of the C-helix at amino acid position 763. While non pathogenic mutations were found in the three amino acids, i.e. positions 764, 765, and 766, located in the C-helix. Most pathogenic exon 20 mutations (75%) are observed in the near loop following the C-helix, comprising six amino acids i.e. positions 767 to 772. The far loop following the C-helix including amino acid positions 773, 774 and 775 harbours 18% of the pathogenic mutations.

The most common mutation is A767_V769dup with 25%, which is followed by S768_D770dup with 18%. The loop following the C-helix with six amino acids is the largest region and harbours 75% of all exon 20 mutations. It can therefore be marked as an important area to bring the EGFR receptor in an active or inactive conformation, implying that mutations within the near loop following the C-helix promote the active conformation of the kinase domain of EGFR, are oncogenic.

Sensitivity of EGFRex20 mutation-positive Ba/F3 cells to TKIs

A total of 11 Ba/F3 *in vitro* studies met the inclusion criteria (Sup Fig. 1, Sup Table 3). In these studies, 12 clinically relevant EGFRex20+ mutations were tested against 21 different EGFR TKIs including four 1st, five 2nd and seven 3rd generation TKIs. Five more recently generated EGFRex20+ active TKIs were also tested *in vitro* on Ba/F3 cells and included in this study (Fig. 3). The A763_Y764insFQEA mutation was sensitive for 17 of the 20 TKIs, while no responses were observed for nazartinib, olmutinib, and rociletinib.

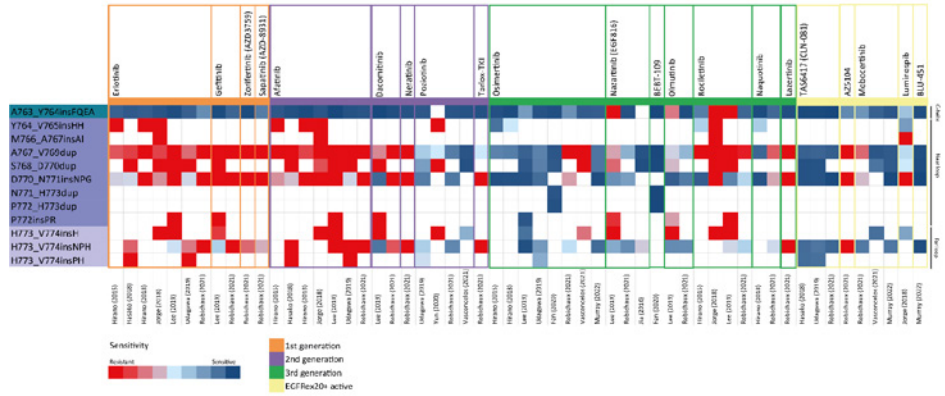


Figure 3. Sensitivity of 12 different EGFRex20+ mutations for different TKIs from 11 in vitro studies. Comparisons of sensitivity are made by calculating the half-maximal inhibitory concentration (IC50) value for each TKI within the various EGFRex20+ Ba/F3 cell lines divided by the IC50 value for the wild type from each study. An EGFRex20+ mutation is sensitive for the tested TKI when the ratio is below one, indicated with blue color and insensitive when the ratio was ≥ 1, meaning the mutant Ba/F3 cells were less likely to be inhibited compared to the WT Ba/F3 cells, indicated with a red color.

Activity of TKIs in EGFRex20 mutation-positive patient-derived models

In total, we found 10 studies using eight different EGFRex20+ patient-derived cell lines (Sup. Fig. 1, Sup. Table 5). These cell lines harbored *EGFR* mutations at the most frequently targeted amino acids, i.e. positions A763, A767, S768, A771, P772, and H773. The patient-derived cell line BID007, harboring the A763_Y764insFQEA mutation, was highly sensitive to afatinib (Fig. 4). Although less potent than afatinib, erlotinib and osimertinib also effectively inhibited proliferation of BID007 cells (Sup. Table 5). Additionally, novel TKIs such as mobocertinib, TAS6417 and poziotinib showed an effective growth inhibition of these cells.

The patient-derived cell lines harboring the A767_V769dup (CUTO14 cells), S768_D770dup (CUTO18), and N771_H773dupNPH (CUTO17 cells) mutations all demonstrated higher sensitivity to tarloxotinib-E (the active metabolite) as compared to gefitinib, afatinib, osimertinib, and the prodrug tarloxotinib. Additional, mobocertinib also had a clear inhibitory potency on CUTO14 cells carrying the A767_V769dupASV mutation. Cells harboring the N771_P772insH mutation (BID019 cells) were not sensitive to afatinib. Only mobocertinib, TAS6417 and poziotinib inhibited growth of BID019 cells.

LU3075 cells, carrying the P772_H773insDNP mutation showed a poor response to most TKIs (Fig. 4). Responses observed for afatinib on cells harboring the H773_V774insNPH mutation (LU0387 cells) were variable. Gonzalez et al showed no inhibition of growth in LU0387 cells, while Yang et al showed a clear inhibitory potency of afatinib (60%), which was similar to the effect of mobocertinib (85%).

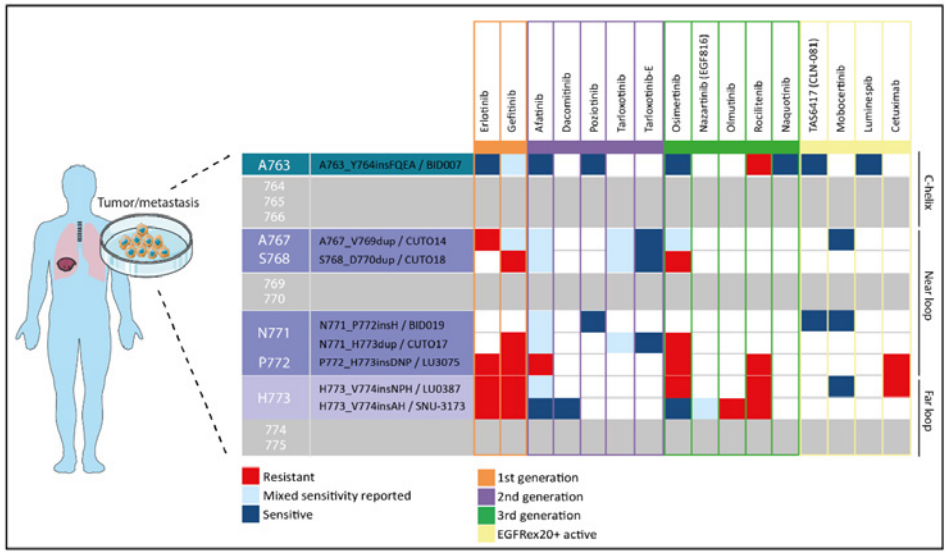


Figure 4. Sensitivity to TKIs of the patient-derived cell lines. The color blue indicates the TKI to be sensitive, the color light blue as variable responses (both inhibitory as sensitive described in different studies), and the color red indicates insensitive.

Treatment responses of EGFRex20 mutation-positive NSCLC patients with first, second, and third generation TKIs

Twenty one studies described clinical responses (BOR and mPFS) with 1st, 2nd and 3rd generation TKIs including 448 patients with different EGFRex20+ mutations or patient groups with mutations in specific regions of the *EGFR* gene (Sup. Fig. 1, Sup. Table 6). Response data from patients with treated with the 1st and 2nd generation TKIs erlotinib, gefitinib and afatinib were generally disappointing (Fig. 5). The A763_Y764insFQEA mutation was sensitive to gefitinib, afatinib and erlotinib. For gefitinib, a patient with this mutation had a partial response (PR) as BOR with a PFS of 4.9 and another patient had stable disease (SD) as BOR with a PFS of 15.3 months. For afatinib, one patient had a PR with a PFS of 24 months. For erlotinib, four patients had PR as BOR, one had SD as BOR and one had progressive disease (PD) as BOR. The mPFS did not exceed over 5 months (Fig. 5).

The 3rd generation TKI poziotinib was designed to overcome the steric hindrance at the active site of the EGFRex20+ mutation, while minimizing toxicity by not targeting the wild type EGFR [18]. So far, five studies reported response data regarding the treatment with poziotinib in EGFRex20+ patients, of which three studies (cohort studies; 2, case study; 1) reported responses according to the different EGFRex20+ mutations (n=27, Fig. 5) [24–26]. The three studies did not indicate the individual EGFRex20+ mutation specific PFS, but only mentioned a general PFS of 5.6 months and 'not met' in the two cohort studies and being 7,3 months in one case study. Two newer studies from Elamin et al (2022) and Le et al (2020), reported response rates for poziotinib in relation to C-helix, near-loop and far-loop region EGFRex20+ mutations. Both studies combined included response data of 165 patients. An inverse correlation was observed between the distance of the insertion from the C-helix and sensitivity to the drug in patients. An ORR of 20.8% and 46% was reported in near-loop mutants compared to 0% and 9.1% in the far-loop mutations (Fig. 6).

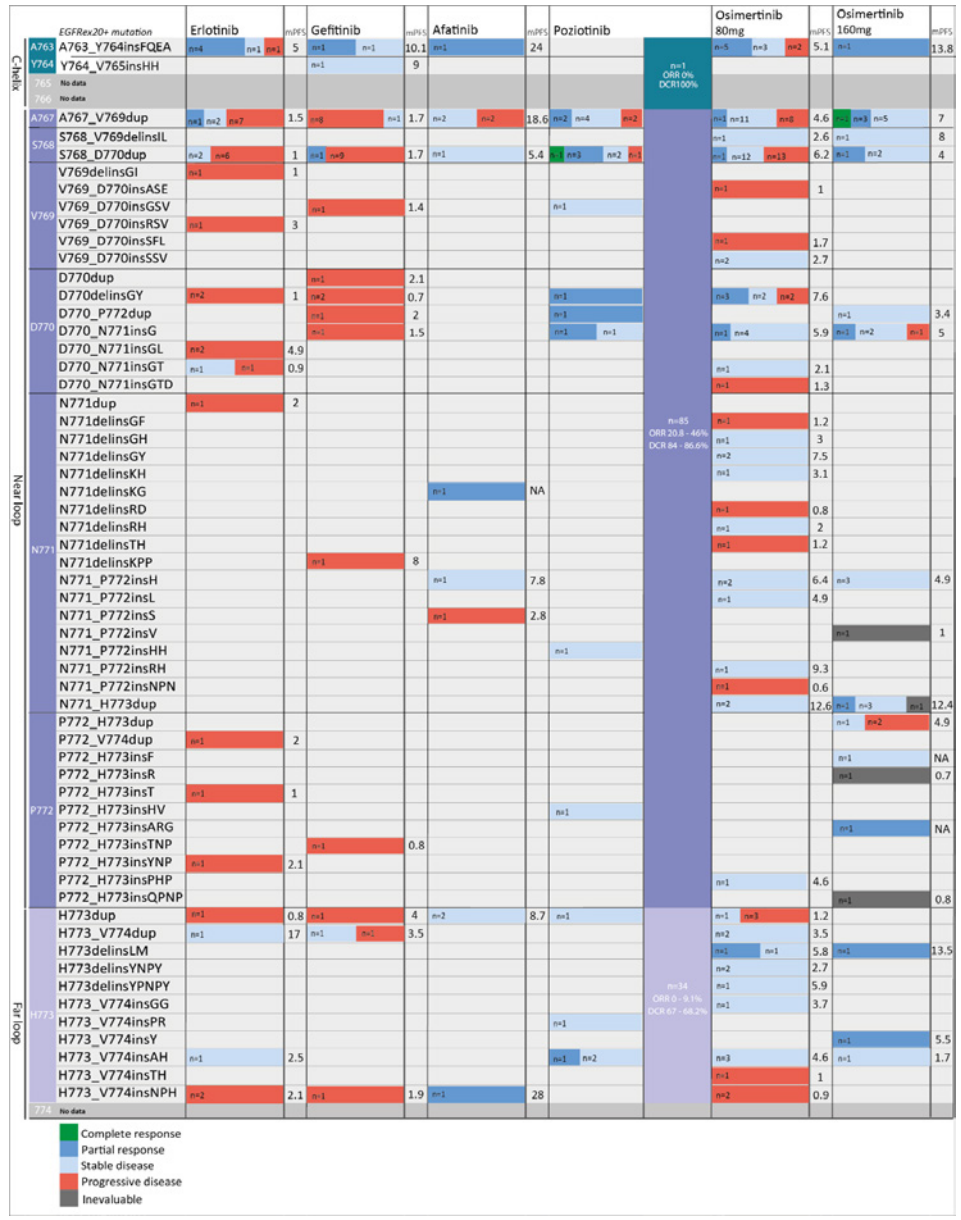


Figure 5. Clinical benefit with 1st, 2nd and 3rd generation TKIs based on tumor responses and mPFS in NSCLC patients across different EGFRex20+ mutations and regions mentioned in different studies found in literature (See also Sup. Table 6). A dark blue bar represents partial response, light blue stable disease, red bar progressive disease and a dark grey bar inevaluable disease, according to RECIST 1.1. If the bar is light grey (empty), there are no responses reported in literature for that TKI for that particular EGFRex20+ mutation. The mPFS is the median progressive free survival of all reported mPFS in the different studies, if available. *Elamin et al. (2022) and Le et al. (2020) did not report responses per EGFRex20+ mutation but looked at responses with DCR dependent on insertion location dividing them between C-helix, near loop or far loop insertions. Abbreviations: DCR = disease control rate, ORR = objective response rate, n = number of patients with that corresponding mutation reported in different studies

The most common treatment-related Grade ≥3 AEs were rash (28-34%) and diarrhea (22-26%) [27,28]. In addition, the FDA indicated that poziotinib could not be approved for the treatment of NSCLC patients (NSCLC) harboring HER2 exon 20 insertion mutations due to the evidence that poziotinib did not yield notable benefit relative to its risks [29].

Osimertinib is a third generation TKI showing promising efficacy *in vitro* in Ba/F3 cells carrying a broad range of EGFRex20+ mutations (Fig. 3). However, at the standard dosage (80mg daily), the clinical evidence regarding efficacy in EGFRex20+ NSCLC patients is disappointing [30,31]. Four retrospective cohort studies have mentioned tumor response to osimertinib 80mg in relation to different EGFRex20+ mutations for a total of 109 patients [31–34] (Fig. 5). Across the whole span of mutations, most patients have SD as BOR and the mPFS did not exceed 12.6. Two large studies have evaluated responses across different EGFRex20+ mutations for osimertinib at a daily dose of 160mg. The phase II ECOG-ACRIN 5162 trial assessed osimertinib in 21 EGFRex20+ patients who previously received at least one line of treatment. Among the 20 eligible patients, the confirmed ORR was 25%, the mPFS was 9.7 months (95% CI, 4.07-NA), and median DoR was 5.7 months (95% CI, 4.73-NA). For 15 patients, the response according to the specific EGFRex20+ mutation was known. All responders harbored an EGFRex20+ mutation in the loop following the C-helix (Fig. 5) [35]. In the POSITION20 trial (including 23 patients with EGFRex20+ insertion mutations, excluding two patients with non EGFRex20+ in-frame insertions) responses were observed and reported across the entire spectrum of EGFRex20+ mutations in a first-line setting (Fig. 5), with an ORR of 28% and a medium PFS of 6.8 months (95% CI, 4.6-9.1) [36]. Treatment related adverse events (trAEs) with osimertinib at a daily dose of 160 mg were obviously higher than with 80 mg daily, and included higher rates of diarrhea (72% any grade), fatigue (44% any grade), and acneiform rash (40% any grade) compared to Osimertinib 80mg [36].

Treatment responses of EGFRex20 *mutation-positive* NSCLC patients with novel compounds and antibody-based combinations

Over the past 10 years, a number of emerging therapies have been developed for patients with EGFRex20+ mutations. A total of 10 studies distinguished targeting EGFRex20+ mutations showing clinical responses (BOR and mPFS) across different EGFRex20+ mutations in 352 patients (Sup. Table 6). The irreversible, oral TKI mobocertinib (TAK-788) was designed to target the active site of the EGFRex20+ mutation. The first dose-escalation phase ½ trial investigated a daily dose of 160mg in 28 EGFRex20+ NSCLC patients [37] (Fig. 6). Overall, Riely et al observed a confirmed ORR of 43% and a mPFS of 7 months in patients with different EGFRex20+ mutations [37]. This study was extended in the EXCLAIM cohort (n=96) in which a pooled platinum-pretreated patient population was analyzed (PPP cohort, n=114). In the EXCLAIM cohort, the ORR was 23% per independent review committee (IRC). Within this cohort, the responses were mentioned across the EGFRex20+ insertion regions (n = 95, Fig. 6). The ORR in the PPP cohort was 26% per IRC. In both groups, the mPFS was 7.3 months [38]. The ORR across both groups were similar, suggesting the responses are not dependent on pretreatment with platinum chemotherapy. The EXCLAIM-2 phase 3 study in which mobocertinib is being evaluated versus chemotherapy as first line treatment is ongoing (NCT04129502).
Another emerging treatment is the bispecific IgG1 antibody amivantamab, targeting both EGFR and MET. The exploratory CHRYSALIS single arm phase II study evaluated the effectivity of amivantamab in 81 patients pre-treated with chemotherapy [39]. This study revealed an ORR of 40% with a median DoR of 11.1 months, a mPFS of 8.3 months. The safety profile associated with dual inhibition of EGFR and MET with grade 3–4 trAEs was reported in 16% of the patients. For 63 patients, antitumor activity was observed across all EGFRex20+ insertion regions, with a higher ORR in patients with mutations in the near loop region (Fig. 6). Based on these results, amivantamab was granted FDA accelerated approval in May 2021 and EMA approval in December 2021 for patients with EGFRex20+ NSCLC with disease progression on or after platinum-based chemotherapy ("FDA grants accelerated approval to amivantamab for metastatic non-small cell lung cancer," 2021). Ongoing phase 3 clinical trials focusing on combination approaches with amivantamab or comparing amivantamab/chemotherapy versus chemotherapy alone as a first-line treatment are under evaluation[41].

The hsp90 inhibitors luminespib and onalespib (tested in combination with erlotinib), showed limited responses in patients with EGFRex20+ NSCLC (Fig. 6). Overall, the ORR of luminespib was 17%, and mPFS was 2.9 months (95% CI, 1.4-5.6) [42]. The trial testing the combination onalespib/erlotinib was closed early due to dose limiting toxicities, mainly diarrhea, and a disappointing clinical activity (Fig. 6) [43].

Previous case series suggested that combined afatinib and cetuximab treatment intensified the EGFR blockade but resulted in increased toxicity. The treatment resulted in responses across all exon 20 mutations [44] (Fig. 6). The results will be validated in a prospective phase II trial (NCT03727724). Interim results of this study look promising, with an ORR of 47% and a DCR of 59% at 18 weeks [45].

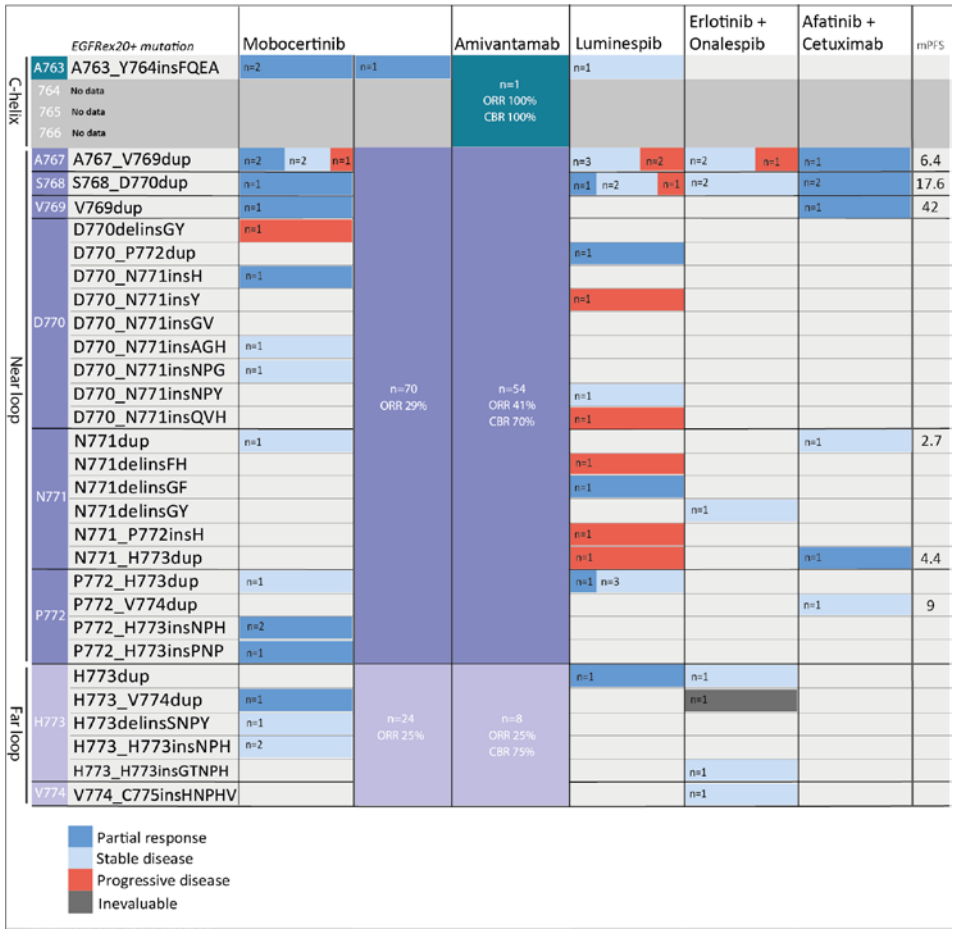


Figure 6. Clinical benefit with different novel compounds and antibody-based combinations based on tumor responses and mPFS in NSCLC patients across different EGFRex20+ mutations (See also Sup. Table 6). A dark blue bar represents partial response, light blue stable disease, red bar progressive disease and a dark grey bar inevaluable disease, according to RECIST 1.1. If the bar is light grey (empty), there are no responses reported in literature for that TKI on that particular EGFRex20+ mutation. Each bar indicates with a number how many responses were found in literature for that EGFRex20+ mutation, also indicated by the width of that color (if mixed responses are reported for that EGFRex20+ mutation). The mPFS is the median progressive free survival of all reported mPFS in the different studies, if applicable.

*Park et al. (2021) and Zhou et al. (2021) did not report responses per EGFRex20+ mutation but looked at responses with DCR dependent on insertion location dividing them between C-helix, near loop or far loop insertions. Abbreviations: DCR = disease control rate, ORR = objective response rate, n = number of patients with that corresponding mutation reported in different studies.

Discussion

EGFRex20+ NSCLC patients are currently treated as a single disease entity, irrespective of the nature and location of the mutation. We have reviewed the literature to determine the likelihood of sensitivity in cell lines and clinical benefit to TKIs and other novel treatments according to the type of the EGFRex20+ mutation. Actionability of individual EGFRex20+ mutations is heterogenous and can be determined by analyzing TKI effectiveness in Ba/F3 and patient derived- cell lines and by reviewing clinical data describing sensitivity to several TKIs. This knowledge can be used as a starting point for treating physicians to select the most optimal drugs to treat individual patients. The A763_Y764insFQEA is a unique EGFR exon 20+ mutation, which shows better responses in comparison to other mutations affecting the near- and far loop of EGFRex20+. It can be treated with TKIs used for classical EGFR mutations, for example erlotinib, gefitinib or (high dose) osimertinib (160mg). For other EGFRex20+ mutations further classifications using a structure based approach and cell line systems are warranted to allow selection of the most optimal treatment. Such new approach can pinpoint why far loop mutations are functionally resistant to TKIs.

Evaluating the whole spectrum of mutations on all available TKI's allows selection of the optimal therapy. It is important to consider that at some amino acid positions, a specific hotspot variant accounts for the vast majority of variants, while at other positions (generally less frequently targeted amino acids), the observed variants are very heterogeneous. The mutation hotspots used in *vitro* studies cover approximately 90% of all EGFRex20+ mutations and results in terms of sensitivity are often in agreement to clinical reports. For example, Ba/F3 cells with mutations located at the commonly affected amino acid position A767 are sensitive for poziotinib, osimertinib, CLN-081 and mobocertinib. Consistent with these findings, patient-derived cell lines and patients carrying these mutations also were sensitive to osimertinib (160mg in patients) and mobocertinib. Thus, osimertinib at a dose of 160mg or mobocertinib represent good treatment options. However, the agreement of the *in vitro* effects and the observed clinical benefits can vary. The H773X EGFRex20+ mutations are sensitive to nine TKIs (Fig. 3) in *in vitro* studies, but show disappointing results in clinical studies. Due to major differences in read-out methods to measure *in-vitro* responses and the different set up of clinical reports (cohort vs. original study vs. case report), preclude a direct comparison between treatment results.

Mutations occurring towards the C-terminal end of the C-helix at position 763 (A763_Y764insFQEA) are structurally different from other exon 20 mutations. This mutation was reported to shift the β3-αC loop and extend the length of this loop. This increased the catalytic activity of the mutated EGFR protein (comparable to the *EGFR* exon 21 L858R mutation). In the 672 patients evaluated in this review, patients with mutations in the C-helix showed increased sensitivity to all EGFR TKIs in comparison to other exon 20 mutations. For the A763_Y764insFQEA mutation, the mPFS ranged from 5 to 24 months while the mPFS ranged from 1.5 to 18.6 months for patients with the near-loop A767_V769dup mutation for 1st, 2nd and 3rd generation TKIs. For patients harbouring the A763_Y764insFQEA mutation new more effective compounds are warranted. Treatment decisions should focus on matching these patients to the best EGFR TKI already used for classical EGFR mutations, for example erlotinib, gefitinib or high dose osimertinib (180mg).

In general, our overview shows that many TKIs (particular earlier generations) show less favorable responses in EGFRex20+ patients. This can partly be explained by the dynamic structural changes of the EGFR protein, especially for mutations of amino acid positions

A767 to V774. Data on uncovering the exact structural implications of EGFRex20+ mutations are scarce. Analysis of D770insNPG and V769insASV in comparison to wild type EGFR showed increased interactions resulting in stabilization of the C-helix structure required for the activation of EGFR [46]. The insensitivity towards TKIs can be explained by the altered structure induced by the mutation of the amino acids close to the ATP-binding pocket, e.g., the phosphate-binding loop (P-loop). A further structural characterisation of other mutations affecting amino acid positions A767 to V774 is required to gain further insight on their functional consequences on the EGFR protein and to explain the differences in responses towards different TKIs.

Although effectivity of TKI's has been studied for EGFRex20+ patients, platinum- based chemotherapy is globally the most commonly used first-line treatment and recommended by the ESMO Clinical Practice Guideline for managing oncogene-addicted NSCLC[47]. Most data regarding chemotherapy are reported in retrospective studies which show less satisfactory results. A review of 105 Chinese NSCLC patients harboring an EGFRex20+ mutation report an ORR 19.2%, with a PFS of 6.4 months (95% CI 5.7-7.1) [48]. Another retrospective collection including 77 NSCLC patients receiving first-line pemetrexed-based chemotherapy reported an ORR 41.6% with a PFS of 5.5 months. No significant differences were found for OS among patients with different EGFRex20+ mutations [49]. Also, treatment with immune checkpoint inhibitors (ICI) as single agents have limited efficacy in oncogene-addicted NSCLC [50]. Data about the immunophenotype and the outcomes of ICI for EGFRex20+ mutations is scarce, as these patients were excluded from most first-line chemotherapy-ICI phase III trials. Only five studies and a case report described responses and PFS of chemotherapy and/or ICI for patients carrying different EGFRex20+ mutations (Sup. Fig. 2) [51,52]. In general, it can be concluded that more recently generated compounds showed better responses across the whole spectrum of EGFRex20+ mutations *in vitro* and in clinical studies. Studies comparing TKIs and chemotherapy are however warranted before first line chemotherapy can be replaced.

Recently, more treatment options are being established for patients with EGFRex20+ NSCLC. TAS6417 (CLN-081) achieved antitumor efficacy in different preclinical models (Fig. 3, Fig. 4). Clinical data from a phase I/II study (ClinicalTrials.gov, Identifier:NCT04036682) evaluating CLN-081 reported 6 confirmed PR and 2 unconfirmed PR among 25 evaluable patients[53]. The most common all-grade trAEs were rash (49%), diarrhea (24%), paronychia (16%), nausea (14%), stomatitis (14%), and dry skin (11%). Another promising breakthrough treatment option is the selective, irreversible *EGFR* inhibitor DZD9008[54]. In the cohort of 59 patients harbouring an EGFRex20+ mutation, 31 patients were treated with the recommended phase 2 dose of 300mg once daily. The ORR was 48.4% (15/31), and disease control rate (DCR) was 90.3% (28/31) across different EGFRex20+ mutations [55]. DZD9008 is currently being evaluated in a phase II clinical study (ClinicalTrials.gov, Identifier: NCT03974022). BLU-451 (formerly known as LNG-451) was designed as a covalent inhibitor for EGFRex20+ mutation while sparing WT EGFR. BLU-451 can penetrate the CNS and showed anti-tumour activity in the brain and spinal cord in preclinical models [56]. A phase I/II clinical trial is ongoing (NCT0521873).

A number of limitations need to be noted regarding the present overview. Established cell lines have undergone unspecified long-term passaging *in vitro*, which makes the cells more homogenous. In the end, the cells represent only a subpopulation of the original tumor due to the selective survival pressures present in culture conditions [57]. As a result, the genetic and epigenetic differences are impossible to mimic and clinical responses are therefore less predictable. It is important to realize that most patients described in the evaluation of clinical treatment results across different insertion regions have been pretreated with chemotherapy (platinum based) or with other TKI's. This may affect the overall response and preconditions of the patient at the start of the specific EGFRex20+ treatment. It should also be noted that for part of the exon 20 mutations only a limited number of patients have been treated. A potential bias in this review is that positive results regarding to the mutated amino acid are more likely to be reported/published as compared to negative results. There is also a bias introduced due to inclusion of case reports, which have a higher probability of exceptional responses as opposed to those published in cohorts. Moreover, earlier studies on EGFRex20+ patients do not mention responses according to the exact EGFRex20+ mutation. Moreover, the exact mutation was not known for all patients who had PD as BOR. Correct annotation according to HGVS nomenclature is warranted to allow a straightforward comparison with cases described in the literature for clinical decision making. Patients with unknown variants have not been included in this report.

Overall, we confirm that the location of the EGFRex20+ mutation is the main determinant for selection of the optimal therapy. It is necessary to focus and test all currently known EGFRex20+ mutations and use the correct HGVS nomenclature. Patients with the A763_Y764insFQEA mutation can be treated with EGFR TKI already used for classical EGFR mutations, for example erlotinib, gefitinib or high dose osimertinib (160mg). The EGFRex20+ mutations should not be analyzed as an exon-based group, but a structural understanding of the impact of mutations and patients-derived cell lines is warranted.

References

1. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non-Small-Cell Lung Cancer to Gefitinib. vol. 21. 2004.
2. Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. . Crit Rev Oncol Hematol 1995;19:183–232.
3. Shaw AT, Kim DW, Nakagawa K, Seto T, Crinó L, Ahn MJ, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. New England Journal of Medicine 2013;368:2385–94. https://doi.org/10.1056/NEJMoa1214886.
4. Hata AN, Engelman JA, Faber AC. The BCL2 family: Key mediators of the apoptotic response to targeted anticancer therapeutics. Cancer Discov 2015;5:475–87. https://doi.org/10.1158/2159-8290.CD-15-0011.
5. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy. Science (1979) 2004;304:1497–500. https://doi.org/10.1126/science.1099314.
6. Soria J-C, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in Untreated EGFR -Mutated Advanced Non–Small-Cell Lung Cancer . New England Journal of Medicine 2018;378:113–25. https://doi.org/10.1056/nejmoa1713137.
7. Sequist L v., Yang JC-H, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III Study of Afatinib or Cisplatin Plus Pemetrexed in Patients With Metastatic Lung Adenocarcinoma With ***EGFR*** Mutations. Journal of Clinical Oncology 2013;31:3327–34. https://doi.org/10.1200/JCO.2012.44.2806.
8. Oxnard GR, Lo PC, Nishino M, Dahlberg SE, Lindeman NI, Butaney M, et al. Natural history and molecular characteristics of lung cancers harboring egfr exon 20 insertions. Journal of Thoracic Oncology 2013;8:179–84. https://doi.org/10.1097/JTO.0b013e3182779d18.
9. Riess JW, Gandara DR, Frampton GM, Madison R, Peled N, Bufill JA, et al. Diverse EGFR Exon 20 Insertions and Co-Occurring Molecular Alterations Identified by Comprehensive Genomic Profiling of NSCLC. Journal of Thoracic Oncology 2018;13:1560–8. https://doi.org/10.1016/j.jtho.2018.06.019.
10. Vyse S, Huang PH. Targeting EGFR exon 20 insertion mutations in non-small cell lung cancer. Signal Transduct Target Ther 2019;4. https://doi.org/10.1038/s41392-019-0038-9.
11. Yasuda H, Park E, Yun CH, Sng NJ, Lucena-Araujo AR, Yeo WL, et al. Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer. Sci Transl Med 2013;5. https://doi.org/10.1126/scitranslmed.3007205.
12. Arcila ME, Nafa K, Chaft JE, Rekhtman N, Lau C, Reva BA, et al. EGFR exon 20 insertion mutations in lung adenocarcinomas: Prevalence, molecular heterogeneity, and clinicopathologic characteristics. Mol Cancer Ther 2013;12:220–9. https://doi.org/10.1158/1535-7163.MCT-12-0620.
13. Cardona AF, Rojas L, Zatarain-Barrón ZL, Freitas HC, Granados ST, Castillo O, et al. EGFR exon 20 insertion in lung adenocarcinomas among Hispanics (geno1.2-CLICaP). Lung Cancer 2018;125:265–72. https://doi.org/10.1016/j.lungcan.2018.10.007.
14. Chouaid C, Filleron T, Debieuvre D, Pérol M, Girard N, Dansin E, et al. A Real-World Study of Patients with Advanced Non-squamous Non-small Cell Lung Cancer with EGFR Exon 20 Insertion: Clinical Characteristics and Outcomes. Target Oncol 2021;16:801–11. https://doi.org/10.1007/s11523-021-00848-9.
15. Leal JL, Alexander M, Itchins M, Wright GM, Kao S, Hughes BGM, et al. EGFR Exon 20 Insertion Mutations: Clinicopathological Characteristics and Treatment Outcomes in Advanced Non–Small Cell Lung Cancer: EGFR Exon 20 Insertion Mutations in NSCLC. Clin Lung Cancer 2021;22:e859–69. https://doi.org/10.1016/j.cllc.2021.04.009.
16. Remon J, Hendriks LEL, Cardona AF, Besse B. EGFR exon 20 insertions in advanced non-small cell lung cancer: A new history begins. Cancer Treat Rev 2020;90. https://doi.org/10.1016/j.ctrv.2020.102105.
17. Tamirat MZ, Koivu M, Elenius K, Johnson MS. Structural characterization of EGFR exon 19 deletion mutation using molecular dynamics simulation. PLoS One 2019;14:e0222814-.
18. Robichaux JP, Elamin YY, Tan Z, Carter BW, Zhang S, Liu S, et al. Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer. Nat Med 2018;24:638–46. https://doi.org/10.1038/s41591-018-0007-9.
19. Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T. Mutations of the Epidermal Growth Factor Receptor Gene in Lung Cancer: Biological and Clinical Implications. vol. 64. 2004.
20. Yosaatmadja Y, Silva S, Dickson JM, Patterson A V., Smaill JB, Flanagan JU, et al. Binding mode of the breakthrough inhibitor AZD9291 to epidermal growth factor receptor revealed. J Struct Biol 2015;192:539–44. https://doi.org/10.1016/j.jsb.2015.10.018.
21. Palacios R, Henson G, Steinmetz M, McKearn JP. Interleukin-3 supports growth of mouse pre-B-cell clones in vitro. Nature 1984;309:126–31. https://doi.org/10.1038/309126a0.
22. Koga T, Suda K, Mitsudomi T. Utility of the Ba/F3 cell system for exploring on-target mechanisms of resistance to targeted therapies for lung cancer. Cancer Sci 2022;113:815–27. https://doi.org/10.1111/cas.15263.
23. Kobayashi S, Ji H, Yuza Y, Meyerson M, Wong K-K, Tenen DG, et al. An Alternative Inhibitor Overcomes Resistance Caused by a Mutation of the Epidermal Growth Factor Receptor. Cancer Res 2005;65:7096–101. https://doi.org/10.1158/0008-5472.CAN-05-1346.
24. Prelaj A, Bottiglieri A, Bhat G, Washington R, Calareso G, Greco GF, et al. Case Report: Exceptional Response to Poziotinib in Patient with Metastatic Non-Small Cell Lung Cancer With EGFR Exon 20 Insertion Mutation. Front Oncol 2022;12. https://doi.org/10.3389/fonc.2022.902967.
25. Prelaj A, Bottiglieri A, Proto C, Lo Russo G, Signorelli D, Ferrara R, et al. Poziotinib for EGFR and HER2 exon 20 insertion mutation in advanced NSCLC: Results from the expanded access program. Eur J Cancer 2021;149:235–48. https://doi.org/10.1016/j.ejca.2021.02.038.
26. Robichaux JP, Elamin YY, Tan Z, Carter BW, Zhang S, Liu S, et al. Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer. Nat Med 2018;24:638–46. https://doi.org/10.1038/s41591-018-0007-9.
27. Elamin YY, Robichaux JP, Carter BW, Altan M, Tran H, Gibbons DL, et al. Poziotinib for EGFR exon 20-mutant NSCLC: Clinical efficacy, resistance mechanisms, and impact of insertion location on drug sensitivity. Cancer Cell 2022;40:754-767.e6. https://doi.org/10.1016/j.ccell.2022.06.006.
28. Le X, Goldman JW, Clarke JM, Tchekmedyian N, Piotrowska Z, Chu D, et al. Poziotinib shows activity and durability of responses in subgroups of previously treated EGFR exon 20 NSCLC patients. Journal of Clinical Oncology 2020;38:9514. https://doi.org/10.1200/JCO.2020.38.15_suppl.9514.
29. Spectrum Pharmaceuticals. Spectrum Pharmaceuticals receives complete response letter from U.S. Food and Drug Administration for poziotinib; reaffirms focus on the commercialization of ROLVEDON™ (eflapegrastim-xnst) injection. 2022.
30. Kim TM, Ock C-Y, Kim M, Kim SH, Keam B, Kim YJ, et al. Phase II study of osimertinib in NSCLC patients with EGFR exon 20 insertion mutation: A multicenter trial of the Korean Cancer Study Group (LU17-19). Annals of Oncology 2019;30:v628. https://doi.org/10.1093/annonc/mdz260.051.
31. van Veggel B, Madeira R Santos JFV, Hashemi SMS, Paats MS, Monkhorst K, Heideman DAM, et al. Osimertinib treatment for patients with EGFR exon 20 mutation positive non-small cell lung cancer. Lung Cancer 2020;141:9–13. https://doi.org/10.1016/j.lungcan.2019.12.013.
32. Yang GJ, Li J, Xu HY, Sun Y, Liu L, Li HS, et al. Osimertinib for Chinese advanced non-small cell lung cancer patients harboring diverse EGFR exon 20 insertion mutations. Lung Cancer 2021;152:39–48. https://doi.org/10.1016/j.lungcan.2020.11.027.
33. Fang W, Huang Y, Hong S, Zhang Z, Wang M, Gan J, et al. EGFR exon 20 insertion mutations and response to osimertinib in non-small-cell lung cancer. BMC Cancer 2019;19. https://doi.org/10.1186/s12885-019-5820-0.
34. Yasuda H, Ichihara E, Sakakibara-Konishi J, Zenke Y, Takeuchi S, Morise M, et al. A phase I/II study of osimertinib in EGFR exon 20 insertion mutation-positive non-small cell lung cancer. Lung Cancer 2021;162:140–6. https://doi.org/10.1016/j.lungcan.2021.10.006.
35. Piotrowska Z, Wang Y, Sequist L V, Ramalingam SS. ECOG-ACRIN 5162: A phase II study of osimertinib 160 mg in NSCLC with EGFR exon 20 insertions. Journal of Clinical Oncology 2020;38:9513. https://doi.org/10.1200/JCO.2020.38.15_suppl.9513.
36. Zwierenga F, van Veggel B, Hendriks LEL, Hiltermann TJN, Hiddinga BI, Hijmering Kappelle LBM, et al. High dose osimertinib in patients with advanced stage EGFR exon 20 mutation-positive NSCLC: Results from the phase 2 multicenter POSITION20 trial. Lung Cancer 2022;170:133–40. https://doi.org/10.1016/j.lungcan.2022.06.012.
37. Riely GJ, Neal JW, Camidge DR, Spira AI, Piotrowska Z, Costa DB, et al. Activity and safety of mobocertinib (Tak-788) in previously treated non–small cell lung cancer with egfr exon 20 insertion mutations from a phase i/ii trial. Cancer Discov 2021;11:1688–99. https://doi.org/10.1158/2159-8290.CD-20-1598.
38. Zhou C, Ramalingam SS, Kim TM, Kim SW, Yang JCH, Riely GJ, et al. Treatment Outcomes and Safety of Mobocertinib in Platinum-Pretreated Patients with EGFR Exon 20 Insertion-Positive Metastatic Non-Small Cell Lung Cancer: A Phase ½ Open-label Nonrandomized Clinical Trial. JAMA Oncol 2021;7:1772–81. https://doi.org/10.1001/jamaoncol.2021.4761.
39. Park K, Haura EB, Leighl NB, Mitchell P, Shu CA, Girard N, et al. Amivantamab in EGFR Exon 20 Insertion-Mutated Non-Small-Cell Lung Cancer Progressing on Platinum Chemotherapy: Initial Results From the CHRYSALIS Phase I Study. J Clin Oncol 2021;39:3391–402. https://doi.org/10.1200/JCO.21.
40. FDA grants accelerated approval to amivantamab-vmjw for metastatic non-small cell lung cancer. US Food & Drug Administration 2021.
41. Agrawal T, Artis E, Xie J, Bhattacharya A, Haddish-Berhane N, Gopen T, et al. P76.74 PAPILLON: Randomized Phase 3 Study of Amivantamab Plus Chemotherapy vs Chemotherapy Alone in EGFR Exon20ins NSCLC. Journal of Thoracic Oncology 2021;16:S621. https://doi.org/10.1016/j.jtho.2021.01.1131.
42. Piotrowska Z, Costa DB, Oxnard GR, Huberman M, Gainor JF, Lennes IT, et al. Activity of the Hsp90 inhibitor luminespib among non-small-cell lung cancers harboring EGFR exon 20 insertions. Annals of Oncology 2018;29:2092–7. https://doi.org/10.1093/annonc/mdy336.
43. Riess JW, Reckamp KL, Frankel P, Longmate J, Kelly KA, Gandara DR, et al. Erlotinib and Onalespib Lactate Focused on EGFR Exon 20 Insertion Non-Small Cell Lung Cancer (NSCLC): A California Cancer Consortium Phase I/II Trial (NCI 9878): Onalespib Plus Erlotinib in EGFR-Mutant NSCLC. Clin Lung Cancer 2021;22:541–8. https://doi.org/10.1016/j.cllc.2021.05.001.
44. Janjigian YY, Smit EF, Groen HJM, Horn L, Gettinger S, Camidge DR, et al. Dual inhibition of EGFR with afatinib and cetuximab in kinase inhibitor-resistant EGFR-mutant lung cancer with and without T790M mutations. Cancer Discov 2014;4:1036–45. https://doi.org/10.1158/2159-8290.CD-14-0326.
45. van Veggel B, van der Wekken AJ, Paats M, Hashemi SMS, Hendriks L, Sikorska K, et al. Interim results of a phase II single arm trial combining afatinib with cetuximab in patients with EGFRex20ins positive NSCLC. Journal of Clinical Oncology 2021;39:9112. https://doi.org/10.1200/JCO.2021.39.15_suppl.9112.
46. Tamirat MZ, Kurppa KJ, Elenius K, Johnson MS. Structural basis for the functional changes by EGFR exon 20 insertion mutations. Cancers (Basel) 2021;13:1–21. https://doi.org/10.3390/cancers13051120.
47. Hendriks LE, Kerr KM, Menis J, Mok TS, Nestle U, Passaro A, et al. Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Annals of Oncology 2023;34:339–57. https://doi.org/10.1016/j.annonc.2022.12.009.
48. Yang G, Li J, Xu H, Yang Y, Yang L, Xu F, et al. EGFR exon 20 insertion mutations in Chinese advanced non-small cell lung cancer patients: Molecular heterogeneity and treatment outcome from nationwide real-world study.

Lung Cancer 2020;145:186–94. https://doi.org/10.1016/j.lungcan.2020.03.014.
49. Xu CW, Wang WX, Wang D, Wang QM, Pu XX, Zhu YC, et al. Pemetrexed-based chemotherapy for non-small-cell lung cancer patients with EGFR exon 20 insertion mutation: A multicenter study. Transl Lung Cancer Res 2020;9:1853–61. https://doi.org/10.21037/tlcr-20-382.
50. Mazieres J, Drilon A, Lusque A, Mhanna L, Cortot AB, Mezquita L, et al. Immune checkpoint inhibitors for patients with advanced lung cancer and oncogenic driver alterations: Results from the IMMUNOTARGET registry. Annals of Oncology 2019;30:1321–8. https://doi.org/10.1093/annonc/mdz167.
51. Byeon S, Kim Y, Lim SW, Cho JH, Park S, Lee J, et al. Clinical outcomes of EGFR exon 20 insertion mutations in advanced non-small cell lung cancer in Korea. Cancer Res Treat 2019;51:623–31. https://doi.org/10.4143/crt.2018.151.
52. Wu JY, Yu CJ, Shih JY. Effectiveness of Treatments for Advanced Non–Small-Cell Lung Cancer With Exon 20 Insertion Epidermal Growth Factor Receptor Mutations. Clin Lung Cancer, vol. 20, Elsevier Inc.; 2019, p. e620–30. https://doi.org/10.1016/j.cllc.2019.06.018.
53. Udagawa H, Hasako S, Ohashi A, Fujioka R, Hakozaki Y, Shibuya M, et al. TAS6417/CLN-081 is a pan-mutation-selective EGFR tyrosine kinase inhibitor with a broad spectrum of preclinical activity against clinically relevant EGFR mutations. Molecular Cancer Research 2019;17:2233–43. https://doi.org/10.1158/1541-7786.MCR-19-0419.
54. Dizal Pharmaceutical Co. Ltd. FDA grants breakthrough therapy designation for dizal pharmaceutical's DZD9008 in patients with locally advanced or metastatic non-small cell lung cancer harboring EGFR exon20 insertion. 2022.
55. Yang JC-H, Wang M, Mitchell P, Fang J, Nian W, Chiu C-H, et al. Preliminary safety and efficacy results from phase 1 studies of DZD9008 in NSCLC patients with EGFR Exon20 insertion mutations. Journal of Clinical Oncology 2021;39:9008. https://doi.org/10.1200/JCO.2021.39.15_suppl.9008.
56. Spira AI, Yu HA, Sun L, Nguyen D, Pearson P, Shim-Lopez J, et al. Phase ½ study of BLU-451, a central nervous system (CNS) penetrant, small molecule inhibitor of EGFR, in incurable advanced cancers with EGFR exon 20 insertion (ex20ins) mutations. Journal of Clinical Oncology 2022;40:TPS9155–TPS9155. https://doi.org/10.1200/JCO.2022.40.16_suppl.TPS9155.
57. Huo KG, D'Arcangelo E, Tsao MS. Patient-derived cell line, xenograft and organoid models in lung cancer therapy. Transl Lung Cancer Res 2020;9:2214–32. https://doi.org/10.21037/tlcr-20-154.

Supplementary Table 1. The EGFRex20+ mutations (original data) from 36 studies and case reports to evaluate the distribution and relative frequency of different NSCLC EGFRex20+ mutations by amino acid position. Some amino acid sequence changes within EGFR exon 20 were 'unknown' because (1) the exact variant was not reported, (2) the insertion mutations were identified just by PCR testing or (3) the exact variant could not be characterized by Sanger sequencing due to very low mutant peaks. The 'other' mutations within EGFR exon 20 are, for example, rare point mutations or coexisting mutations together with EGFR exons other than exon 20 and where therefore excluded for table 1. The point mutation p.S768I [a substitution at codon 768 of exon 20 (c.2303G>T, p.S768I)] was also excluded from table 1, since it is not a insertion/deletion.

Jose Luis Leal et al, 2021	#	Cardona et al, 2018	#	Beau Faller et al, 2014	#	Oxnard et al, 2013	#	Naidoo et al, 2015	#	Koopman et al, 2021	#	Wu et al, 2019	#	Yang et al, 2021	#	Arcila et al, 2013	#	Prelaj et al, 2021	#	Riess et al, 2018	#	Riely et al, 2022	#
V769_D770insASV	11	H773insH	19	A767 V769dupASV	5	V769_D770insASV	6	D770_N771insSVD	11	A763_Y764insFQEA	1	A763_Y764insFQEA	9	D770_N771insSVD	17	D770_N771insSVD	7	H773_V774insH	1	A767_V769dupASV	55	A763_Y764insFQEA	2
D770_N771insSVD	7	V769_D770insASV	16	D761_E762insEAFQ	1	H773insH	3	V769_D770insASV	10	A767_V769dup	16	A767_V769dupASV	30	V769_D770insASV	11	V769_D770insASV	4	H773_V774insAH	2	S768_D770dupSVD	52	A767_V769dup	5
N771_P772insPH	4	A763_Y764insFQEA	13	A763_Y764insFQEA	1	A763_Y764insFQEA	3	H773_v774insNPH	4	S768_D770dup	9	S768_D770dupSVD	17	A763_Y764insFQEA	5	A767_S768insTLA	1	D770_N771insSVD	4	N771_H773dupNPH	20	S768_D770dup	1
D770insG	3	H773_V774insPH	13	M766_V769insWPA	1	H773_V774PH	2	H773_V774insH	4	D770_H773dup	1	V769_D770insGSV	1	D770delinsGY	3	H773_V774insNPH	2	V769_D770insASV	1	A763_Y764insFQEA	15	V769dup	1
H773_V774insPHPH	3	H773_V774insNPH	9	A767_S768insSVR	3	H773_V774NPH	2	H773_V774insPH	2	D770_N771insG	3	D770delinsGY	3	D770_N771insG	3	V774_C775insHV	2	A767_V769dup	2	D770_N771insG	10	D770delinsGY	1
H773dup	3	H773_V774insSVD	5	S768_D770dupSVD	3	V774_C775insHV	2	H773_V774insAH	2	D770_N771insGF	1	D770_N771insG	4	H773_V774insNPH	2	H773_V774insPH	3	M766_A767insASV	4	P772_H773dupPH	9	D770_N771insH	1
D770delinsGY	1	V774_C775insHV	4	V769_D770delinsGI	1	D770_N771insSVD	2	A763_Y764insFQEA	1	D770_N771insSVA	2	D770_N771insGTT	1	V769_D770insSSV	2	H773_V774insAH	1	S768_D770dup	1	H773dupH	8	D770_N771insAGH	1
H773_V774dup	1	D770delinsGY	4	V769_D770insL	1	D770delinsGY	2	A767_S768insTLA	1	D770_N771insT	1	D770_N771insY	1	V769_D770insASE	1	D770_N771insGT	1	P772_H773insHV	1	D770_N771>GYN	6	D770_N771insNPG	1
H773_V774insNPH	1	N771_P772insV	2	D770_H773dupTTP	1	N771_P772insV	1	V769_D770insGE	1	D770_N771insY	1	N771delinsTH	1	D770_N771insGTD	1	D770_N771insGF	1	D770_N771insG	1	H773_V774dupHV	6	N771dup	1
M766_A767insASV	1	N771delinsGY	2	D770_N771insSVD	4	P772_H773insPNP	1	V774_C775insHV	1	D770_P772dup	1	N771delinsKH	1	D770delinsDGT	1	D770>GY	1	Other	4	V774_C775>AHVC	6	P772_H773dup	1
N771_delinsGF	1	H773_V774insAH	1	N771dupN	1	D770_N771insGL	1	N771_P772insH	1	D770delinsEQPP	1	N771_P772insS	1	N771delinsGY	1	D770_N771insY	1			N771_P772>GYP	5	P772_H773insNPH	2
N771_H773dup	1			N771delinsKPP	1	N771delinsGY	1	N771_P772insN	1	D770delinsGY	3	N771_H773dupNPH	6	N771delinsRD	1	H773_V774insH	3	Zwierenga et al, 2021	#	D770_N771>GSVDN	4	P772_H773insPNP	1
N771_P772insT	1	Yang et al, 2020	#	P772_C775dupPHVC	1	H773_V774insAH	1	P772_H773insNP	11	N771_H773dup	2	P772_H773 insGHP	1	N771delinsRH	1	N771_P772insH	1	A763_Y764insFQEA	1	N771_P772insH	3	H773_V774dup	1
P772_H773dup	1	V769_D770insASV	38	P772_H773insLGNP	1			P772_H773insNPH	1	N771_P772insH	1	P772_H773dup	1	N771delinsKH	1	A763_Y764insFQEA	3	A767_V769dup	3	Other	64	H773delinsSNPY	1
P772_H773insHV	1	D770_N771insSVD	29	P772_H773insDNP	1	Byeon et al, 2019	#	D770_N771insGV	1	N771_P772insR	1	P772_H773insTNP	1	N771delinsGF	1	Other	1	S768_D770dup	2			H773_V774insNPH	2
P772_H773insPHPH	1	H773_V774insNPH	11	P772_H773insT	1	His-773_Val774insHis	7	D770_N771insGT	1	N771delinsGF	1	H773_V774insH	1	N771_P772insH	1			S768_V769delinsIL	1	Shi et al, 2022	#		
P772_H773insRCP	1	D770_N771insG	8	H773_V774dupH	3	Val774_Cys775insHisVal	3	H773_V774insY	1	N771delinsGY	1	H773_V774dup	1	N771_P772insNPN	1	Geng et al, 2022	#	D770_N771insG	2	N771_H773dup	1	Prelaj et al, 2022	#
S768_V769delinsIL	1	H773_V774insH	6	H773_V774insPH	2	His773_Val774insProHis	2	Unknown	2	N771delinsKG	1	N771dup	2	H773dup	1	S768_D770dup	1	D770_P772dup	1	D770_P772dup	2	D770_N771insSVD	1
V769_P772dup	1	A763_Y764insFQEA	5	Unknown	11	His773_Val774insAsnProHis	1			N771delinsTH	1	P772_C775 dupPHVC	1	P772_H773insPHP	1	N771_H773dup	1	N771_P772insH	2	A767_S768insTLA	1		
S786I	27	V774_C775insHV	4	Other	7	Pro772_His773insThrThrPro	1	Fang en al, 2019	#	P772 P772_C775dup	1	H773_V774 ins GTNPH	1	H773_V774insTH	1	A767_V769dup	2	N771_P772insV	1	A767_V769dup	3	Fang et al, 2019	#
Unknown	47	D770delinsGY	3			Pro772_His773insGlyAsnPro	1	A767_V769dup	1	P772_H773dup	3			H773_V774insPH	1	A763_Y764insFQEA	1	N771_H773dup	3	S768_D770dup	1	A767_S768insSVD	1
		H773_V774insTH	3	Jenn-Yu Wu et al, 2008	#	Val769_Asn771insValGlyVal	1	S768_D770dup	2	P772_H773insANP	1	Takeda et al, 2018	#	H773delinsYNPY	1	H773delinsQY	1	P772_H773dup	2	H773dup	1		
Piotrowska et al, 2020	#	P772_H773insGHP	2	A767_V769dupASV	3	Asp-770_Asn771insGly	1	N771_P772insL	1	H773_V774dup	2	M766_A767insASV	2	H773delinsYPNPY	1	S768_D770dup	1	P772_H773insR	1	N771delinsSVDS	1	Chan et al, 2018	#
A767_V769dupASV	5	H773delinsPNPY	2	S768_D770dupSVD	4	S768I	17	D770_N771insG	1	H773_V774insAH	2	A767_S768insSVD	3	V774_C775insHV	1	N771delinsGY	1	Other	2			A767_V769dup	1
D770_N771insG	2	N771delinsGY	2	D770_N771insD	1	Other	4	A763_Y764insFQEA	1	H773delinsYNPY	1	V769_D770insASV	1	C775_R776insPHVC	1	D770_N771insGF	1			Piotrowska et al, 2018	#		
N771_P772insH	1	V774_C775insCPHV	2	D770_N771insG	2	Unknown	29			V774delinsHC	1	D770_N771insGL	1	Unknown	1	H773insHA	1	Riess et al, 2021	#	A763_Y764insFQEA	1	Lin et al, 2020	#
P772_H773insF	1	Unknown	24	N771_H773dupNPH	1			Tsigelny et al, 2015	#	H773dup	5	P772_H773insGNP	1			Unknown	2	A767_V769dup	3	A767_V769dup	5	N771delinsKG	1
D770_N771insSVD	1	Other	26	Other	9	Robichaux et al, 2018	#	exon 20 (D770>GY)	1	S768I	5	S768I	2	Van Veggel et al, 2018	#			S768_D770dup	2	S768_D770dup	4		
N771_H773dup	1					V769_D770insGSV	1	D770_P772del_insKG	1	Unknown	4	Other	2	Ser768_Asp770dup	1	Yang ey al, 2022	#	N771delinsGY	1	D770_P772dup	1	Zöchbauer-Müller et al, 2021	#
V769_D770insASV	1	Van Veggel et al, 2020	#	Yasuda et al, 2013	#	H773_V774insAH	1	Other	1	Other	1			Asn771_His773dup	1	A763_Y764insFQEA	8	H773dup	1	D770_N771insY	1	H773dup	1
H773_V774insNPH	1	H773delinsLM	2	A763_Y764insFQEA	3	H773_V774insPR	1							His773dup	1	D770delinsGY	4	H773_V774dup	1	D770_N771insGV	1		
H773_V774insPH	1	H773delinsYNPY	1	Y764_V765insHH	1	N771_P772insHH	1							Ala767_Val769dup	1	V769_D770insASV	7	H773_V774insGTNPH	1	D770_N771insNPY	1	Urbán et al, 2021	#
P772_H773insARG	1	N771delinsTH	1	M766_A767insASV	1	D770delinsGY	1									D770_N771insSVD	9	V774_C775insHNPHV	1	D770_N771insQVH	1	N771_H773dup	1
		H773_V774insAH	2	A767_V769dupASV	1	D770_N771insG	1									P772_H773insH	1			N771delinsFH	1		
		A767_V769dup	4	V769_D770insASV	1	A767_V769dupASV	1									P772_H773insGHP	1			N771delinsGF	1	Zhu et al, 2022	#
		N771_H773dup	2	D770_N771insGL	2	S768_D770dupSVD	1									H773delinsRY	1			N771_H773dup	1	N771delinsGF	1
		S768_D770dup	1	D770_N771insGT	1	S768I	1									D770_N771insGD	1			N771_P772insH	1		
		N771_P772insH	1	D770_N771insSVD	2	P772_H773insDNP	1									H773_V774insAH	1			P772_H773dup	4		
		N771delinsGY	1	D770delinsGY	2											N771_P772insH	1			H773dup	1		
		S768_V769delinsIL	1	P772_H773insYNP	1											P772_H773insH	1						
		V769_D770insGG	1	P772_H773dup	1																		
		N771delinsGH	1	H773_V774insH (H773dup)	2																		
		V769_D770insSFL	1	H773_V774insNPH	1																		

#; represents the count of each EGFRex20+ mutation present in the study

Supplementary Table 2. Overview of the original mutations (see supplementary table 1) and the annotation after converting according to the HGVS using the NP_005219.2 EGFR transcript. All variants were manually checked by adding the mutated amino acids into the *EGFR* exon 20 wild type amino acid position, as highlighted in yellow per variant. The *EGFR* exon 20 mutations variants M766_V769insWPA and D770_H773dupTTP (Beau Faller et al, 2014) were not recognized by the ncbi isoform and could not be manually reproduced, and are therefore excluded from the overview mentioned in supplementary Figure 1.

		Aminoacid position EGFR exon 20	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776			
			D	E	A	Y	V	M	A	S	V	D	N	P	H	V	C	R			
Input Status Normalized DNA	g. RNA Protein, mentioned in source article	EGFR mutation variant (protein abbreviation, after mutalyzer batch checker)																			
NP_005219.2:p.D761_E762insEAFQ	NP_005219.2:p.(Ala763_Tyr764insPheGlnGluAla)	A763_Y764insFQEA	D	E	A	F	Q	E	A	Y	V	M	A	S	V	D	N	P			
NP_005219.2:p.M766_A767insASV	NP_005219.2:p.(Ala767_Val769dup)	A767_V769dup	D	E	A	Y	V	M	A	S	V	A	S	V	D	N	P	H	V	C	R
NP_005219.2:p.M766_V769insWPA	N/A N/A N/A	EXCLUDE	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	C	R			
NP_005219.2:p.A767_S768insSVR	NP_005219.2:p.(Val769_Asp770insArgSerVal)	V769_D770insRSV	D	E	A	Y	V	M	A	S	V	R	S	V	D	N	P	H	V	C	R
NP_005219.2:p.A767_V769dupASV	NP_005219.2:p.(Ala767_Val769dup)	A767_V769dup	D	E	A	Y	V	M	A	S	V	A	S	V	D	N	P	H	V	C	R
NP_005219.2:p.S768_D770dupSVD	NP_005219.2:p.(Ser768_Asp770dup)	S768_D770dup	D	E	A	Y	V	M	A	S	V	D	S	V	D	N	P	H	V	C	R
NP_005219.2:p.A767_S768insSVD	NP_005219.2:p.(Ser768_Asp770dup)	S768_D770dup	D	E	A	Y	V	M	A	S	V	D	S	V	D	N	P	H	V	C	R
NP_005219.2:p.V769_D770insASV	NP_005219.2:p.(Ala767_Val769dup)	A767_V769dup	D	E	A	Y	V	M	A	S	V	A	S	V	D	N	P	H	V	C	R
NP_005219.2:p.D770_H773dupTTP	N/A N/A N/A	EXCLUDE	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	C	R			
NP_005219.2:p.D770_N771>GSVDN	N/A N/A N/A	D770_N771insGSVDN	D	E	A	Y	V	M	A	S	V	D	G	S	V	D	N	N	P	H	V C R
NP_005219.2:p.D770_N771>GYN	N/A N/A N/A	D770_N771insGYN	D	E	A	Y	V	M	A	S	V	D	G	Y	N	N	P	H	V	C	R
NP_005219.2:p.D770_N771insD	NP_005219.2:p.(Asp770dup)	D770dup	D	E	A	Y	V	M	A	S	V	D	D	N	P	H	V	C	R		
NP_005219.2:p.D770insG	NP_005219.2:p.(Asp770_Asn771insGly)	D770_N771insG	D	E	A	Y	V	M	A	S	V	D	G	N	P	H	V	C	R		
NP_005219.2:p.D770_N771insSVD	NP_005219.2:p.(Ser768_Asp770dup)	S768_D770dup	D	E	A	Y	V	M	A	S	V	D	S	V	D	N	P	H	V	C	R
NP_005219.2:p.D770>GY	N/A N/A N/A	D770_N771insGY	D	E	A	Y	V	M	A	S	V	D	G	Y	N	P	H	V	C	R	
NP_005219.2:p.N771_H773dupNPH	NP_005219.2:p.(Asn771_His773dup)	N771_H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	N	P	H	V	C	R
NP_005219.2:p.N771_P772>GYP	N/A N/A N/A	N771_P772insGYP	D	E	A	Y	V	M	A	S	V	D	N	G	Y	P	P	H	V	C	R
NP_005219.2:p.N771_P772insN	NP_005219.2:p.(Asn771dup)	N771dup	D	E	A	Y	V	M	A	S	V	D	N	N	P	H	V	C	R		
NP_005219.2:p.N771_P772insPH	NP_005219.2:p.(Pro772_His773dup)	P772_H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	P	H	V	C	R	
NP_005219.2: p.D770_P772del771NinsTH	NP_005219.2:p.(Asp770_Pro772delAsn771insThrHis)	N771delinsTH	D	E	A	Y	V	M	A	S	V	D	T	H	P	H	V	C	R		
NP_005219.2:p.N771dupN	NP_005219.2:p.(Asn771dup)	N771dup	D	E	A	Y	V	M	A	S	V	D	N	N	P	H	V	C	R		
NP_005219.2:p.P772_C775dupPHVC	NP_005219.2:p.(Pro772_Cys775dup)	P772_C775dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	C	P	H	V	C R
NP_005219.2:p.P772_H773dupPH	NP_005219.2:p.(Pro772_His773dup)	P772_H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	P	H	V	C	R	
NP_005219.2:p.P772_H773insDNP	NP_005219.2:p.(Asp770_Pro772dup)	D770_P772dup	D	E	A	Y	V	M	A	S	V	D	N	P	D	N	P	H	V	C	R
NP_005219.2:p.P772_H773insNP	NP_005219.2:p.(Asn771_Pro772dup)	N771_P772dup	D	E	A	Y	V	M	A	S	V	D	N	P	N	P	H	V	C	R	
NP_005219.2:p.H773_V774dupHV	NP_005219.2:p.(His773_Val774dup)	H773_V774dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	H	V	C	R	
NP_005219.2:p.H773_V774insH	NP_005219.2:p.(His773dup)	H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	H	V	C	R		
NP_005219.2:p.H773_V774dupH	NP_005219.2:p.(His773dup)	H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	H	V	C	R		
NP_005219.2:p.H773_V774insPH	NP_005219.2:p.(Pro772_His773dup)	P772_H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	P	H	V	C	R	
NP_005219.2:p.H773dupH	NP_005219.2:p.(His773dup)	H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	H	V	C	R		
NP_005219.2:p.H773insH	N/A N/A N/A	H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	H	V	C	R		
NP_005219.2:p.V774_C775>AHVC	N/A N/A N/A	V774_C775insAHVC	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	A	H	V	C	C R
NP_005219.2:p.V774_C775insCPHV	NP_005219.2:p.(Pro772_Cys775dup)	P772_C775dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	C	P	H	V	C R
NP_005219.2:p.V774_C775insHV	NP_005219.2:p.(His773_Val774dup)	H773_V774dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	H	V	C	R	
NP_005219.2:p.C775_R776insPHVC	NP_005219.2:p.(Pro772_Cys775dup)	P772_C775dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	C	P	H	V	C R

Supplementary Table 3. Overview of the Ba/F3 cell line models used for the *in vitro* TKI sensitivity studies.

Reference	Tested EGFRex20+ mutational variants	TKI	Cell viability instrument	Reported value	Year published	DOIs
Hasako et al, 2018	Wild type EGFR A763_Y764insFQEA A767_V769dup S768_D770dup D770_N771insG H773_V774insNPH H773_V774insPH	Erlotinib Afatinib TAS6417	CellTiter-Glo luminescent cell viability assay (Promega)	IC_{50}	2018	10.1158/1535-7163.MCT-17-1206
Lee et al, 2019	Wild type EGFR A763_Y764insFQEA A767_V769dup S768_D770dup D770_N771insNPG P772insPR H773insH H773insNPH	Erlotinib Gefitinib Afatinib Dacomitinib Rociletinib Olmutinib Nazartinib	CellTiter-Glo luminescent cell viability assay (Promega)	IC_{50}	2019	10.1016/j.jtho.2019.05.006
Hirano et al, 2015	Wild type EGFR A763_Y764insFQEA A767_V769dup D770_N771insNPG Y764_V765insHH	Erlotinib Afatinib Osimertinib Rociletinib	CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega)	IC_{50}	2015	10.18632/oncotarget.5887
Udagawa et al, 2019	Wild type EGFR A763_Y764insFQEA A767_V769dup S768_D770dup D770_N771insG H773_V774insNPH H773_V774insPH	Erlotinib Afatinib Osimertinib TAS6417 Poziotinib	CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega)	IC_{50}	2019	10.1158/1541-7786.MCR-19-0419
Hirano et al, 2018	Wild type EGFR A763_Y764insFQEA Y764_V765insHH A767_V769dup D770_N771insNPG	Erlotinib Afatinib Osimertinib Naquotinib	CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega)	IC_{50}	2018	10.1158/1535-7163.MCT-17-1033
Jia et al, 2016	Wild type EGFR A767_V769dup S768_D770dup H773_V774insNPH	EGF816	Bright-Glo Luciferase Assay System (Promega)	EC_{50}	2016	10.1158/0008-5472
Fan et al, 2020	Wild type EGFR A763_Y764insFQEA A767_V769dup S768I S768_D770dup N771_H773dup P772_H773dup	Osimertinib BEBT-109	CellTiter-Glo luminescent cell viability assay (Promega)	IC_{50}	2020	10.1016/j.tranon.2020.100961
Yun et al, 2020	Wild type EGFR Y764_V765insHH A767_V769dup D770delinsGY S768_D770dup H773dup	Poziotinib	CellTiter-Glo 2.0 Assay Kit (Promega)	IC_{50}	2020	10.1158/2159-8290.CD-20-0116
Vasconcelos et al, 2021	Wild type EGFR A763_Y764insFQEA A767_V769dupA S768_D770dup H773dup	Osimertinib Pozitionib Mobocertinib	CellTiter 96 AQueous One Solution proliferation kit (Promega) and/or Cell Counting Kit-8 (Dojindo Molecular Technologies)	IC_{50}	2021	10.1016/j.jtocrr.2020.100105
Jorge et al, 2018	Wild type EGFR A763_Y764insFQEA Y764_V765insHH M766_A767insAI A767_V769dup S768_D770dup D770_N771insNPG H773dup	Erlotinib Afatinib Rociletinib Luminespib	CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega)	IC_{50}	2018	10.1158/1078-0432.CCR-18-1541
Robichaux et al, 2021	A763_Y764insFQEA A767_V769dup S768I D770_ N771insNPG H773_V774insNPH	Erlotinib Afatinib Gefitinib Osimertinib AZD3759 Sapatinib Dacomitinib Neratinib Poziotinib Tarlox-TKI CLN-081 AZ5104 Mobocertinib Nazartinib Olmutinib Rociletinib Naquotinib Lazertinib	CellTiter-Glo luminescent cell viability assay (Promega)	Mutant to WT ratios (dividing the IC50 values of mutant cell lines by the average IC50 value of Ba/F3 cells expressing WT EGFR)	2021	10.1038/s41586-021-03898-1

Supplementary Table 4. IC_{50} values (recalculated to nmol/L) and mutant to wild type ratios for the published models mentioned in Supplementary Table 3.

Reported IC_{50} values (unit: nmol/L) per inhibitory compound

Reference	Tested EGFRex20+ mutational variants	Erlotinib	Afatinib	Gefitinib	Osimertinib	Olmutinib	Nazartinib	Rociletinib	BEBT-109	Naquotinib	TAS6417	Luminespib	EGF816	Dacomitinib	Poziotinib	Mobocertinib
Hasako et al, 2018	Wild type EGFR	919	34								676					
	A763_Y764insFQEA	100	2								5.05					
	A767_V769dup	1591	93								103					
	S768_D770dup	2063	121								38					
	D770_N771insG	1012	41								38					
	H773_V774insNPH	1459	230								145					
	H773_V774insPH	2675	325								150					
Lee et al, 2019	Wild type EGFR	1333	7	1127	259	236	82	263						39		
	A763_Y764insFQEA	33	0	22	132	352	352	1022						0		
	A767_V769dup	5051	54	4870	41	262	108	1325						55		
	S768_D770dup	5180	64	3479	28	288	129	732						86		
	D770_N771insNPG	3240	17	2422	15	30	36	97						8		
	P772insPR	5391	200	3611	22	314	148	3035						162		
	H773insNPH	1391	20	949	11	97	50	288						11		
	H773insH	2859	353	1980	66	498	510	2309						320		
Hirano et al, 2015	Wild type EGFR	1020	31		938			2052								
	A763_Y764insFQEA	154	3		44			673								
	A767_V769dup	1679	158		333			5290								
	D770_N771insNPG	1146	43		42			262								
	Y764_V765insHH	>10000	134		237			1730								
Udagawa et al, 2019	Wild type EGFR	980	48		688						702				6	
	A763_Y764insFQEA	3145	279		10						4				22	
	A767_V769dup	91	69		77						26				41	
	S768_D770dup	7	0		4						1				0	
	D770_N771insG	2970	270		10						3				30	
	H773_V774insNPH	138	3		45						5				1	
	H773_V774insPH	1511	100		244						121				5	
Hirano et al, 2018	Wild type EGFR	667	28		704					830						
	A763_Y764insFQEA	84	0.6		16					19						
	Y764_V765insHH	>10000	734		701					681						
	A767_V769dup	2021	48		230					148						
	D770_N771insNPG	1835	92		38					56						
Jia et al, 2016	Wild type EGFR												166			
	A767_V769dup												11			
	S768_D770dup												7			
	H773_V774insNPH												190			
Fan et al, 2020	Wild type EGFR				1112				3415							
	A763_Y764insFQEA				45				11							
	A767_V769dup				101				21							
	S768I				198				52							
	S768_D770dup				110				32							
	N771_H773dup				89				21							
	P772_H773dup				59				15							
Yun et al, 2020	Wild type EGFR														0.8	
	Y764_V765insHH														6.0	
	A767_V769dup														1.3	
	D770delinsGY														0.8	
	S768_D770dup														1.5	
	H773dup														10.4	
Vasconcelos et al, 2021	Wild type EGFR				65.83										28	763
	A763_Y764insFQEA				30										2	109
	A767_V769dup				179										2	63
	S768_D770dup				133										2	203
	H773dup				100										18	180
Jorge et al, 2018	Wild type EGFR	1535	15					119				3				
	A763_Y764insFQEA	111	1					1053				3				
	Y764_V765insHH	3400	99					1035				2				
	M766_A767insAI	3276	36					1762				8				
	A767_V769dup	2628	34					1210				3				
	S768_D770dup	3187	25					1237				4				
	D770_N771insNPG	2138	31					341				8				
	H773dup	3884	161					1194				2				

Reported mutant to wild type ratios per inhibitory compound

Reference	Tested EGFRex20+ mutational variants	Erlotinib	Afatinib	Gefitinib	Osimertinib	Olmutinib	Rociletinib	Lazertinib	Naquotinib	AZD3759	Sapatinib	Dacomitinib	Neratinib	Poziotinib	Tarlox-TKI	CLN-081	AZ5104	Mobocertinib	Nazartinib
Robichaux et al, 2021	A763_Y764insFQEA	0,42	0,07	0,17	0,12	0,03	0,18	0,04	0,19	0,02	0,31	0,04	0,07	0,07	0,05	0,00	0,40	0,47	0,06
	A767_V769dup	60,28	4,07	30,47	2,39	0,68	1,86	10,93	1,61	4,15	2,23	3,06	3,52	0,32	0,99	0,14	4,66	1,55	0,64
	S768I	1,57	42,75	3,30	0,37	0,01	0,85	1,26	1,09	0,14	0,19	17,66	10,70	4,86	0,39	0,08	1,36	0,78	0,53
	D770_ N771insNPG	16,70	6,26	26,32	1,33	0,31	0,69	4,93	1,30	3,84	5,02	5,40	27,86	0,29	5,02	0,02	1,98	1,21	0,53
	H773_V774insNPH	5,55	2,05	28,96	0,93	0,43	0,87	5,10	1,08	1,56	1,26	1,65	7,72	0,39	11,56	0,18	4,00	0,30	0,42

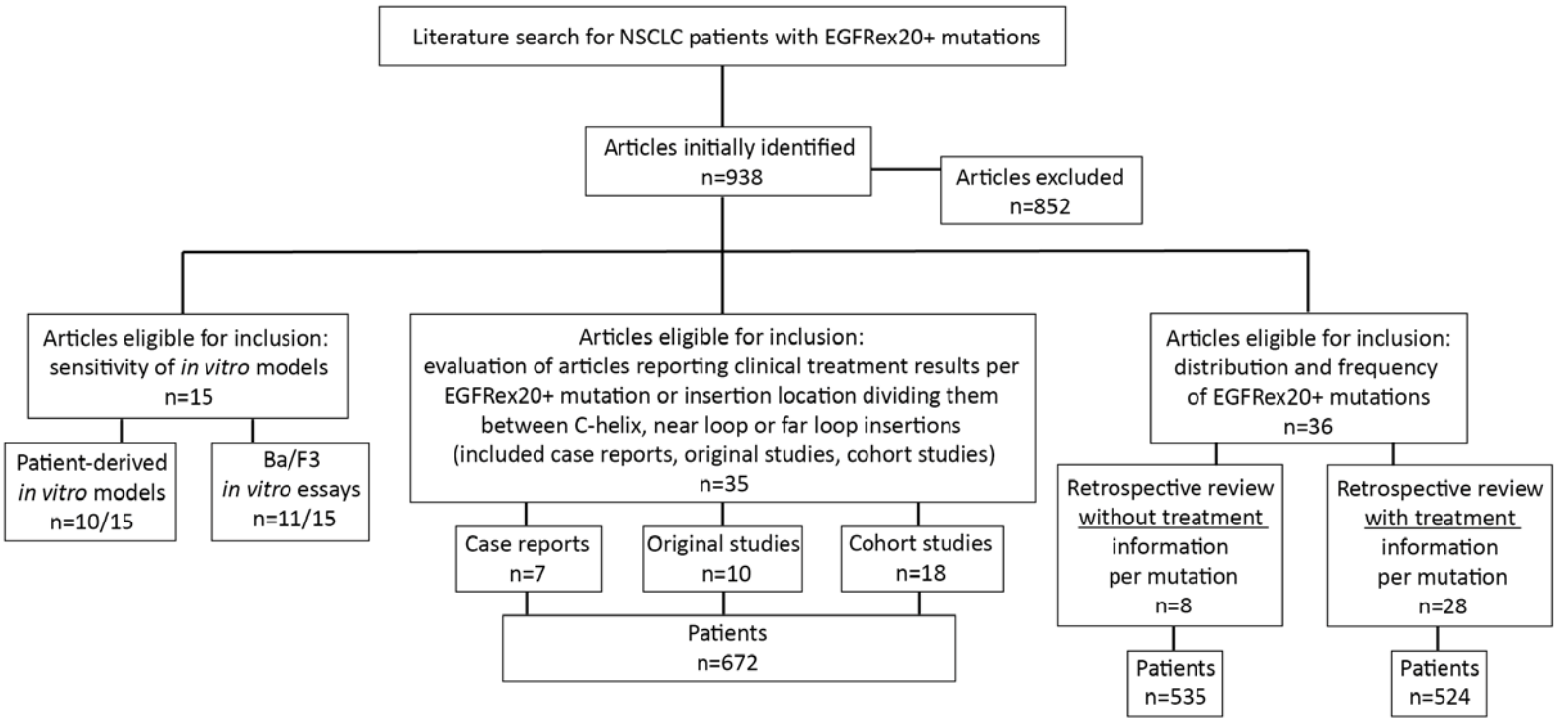
Supplementary Table 5. Published EGFRex20+ patient derived cell lines with IC_{50} values (calculated to nmol/L) of several inhibitory compounds and the IC_{50} values of the control matching mutational cellines (if applicable). All experiments were performed in triplo. *; When experiments were perfomed twice instead of in triplo, both values were mentioned. #; Ba/F3 cell lines were used as a control. $; A431 cell line was used as a control.

			Reported IC_{50} values (calculated to nmol/L) per inhibitory compound																
Reference	**Patient-derived cell line**	**Mutational status**	**Erlotinib**	**Afatinib**	**Gefitinib**	**Osimertinib**	**Rocelitenib**	**Poziotinib**	**Naquotinib**	**Luminespib**	**TAS6417**	**Mobocertinib**	**Cetuximab**	**Dacometinib**	**Nazartinib**	**Olmutinib**	**Tarloxotinib**	**Tarlox-E**
Yesuda et al, 2013	BID007	A763_Y764insFQEA	82	5	565													
	#	A763_Y764insFQEA	48	4	174													
Hirano et al, 2015	BID007	A763_Y764insFQEA	45	8		40	1278											
	#	A763_Y764insFQEA	154	3		44	673											
	#	Wild type EGFR	1020	31		938	2052											
Hirano et al, 2018	BID007	A763_Y764insFQEA	233	13		108			52									
	#	A763_Y764insFQEA	84	0.6		16			19									
	#	Wild type EGFR	667	28		704			830									
Udagawa et al, 2019	BID007	A763_Y764insFQEA	53	3		87		1			3							
	BID019	N771_P772insH		221				1			21							
	#	A763_Y764insFQEA	138	3		45		1			5							
	#	Wild type EGFR	980	48		688		6			702							
Jorge et al, 2018	BID007	A763_Y764insFQEA								7								
	#	A763_Y764insFQEA								3								
	#	Wild type EGFR								3								
Vasconcelos et al, 2021	BID007	A763_Y764insFQEA										60.36						
	BID019	N771_P772insH										109.70						
	#	A763_Y764insFQEA										109						
	#	Wild type EGFR										763,4						
Yang et al, 2016*	LU0387	H773_V774insNPH	11621 >10000	10823 >10000	>10000 >10000	3298 5721	2636 3442						Insensitive Insensitive					
	LU3075	P772_H773insDNP	96665 >100000	1230 1816	4369 6790	1591 2570	2095 2501						Insensitive Insensitive					
Gonzalez et al, 2021	LU0387	H773_V774insNPH	2793	20	364	195						21						
	CUTO14	A767_V769dup	2679	66	1021	575						33						
	#	H773_V774insNPH	2764	103	4054	469						18						
	#	A767_V769dup	1340	46	369	173						11						
	$	Wild type EGFR	71	3.9	56	-						35						
Lee et al, 2019	SNU-3173	H773_V774insAH	4535	16.7	1050	62.7	1202.5							13.7	139.7	627		
	#	Wild type EGFR	1333	7	1127	259								39	82	236		
Estrada et al, 2021	CUTO14	A767_V769dup		110.9	374	303											4645	72.2
	CUTO17	N771_H773dup		219.7	4197	426											3090	48.1
	CUTO18	S768_D770dup		841.3	>10000	647											>10000	158.4

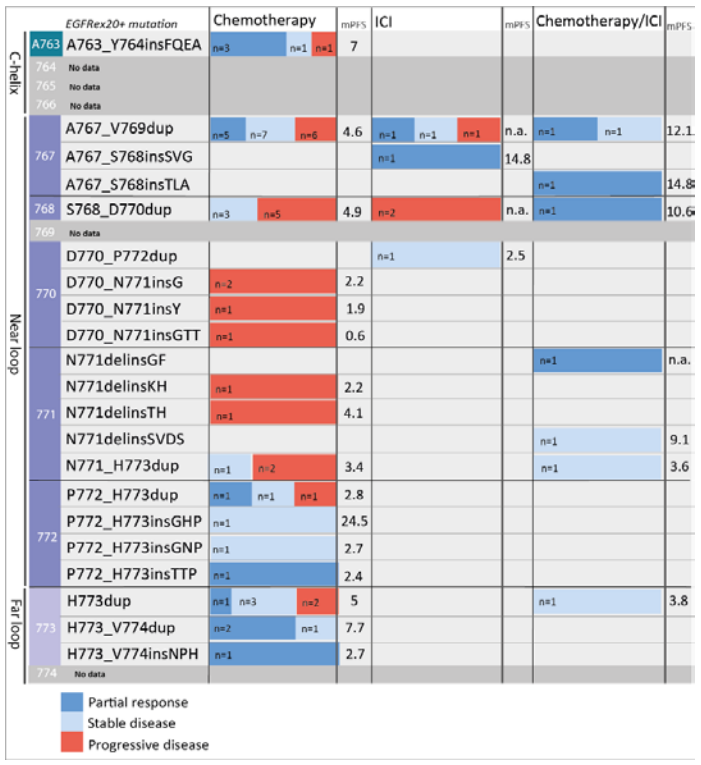
Supplementary Table 6. Articles reporting clinical treatment results (ORR ans PFS if applicable) of in total 672 patients per EGFRex20+ mutation or insertion location dividing them between C-helix, near loop or far loop insertions. *Platinum based chemotherapy regim.
Abbreviations: ICI = immune checkpoint inhibitor (ICI)-based treatment.

Reference	Type of study	Treatment	Year published	DOIs
Wu, Jenn-Yu et al. "Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response." Clinical cancer research : an official journal of the American Association for Cancer Research vol. 14,15 (2008): 4877-82.	Cohort	Gefitinib	2008	10.1158/1078-0432.CCR-07-5123
Yasuda, Hiroyuki et al. "Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer." Science translational medicine vol. 5,216 (2013): 216ra177.	Cohort	Erlotinib Geftinib	2013	10.1126/scitranslmed.3007205
Beau-Faller, M et al. "Rare EGFR exon 18 and exon 20 mutations in non-small-cell lung cancer on 10 117 patients: a multicentre observational study by the French ERMETIC-IFCT network." Annals of oncology : official journal of the European Society for Medical Oncology vol. 25,1 (2014): 126-31.	Cohort	Erlotinib Gefitinib	2014	10.1093/annonc/mdt418
Naidoo, J et al. "Epidermal growth factor receptor exon 20 insertions in advanced lung adenocarcinomas: Clinical outcomes and response to erlotinib." Cancer vol. 121,18 (2015): 3212-3220.	Cohort	Erlotinib	2015	10.1002/cncr.29493
Tsigelny, Igor F et al. "Molecular determinants of drug-specific sensitivity for epidermal growth factor receptor (EGFR) exon 19 and 20 mutants in non-small cell lung cancer." Oncotarget vol. 6,8 (2015): 6029-39.	Cohort	Erlotinib/cetuximab	2015	10.18632/oncotarget.3472
Chan, Raymond Tsz-Tong. "Afatinib for an EGFR exon 20 insertion mutation: A case report of progressive stage IV metastatic lung adenocarcinoma with 54 months' survival." Asia-Pacific journal of clinical oncology vol. 14 Suppl 1 (2018): 7-9.	Case report	Afatinib	2018	10.1111/ajco.12853
van Veggel, Bianca et al. "Afatinib and Cetuximab in Four Patients With EGFR Exon 20 Insertion-Positive Advanced NSCLC." Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer vol. 13,8 (2018): 1222-1226.	Cohort	Afatinib/cetuximab	2018	10.1016/j.jtho.2018.04.012
Takeda, Masayuki et al. "Clinical characteristics of non-small cell lung cancer harboring mutations in exon 20 of EGFR or HER2." Oncotarget vol. 9,30 21132-21140. 20 Apr. 2018.	Cohort	ICI	2018	10.18632/oncotarget.24958
Piotrowska, Z et al. "Activity of the Hsp90 inhibitor luminespib among non-small-cell lung cancers harboring EGFR exon 20 insertions." Annals of oncology : official journal of the European Society for Medical Oncology vol. 29,10 (2018): 2092-2097.	Original study	Luminespib	2018	10.1093/annonc/mdy336
Robichaux, Jacqulyne P et al. "Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer." Nature medicine vol. 24,5 (2018): 638-646.	Original study	Poziotinib	2018	10.1038/s41591-018-0007-9
Fang, Wenfeng et al. "A Patient with EGFR Exon 20 Insertion-Mutant Non-Small Cell Lung Cancer Responded to Osimertinib plus Cetuximab Combination Therapy." Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer vol. 14,9 (2019): e201-e202.	Case report	Afatinib/cetuximab	2019	10.1016/j.jtho.2019.04.013
Wu, Jenn-Yu et al. "Effectiveness of Treatments for Advanced Non-Small-Cell Lung Cancer With Exon 20 Insertion Epidermal Growth Factor Receptor Mutations." Clinical lung cancer vol. 20,6 (2019): e620-e630.	Cohort	Chemotherapy* Gefitinib Erlotinib Afatinib	2019	10.1016/j.cllc.2019.06.018
Byeon, Seonggyu et al. "Clinical Outcomes of EGFR Exon 20 Insertion Mutations in Advanced Non-small Cell Lung Cancer in Korea." Cancer research and treatment vol. 51,2 (2019): 623-631.	Cohort	Chemotherapy*	2019	10.4143/crt.2018.151
Fang, Wenfeng et al. "EGFR exon 20 insertion mutations and response to osimertinib in non-small-cell lung cancer." BMC cancer vol. 19,1 595. 17 Jun. 2019.	Cohort	Osimertinib 80mg	2019	10.1186/s12885-019-5820-0
Lin, Ling et al. "Response to Afatinib in a Patient with NSCLC Harboring Novel EGFR Exon 20 Insertion Mutations." OncoTargets and therapy vol. 13 9753-9757. 30 Sep. 2020.	Case report	Afatinib	2020	10.2147/OTT.S268694
Piotrowska, Zofia et al. "ECOG-ACRIN 5162: A phase II study of osimertinib 160 mg in NSCLC with EGFR exon 20 insertions.," Journal of Clinical Oncology, vol. 38, no. 15_suppl, p. 9513, May 2020.	Cohort	Osimertinib 160mg	2020	10.1200/JCO.2020.38.15_suppl.9513
Yang, Guang-Jian et al. "Osimertinib for Chinese advanced non-small cell lung cancer patients harboring diverse EGFR exon 20 insertion mutations." Lung cancer (Amsterdam, Netherlands) vol. 152 (2021): 39-48.	Cohort	Osimertinib 80mg and 160mg	2020	10.1016/j.lungcan.2020.11.027
van Veggel, B et al. "Osimertinib treatment for patients with EGFR exon 20 mutation positive non-small cell lung cancer." Lung cancer (Amsterdam, Netherlands) vol. 141 (2020): 9-13.	Cohort	Osimertinib 80mg and 160mg	2020	10.1016/j.lungcan.2019.12.013
Le, Ziuning et al. "Poziotinib shows activity and durability of responses in subgroups of previously treated EGFR exon 20 NSCLC patients." Journal of Clinical Oncology, vol. 38, no. 15_suppl, p. 9514, May 2020.	Original study	Poziotinib	2020	10.1200/JCO.2020.38.15_suppl.9514
Zöchbauer-Müller, Sabine et al. "Case Report: Afatinib Treatment in a Patient With NSCLC Harboring a Rare EGFR Exon 20 Mutation." Frontiers in oncology vol. 10 593852. 26 Jan. 2021.	Case report	Afatinib	2021	10.3389/fonc.2020.593852
Urbán, László et al. "Major Clinical Response to Afatinib Monotherapy in Lung Adenocarcinoma Harboring EGFR Exon 20 Insertion Mutation." Clinical lung cancer vol. 22,1 (2021): e112-e115.	Case report	Afatinib	2021	10.1016/j.cllc.2020.09.005
Park, Keunchil et al. "Amivantamab in EGFR Exon 20 Insertion-Mutated Non-Small-Cell Lung Cancer Progressing on Platinum Chemotherapy: Initial Results From the CHRYSALIS Phase I Study." Journal of clinical oncology : official journal of the American Society of Clinical Oncology vol. 39,30 (2021): 3391-3402.	Original study	Amivantamab	2021	10.1200/JCO.21.00662
Riely, Gregory J et al. "Activity and Safety of Mobocertinib (TAK-788) in Previously Treated Non-Small Cell Lung Cancer with EGFR Exon 20 Insertion Mutations from a Phase I/II Trial." Cancer discovery vol. 11,7 (2021): 1688-1699.	Original study	Mobocertinib	2021	10.1158/2159-8290.CD-20-1598
Zhou, Caicun et al. "Treatment Outcomes and Safety of Mobocertinib in Platinum-Pretreated Patients With EGFR Exon 20 Insertion-Positive Metastatic Non-Small Cell Lung Cancer: A Phase 1/2 Open-label Nonrandomized Clinical Trial." JAMA oncology vol. 7,12 (2021): e214761.	Original study	Mobocertinib	2021	10.1001/jamaoncol.2021.4761
Riess, Jonathan W et al. "Erlotinib and Onalespib Lactate Focused on EGFR Exon 20 Insertion Non-Small Cell Lung Cancer (NSCLC): A California Cancer Consortium Phase I/II Trial (NCI 9878)." Clinical lung cancer vol. 22,6 (2021): 541-548.	Original study	Onalespib Erlotinib	2021	10.1016/j.cllc.2021.05.001
Yasuda, Hiroyuki et al. "A phase I/II study of osimertinib in EGFR exon 20 insertion mutation-positive non-small cell lung cancer." Lung cancer (Amsterdam, Netherlands) vol. 162 (2021): 140-146.	Original study	Osimertinib 80mg	2021	10.1016/j.lungcan.2021.10.006
Prelaj, Arsela et al. "Poziotinib for EGFR and HER2 exon 20 insertion mutation in advanced NSCLC: Results from the expanded access program." European journal of cancer (Oxford, England : 1990) vol. 149 (2021): 235-248.	Cohort	Poziotinib	2021	10.1016/j.ejca.2021.02.038
Nikanjam, Mina et al. "Cetuximab in Patients with Non-Small Cell Lung Cancer and EGFR Exon 20 Insertion Alterations." Clinical oncology, case reports vol. 5,1 (2022): 210.	Cohort	Afatinib/cetuximab	2022	
Geng, D et al. "Clinical and molecular characteristics of epidermal growth factor receptor exon 20 insertion mutations in non-small-cell lung cancer." Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico vol. 24,2 (2022): 379-387.	Cohort	ICI	2022	10.1007/s12094-021-02701-x
Zhu, Lingling et al. "Case Report: Partial Response Following Nivolumab Plus Docetaxel in a Patient With EGFR Exon 20 Deletion/Insertion (p.N771delinsGF) Mutant Lung Adenocarcinoma Transdifferentiated From Squamous Cell Carcinoma." Frontiers in cell and developmental biology vol. 9 755135. 10 Jan. 2022.	Case report	ICI/chemotherapy*	2022	10.3389/fcell.2021.755135
Shi, Chao et al. "Real-world clinical treatment outcomes in Chinese non-small cell lung cancer with EGFR exon 20 insertion mutations." Frontiers in oncology vol. 12 949304. 2 Sep. 2022.	Cohort	ICI/chemotherapy*	2022	10.3389/fonc.2022.949304
Zwierenga, Fenneke et al. "High dose osimertinib in patients with advanced stage EGFR exon 20 mutation-positive NSCLC: Results from the phase 2 multicenter POSITION20 trial." Lung cancer (Amsterdam, Netherlands) vol. 170 (2022): 133-140.	Original study	Osimertinib 160mg	2022	10.1016/j.lungcan.2022.06.012
Yang, Guangjian et al. "*EGFR* exon 20 insertion variants A763_Y764insFQEA and D770delinsGY confer favorable sensitivity to currently approved EGFR-specific tyrosine kinase inhibitors." *Frontiers in pharmacology* vol. 13 984503. 8 Nov. 2022.	Cohort	Osimertinib 80mg Gefitinib Erlotinib Afatinib	2022	10.3389/fphar.2022.984503
Prelaj, Arsela et al. "Case Report: Exceptional Response to Poziotinib in Patient with Metastatic Non-Small Cell Lung Cancer With EGFR Exon 20 Insertion Mutation." Frontiers in oncology vol. 12 902967. 8 Jun. 2022.	Case report	Poziotinib	2022	10.3389/fonc.2022.902967
Elamin, Yasir Y et al. "Poziotinib for EGFR exon 20-mutant NSCLC: Clinical efficacy, resistance mechanisms, and impact of insertion location on drug sensitivity." Cancer cell vol. 40,7 (2022): 754-767.e6.	Original study	Poziotinib	2022	10.1016/j.ccell.2022.06.006

Supplementary Figure 1. Flowchart on number of articles selected from literature search with results on in vitro experiments and clinical treatment and how patient selection was performed. Fifteen articles were found to be eligible for reporting sensitivity of in vitro models. Six articles reported data both on patient derived in vitro models and Ba/F3 in vitro essays. Thirty-five articles were found to report an evaluation on clinical treatment per EGFRex20+ mutation location, including articles reporting results only dividing the location between C-helix, near loop or far loop insertions. For the distribution and frequency of EGFRex20+ mutations, 36 articles were retrospectively analyzed. The 28 articles founded which contained treatment information, are also included within the 35 articles for the evaluation of clinical treatment per EGFRex20+ mutation.
Abbreviations: NSCLC = non-small cell lung cancer; EGFRex20+ = in-frame deletion and/or insertion mutations clustering within the EGFR exon 20 region.

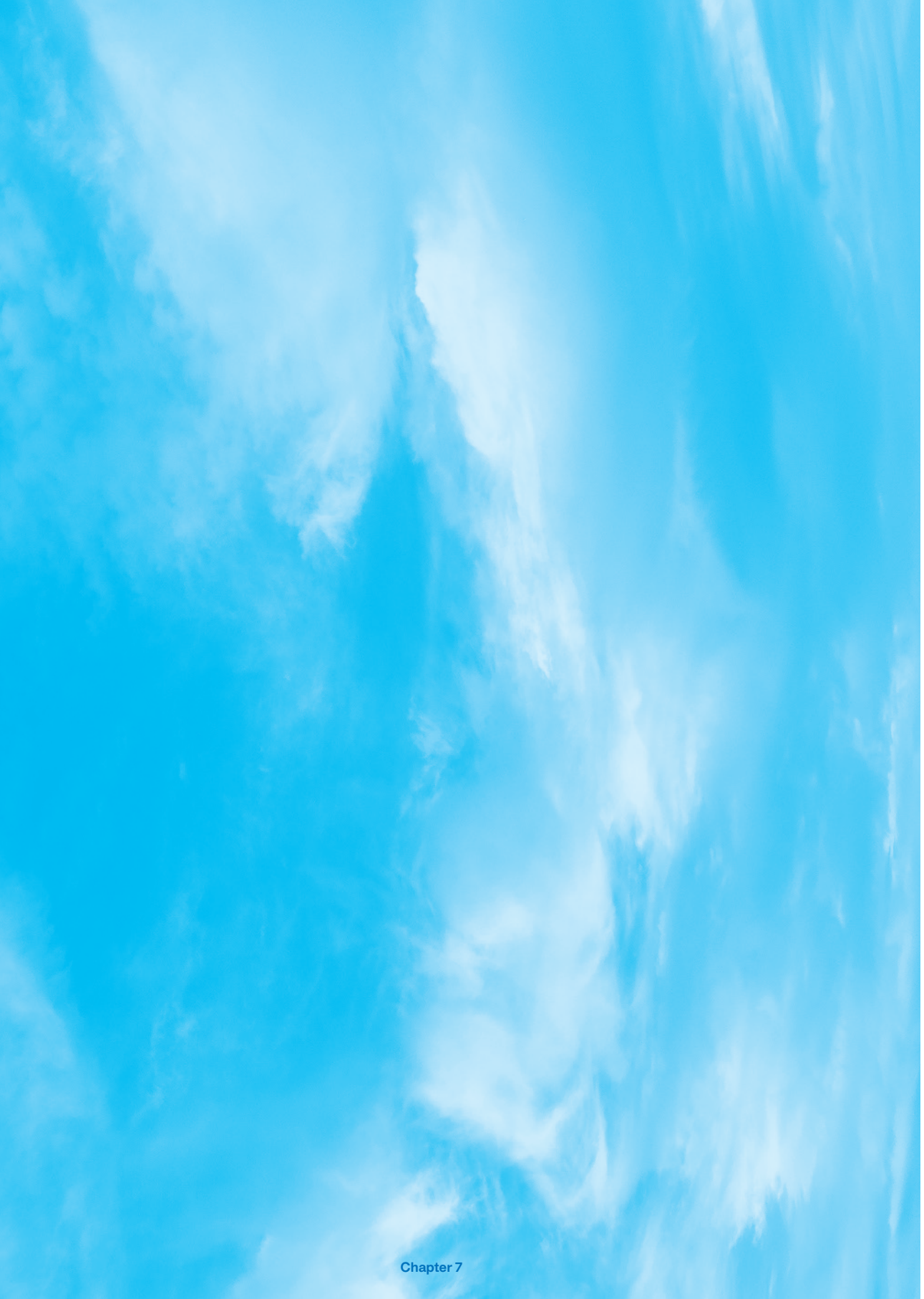


Supplementary Figure 2. A cross-tabulation comparing observed clinical benefit with chemotherapy, ICI and chemotherapy/ICI combination therapy based on tumor responses in NSCLC patients across different EGFRex20+ mutations (Sup. Table 6). The size of the bar representing the responses reflects the relative frequency of all responses mentioned for the corresponding mutation. Used chemotherapy was platinum-based and the used anti PD1 are camrelizumab, sintilimab, pembrolizumab and nivolumab. The mPFS is the median progressive free survival of all reported mPFS in the different studies, if applicable. Abbreviations: ICI = immune checkpoint inhibitor (ICI)-based treatment, PD1 = programmed death protein 1.



Part II

Analysis and treatment of resistance mechanisms after third generation EGFR TKI treatment



Chapter 7

Genomic Profiling Identifies Outcome-Relevant Mechanisms of Innate and Acquired Resistance to Third-Generation Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor Therapy in Lung Cancer

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*These authors contributed equally to this work.

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Abstract

***Purpose*: Third-generation epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitors (TKIs) are effective in acquired resistance (AR) to early-generation EGFR TKIs in EGFR-mutant lung cancer. However, efficacy is marked by interindividual heterogeneity. We present the molecular profiles of pretreatment and post-treatment samples from patients treated with third-generation EGFR TKIs and their impact on treatment outcomes.**

***Patients and methods*: Using the databases of two lung cancer networks and two lung cancer centers, we molecularly characterized 124 patients with *EGFR* p.T790M-positive AR to early-generation EGFR TKIs. In 56 patients, correlative analyses of third-generation EGFR TKI treatment outcomes and molecular characteristics were feasible. In addition, matched post-treatment biopsy samples were collected for 29 patients with progression to third-generation EGFR TKIs.**

***Results*: Co-occurring genetic aberrations were found in 74.4% of *EGFR* p.T790-positive samples (n = 124). Mutations in *TP53* were the most frequent aberrations detected (44.5%; n = 53) and had no significant impact on third-generation EGFR TKI treatment. Mesenchymal-epithelial transition factor (*MET*) amplifications were found in 5% of samples (n = 6) and reduced efficacy of third-generation EGFR TKIs significantly (eg, median progression-free survival, 1.0 months; 95% CI, 0.37 to 1.72 v 8.2 months; 95% CI, 1.69 to 14.77 months; P ≤ .001). Genetic changes in the 29 samples with AR to third-generation EGFR TKIs were found in EGFR (eg, p.T790M loss, acquisition of p.C797S or p.G724S) or in other genes (eg, *MET* amplification, *KRAS* mutations).**

***Conclusion*: Additional genetic aberrations are frequent in EGFR-mutant lung cancer and may mediate innate and AR to third-generation EGFR TKIs. *MET* amplification was strongly associated with primary treatment failure and was a common mechanism of AR to third-generation EGFR TKIs. Thus, combining EGFR inhibitors with TKIs targeting common mechanisms of resistance may delay AR.**

Introduction

Treatment with selective early-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) has demonstrated high efficacy in patients with lung cancer harboring activating *EGFR* mutations. However, because of a Darwinian-like selection of drug desensitized tumor cells, resistance inevitably develops. [1-6]

In 60% of patients, acquired resistance (AR) is mediated through a mutation in the gatekeeper threonine of EGFR exon 20—p.T790M. [7,8] Third- generation EGFR TKIs have been designed to overcome p.T790M-driven resistance, and confirmed response rates (RRs) range from 61% for osimertinib to 45% for rociletinib (CO-1686) and 55% for nazartinib (EGF816). [9-15]

Apart from monogenetically driven resistance, patients with tumor heterogeneity have been reported, including co- occurrence of p.T790M and amplifications of the mesenchymal-epithelial transition factor (MET) proto-oncogene (*MET*) or the human epidermal growth factor receptor 2 gene (*ERBB2*), as well as mitogen-activated protein kinase/extracellular regulated kinase pathway activation.[17-25] The combination of EGFR TKIs with other inhibitors may restore EGFR dependency and response to EGFR inhibition.[17-19,21-28] Thus, the effects of co-occurring factors of resistance detected before third-generation EGFR TKI treatment and their impact on efficacy has been the focus of research. [19,24, 25] However, most reports are based on the analysis of cell-free DNA, and the numbers of matched pretreatment and post-treatment tumor samples are usually low. Apart from that, only a few studies have been performed that systematically investigated the impact of co-occurring aberrations on third-generation EGFR TKIs. We present a comprehensive analysis of co-occurring genetic aberrations in pretreatment and post-treatment tumor tissue and their contribution to innate resistance (IR) and AR to three third-generation EGFR TKIs.

Methods

Study Design, Patient Selection, and Tumor Tissue Collection

To determine the frequency of co-occurring genetic aberrations in samples of *EGFR* p.T790M-mediated resistance to early-generation EGFR TKIs, we systematically searched the databases of the Network Genomic Medicine, the NOWEL network, the Department of Thoracic Oncology of the Netherlands Cancer Institute, and the Institute of Oncology at the Vall d'Hebron University Hospital for patients with non–small-cell lung cancer (NSCLC) who fulfilled the following selection criteria (cohort A; patients a1 to a68/b1 to b56; Fig 1; Data Supplement): (1) presence of *EGFR* p.T790M and (2) progression while receiving treatment with first- or second-generation EGFR TKIs.

To assess the effect of molecular aberrations on third- generation EGFR TKI efficacy in pretreatment and post- treatment samples, we selected patients from cohort A according to the following criteria (cohort B; patients b1 to b56; Fig 1; Data Supplement): (1) locally advanced/metastasized NSCLC harboring activating *EGFR* mutations and *EGFR* p.T790M, (2) third-generation EGFR TKI treatment in the setting of AR, and (3) sufficient imaging data for efficacy assessments according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. Patients were treated in the AURA 1/3 trials (osimertinib; NCT01802632/ NCT02151981), Tiger-2/-3 trials (rociletinib; NCT02147990/NCT02322281), CEGF816X2101 trial (nazartinib; NCT 02108964), osimertinib compassionate use program

(CUP), or clinical routine. Patients treated in trials or the CUP were selected according to the specific eligibility criteria.

In a subset of patients from cohort B, a rebiopsy was performed at disease progression for identification of mecha- nisms of AR. These patients were grouped in cohort C (Fig 1).

In all patients, tumor tissue was collected in growing lesions by aspiration biopsy, core needle biopsy, or excisional biopsy (Data Supplement). All patients consented to the procedures according to local and Good Clinical Practice standards. Procedures were approved by the local ethics committees or review boards.

We identified three patients with *EGFR* p.G724S mutations (see Results). A more detailed description will be published elsewhere.[29]

Efficacy Assessments

Patients treated within the osimertinib CUP or in clinical routine received scans as clinically indicated and per local practice. In patients treated within clinical trials, scans were performed according to the protocols. Scans were evaluated according to RECIST 1.1.[30] Partial responses (PRs) were confirmed at least 4 weeks after the first scan showing a PR. IR was defined as progressive disease, (PD) as best response.

Detection of EGFR p.T790M and Targeted Massively Parallel Sequencing

Tumor samples were formalin fixed paraffin embedded. Tumor tissue of patients was genomically characterized by massively parallel sequencing (MPS), if feasible. Four different MPS technologies and panels were used and are described in the Data Supplement in detail. In patients screened within Network Genomic Medicine (a1 to a68/b1 to b31/b37 to b43), MPS was performed with an Ion AmpliSeq Custom DNA Panel (Thermo Fisher Scientific, Waltham, MA) and a MiSeq benchtop sequencer (Illumina, San Diego, CA) or with a GeneRead DNAseq Custom Panel V2 (Qiagen, Santa Clarita, CA) consisting of 205 amplicons.[31]

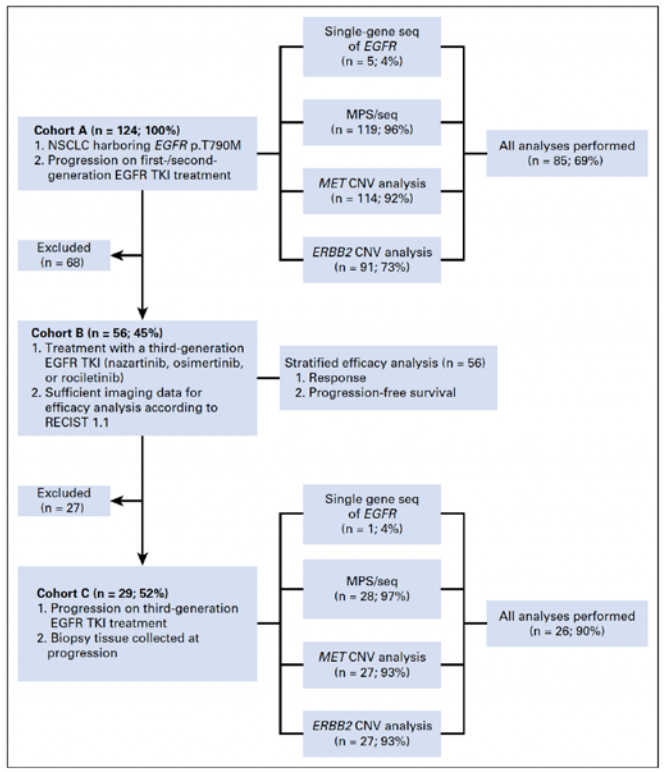


Fig 1. Flowchart of the study population and cohorts. CNV, copy number variation; *EGFR*, epidermal growth factor receptor; *ERBB2*, human epidermal growth factor receptor 2 gene; *MET*, mesenchymalepithelial transition factor; MPS, massively parallel sequencing; NSCLC, non–small-cell lung cancer; RECIST, Response Evaluation Criteria in Solid Tumors; seq, sequencing; TKI, tyrosine kinase inhibitor."

In patients screened within the NOWEL network (a33 to a36), sequencing was performed using the NEOPlus hybrid-capture–based approach (NEO New Oncology, Cologne, Germany). Samples of patients from the Netherlands Cancer Institute (b44 to b56) were analyzed on a MiSeq benchtop sequencer (Illumina) using the TruSeq Amplicon Cancer Panel v1.0 (Illumina). For patients in which MPS was not feasible, *EGFR* status was determined by Sanger sequencing or digital droplet polymerase chain reaction. The molecular analyses performed in each sample are available in the Data Supplement.

Determination of Copy Number Variations and Small-Cell Lung Cancer Transformation

MET copy number variation (CNV) analysis was performed by fluorescence in situ hybridization using the ZytoLight SPEC *MET/CEN7* Dual Color Probe (ZytoVision, Bremerhaven, Germany).[20] Samples were classified as *MET* amplified if fulfilling the criteria for high-level amplification established by Schildhaus et al[20] (ie, *MET/CEN7* ratio greater than or equal to 2.0 or an average *MET* gene copy number [GCN] per cell of greater than or equal to 6.0).[23] All other tumors were classified as *MET* wild type (WT).

ERBB2 CNV status was determined using the ZytoLight SPEC *ERBB2/CEP17* Dual Color Probe (ZytoVision) or the INFORM HER2 Dual ISH DNA Probe (Ventana, Tucson, AZ).[17] Amplification of *ERBB2* was positive if the *ERBB2/ CEP17* ratio was greater than or equal to 2.0 or the average ERBB2 GCN per cell was greater than or equal to 6.0. In the post-treatment samples (cohort C) of b41 to b56, *MET* and *ERBB2* status was assessed by fluorescence in situ hy- bridization or chromogen in situ hybridization only if CNVs were detected by MPS.

Small-cell lung cancer transformation was assessed using microscopy by experienced pathologists. Transformation was defined by the occurrence of small-cell lung cancer histology.

Statistical Analyses

RR was defined as the percentage of complete remissions and PR as best response. Progression-free survival (PFS) indicated the time from treatment start until PD or death. Overall survival (OS) was defined as the time from first diagnosis until death. Time-to-event end points were analyzed using the Kaplan-Meier estimator. Qualitative variables were summarized by count and percentage; quantitative variables were summarized by mean, median, and range. Differences in time-to-event distribution were evaluated by the log-rank test, and statistical association between any two categorical variables was assessed by Fisher's exact test; 95% CIs for proportions were calculated using the Clopper-Pearson (binominal) formula. P values less than or equal to .05 were considered statistically significant. The frequencies of the genetic changes were calculated on the basis of the number of patients screened for each aberration. Calculations were performed in Excel (Microsoft, Redmond, WA) and SPSS Statistics version 24 (SPSS, Chicago, IL).

Table 1. Summary of Efficacy Analyses by Genetic Alterations and Background of Patients in Cohort B (n = 56)

		OS		PFS		RR	
Genetic Alteration/Patient Characteristic	**No. (%)**	**OS, months (95% CI)**	***P***	**PFS, months (95% CI)**	***P***	**RR, % (95% CI)**	***P***
All patients	56 (100)	54.0 (46.0 to 61.9)		8.0 (6.9 to 9.1)		60.7 (46.8 to 73.5)	
Baseline *EGFR* status							
Del19 (1)	41 (73.2)	54.7 (8.6 to 100.8)	1 *v* 2: .91	8.2 (5.9 to 10.4)	1 *v* 2: .096	68.3 (51.9 to 81.9)	1 *v* 2: .117
L858R (2)	14 (25)	54.0 (45.6 to 62.4)		6.8 (3.7 to 9.9)		42.9 (17.7 to 71.1)	
Other (3)	1 (1.8)	16 (-)		4.2 (-)		0.0 (0.0 to 97.5)	
TP53 status							
WT	27 (52.9)	55.3 (48.9 to 61.7)	.307	8.1 (6.5 to 9.7)	.354	70.4 (49.8 to 86.3)	.261
Mutation	24 (47.1)	47.0 (27.2 to 66.8)		7.3 (1.3 to 13.3)		54.2 (32.8 to 74.5)	
MET status							
WT	43 (91.5)	55.3 (43.1 to 67.5)	< .001	8.0 (6.9 to 9.1)	< .001	62.8 (46.7 to 77.0)	.027
Amplification	4 (8.5)	16.0 (8.8 to 23.5)		1.0 (0.3 to 1.7)		0.0 (0.0 to 60.2)	
ERBB2 status							
WT	40 (93.0)	56.6 (41.9 to 71.2)	.825	8.0 (6.7 to 9.3)	.933	62.5 (45.8 to 77.3)	.552
Amplification	3 (7.0)	26.6 (9.6 to 43.6)		4.2 (0.4 to 8.0)		33.3 (0.8 to 90.6)	
CTNNB1 status							
WT	48 (94.1)	54.0 (47.5 to 60.5)		8.0 (5.8 to 10.2)	.271	62.5 (47.4 to 76.1)	.691
Mutation	3 (5.9)	All patients censored		14.7 (2.9 to 26.5)		66.7 (9.4 to 99.2)	
PTEN status							
WT	49 (96.1)	54.7 (48.4 to 61.0)	.475	8.0 (6.4 to 9.6)	.64	63.3 (48.3 to 76.6)	.611
Mutation	2 (3.9)	13.2 (-)		1.8 (-)		50.0 (1.3 to 98.7)	
PIK3CA status							
WT	49 (96.1)	54.7 (48.2 to 61.1)	.906	8.0 (6.2 to 9.8)	.327	61.2 (46.2 to 74.8)	.389
Mutation	2 (3.9)	27.9 (-)		4.3 (-)		100 (15.8 to 100.0)	
Sex							
Female	37 (66.1)	55.3 (45.7 to 64.9)	.356	8.0 (6.9 to 9.1)	.953	59.5 (42.1 to 75.3)	.511
Male	19 (33.9)	44.8 (31.7 to 57.9)		7.3 (1.6 to 13.0)		63.2 (38.4 to 83.7)	
Stage at diagnosis							
I	1 (1.8)	54.7 (-)	.650	6.8 (-)	.807	100 (2.5 to 100.0)	.226
II	2 (3.6)	49.3 (-)		9.0 (-)		100 (15.8 to 100.0)	
III	3 (5.4)	72.7 (-)		8.2 (8.0 to 8.4)		100 (29.2 to 100.0)	
IV	50 (89.3)	51.0 (35.4 to 66.6)		7.3 (6.8 to 9.1)		56.0 (41.3 to 70.0)	
Smoking status							
Never	43 (78.2)	54.0 (46.3 to 61.7)	.650	8.2 (6.3 to 10.1)	.126	67.4 (51.5 to 80.9)	.100
Ever	12 (21.8)	104.0 (0.0 to 212.9)		4.8 (3.7 to 8.0)		41.7 (15.2 to 72.3)	
EGFR TKI							
Osimertinib (1)	37 (66.1)	54.0 (38.1 to 69.9)	All: .247	8.1 (6.2 to 14.7)	All: .04	73.0 (55.9 to 86.2)	All: .006
Rociletinib (2)	8 (14.3)	30.4 (-)		3.7 (0 to 7.9)	1 vs 3: .669	12.5 (0.3 to 52.7)	1 *v* 3: .283
Nazartinib (3)	11 (19.6)	62.3 (43.0 to 81.6)		9.2 (7.0 to 11.4)		54.5 (23.4 to 83.3)	
No. of prior EGFR TKIs							
1	40 (71.4)	49.3 (40.7 to 57.9)	.049	8.0 (6.9 to 9.1)	.959	57.5 (40.9 to 73.0)	.55
≥ 2	16 (28.6)	76.4 (23.2 to 129.6)		4.8 (0.0 to 10.1)		68.8 (41.3 to 89.0)	

Abbreviations: *EGFR*, epidermal growth factor receptor; *ERBB2*, human epidermal growth factor receptor 2 gene; *MET*, mesenchymal-epithelial transition factor; OS, overall survival; PFS, progression-free survival; RR, response rate; TKI, tyrosine kinase inhibitor; WT, wild type.

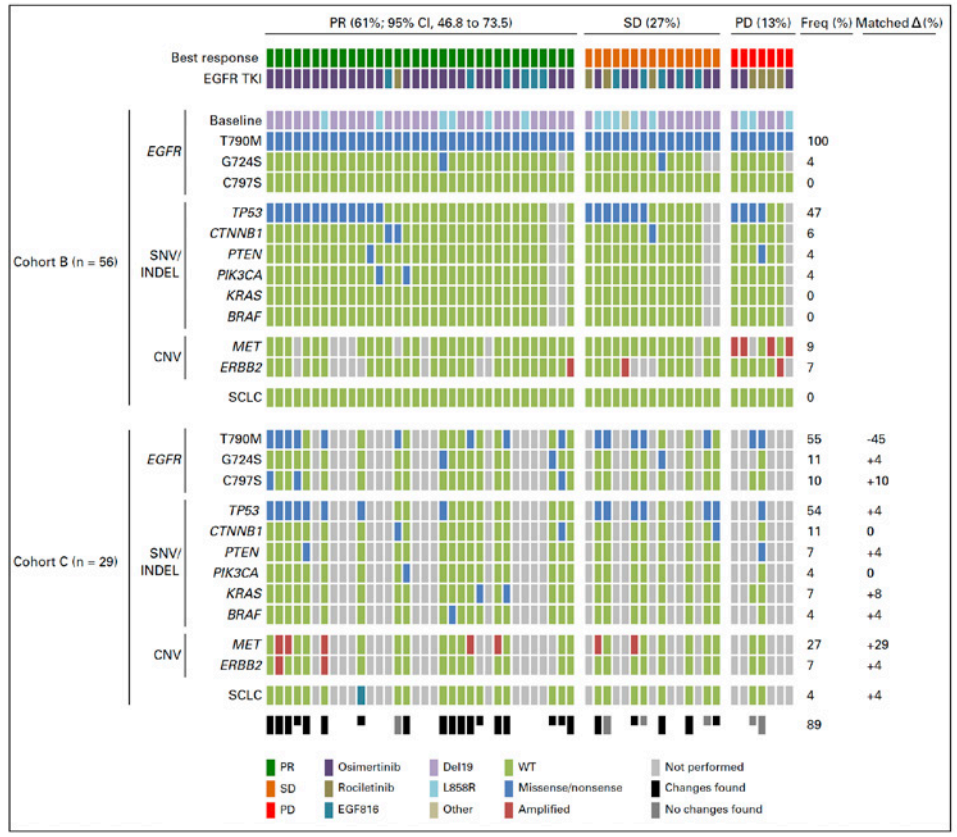


Fig 2. Map of genetic aberrations detected by sequencing (single nucleotide variant [SNV] and insertion/deletion [INDEL]) and copy number variation (CNV) analyses in biopsy specimens of epidermal growth factor receptor (EGFR) p.T790M-positive patients before treatment with a third-generation *EGFR* tyrosine kinase inhibitor (TKI; ie, osimertinib, rociletinib, nazartinib; upper block; cohort B; n = 56) and at progression to the specific treatment (lower block; cohort C; n = 29). The change in the frequency of specific aberrations during the course of treatment in matched samples is indicated in the lower block on the far right (Matched Δ). Half boxes indicate incomplete molecular work-up. Freq, frequencies; PD, progressive disease; PR, partial response; SCLC, small-cell lung cancer; SD, stable disease; WT, wild type.

Results

Clinical and Molecular Characteristics of Patients With p.T790M-Positive AR to Early-Generation EGFR TKI Therapy (cohort A) and Impact on Outcome of Third-Generation EGFR TKI Treatment (cohort B)

The molecular characteristics of cohort A (n = 124) and the impact on OS are illustrated in the Data Supplement. A total of 56 patients (45%) from cohort A fulfilled the selection criteria for cohort B and showed the clinical characteristics outlined in the Data Supplement. Patients received third-generation EGFR TKI treatment with osimertinib (n = 37; 66.1%), nazartinib (n = 11; 19.6%), and rociletinib (n = 8; 14.3%).

The RR in the overall population was 61% (95% CI, 46.8% to 73.5%), and median PFS was 8.0 months (95% CI, 6.9 to 9.1 months; Table 1). Efficacy of osimertinib and nazartinib treatment was not significantly different. One PR was confirmed while the patient was taking rociletinib, and RR was 12.5% (95% CI, 0.3% to 52.7%). Median PFS with rociletinib was 3.7 months (95% CI, 0.0 to 7.9 months).

Initial tumor stage, gender, smoking status, and the number of prior EGFR TKIs had no significant impact on treatment outcomes (Table 1). A map of molecular aberrations found in patients from cohort B is displayed in Figure 2 (Data Supplement). OS (47.0 months; 95% CI, 27.2 to 66.8 v 55.3 months; 95% CI, 48.9 to 61.7 months; P =.307), PFS (7.3 months; 95% CI, 1.3 to 13.3 v 8.1 months; 95% CI, 6.5 to 9.7 months; P = .354), and RR (54.2%; 95% CI, 32.8% to 74.5% v. 70.4%; 95% CI, 49.8% to 86.3%; P = .261) were not significantly different in patients with TP53 mutations compared with patients with TP53 WT (Table 1). Only one of three (33.3%) *ERBB2*-amplified patients responded to treatment (P = .552). PFS and OS were 4.2 months (95% CI, 0.4 to 8.0 months) and 26.6 months (95% CI, 9.6 to 43.6 months) for *ERBB2*-amplified patients compared with 8.0 months (95% CI, 6.7 to 9.3 months; P = .933) and 56.6 months (95% CI, 41.9 to 71.2 months; P = .825) in patients with ERBB2 WT (Table 1). Similarly, in patients with mutations in *PTEN* and *PIK3CA*, OS, PFS, and RR were nonsignificantly reduced (Table 1).

The RR in patients with *MET* amplifications (n = 4; 9%) was 0% (PD rate, 100%) compared with 62.8% in patients with no *MET* amplification (P = .027; Table 1; Fig 3; Data Supplement). Similarly, PFS (1.0 month; 95% CI, 0.3 to 1.7 v 8.0 months; 95% CI, 6.9 to 9.1 months; P < .001) and OS (16.0 months; 95% CI, 8.8 to 23.5 v 55.3 months; 95% CI, 43.1 to 67.5 months; P < .001) were significantly shorter in *MET*-amplified patients (Table 1; Fig 3).

Mechanisms of AR to Third-Generation EGFR TKI Therapy (cohort C)

In total, 44 patients (79%) in cohort B had disease progression, and tumor samples were available from 29 patients (52%; cohort C; Figs 1 and 2). The results of the molecular analyses were matched with pretreatment samples and one earlier sample, if possible, to distinguish between passenger and acquired aberrations. The calculation of the frequency of changes in a gene compared with the pretreatment sample was performed in matched samples only (Fig 2; Data Supplement). The overall percentage of samples in which we detected acquired changes in the molecular pattern was 89% (n = 23). Loss of *EGFR* p.T790M was by far the most common molecular change (n = 13 of 29; 45%). Isolated loss of p.T790M without any other genetic change was detected in four samples (n = 4 of 26; 15%). However, we found small-cell lung cancer transformation in one sample (4%), which showed loss of p.T790M. Acquisition of high-level *MET* amplification was detected in seven samples (n = 7 of 25; 29%), and the mean *MET* copy number increased significantly between pretreatment and post-treatment biopsies (GCN mean, 2.8 v 6.3; two-tailed, pairwise *t* test P =.02; Data Supplement). The third most common genetic changes in cohort C were acquisition of *EGFR* p.C797S (n=3 of 29; 10%), of which two were in *cis* and one in *trans* position, and loss of p.T790M with acquisition of p.G724S (n = 3 of 28; 11%). Amplification of *ERBB2* was observed in two samples (7%) and occurred together with *MET* amplification. Both patients were *MET* and *ERBB2* WT in pretreatment samples and had a long PFS of 15.1 and 19.7 months, respectively. Common *KRAS* mutations were detected in two samples (7%)—*KRAS* p.G12S and p.G12C. The *KRAS* p.G12C mutation involved the change of two consecutive nucleotides c.33_34delinsCT on the same allele, with an allelic fraction of 2.7%. Both patients are illustrated in Figure 4. Acquired mutations in *BRAF* (p.V600E), *TP53* (p.E180*), and *PTEN* (p.S229*) were detected in one sample each (4%). Mutations in *PIK3CA* and changes in *EGFR* and outside of *EGFR*, (III) changes in EGFR only, and (IV) no changes found. The change in the frequency of specific aberrations during the course of treatment in matched samples is indicated in the lower block on the far right (Matched Δ). Half boxes indicate incomplete molecular work-up. (B) Progression-free survival of patients by cluster. Median progression-free survival (95% CI): I, 9.6 months (6.7 to 12.6 months); II, 7.3 (3.7 to 11.0 months); III, 8.2 (6.5 to 9.9 months); and IV, 4.8 (0.0 to 9.6 months). Levels of *CTNNB1* were already present in pretreatment samples in patients where matched samples were available and were considered as passenger mutations.

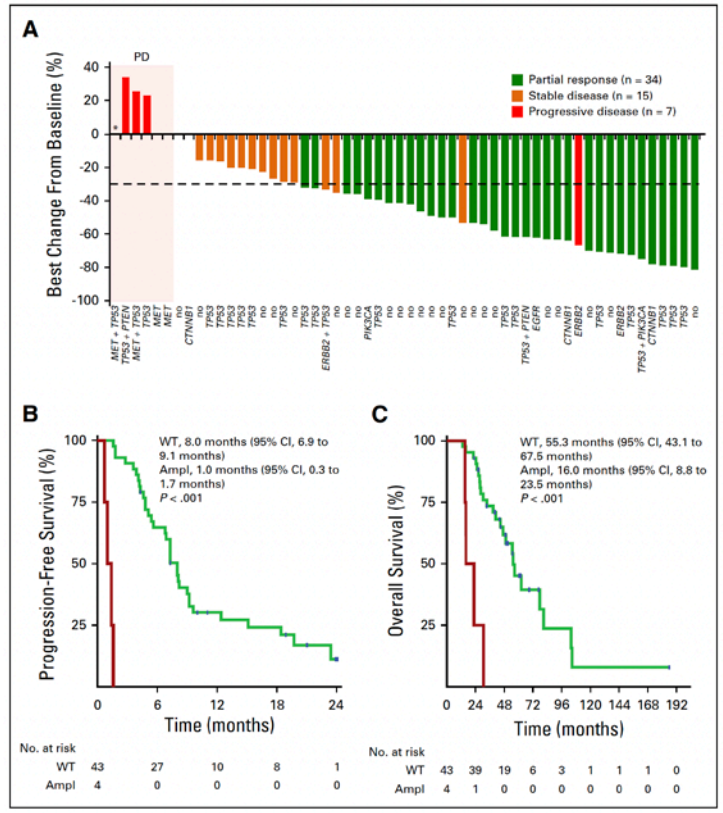


Fig 3. (A) Waterfall plot showing the best change in percent of the target lesions according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 per patient during treatment with a third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI; n = 56; cohort B). (*) Patient with progressive disease (PD) as best response but no target lesion measurement possible. Kaplan-Meier graphs displaying (B) progression free survival and (C) overall survival for patients with EGFR p.T790M-positive non–small-cell lung cancer (NSCLC) with and without mesenchymalepithelial transition factor (MET) amplification (ampl), who received treatment with third-generation EGFR TKIs. Both median overall survival and progression-free survival are dramatically reduced in the presence of MET amplifications. ERBB2, human epidermal growth factor receptor 2 gene; WT, wild type.

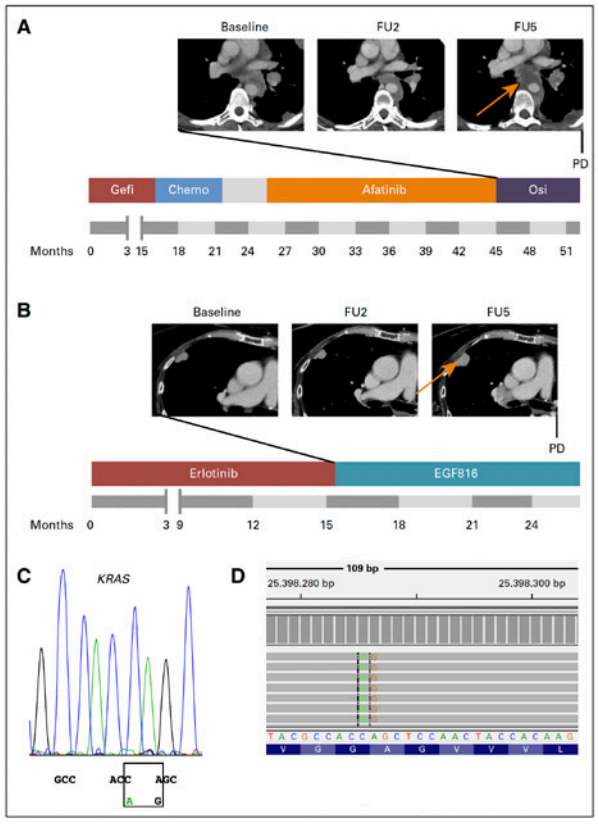


Fig 4. (A) Timeline showing the course of treatment of a female patient diagnosed with stage IV at 51 years of age. After treatment with gefitinib (gefi), platinum-doublet chemotherapy (chemo), and afatinib, the patient received osimertinib (osi; progression-free survival, 7.3 months). A progressive paraesophageal lesion was biopsied and revealed a KRAS p.G12S mutation and loss of p.T790M. The patient received local radiotherapy and died approximately 1.5 months later. (B) Timeline showing the course of treatment of a 76-year-old female patient initially diagnosed at stage II. Treatment with erlotinib was initiated once an epidermal growth factor receptor (EGFR) del19 was detected at recurrence of the dis-ease. At progression, a p.T790M mutation was detected, and treatment with nazartinib was started, resulting in a good partial response. At progression, another biopsy at the spot indicated by the yellow arrow was collected, revealing a KRAS p.G12C mutation. (C) Analysis of the KRAS p.G12C mutation by Sanger sequencing. Electropherogram of the reverse sequencing reaction showing the nucleotide change c.33_34delinsCT.(D) Detection of the KRAS p.G12C mutation by massively parallel sequencing. The nucleotide change c.33_34delinsCT is visualized by the integrative genomics viewer. FU, follow-up; PD, progressive disease.

Genetic Clustering of AR Mechanisms to Third-Generation EGFR TKIs and Impact on Third-Generation EGFR TKI Efficacy (cohort C)

Occurrence of multiple mechanisms of AR followed a distinctive pattern (Fig 5A). Changes in *EGFR*, such as loss of p.T790M and acquisition of p.C797S, were mutually exclusive. Except for one patient, CNV in *MET* and/or *ERBB2* did not occur together with p.C797S or loss of p.T790M. In the samples with new *BRAF* and *TP53* mutations, as well as in one of the patients with *KRAS*-mutant disease, p.T790M was lost. *ERBB2* amplifications were all found in samples that also harbored amplifications of *MET*.

We therefore clustered the patients in four groups: (I) changes outside of *EGFR* only, (II) changes in *EGFR* and outside of *EGFR*, (III) changes in EGFR only, and (IV) no changes found (Fig 5A). Seven patients (24%) belonged to cluster I, and 11 belonged to cluster III (38%). Five patients (17%) had changes in and off the target at the same time (cluster II). No changes were found in six patients (21%; cluster IV). In patients treated with osimertinib, a larger fraction belonged to cluster III than cluster I or II (n = 10; 47. 6% for III v n = 5; 23.8% for I and II). In patients treated with rociletinib, this trend was inversed (changes in *EGFR*,n= 0; 0% v no changes found, n = 4; 100%). Of the four patients treated with nazartinib, two (50%) displayed changes outside of *EGFR*. In one patient (25%), changes in *EGFR* were found. No changes were found in another patient (25%). The statistical significance for a

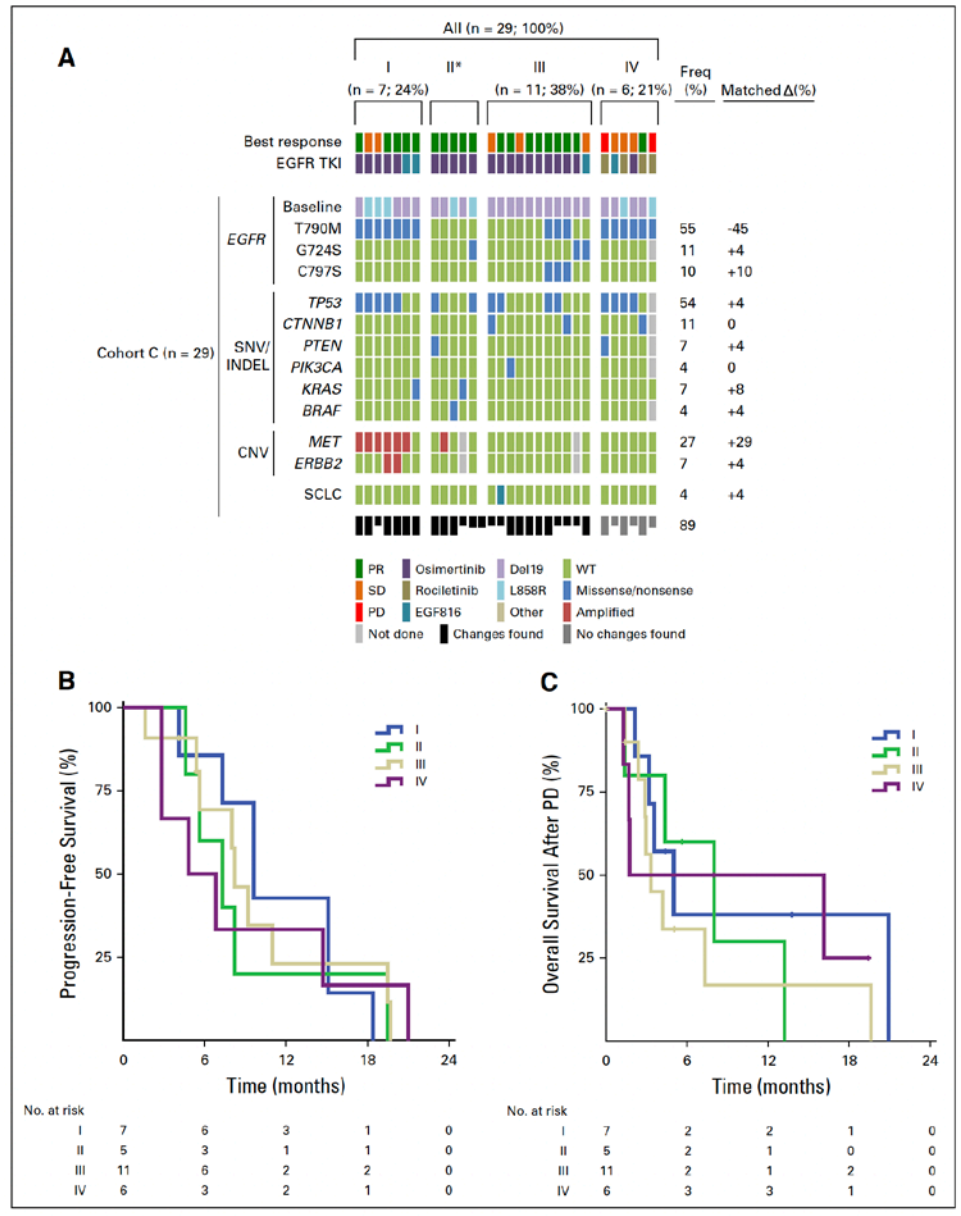


Fig 5. (A) Map of genetic aberrations detected by sequencing (single nucleotide variant [SNV] and insertion/deletions [INDELs]) and copy number variation (CNV) analyses in biopsy specimens collected after treatment with a third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI; cohort C; n = 29). Patients were clustered in four groups: (I) changes outside of EGFR only, (II) changes in EGFR and outside of EGFR, (III) changes in EGFR only, and (IV) no changes found. The change in the frequency of specific aberrations during the course of treatment in matched samples is indicated in the lower block on the far right (Matched Δ). Half boxes indicate incomplete molecular work-up. (B) Progression-free survival of patients by cluster. Median progression-free survival (95% CI): I, 9.6 months (6.7 to 12.6 months); II, 7.3 (3.7 to 11.0 months); III, 8.2 (6.5 to 9.9 months); and IV, 4.8 (0.0 to 9.6 months). Levels of statistical significance for comparison of clusters were P < .001 (C) Overall survival by cluster from progressive disease (PD) on third-generation EGFR inhibitor treatment until death. Median overall survival (95% CI): I, 5 months (2.1 to 8.0 months); II, 8 (2.3 to 13.7 months); III, 3.3 (2.3 to 4.4 months); and IV, 1.7 (0.0 to 15.6 months). Levels of statistical significance for comparison of clusters were P < .001 ERBB2, human epidermal growth factor receptor 2 gene; freq, frequencies; MET, mesenchymal-epithelial transition factor; PR, partial response; SCLC, small-cell lung cancer; SD, stable disease; WT, wild type. (*) n = 5; 17%.

cross table stratified by cluster and type of EGFR TKI was P = .002 (Fisher's exact test). Differences in PFS by cluster were not statis- tically significant (Fig 5B). Similarly, OS after PD was also not significantly different between the clusters (Fig 5C). Overall response rate (ORR) was 71.4% (n = 5) for patients in cluster I, 100% (n = 5) in cluster II, 72.2% (n = 8) in cluster III, and 16.7% (n = 1) in cluster IV (Fisher's exact test for comparison of all clusters, P = .022).

Nine patients (31%) received a treatment trying to match the targets identified in the molecular analysis. Median duration of treatment was 1.8 months (95% CI, 0.3 to 3.3 months) for targeted approaches versus 2.6 months (95% CI, 0.0 to 5.2 months) for chemotherapy (n = 4; P = .891; Data Supplement).

Discussion

Tumor heterogeneity turns out to be one of the key mechanisms underlying resistance to EGFR-targeted therapies.[17-19,21-28] In this study, we analyzed pretreatment and post-treatment biopsy samples and clinical features of patients with NSCLC treated with third-generation EGFR inhibitors to assess determinants of IR and AR.

Our first analysis revealed a high genomic heterogeneity in patients with p.T790M-positive resistance to early- generation EGFR inhibitors. Some of these aberrations, for example, amplifications of *MET*, are known to cause AR to any EGFR TKI.[17-19] The role of others, such as *TP53*, *PTEN*, *PIK3CA*, and *CTNNB1*, however, is still not well characterized.

We therefore sought to determine the effect of these aberrations on third-generation EGFR TKI treatment outcomes. Overall efficacy and OS were similar in patients treated with osimertinib and nazartinib and in concordance with the data reported so far. However, patients treated with rociletinib had a worse outcome than reported previously, which may be caused by the low patient number. Several groups have reported on an association of *TP53* mutations and shorter OS in patients with *EGFR*-mutant NSCLC. However, most of these reports were not statistically significant, and similarly, OS, RR, and PFS were only numerically reduced in patients with *TP53* mutations in our study.[32-38] Patient numbers with aberrations in *PTEN, PIK3CA*, and *ERBB2* were low, and the differences in treatment efficacy were not statistically significant. However, preclinical models and reports on small patient series suggest a negative impact of these aberrations on EGFR TKI therapy. [7,17,19,39,40] In contrast, survival and treatment efficacy were dramatically impaired in patients with *MET*-amplified tumors, putting *MET* in the front line of potential mechanisms of IR.

To define mechanisms of AR to third-generation EGFR TKIs, we analyzed post-treatment biopsies of 29 patients (cohort C) and found that loss of p.T790M was by far the most frequent genetic change. However, only a small fraction of patients had an isolated loss of p.T790M. It is likely that other genetic changes that we did not detect with our analysis may contribute to AR in these patients with a loss of p.T790M and no other genetic change. [23] The acquisition of p.C797S was detected in three patients, and several studies have confirmed the resistance-mediating effect of this substitution to osimertinib treatment. [23,41] In addition, we found the secondary *EGFR* mutation p.G724S in three samples. In contrast to p.C797S, p.G724S was also in part detected in the samples collected at progression to early-generation EGFR TKIs.[29,42] However, after failure of third-generation EGFR TKI treatment, p.G724S was always co-occurring with loss of p.T790M, suggesting the treatment-induced selection of this mutation. Acquisition of *MET* amplification was the second most frequent event associated with AR to third-generation EGFR inhibition, and similar frequencies have been described in the literature.[19,23] The high prevalence of *MET* amplification in IR and AR points out the crucial role of *MET* in EGFR inhibitor resistance. Interestingly, amplifications of *MET* and *ERBB2* occurred together in two patients. It is unclear whether this reflects the existence of two independent tumor clones or whether both aberrations are acquired in the same clone and how they influence therapy outcome. We also found acquired mutations in *KRAS* in two patients and a *BRAF* p.V600E mutation in one patient. Activation of the MEK/ extracellular regulated kinase pathway through *KRAS* mutations as an escape mechanism and efficacy of the combined EGFR and MEK inhibition was reported previously.[17,26,27] Thus, taken together, treatment of *EGFR*- mutant NSCLC with TKIs targeting EGFR as well as MET and MEK may delay the development of AR and prevent IR in selected patients.

By clustering the genetic findings at AR into four groups— mechanism of resistance off target (I), on target (III), or in both (II), and no changes detected (IV)—we found a distinct molecular pattern depending on the EGFR TKI applied. Changes in EGFR were almost exclusively found in patients treated with osimertinib. In contrast, no patient treated with rociletinib displayed changes in EGFR, and other studies have confirmed the absence of secondary *EGFR* mutations in patients with progression while taking rociletinib.[19,43] It is conceivable that this effect may be caused by a lower selection pressure of rociletinib on cells with on-target aberrations. We also found a statistically significant association between cluster and ORR, because patients in cluster IV had a markedly reduced ORR to third- generation EGFR treatment. However, differences in PFS or OS after PD were not significant.

In summary, our study first shows that molecular heterogeneity of p.T790M-mutant lung cancer with AR to early- generation EGFR TKIs influences efficacy of third generation inhibitors. Our observations also show the need to integrate information on co-occurring alterations in the design of clinical trials, aiming at a more precise identification of patients who benefit from combined tar- geted treatment. Because osimertinib has been approved for first-line treatment of *EGFR*-mutant NSCLC in many countries, our analysis may be of relevance to a decreasing subgroup. But mechanisms of resistance to first-line osimertinib have not been well characterized, and it is conceivable that recurrent mechanisms of resistance to EGFR inhibition such as *MET* amplification, *MET* activation, and *EGFR* p.C797S may also play a major role in this setting.

References

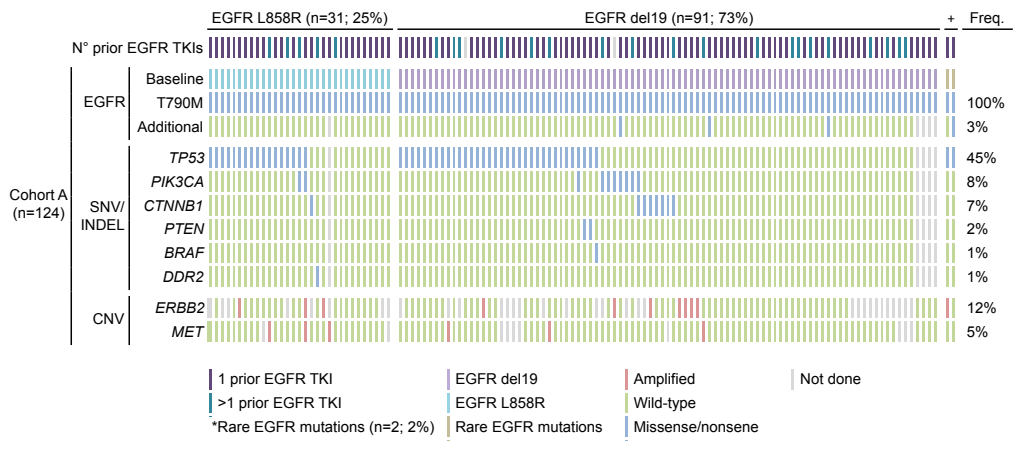
1. Mok TS, Wu YL, Thongprasert S, et al: Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 361:947-957, 2009
2. Maemondo M, Inoue A, Kobayashi K, et al: Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 362:2380-2388, 2010
3. Han JY, Park K, Kim SW, et al: First-SIGNAL: First-line single-agent Iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. J Clin Oncol 30:1122-1128, 2012
4. Rosell R, Carcereny E, Gervais R, et al: Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation- positive non-small-cell lung cancer (EURTAC): A multicentre, open-label, randomised phase 3 trial. Lancet Oncol 13:239-246, 2012
5. Zhou C, Wu YL, Chen G, et al: Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): A multicentre, open-label, randomised, phase 3 study. Lancet Oncol 12:735-742, 2011
6. Sequist LV, Yang JCH, Yamamoto N, et al: Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol 31:3327-3334, 2013
7. Sequist LV, Waltman BA, Dias-Santagata D, et al: Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 3:75ra26, 2011
8. Yu HA, Arcila ME, Rekhtman N, et al: Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. Clin Cancer Res 19:2240-2247, 2013
9. Cross DAE, Ashton SE, Ghiorghiu S, et al: AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. Cancer Discov 4:1046-1061, 2014
10. Jia Y, Juarez J, Li J, et al: EGF816 exerts anticancer effects in non-small cell lung cancer by irreversibly and selectively targeting primary and acquired activating mutations in the EGF receptor. Cancer Res 76:1591-1602, 2016
11. Kasibhatla S, Li J, Tompkins C, et al: EGF816, a novel covalent inhibitor of mutant-selective epidermal growth factor receptor, overcomes T790M-mediated resistance in NSCLC. Cancer Res 74, 2014 (suppl; abstr 1733)
12. Ja¨ nne PA, Yang JCH, Kim DW, et al: AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. N Engl J Med 372:1689-1699, 2015
13. Sequist LV, Soria JC, Goldman JW, et al: Rociletinib in EGFR-mutated non-small-cell lung cancer. N Engl J Med 372:1700-1709, 2015
14. Sequist LV, Soria JC, Camidge DR: Update to rociletinib data with the RECIST confirmed response rate. N Engl J Med 374:2296-2297, 2016
15. Fassunke J, Müller F, Keul M, et al: Overcoming EGFRG724S-mediated osimertinib resistance through unique binding characteristics of second-generation EGFR inhibitors. Nat Commun 9:4655, 2018
16. Tan DSW, Seto T, Leighl NB, et al: First-in-human phase I study of EGF816, a third-generation, mutant-selective EGFR tyrosine kinase inhibitor, in advanced non-small cell lung cancer (NSCLC) harboring T790M. J Clin Oncol 33, 2015 (suppl; abstr 8013)
17. Ortiz-Cuaran S, Scheffler M, Plenker D, et al: Heterogeneous mechanisms of primary and acquired resistance to third-generation EGFR inhibitors. Clin Cancer Res 22:4837-4847, 2016
18. Bean J, Brennan C, Shih JY, et al: MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci USA 104:20932-20937, 2007
19. Chabon JJ, Simmons AD, Lovejoy AF, et al: Circulating tumour DNA profiling reveals heterogeneity of EGFR inhibitor resistance mechanisms in lung cancer patients. Nat Commun 7:11815, 2016 [Erratum: Nat Commun 7:13513, 2016],
20. Schildhaus HU, Schultheis AM, Rüschoff J, et al: MET amplification status in therapy-nai¨ve adeno- and squamous cell carcinomas of the lung. Clin Cancer Res 21:907-915, 2015
21. Planchard D, Loriot Y, Andre´ F, et al: EGFR-independent mechanisms of acquired resistance to AZD9291 in EGFR T790M-positive NSCLC patients. Ann Oncol 26:2073-2078, 2015
22. Scheffler M, Merkelbach-Bruse S, Bos M, et al: Spatial tumor heterogeneity in lung cancer with acquired epidermal growth factor receptor-tyrosine kinase inhibitor resistance: Targeting high-level MET-amplification and EGFR T790M mutation occurring at different sites in the same patient. J Thorac Oncol 10:e40-e43, 2015
23. Lin CC, Shih JY, Yu CJ, et al: Outcomes in patients with non-small-cell lung cancer and acquired Thr790Met mutation treated with osimertinib: A genomic study. Lancet Respir Med 6:107-116, 2017
24. Blakely CM, WaTKIns TBK, Wu W, et al: Evolution and clinical impact of co-occurring genetic alterations in advanced-stage EGFR-mutant lung cancers. Nat Genet 49:1693-1704, 2017
25. Yang Z, Yang N, Ou Q, et al: Investigating novel resistance mechanisms to third-generation EGFR tyrosine kinase inhibitor osimertinib in non-small cell lung cancer patients. Clin Cancer Res 24:3097-3107, 2018
26. Eberlein CA, Stetson D, Markovets AA, et al: Acquired resistance to mutant-selective EGFR inhibitor AZD9291 is associated with increased dependence on RAS signaling in preclinical models. Cancer Res 75:2489-2500, 2015
27. Ercan D, Xu C, Yanagita M, et al: Reactivation of ERK signaling causes resistance to EGFR kinase inhibitors. Cancer Discov 2:934-947, 2012
28. Tricker EM, Xu C, Uddin S, et al: Combined EGFR/MEK inhibition prevents the emergence of resistance in EGFR-mutant lung cancer. Cancer Discov 5:960-971, 2015
29. Reference deleted.
30. Eisenhauer EA, Therasse P, Bogaerts J, et al: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45:228-247, 2009
31. Ko¨ nig K, Peifer M, Fassunke J, et al: Implementation of amplicon parallel sequencing leads to improvement of diagnosis and therapy of lung cancer patients. J Thorac Oncol 10:1049-1057, 2015
32. Clinical Lung Cancer Genome Project (CLCGP), et al: A genomics-based classification of human lung tumors. Sci Transl Med 5:209ra153, 2013
33. Molina-Vila MA, Bertran-Alamillo J, Gasco´ A, et al: Nondisruptive p53 mutations are associated with shorter survival in patients with advanced non-small cell lung cancer. Clin Cancer Res 20:4647-4659, 2014
34. Aisner DL, Sholl LM, Berry L, et al: The impact of smoking and TP53 mutations in lung adenocarcinoma patients with targetable mutations-the Lung Cancer Mutation Consortium (LCMC2). Clin Cancer Res 24:1038-1047, 2018
35. Labbe´ C, Cabanero M, Korpanty GJ, et al: Prognostic and predictive effects of TP53 co-mutation in patients with EGFR-mutated non-small cell lung cancer (NSCLC). Lung Cancer 111:23-29, 2017
36. VanderLaan PA, Rangachari D, Mockus SM, et al: Mutations in TP53, PIK3CA, PTEN and other genes in EGFR mutated lung cancers: Correlation with clinical outcomes. Lung Cancer 106:17-21, 2017
37. Shepherd FA, Lacas B, Le Teuff G, et al: Pooled analysis of the prognostic and predictive effects of TP53 comutation status combined with KRAS or EGFR mutation in early-stage resected non-small-cell lung cancer in four trials of adjuvant chemotherapy. J Clin Oncol 35:2018-2027, 2017
38. Canale M, Petracci E, Delmonte A, et al: Impact of TP53 mutations on outcome in EGFR-mutated patients treated with first-line tyrosine kinase inhibitors. Clin Cancer Res 23:2195-2202, 2017
39. Kinross KM, Montgomery KG, Kleinschmidt M, et al: An activating Pik3ca mutation coupled with Pten loss is sufficient to initiate ovarian tumorigenesis in mice. J Clin Invest 122:553-557, 2012
40. Wu X, Renuse S, Sahasrabuddhe NA, et al: Activation of diverse signalling pathways by oncogenic PIK3CA mutations. Nat Commun 5:4961, 2014
41. Oztan A, Fischer S, Schrock AB, et al: Emergence of EGFR G724S mutation in EGFR-mutant lung adenocarcinoma post progression on osimertinib. Lung Cancer 111:84-87, 2017
42. Thress KS, Paweletz CP, Felip E, et al: Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. Nat Med 21:560-562, 2015
43. Piotrowska Z, Niederst MJ, Karlovich CA, et al: Heterogeneity underlies the emergence of EGFRT790 wild-type clones following treatment of T790M-positive cancers with a third-generation EGFR inhibitor. Cancer Discov 5:713-722, 2015

Supplementary information

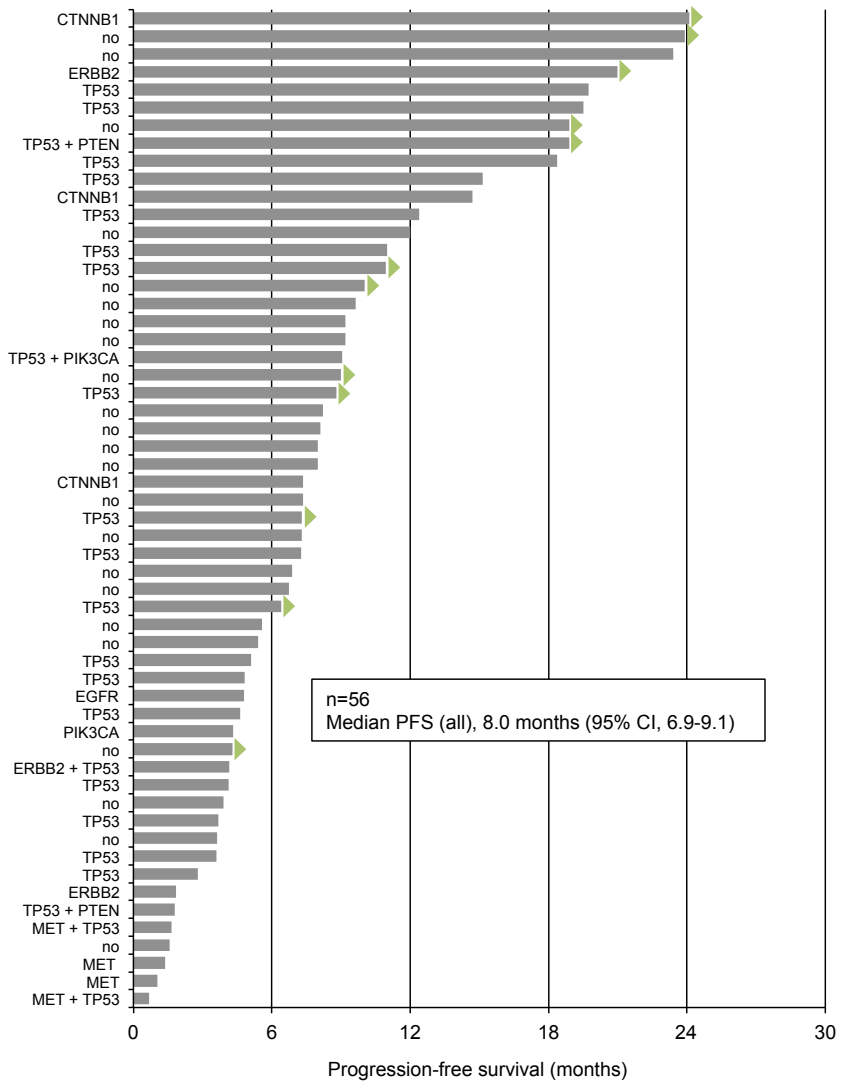
Genomic profiling identifies outcome-relevant mechanisms of innate and acquired resistance to third-generation EGFR TKI therapy in lung cancer

Sebastian Michels, Carina Heydt, Bianca van Veggel, et al.

Suppl. figure 1: Map of genetic aberrations detected by sequencing (SNV and INDEL) and CNV analyses in biopsy specimens of *EGFR* p.T790M-positive patients with resistance to early-generation EGFR TKI treatment (Cohort A; n=124). Every column represents a single patient. Columns were clustered according to the underlying baseline *EGFR* mutation (i.e. *EGFR* del19, *EGFR* p.L858R, rare *EGFR* mutations) and the co-occurring SNV. Frequencies of the specific aberration are indicated on the right. Freq., frequencies; SNV, single nucleotide variant; INDEL, insertion/deletion; CNV, copy number variant



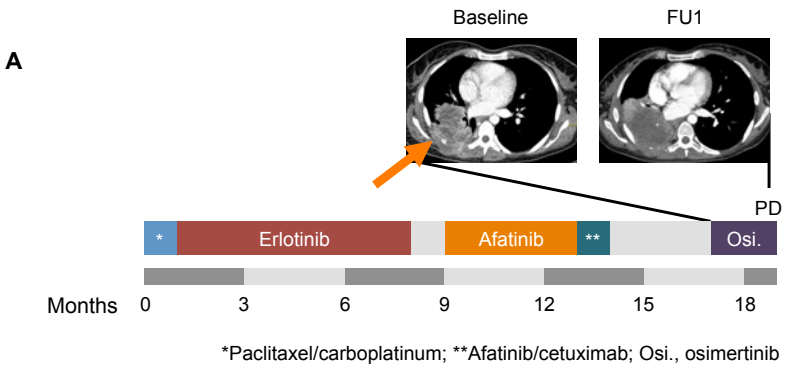
Suppl. figure 2: Swimmer plot of progression-free survival by co-occurring genetic aberrations.



Suppl. figure 3: Map of genetic aberrations detected by sequencing (SNV and INDEL) and CNV analyses in matched biopsy specimens of *EGFR* p.T790M-positive patients with resistance to early-generation EGFR TKI treatment collected at baseline (1) and prior to third-generation EGFR TKI treatment (2). Every row represents a single patient. Unmatched freq., unmatched frequencies at the specific time-point; Matched Δ, differences in frequencies in matched samples; SNV, single nucleotide variant; INDEL, insertion/deletion; CNV, copy number variant

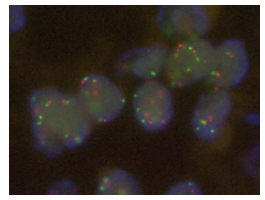
Suppl. figure 4: (A) Panel showing the course of treatment (timeline) of a 36-year old female patient who was diagnosed with an advanced *EGFR* del19-positive NSCLC. On the left CT scan (baseline), the yellow arrow indicates the spot of biopsy. The right CT is representative for the systemic progression of the disease while on treatment with osimertinib for 1.6 months only. The patients died rapidly thereafter (OS, 22.8 months). (B) Magnification of dual-color break-apart FISH (ZytoLight SPEC *MET/CEN7* Dual Color Probe, ZytoVision) of the biopsy showing *MET* amplification (GCN, 7.33; *MET/CEP7* ratio, 1.1). (C and D) Changes of *MET* GCN in tumor samples at baseline (prior to treatment with a third-generation EGFR TKI) compared to post-treatment samples.

A



*Paclitaxel/carboplatinum; **Afatinib/cetuximab; Osi., osimertinib

B



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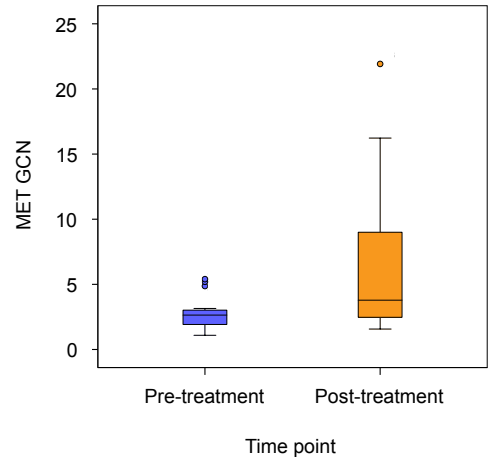
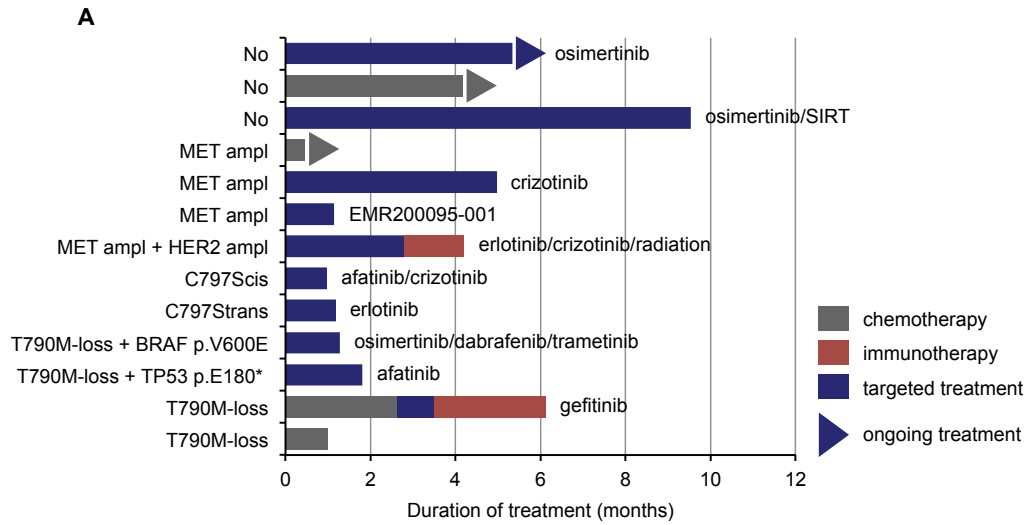


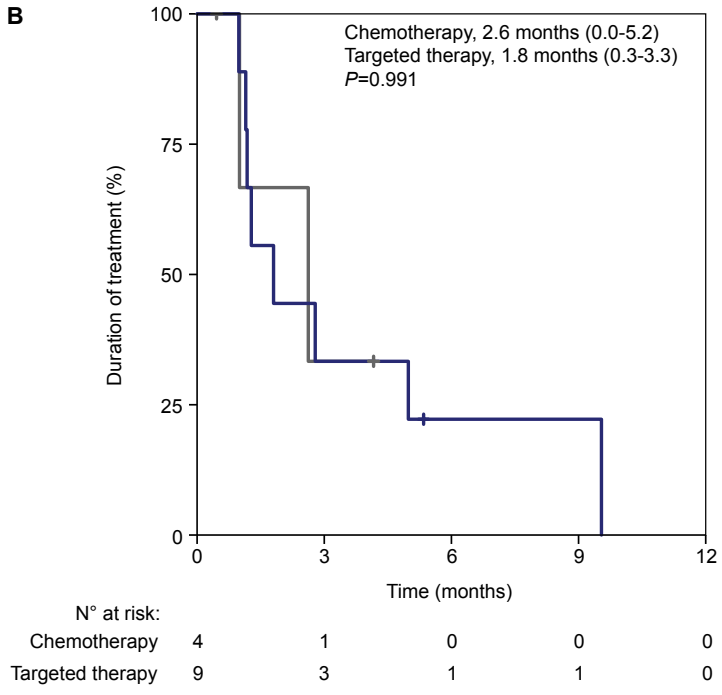
D

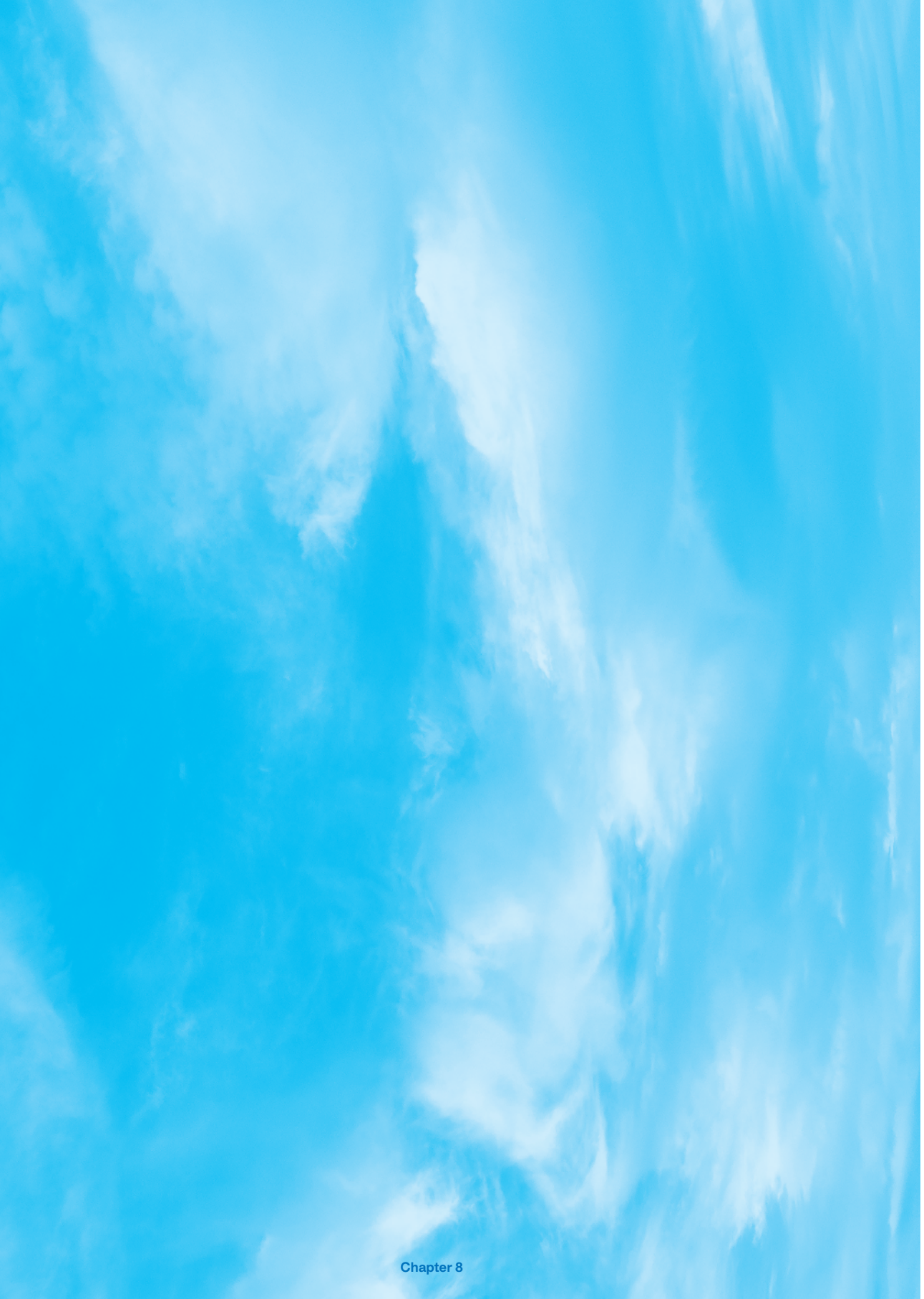
	Time point	n	Mean	Std. deviation	p (2-tailed)
MET GCN	Baseline	18	2.8367	1.20610	0.02
	Post-treatment	18	6.3683	5.64034	

Suppl. figure 5: Map of genetic aberrations detected by sequencing (SNV and INDEL) and CNV analyses in matched biopsy specimens of *EGFR* p.T790M-positive patients with resistance to early-generation EGFR TKI treatment collected at baseline (1), prior to third-generation EGFR TKI treatment (2) and at progression (3). Every row represents a single patient. Unmatched freq., unmatched frequencies at the specific time-point; Matched Δ, differences in frequencies from matched samples; SNV, single nucleotide variant; INDEL, insertion/deletion; CNV, copy number variant

Suppl. figure 6: (A) Swimmer plot of duration of treatment of patients who received a treatment after progression on third-generation EGFR inhibitor therapy. Patients are grouped by the underlying genetic aberration. The targeted treatment applied is indicated at the end of the row. (B) Kaplan-Meier chart of the duration of treatment. Grey line, chemotherapy; blue line, targeted treatment.







Chapter 8

Crizotinib treatment for patients with *EGFR* mutation positive NSCLC that acquire *cMET* amplification after EGFR TKI therapy results in short-lived and heterogeneous responses

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Lung Cancer. 2018 Oct; 124:130-134

Abstract

Purpose: **Next to secondary epidermal growth factor receptor (EGFR) mutations, cMET amplification plays an important role in mediating acquired resistance to EGFR tyrosine kinase inhibitors (TKI) treatment. Crizotinib, a dual ALK and cMET inhibitor, can induce responses in patients with EGFR mutation positive non-small cell lung cancer (NSCLC) that acquire cMET amplification after EGFR TKI treatment. However, little is known about the duration of response and post-progression resistance mechanisms. Here, we report on the clinical outcome of a series of patients with cMET-driven resistance to EGFR TKIs, treated with crizotinib.**

Materials and methods: **Eight patients with EGFR mutation positive NSCLC that acquired cMET amplification after EGFR TKI treatment were treated with crizotinib 250 mg twice daily, as monotherapy (n=2) or in combination with an EGFR TKI (n=6).**

Results: **Four out of eight patients (50%) showed a partial response (PR) according to RECIST 1.1. Median progression-free survival (PFS) was 1.4 (95% CI 1.2 – 5.0) months. Responses were short-lasting with a median PFS of 3.5 (95% CI 1.4 – 5.2) months in patients with a PR. Median overall survival was 5.9 (95% CI 1.3 – 6.0) months and not statistically different between responders and non-responders (p=0.37). All but one patient tolerated crizotinib treatment well. Heterogeneous responses were seen in patients with progressive disease as best response with a marked size decrease of the biopsied (cMET amplification positive) lesion and progression of other lesions. cMET amplification was not always mutually exclusive with other EGFR TKI resistance mechanisms. Post-progression biopsies were negative for cMET amplification.**

Conclusion: **Crizotinib treatment for patients with EGFR mutation positive NSCLC that acquire cMET amplification after EGFR TKI treatment results in short-lived and often heterogeneous responses, possibly due to subclonality of cMET-driven resistance and co-occurrence of other EGFR TKI resistance mechanisms.**

1. Introduction

Patients with epidermal growth factor receptor (EGFR) mutation positive non-small-cell lung cancer (NSCLC) are highly sensitive to treatment with EGFR tyrosine kinase inhibitors (TKIs) (1). However, despite this initial response, resistance eventually occurs in all patients. Different molecular events underlie the acquired resistance (2, 3). The most well described mechanism of resistance is the occurrence of secondary EGFR mutations, such as T790M after treatment with first and second generation EGFR TKIs and C797S after treatment with third generation EGFR TKIs. These mutations negatively alter the ratio between EGFR TKI and ATP affinity for the EGFR kinase domain. Amongst other resistance mechanisms, bypass track activation can occur. In this situation, amplification of a different transmembrane receptor, such as cMET, FGFR-1 or HER2, results in increased downstream signaling in the presence of continued EGFR suppression by the EGFR TKI (4). Amplification of the cMET gene has been observed in approximately 5-20% of patients with acquired resistance to first- and second generation EGFR TKIs (2, 3, 5) and up to 30% after progression on third generation EGFR TKIs (6, 7). Here, we report on the clinical outcome of a retrospective series of patients treated with crizotinib with or without an EGFR TKI, for EGFR mutation positive NSCLC with acquired cMET amplification after EGFR TKI treatment.

2. Material and methods

Eight patients with advanced EGFR mutation positive NSCLC that acquired cMET amplification after EGFR TKI treatment were treated with crizotinib in two institutions in The Netherlands (The Netherlands Cancer Institute and VU University Medical Center). Data were obtained retrospectively by medical record review. Mutation- and copy number variation (CNV) analyses were assessed by next-generation sequencing (NGS) and in-situ hybridization (ISH) on the post-EGFR TKI progression and pre-crizotinib treatment tumor samples and, whenever available, on the treatment-naïve tumor samples, as well as on the post-crizotinib progression tumor samples. Targeted NGS was performed using the TruSeq Amplicon – Cancer Panel (Illumina). This amplicon-based panel detects mutations in hotspot regions of 48 cancer related genes (ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, VHL), as well as amplifications of most of the reported genes, including EGFR, HER2 and cMET. NGS was used to screen for cMET amplification. In case of a positive finding, ISH was used for confirmation (8). ISH was performed for determination of cMET gene copy number (GCN) using cMET amplification MET/CEP7 Dual Color Probe (Cytocell or Ventana) according to manufacturer's recommendations. cMET amplification was defined by MET/CEP7 ratio ≥2, presence of cMET clusters or cMET GCN > 5 (9, 10).
Patients were treated with crizotinib 250 mg twice daily in combination with an EGFR TKI or as monotherapy. Response evaluation was performed every six weeks and scored according to Response Criteria In Solid Tumors (RECIST) version 1.1. Treatment was continued until disease progression according to RECIST or unacceptable toxicity. Progression-free survival (PFS) was defined as the interval between crizotinib initiation and the date of radiological progression or death, whichever came first.

Patient	1	2	3	4	5	6	7	8
Baseline characteristics								
Gender	Female	female	female	male	female	female	female	female
Age (years)	67	60	62	70	79	37	66	72
Initial EGFR mutation	exon 19 deletion	exon 19 deletion	exon 19 deletion	exon 21 L858R	exon 19 deletion	exon 19 deletion	exon 19 deletion	exon 21 L858R
Date first diagnosis stage IV	05-2016	07-2014	11-2014	08-2014	10-2015	01-2016	10-2012	01-2016
Prior treatments	Gefitinib; cisplatin-pemetrexed	Gefitinib; carboplatin-pemetrexed	Erlotinib; cisplatin-pemetrexed; rociletinib; osimertinib	Cisplatin-pemetrexed, erlotinib; osimertinib	Erlotinib; osimertinib; osimertinib + dabrafenib/trametinib	Afatinib; erlotinib; carboplatin-pemetrexed	Erlotinib; cisplatin-pemetrexed; osimertinib	Erlotinib; osimertinib
Baseline biopsy								
cMET Z-score(NGS)	0	0	NA	NA	0.1	-0.2	-1.0	-0.1
cMET/CEP (CISH)	NA	NA	3/3 (1)	NA	2.5/2.8 (0.9)	2.3/2.5 (0.9)	3/2.8 (1.1)	2/2 (1)
Other molecular alterations (NGS)	TP53	EGFR amplification; TP53	NA	NA	TP53	EGFR amplification; STK11; TP53	TP53	TP53
Biopsy after first generation EGFR TKI								
cMET Z-score(NGS)	0	7.3	1.7	0.5	NA	5.1	NA	-0.4
cMET/CEP (CISH)	Clusters	Clusters	4/4 (1)	NA	2.1/2.7 (0.8)	7/4.9 (1.4)	2.6/1.3 (2)	2/2 (1)
Other molecular alterations (NGS)	TP53	EGFR amplification; TP53	T790M; EGFR amplification; TP53	T790M; EGFR amplification; TP53	T790M; BRAF V600E; EGFR amplification; TP53	EGFR and cMET amplification; TP53	T790M (HRM)	T790M; TP53; HRAS; APC
Biopsy after third generation EGFR TKI								
cMET Z-score(NGS)			21.5	8.4	9.8		6.2	12.8
cMET/CEP (CISH)			12/3 (4)	Clusters	Clusters		Clusters	12/2 (6)
Other molecular alterations (NGS)			TP53	EGFR amplification; TP53	EGFR amplification; TP53		TP53	TP53
Treatment	Crizotinib + erlotinib	crizotinib	Crizotinib + osimertinib	Crizotinib + osimertinib	Crizotinib + erlotinib	Crizotinib + erlotinib	Crizotinib	Crizotinib + osimertinib
RECIST response	PR	PR	PD	PD	Unknown	PR	PD	PR
PFS (months)	5.0	5.2	1.2	1.4	1.3	1.4	1.4	2.1
Toxicity	Diarrhea grade I; Skin toxicity grade I; Hepatitis grade I	Nausea grade III; vomiting grade II	Diarrhea grade I; vomiting grade I	None	Oedema grade II	None	Nausea grade III	Nausea grade I, fatigue grade II
Post-progression biopsy								
cMET Z-score(NGS)	1.6			1.9		NA		
cMET/CEP (CISH)	7.52/6.2 (1.2)			5.08/4.16 (1.2)		2.5/1.9 (1.3)		
Other molecular alterations (NGS)	TP53			EGFR amplification; TP53; cMET (p.Y1248)		T790M (HRM)		
Post progression treatment	Osimertinib (PD); pembrolizumab (PD)	None	None	Carboplatin-pemetrexed (PD); carboplatin-placlitaxel-bevacizumab (PD); pembrolizumab (PD); osimertinib + cabozantinib (PD)	None	Osimertinib (PD); carboplatin-paclitaxel-bevacizumab (PD); nivolumab (PD)	Erlotinib (PD); nivolumab (PD); osimertinib (PD)	Pembrolizumab (PD); carboplatin-pemetrexed (ongoing treatment)

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; NGS, next generation sequencing; CISH, chromogenic in situ hybridization; NA, not applicable; RECIST: Response Evaluation Criteria in Solid Tumors; PR, partial response; PD, progressive disease; PFS, progression free survival; HRM, high resolution melting

3. Results

3.1 Patient characteristics and molecular profiling

Patient characteristics and prior treatments are listed in Table 1. Six patients had an EGFR exon 19 deletion and two patients an EGFR exon 21 L858R mutation. All patients were treated with a first or second generation EGFR TKI as initial EGFR TKI treatment and six of eight patients (75%) were treated with platinum doublet chemotherapy prior to crizotinib treatment. One patient received afatinib as first EGFR TKI, but due to development of pneumonitis the treatment was changed to erlotinib. Five patients were subsequently treated with a third generation EGFR TKI, after acquiring an EGFR T790M mutation. One of these patients was treated with rociletinib for seven months, before switching to osimertinib treatment, while the other four were treated with osimertinib directly.

Molecular analyses of the tumors at the different time points are listed in Table 1. cMET amplification was absent in the treatment-naïve tumor samples of 7/8 patients. In one patient no NGS or ISH was performed at baseline. For the five patients that acquired the T790M mutation, cMET/CEP7 ratios after progression on the first generation EGFR TKI were low in four, while it could not be calculated in one patient. Results of cMET amplification testing of the tumor samples that were obtained after progression on the last EGFR TKI can be found in Table 1.

3.2 Crizotinib treatment, adverse events and response

Patients were treated with crizotinib monotherapy (n=2) or in combination with a first (n=3) or third (n=3) generation EGFR TKI. Toxicity was manageable in most patients (Table 1). Dose modification of crizotinib was necessary for three patients of which two were treated with crizotinib monotherapy. One patient experienced grade III nausea and grade II vomiting that resolved upon crizotinib dose reduction to 200 mg twice-daily. The second patient experienced grade I vomiting and diarrhea that resolved upon crizotinib dose reduction to 200 mg twice-daily. The third patient experienced grade III nausea, that persisted upon dose reduction up to 200 mg once daily, after which treatment was discontinued. Except for grade I liver test elevations, no hepatotoxicity and pneumonitis were observed.

Among the eight patients treated with crizotinib, four (50%) experienced a partial response (PR) according to RECIST v 1.1. Median PFS in all patients was 1.4 (95% CI 1.2 – 5.0) months. For patients with a PR to crizotinib treatment this was 3.5 (95% CI 1.4 – 5.2) months. Patients on crizotinib monotherapy (n=2) did not appear to fare better or worse than patients that received crizotinib and EGFR TKI combination therapy (n=6; Table 1). Median overall survival (OS) for all patients was 5.9 (95% CI 1.3 – 6.0) months. The median OS was not statistically different between patients with and patients without a PR to crizotinib treatment (median OS 5.9 [95% CI 4.7 – 7.9] months vs 4.0 [95% CI 1.3 – 6.0] months; p=0.372). Mixed responses were observed in three of four non-responding patients. In these patients the biopsied (cMET amplification positive) lesion showed a ≥30% size decrease, together with progression of other existing lesions or the appearance of new lesions. Only the patient that did not tolerate crizotinib showed progression of all target lesions, including the biopsied one. Figure 1 shows examples of the different response patterns.

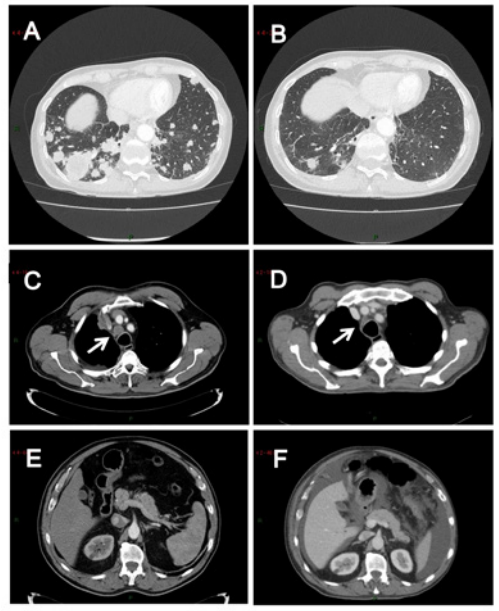


Fig 1. Different response patterns after treatment with crizotinib in patients with EGFR mutation positive non-small cell lung cancer and acquired cMET amplification after EGFR TKI treatment. **Patient 1:** Partial response to crizotinib treatment. **A:** Baseline CT scan demonstrating pulmonary metastases. **B:** Partial response on CT scan after treatment. Patient 4: Mixed response to crizotinib treatment. **C:** Baseline CT scan demonstrating the biopsied, cMET amplification positive, mediastinal lymph node metastasis. **D:** Size decrease of mediastinal and pleural metastases after treatment. **E and F:** Progression of peritoneal metastases and occurrence of a malignant peritoneal effusion.

3.3 Post-crizotinib treatment

Post-crizotinib progression biopsies were available for three patients. The activating EGFR mutation was present in all biopsies. cMET/CEP7 ratio was <2 for all patients and all showed a decrease in cMET copy number (Table 1).

In patient number 4, a cMET mutation (p.Y1248H) was found that is known to result in resistance to type I cMET inhibitors, such as crizotinib, and that might be sensitive to type II cMET inhibitors, such as cabozantinib (11). Post-crizotinib progression, this patient was treated with platinum based chemotherapy and pembrolizumab and upon radiological progression with cabozantinib. Unfortunately after 5 days of cabozantinib, there was clinical progression and treatment was discontinued. In the patient number 6, a T790M mutation was found in the post-crizotinib progression biopsy, while the pre-crizotinib tumor sample was T790M negative, as was the ctDNA using digital droplet PCR. This patient had a PR on erlotinib and crizotinib. However, one lesion did not respond and eventually progressed. Biopsy of this gluteal metastasis was T790M positive. Unfortunately, subsequent osimertinib was not effective. In patient number 1, no known EGFR TKI resistance mechanism was found.

PD-L1 status was known for five patients. Four of five patients (80%) showed ≥50% tumor cell membrane PD-L1 expression by IHC (22C3 antibody). Five of eight patients, including all patients with high PD-L1 expression, received a PD-1 inhibitor, but none of the patients responded.

4. Discussion

In this series, eight patients with acquired cMET amplification after first- or third generation EGFR TKI treatment were treated with crizotinib, a type I cMET inhibitor that preferentially binds the active conformation of cMET. Four of eight patients (50%) had a partial response. Responses were not durable with a median PFS of 3.5 (95% CI 1.4 – 5.2) months in responders and OS not being different between responders and non-responders. Except for one patient that did not tolerate crizotinib treatment, the biopsied lesion showed a size decrease of ≥30% in all patients, even in those with progressive disease as best response. cMET amplification was not always mutual exclusive with other mechanisms of EGFR TKI resistance within patients, both in, as well as over time.

In vitro experiments with tumor cell lines with a sensitizing EGFR mutation and cMET amplification showed that dual EGFR and cMET TKI treatment can result in complete tumor cell kill (12). Our results support this data as responses were observed and after crizotinib treatment cMET/CEP7 ratios were not increased anymore and cMET copy numbers decreased as compared to the pre-crizotinib tumor sample. However, our data also shows that *in vivo* cMET amplification might be a subclonal event, which explains why cMET TKI treatment results in short-lasting responses that are often heterogeneous. Subsequent therapies post-crizotinib progression were generally unsuccessful and no responses were observed with PD-1 directed immunotherapy, despite high PD-L1 levels.

In one patient a cMET mutation (p.Y1248H) was encountered that is known to cause resistance to type I cMET inhibitor treatment (11). Altogether, cMET amplification seems to be an acquired resistance mechanism with an unfavorable prognosis, irrespective of the type of treatment.
Multiple case reports have described tumor responses with crizotinib, as monotherapy or in combination with an EGFR TKI, in patients with acquired cMET amplification after EGFR TKI treatment (13-15). However, little has been reported about the duration of response, post-progression resistance mechanisms and efficacy of post-crizotinib treatment. Wang et al. (14) investigated tissue biopsies from thirteen patients with acquired resistance to osimertinib. Four patients harbored cMET amplification and they observed a median PFS of 3.5 months. Baldacci et al. (16) reported on 19 patients with cMET amplification after first- or second generation EGFR TKI treatment. Thirteen patients received a cMET TKI, as monotherapy (n=12) or in combination with an EGFR TKI (n=1). Two patients had a partial response with a median duration of 3 months.

Two reports suggested that cMET amplification is a subclonal EGFR TKI resistance mechanism. Chabon et al. (6) reported on 16 patients with concomitant T790M mutations and cMET copy number gains in tumor samples after first/second generation EGFR TKI resistance development and before treatment with the third generation EGFR TKI rociletinib. Patients with pre-rociletinib cMET copy number gains had a significantly shorter median PFS than patient without cMET copy number gain. Minari et al. (17) reported on the co-occurrence of a BRAF V600E mutation and cMET amplification as resistance mechanism after osimertinib treatment. In our series, serial biopsies during EGFR TKI treatment revealed a co-occurring EGFR T790M and BRAF V600E mutation in one patient after first generation EGFR TKI treatment. After osimertinib treatment, a repeated biopsy in this patient showed absence of the BRAF V600E mutation and occurrence of cMET amplification.

Prospective trials are needed to investigate optimal treatment combinations to overcome acquired cMET-driven resistance. Phase Ib/II trials of the cMET inhibitors savolitinib and capmatinib alone or in combination with first or third generation EGFR TKIs, are currently active. Preliminary activity of savolitinib in combination with gefitinib (PR of 31% in 51 patients) or osimertinib (PR of 40% in 47 patients) was observed in EGFR-mutated NSCLC patients with cMET-amplification after progressing on prior EGFR TKI treatment. These data are encouraging and the final results are eagerly awaited.

5. Conclusion

In conclusion, this case series suggests that crizotinib treatment in patients with EGFR mutation positive NSCLC that acquired cMET amplification after EGFR TKI treatment results in short-lived and heterogeneous responses, possibly due to subclonality of cMET-driven resistance and co-occurrence of other EGFR TKI resistance mechanisms.

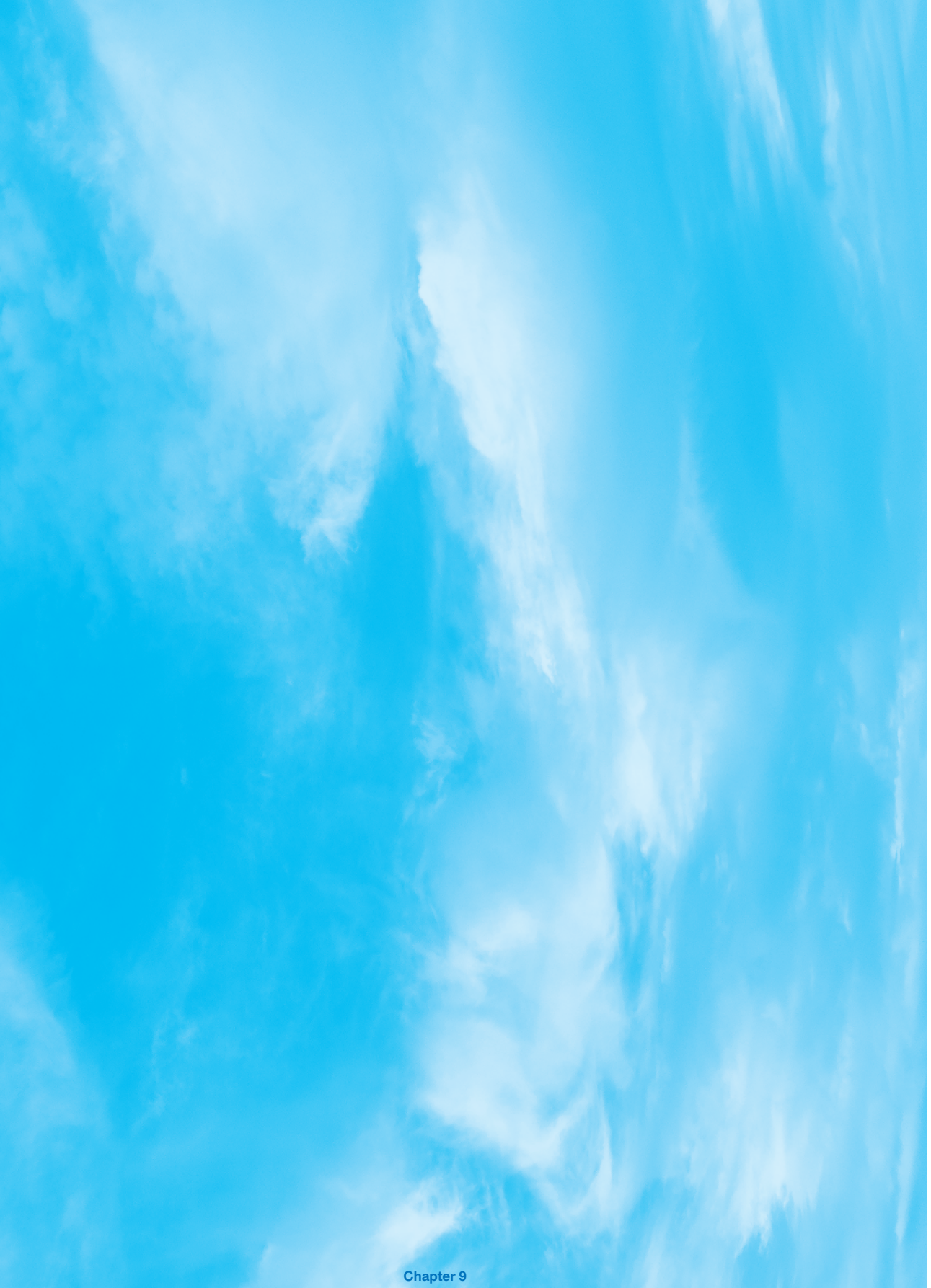
References

1. Lee CK, Brown C, Gralla RJ, Hirsh V, Thongprasert S, Tsai CM, et al. Impact of EGFR inhibitor in non-small cell lung cancer on progression-free and overall survival: a meta-analysis. Journal of the National Cancer Institute. 2013;105(9):595-605.
2. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Science translational medicine. 2011;3(75):75ra26.
3. Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. Clinical cancer research : an official journal of the American Association for Cancer Research. 2013;19(8):2240-7.
4. Rotow J, Bivona TG. Understanding and targeting resistance mechanisms in NSCLC. Nature reviews Cancer. 2017;17(11):637-58.
5. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science. 2007;316(5827):1039-43.
6. Chabon JJ, Simmons AD, Lovejoy AF, Esfahani MS, Newman AM, Haringsma HJ, et al. Circulating tumour DNA profiling reveals heterogeneity of EGFR inhibitor resistance mechanisms in lung cancer patients. Nature communications. 2016;7:11815.
7. Piotrowska Z, Thress KS, Mooradian M, Heist RS, Azzoli CG, Temel JS, et al. MET amplification (amp) as a resistance mechanism to osimertinib. Journal of Clinical Oncology. 2017;35(15_suppl):9020-.
8. Hoogstraat M, Hinrichs JW, Besselink NJ, Radersma-van Loon JH, de Voijs CM, Peeters T, et al. Simultaneous detection of clinically relevant mutations and amplifications for routine cancer pathology. J Mol Diagn. 2015;17(1):10-8.
9. Bubendorf L, Dafni U, Schöbel M, Finn SP, Tischler V, Sejda A, et al. Prevalence and clinical association of MET gene overexpression and amplification in patients with NSCLC: Results from the European Thoracic Oncology Platform (ETOP) Lungscape project. Lung cancer. 2017;111:143-9.
10. Wu Y-L, Yang JC-H, Kim D-W, Su W-C, Ahn M-J, Lee DH, et al. Safety and efficacy of INC280 in combination with gefitinib (gef) in patients with EGFR-mutated (mut), MET-positive NSCLC: A single-arm phase Ib/II study. Journal of Clinical Oncology. 2014;32(15_suppl):8017-.
11. Li A, Yang JJ, Zhang XC, Zhang Z, Su J, Gou LY, et al. Acquired MET Y1248H and D1246N Mutations Mediate Resistance to MET Inhibitors in Non-Small Cell Lung Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2017;23(16):4929-37.
12. Turke AB, Zejnullahu K, Wu YL, Song Y, Dias-Santagata D, Lifshits E, et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer cell. 2010;17(1):77-88.
13. Ou SI, Agarwal N, Ali SM. High MET amplification level as a resistance mechanism to osimertinib (AZD9291) in a patient that symptomatically responded to crizotinib treatment post-osimertinib progression. Lung cancer. 2016;98:59-61.
14. Wang Y, Li L, Han R, Jiao L, Zheng J, He Y. Clinical analysis by next-generation sequencing for NSCLC patients with MET amplification resistant to osimertinib. Lung cancer. 2018;118:105-10.
15. Yoshimura K, Inui N, Karayama M, Inoue Y, Enomoto N, Fujisawa T, et al. Successful crizotinib monotherapy in EGFR-mutant lung adenocarcinoma with acquired MET amplification after erlotinib therapy. Respiratory medicine case reports. 2017;20:160-3.
16. Baldacci S, Mazieres J, Tomasini P, Girard N, Guisier F, Audigier-Valette C, et al. Outcome of EGFR-mutated NSCLC patients with MET-driven resistance to EGFR tyrosine kinase inhibitors. Oncotarget. 2017;8(62):105103-14.
17. Minari R, Bordi P, La Monica S, Squadrilli A, Leonetti A, Bottarelli L, et al. Concurrent acquired BRAF V600E mutation and MET amplification as resistance mechanism of first-line osimertinib treatment in NSCLC EGFR-mutated patient. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer. 2018.

Part III

General discussion and future perspectives

Summary/ Samenvatting



Chapter 9

General discussion and future perspectives

PART I. Targeted treatment of *EGFR* exon 20 insertion mutations

When this thesis started in 2017, no targeted treatment options for *EGFR* ex20ins were available and they were considered intrinsically resistant to EGFR TKIs (1). Like *EGFR* wild-type metastatic NSCLC patients, platinum-based chemotherapy was the only registered systemic treatment available for these patients. This was disappointing, considering EGFR TKIs dramatically changed the clinical landscape of *EGFR*-mutated advanced NSCLC with median overall survival (OS) rates of approximately three years (2). New targeted treatment options were an unmet need for these patients. *EGFR* ex20ins mutations were thought to be undruggable due to structural differences compared to classic *EGFR* mutations, resulting in steric hindrance of the drug-binding pocket. In addition, *EGFR* ex20ins mutations are difficult to selectively target, due to similarities with wild-type *EGFR* (3, 4). However, there is also some good news. In recent years, the targeted therapy landscape for *EGFR* ex20ins mutations has finally begun to change.

The role of osimertinib

At the start of this thesis, it was clear that early generation TKIs were not effective in patients with NSCLC harboring an *EGFR* exon20ins mutation. However, the role of osimertinib had not yet been established (3-7). Based on preclinical data, the role of osimertinib for this patient category was unclear. Clinical data on the effectiveness of osimertinib in patients harboring an *EGFR* ex20ins mutation were limited.

We collected clinical data of 21 patients with advanced stage NSCLC harboring an *EGFR* ex20ins mutation who were treated with osimertinib 80 mg once daily (**chapter 2**). We concluded that standard-dose osimertinib has limited antitumor activity with an ORR of 5%. Despite the low response rate, 67% experienced a small decrease in tumor size. In addition, a subgroup of patients (38%) derived durable disease stabilization of five months and beyond. Therefore, osimertinib seems only partially capable of blocking the *EGFR* ex20ins mutation. These findings were in line with following studies in which the ORR varied from 0% to 6.5% (8-10). Based on the above-mentioned data, osimertinib 80 mg once daily has no significant effectiveness in metastatic NSCLC patients harboring an *EGFR* ex20ins mutation.

Does 160 mg do the job?

In vitro models to determine the therapeutic window of EGFR TKIs revealed that the IC50 values of osimertinib for *EGFR* ex20ins were 10 times lower than those for the wild type *EGFR*, indicating that osimertinib may present a wide therapeutic window (4). However, the IC50 values of osimertinib for EGFR ex20ins were 10 to 100 fold higher compared to classic mutations. Thus, a higher dose of osimertinib may be required to effectively treat these patients (4, 6).

We initiated a single-arm, phase II trial to evaluate the efficacy of high-dose osimertinib (160 mg) once daily. In this thesis, we described the results of the largest phase II study that investigated the effects of osimertinib 160 mg once daily in 25 patients with an *EGFR* exon

20 mutation (**chapter 3**). Treatment-naïve patients and patients with asymptomatic brain metastases were allowed. The confirmed ORR was 28% and 80% of patients experienced a decrease in tumor size. It should be mentioned that we also included patients with *EGFR* ex20 point mutations in our trial. Two of three patients with an *EGFR* ex20 point mutation showed a partial response. If you only include the patients with an insertion mutation, the ORR would be slightly lower (22%). Our findings were in line with another trial that investigated the role of high-dose osimertinib. In the ECOG-ACRIN trial, 21 patients with advanced NSCLC harboring an *EGFR* ex20ins mutation who had at least one prior line of therapy were treated with high-dose osimertinib. They reported a confirmed ORR of 24% and a median PFS of 9.6 months (11). Although both studies did not meet their primary endpoint, approximately a quarter of the patients showed a confirmed partial response and a large proportion of patients experienced disease stabilization.

To summarize, the clinical efficacy of high-dose osimertinib was improved compared to the trials who tested the efficacy of osimertinib 80 mg daily. Therefore, there seems to be a relationship between dose and response. Although the results of high-dose osimertinib treatment in patients with an *EGFR* ex20ins mutation are not as expected, further research aimed at identifying subgroups who derive benefit from this treatment can be interesting considering its favorable toxicity profile. Since the maximum tolerated dose of osimertinib could not be determined, a higher dose of 240 mg once daily may be more effective (12). However, this would lead to a very expensive and potentially more toxic treatment option.

Furmonertinib (Alflutinib, AST2818) is another irreversible, selective, third-generation EGFR TKI that is very similar to osimertinib in terms of dosing regimen, efficacy and toxicity (13). Furmonertinib has a modified structure of the methoypyridine ring and adjacent benzene ring of osimertinib to improve binding to the hydrophobic pocket within the EGFR kinase domain (14, 15). For *EGFR* ex20ins mutations, furmonertinib is currently being tested in high doses, 160 mg and 240 mg once daily respectively (16, 17). Recently, interim-data of the ongoing phase Ib FAVOUR trial were presented at WCLC. The confirmed ORR in the treatment-naïve furmonertinib 240 mg cohort was 78.6% and 46.2% and 38.5% in previously treated furmonertinib 240 and 160 mg cohorts, respectively (18). A real-world study of the efficacy and safety of furmonertinib for patients with an *EGFR* ex20ins mutation showed an ORR of 42.9% in the 240 mg once-daily dosage group compared to 39.5% in the 160 mg group and 25% in the 80 mg group. The efficacy of furmonertinib appeared to increase with increasing dosage similar to osimertinib (17). A phase III study in first-line patients with stage IV *EGFR* ex20ins mutated NSCLC is currently ongoing (FURVENT; NCT05607550). Based on the data mentioned above, furmonertinib seems to perform better in *EGFR* ex20ins mutations compared to osimertinib, regardless of dosage, probably due to the optimized binding capabilities.

Antibody-based combinations

One of the other attempts to tackle the primary resistance of *EGFR* ex20ins mutants to early generation EGFR TKIs was a combination strategy of afatinib and anti-EGFR mAbs. In **chapter 4**, we showed that dual EGFR blockade with afatinib and cetuximab was able to induce tumor responses in patients harboring an *EGFR* ex20ins mutation. Three out of four patients had a partial response with a median PFS of 5.4 months. These findings led to a dedicated clinical trial. The results of the AFACET study, a phase II trial investigating the efficacy of afatinib and cetuximab in patients with *EGFR* ex20ins positive advanced NSCLC, are presented in **chapter 5**. The study achieved its primary endpoint, with a disease control rate (DCR) of 54% at 18 weeks of treatment. The median PFS was 5.5 months and a confirmed ORR of 32%.

Regarding efficacy, combination treatment with afatinib and cetuximab seems slightly more effective compared to mobocertinib or poziotinib and quite similar to amivantamab. However, these studies are difficult to compare due to varying inclusion criteria. In our study, approximately half of the patients were treatment naïve. Most other studies included patients after progression on platinum-based chemotherapy. Afatinib and cetuximab also provide no benefit in brain metastases since the intracranial activity turned out to be limited. However, EGFR related toxicity remains a major limitation of this treatment combination. In our trial, grade three or higher TRAEs were reported in 54% of patients. In addition, 68% of patients required a dose reduction and 16% discontinued treatment due to adverse events. Previous studies with afatinib and cetuximab reported similar toxicity rates (19, 20). Timely referral to a dermatologist and dose reductions are needed. A lower starting dose of 30 mg afatinib is not inferior to afatinib 40 mg daily (21) and may lead to less toxicity.

In conclusion, the synergism of combining EGFR TKIs with mAbs must be balanced with toxicity. If other compounds such as furmonertinib prove to be more effective in the (intracranial) treatment of *EGFR* ex20ins mutated NSCLC with significantly less toxicity, the role of combination strategies like afatinib and cetuximab will remain limited in this specific patient group.

Another hypothesis could be combining a less toxic and potentially more effective third generation EGFR TKI with cetuximab. *In vitro*, this combination therapy induced a more potent inhibitory effect against several *EGFR* ex20ins mutations compared to osimertinib alone (22). To my knowledge, no clinical trials have been conducted with this combination, only case reports are available. However, a recent phase I study tested osimertinib 80 mg once daily combined with necitumumab (another EGFR mAb). In the subgroup of patients harboring an *EGFR* ex20ins mutation, four partial responses were described. In the total study population (n = 100), a significant amount (38%) of grade 3 toxicity was still seen (23). Furthermore, the results of a phase 1b trial of anti-EGFR antibody JMT101 and high-dose osimertinib in patients with *EGFR* ex20ins positive NSCLC were published recently. JMT101 is an anti-EGFR mAb similar to cetuximab but with a higher target affinity and favorable safety profiles (24). With an ORR of 44.6%, it appears to be an effective treatment option. In this study 68% of patients reported grade three toxicity or higher. Although unexpected, toxicity rates are even higher compared to afatinib and cetuximab combination treatment. Given the recent development of less toxic compounds, this does not seem to be the most promising treatment option for the future.

New targeted treatment options

Several new targeted treatment options, specifically designed to target *EGFR* ex20ins mutations, are currently being investigated. Firstly, poziotinib, an irreversible TKI that covalently binds both *EGFR* and *HER2* (3). Recent data of poziotinib showed only modest activity (ORR 14.8%) and it fails to selectively inhibit ex20ins mutants, with 28% grade ≥ 3 rash and 26% grade ≥ 3 diarrhea, often leading to treatment discontinuation (25). Despite attempts to reduce toxicity with twice daily dosing, this does not seem to be a desirable treatment option for this patient group (26).

Secondly mobocertinib, a highly selective EGFR and HER2 TKI. Despite its low affinity for wild-type *EGFR*, treatment also led to significant typical EGFR TKI related toxicity (mainly rash and diarrhea). The confirmed RR among platinum-pretreated patients was 28%. In addition, after treatment with mobocertinib, grade ≥ 3 treatment-related adverse events (TRAEs) occurred in 40% of patients, most commonly diarrhea (27, 28). Based on this data, the Food

and Drug Administration (FDA) granted accelerated approval to mobocertinib for patients with *EGFR* ex20ins positive NSCLC, whose disease has progressed on or after platinum-based chemotherapy (29). However, the European Medicines Agency (EMA) had concerns regarding the efficacy and toxicity of mobocertinib. In particular, regarding the small percentage of patients who responded and lack of comparison with other treatment options. As a result, the application for a conditional marketing authorization in Europe was withdrawn.

Thirdly, amivantamab, a monoclonal bispecific anti-EGFR-MET antibody with immune cell-directing activity, has shown clinical activity and has granted FDA approval for treatment of *EGFR* ex20ins positive NSCLC after platinum-based chemotherapy (30, 31). Recent data on long-term efficacy showed an ORR of 37%, median PFS of 6.9 months and median OS of 23 months. Some patients received amivantamab for more than 2.5 years. Responses were also seen in heavily pretreated patients. This makes it a suitable treatment option after failure of chemo(immuno)therapy and EGFR TKI's (32). In the meantime, the use of amivantamab combined with platinum-based chemotherapy as first-line treatment compared to chemotherapy alone resulted in superior efficacy regarding PFS (33). The OS data were not mature but showed evidence of improved survival. Enthusiasm has been tempered due to its side effects. Treatment with amivantamab is associated with significant rash (89%) and diarrhea. Thereby, treatment is less convenient as it requires intravenous administration every two weeks and regularly causes infusion reactions (67%).

Lastly, zipalertinib (formerly known as CLN-081, TAS6417) was specifically designed to fit into the ATP-binding pocket of the *EGFR* ex20ins kinase while sparing wild-type *EGFR* (34). It covalently modifies C797 in the ATP-binding site of the *EGFR* hinge region. The results of a phase ½a study of pretreated patients showed a confirmed RR of 41% at the dose of 100 mg twice daily. The safety profile appears similar and possibly even slightly more favorable compared to sunvozertinib. No grade III or more EGFR related toxicity was observed (35).

More promising are the data of Sunvozertininb, an irreversible and selective EGFR TKI with activity against *EGFR* ex20ins as well as T790M, uncommon and classical *EGFR* mutations (36). This drug was designed to optimize binding capacity compared to osimertinib by replace the less flexible methylindole on osimertinib with a more flexible anilinophenyl moiety (36). At the 2023 ASCO Annual Meeting, results from the phase 2 WU-KONG6 study were presented. Ninety-seven pre-treated patients harboring an *EGFR* ex20ins mutation were treated with sunvozertinib 300 mg once daily with an impressive ORR of 60.8% and median duration of treatment of 7.0 months (37). In the preliminary safety results of the phase I trial, 33.3% grade 3 or higher toxicity was seen across all dose levels up to 400 mg. In first-line setting sunvozertinib is even more effective with an ORR of 77.8% in the 300 mg dosing cohort, similar to presented furmonertinib data. No detailed safety data were reported (38). Toxicity of furmonertinib appears to increase with higher doses with 13% grade 3 or higher TRAEs in the treatment-naïve 240 mg cohort and 29% in the previously treated 240 mg cohort.

Intracranial activity

An interesting subgroup concerns patients with intracranial metastases. Approximately half of all patients with *EGFR* mutated NSCLC will develop cerebral metastases (39). So besides toxicity, intracranial activity will also play an important role in defining the optimal treatment for *EGFR* ex20ins mutated NSCLC. In our study with high-dose osimertinib, all three patients with baseline brain metastases showed a decrease in brain lesion size (40). The robust intracerebral activity of osimertinib has already been demonstrated in previous trials (41). Not much is known about the intracranial activity of the other new compounds, but available data are not yet promising. Mobocertinib demonstrated limited intracranial activity in patients with baseline brain metastases (27). The CHRYSALIS study investigating the safety and efficacy of amivantamab, included only patients with treated and asymptomatic brain metastases. Thirty-two percent of patients with treated brain metastasis still developed intracranial progression on amivantamab (30). Due to its large molecular size, amivantamab is expected to have poor activity in the brain. In conclusion, there could be a role for osimertinib or other brain-penetrant third generation EGFR TKIs like furmonertinib in the treatment of *EGFR* ex20ins mutated NSCLC patients with brain metastases.

It will be interesting to see the results of the new small-molecule inhibitors, especially with regard to CNS-penetrating activity. Specific data regarding intracranial efficacy of furmonertinib in *EGFR* ex20ins mutated patients are not reported yet. The phase Ib FAVOUR trial included patients with asymptomatic brain metastases (39%). The efficacy of zipalertinib in untreated brain metastases will be investigated in cohort C of the REZILIENT2 trial (NCT05967689). A phase III study with sunvozertinib also allows patients with untreated brain metastases, only leptomeningeal disease is excluded (NCT05668988). The ongoing phase I/II CONCERTO trial (NCT05241873) is investigating the efficacy and tolerability of BLU-451, a CNS-penetrant, potent, selective, and wild-type sparing EGFR TKI. Preliminary data show responses, also intracranial, without dose-limiting toxicities and only grade 1 or 2 toxicity (42).

In **chapter 6**, we present a comprehensive overview of the (pre)clinical activity of currently available treatments across different insertion regions. More than 60 unique variants are identified to date. Marked differences in treatment responses in this heterogeneous group have been reported and are poorly understood. Further research is needed to better understand the heterogeneity of *EGFR* exon 20 variants. Probably we should not only focus on exon-based data, but also define the role of co-mutations or its tumor microenvironment. Kirchner et al. found significant gene expression changes between *EGFR* ex20ins mutated tumors, classical *EGFR* mutations, and *EGFR* wild-type variants (43). We also need to find out whether the use of a structure-function modeling approach in addition to the more common exon-based classification is able to better predict drug sensitivity and patient outcomes.

Lastly, further research is required to determine optimal sequencing or combining of treatments. Trials assessing the effectiveness of combination strategies with platinum-based chemotherapy in first-line setting are ongoing. The phase III PAPILLON study has investigated amivantamab plus chemotherapy versus chemotherapy alone as first-line treatment option (33). The OS data are not yet available. The REZILIENT3 study is testing zipalertinib and chemotherapy in first-line (NCT05973773). It should be noted that combination treatments are associated with more toxicity. Therefore, more research would be desirable into which subgroup of patients derive benefit from combined treatments.
If we prove better able to effectively treat *EGFR* ex20ins mutations with targeted agents, more research will be needed into possible resistance mechanisms to determine treatment options after the development of acquired resistance. Mechanisms of acquired resistance after poziotinib treatment included *EGFR* T790M, MET amplifications, and epithelial to mesenchymal transition (44).

Future perspectives on *EGFR* exon 20 insertion mutations

The above-mentioned new treatment options represent an important step forward in the treatment of patients harboring an *EGFR* ex20ins mutation. It remains a challenge to design novel agents with a wide therapeutic window that maximize clinical efficacy by reaching high enough doses while maintaining good tolerability.

The ideal compound is central nervous system (CNS)-penetrant, potent, selective and wild-type sparing to reduce EGFR-related toxicity. In addition to the most promising agents furmonertinib and sunvozertinib, several new compounds like BLU-451, BAY-568, YK-029A and ORIC-114 are currently in development (42, 45-47). Furthermore, we await long-term efficacy data and more detailed toxicity data of furmonertinib and sunvozertinib. Mobocertinib (only in USA) and amivantamab are currently approved in post-platinum setting. We know little about their effectiveness in first line. The efficacy of furmonertinib and sunvozertinib in treatment-naïve patients seems very promising. Although platinum-based chemotherapy is still the first-line treatment of choice, this is expected to change in the short term (48). The role of immunotherapy in this patient group is unclear and requires further investigation. Also taking into account a possible increased risk of toxicity in combination with or prior to targeted therapy.

PART II. Analysis and treatment of resistance mechanisms after third generation EGFR TKI treatment

Almost all patients will eventually develop resistance after EGFR-directed targeted treatment. Acquired resistance mechanisms post-osimertinib treatment are more diverse and heterogeneous and compromise both *EGFR*-dependent and *EGFR*-independent mechanisms. Available data for resistance mechanisms after osimertinib treatment were limited, with most studies focusing on circulating tumor (ct) DNA versus tissue analysis. Understanding resistance mechanisms is important to define new treatment options after disease progression or to prevent the development of resistance.

In **chapter 7**, we investigated innate and acquired resistance mechanisms by analyzing the molecular profiles of pre- and post-treatment samples of *EGFR* T790M positive NSCLC patients. After third-generation EGFR TKI treatment, loss of *EGFR* T790M was the most common molecular change in 45% of patients, followed by *cMET* amplification (29%), *EGFR* p.C797S (10%), amplification of ERBB2 or *KRAS* mutations (7%) and small-cell lung cancer transformation or *BRAF* mutations in 4% of patients. We concluded that tumor heterogeneity is one of the key mechanisms of underlying resistance to EGFR-targeted therapies.

In the meantime, more research has been done. The most common on-target resistance mutations after first-line osimertinib treatment are C797X mutations, followed by L718X, L792 and G724S mutations (49-51). Fourth-generation EGFR TKIs are currently being tested, preempting the development of C797S, and can be active against C797S alone or in combination with T790M mutations (52). Alterations in L718 and G724 are sensitive to early generation EGFR inhibitors (53, 54). However, osimertinib resistance is associated with predominantly *EGFR*-independent genomic alterations. The most common off-target resistance observed post-osimertinib is *cMET* amplification (51).

In **chapter 8**, we presented a retrospective case series about eight *EGFR*-mutated NSCLC patients who acquired *cMET* amplification after EGFR TKI treatment and were treated with crizotinib. Adding crizotinib to EGFR-directed treatment can be effective since 50% showed a PR. However, responses were short-lasting and often heterogeneous. Tumor heterogeneity is common after osimertinib treatment, not just among on- and off-target resistance, but also within on-target resistance mechanisms (50). This may lead to inferior responses after targeted treatment.

Other combinations of cMET inhibitors like savolitinib and EGFR TKIs have since been investigated. The phase Ib TATTON trial (savolitinib and osimertinib) reported an ORR of 33% and a median PFS of 5.5 months (55). cMET inhibitors seem more effective in high-level *cMET* amplified patients (gene copy number (GCN) ≥ 10). Similar interim results of the ORCHARD trial were presented with a response rate of 41%. Data regarding duration of response has not yet been reported (56).

In addition, amivantamab in combination with lazertinib has proven to be effective in the treatment of osimertinib resistance with an ORR of 36% and median PFS of 4.9 months (57). Interestingly also patients without known (cMET) resistance mechanisms did respond. Lastly, telisotuzumab vedotin (Teliso-V), an antibody-drug conjugate (ADC) directed against the cMET extracellular domain, shows promising efficacy in cMET overexpressed and *cMET* amplified patients (58). Further research is needed to define which patients derive long-lasting benefit and with cut-off regarding GCN should be used.

Future perspectives after progression on third-generation EGFR TKI

To date, no approved targeted therapies on progression after third-generation EGFR TKI treatment are available. Platinum-based chemotherapy is the standard treatment option after failure of osimertinib treatment. However, chemotherapy has limited (real-world) efficacy and we prefer a more personalized treatment based on the underlying resistance mechanism, if possible (59). Therefore, biomarker analysis is necessary. We should strive to obtain sufficient tissue for molecular analysis, also to detect SCLC or squamous carcinoma transformation. In case a tissue biopsy is not possible, ctDNA analysis can be useful. More research is needed to determine the additive effect of combining those strategies. Additionally biomarker research will hopefully lead to a more personalized treatment after EGFR TKI resistance.

Targeted treatment of *EGFR*-dependent or *EGFR*-independent resistance mechanisms can be effective, only generally with limited duration of response due to subclonality and co-occurrence of other EGFR TKI resistance mechanisms. One of the attempts to improve the duration of response is addition of chemotherapy. Recently, the results of the MARIPOSA-2 study were published (60). In this trial chemotherapy and amivantamab combination with or without lazertinib versus chemotherapy alone were tested after disease progression on osimertinib. The PFS doubled after chemotherapy – amivantamab – lazertinib treatment versus chemotherapy and ORR was 64% for the triplet combination versus 36% for chemotherapy, respectively. However, this treatment comes at a cost, with 92% grade 3 or higher toxicity. Thirty-four percent of patients discontinued chemotherapy-amivantamab-lazertinib treatment due to adverse events. Another strategy is to prevent the development of osimertinib-resistance by combining therapies in first-line setting, like amivantamab and lazertinib, resulting in superior PFS compared to osimertinib (61, 62). Addition of chemotherapy to osimertinib in treatment-naïve patients can also delay the onset of acquired resistance based on the FLAURA2 data, especially in patients with brain metastases, however it is not known whether all the options mentioned above also lead to a survival advantage.

In conclusion, more research needs to be done of appropriate sequencing of therapy. Until there is more data available regarding overall survival, we must ask ourselves if the benefits outweigh the risks of higher toxicity rates and the inconvenience of intravenous administration every two or three weeks for years. Probably subgroups like patients with brain metastases who benefit the most could be eligible.

Another interesting development in the treatment of EGFR resistance are the ADCs. This targeted delivery of cytotoxic chemotherapy enables the administration of higher doses compared to conventional chemotherapy and does not rely on targeting the genomic resistance mechanism, but on the expression of target proteins in the cell membrane (63, 64).

The combination of osimertinib and PD-1/PD-L1 therapy was not effective and was associated with a higher toxicity risk, however the combination of osimertinib with CTLA-4 inhibitor ipilimumab seems tolerable based on recently published data (65). It would be preferable to get immunotherapy working; also in *EGFR*-mutated NSCLC as this can lead to more durable responses as seen in smoking-related NSCLC. In addition, the use of adoptive cell therapy using tumor infiltrating lymphocytes can be active, also in *EGFR*-mutated NSCLC patients (66). Further exploration of the role of cell therapy in NSCLC patients harboring a driver alteration is needed.

Co-occurring alterations like TP53 mutations may contribute to tumor heterogeneity and are associated with poor outcomes, especially with additional tumor suppressor gene alterations (67, 68). This subset of patients may benefit from a more aggressive regimen, like combining an EGFR TKI with chemotherapy in first-line setting.

In conclusion, the challenges for acquired resistance in *EGFR*-mutated NSCLC are defining the best place in the sequence of treatment and the role of combination treatments of targeted therapy and chemotherapy/ADCs and immunotherapy, ideally after thorough molecular analysis to make optimal use of personalized care.

References

1. Burnett H, Emich H, Carroll C, Stapleton N, Mahadevia P, Li T. Epidemiological and clinical burden of EGFR Exon 20 insertion in advanced non-small cell lung cancer: A systematic literature review. PLoS One. 2021;16(3):e0247620.
2. Ramalingam SS, Vansteenkiste J, Planchard D, Cho BC, Gray JE, Ohe Y, et al. Overall Survival with Osimertinib in Untreated, EGFR-Mutated Advanced NSCLC. N Engl J Med. 2020;382(1):41-50.
3. Robichaux JP, Elamin YY, Tan Z, Carter BW, Zhang S, Liu S, et al. Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer. Nat Med. 2018;24(5):638-46.
4. Hirano T, Yasuda H, Tani T, Hamamoto J, Oashi A, Ishioka K, et al. In vitro modeling to determine mutation specificity of EGFR tyrosine kinase inhibitors against clinically relevant EGFR mutants in non-small-cell lung cancer. Oncotarget. 2015;6(36):38789-803.
5. Fang W, Huang Y, Hong S, Zhang Z, Wang M, Gan J, et al. EGFR exon 20 insertion mutations and response to osimertinib in non-small-cell lung cancer. BMC Cancer. 2019;19(1):595.
6. Floc'h N, Martin MJ, Riess JW, Orme JP, Staniszewska AD, Ménard L, et al. Antitumor Activity of Osimertinib, an Irreversible Mutant-Selective EGFR Tyrosine Kinase Inhibitor, in NSCLC Harboring EGFR Exon 20 Insertions. Mol Cancer Ther. 2018;17(5):885-96.
7. Piotrowska Z, Fintelmann FJ, Sequist LV, Jahagirdar B. Response to Osimertinib in an EGFR Exon 20 Insertion-Positive Lung Adenocarcinoma. J Thorac Oncol. 2018;13(10):e204-e6.
8. Ichihara E, Yasuda H, Takashima Y, Zenke Y, Takeuchi S, Morise M, et al. Abstract CT106: Phase I/II study of osimertinib in EGFR exon 20 insertion mutations in non-small cell lung cancer patients: AEX20. Cancer Research. 2021;81(13_Supplement):CT106-CT.
9. Kim TM, Ock CY, Kim M, Kim SH, Keam B, Kim YJ, et al. 1529P - Phase II study of osimertinib in NSCLC patients with EGFR exon 20 insertion mutation: A multicenter trial of the Korean Cancer Study Group (LU17-19). Annals of Oncology. 2019;30:v628.
10. Yang GJ, Li J, Xu HY, Sun Y, Liu L, Li HS, et al. Osimertinib for Chinese advanced non-small cell lung cancer patients harboring diverse EGFR exon 20 insertion mutations. Lung Cancer. 2021;152:39-48.
11. Piotrowska Z, Wang Y, Sequist L, Ramalingam S. ECOG-ACRIN 5162: A phase II study of osimertinib 160 mg in NSCLC with EGFR exon 20 insertions. Journal of Clinical Oncology. 2020;38:9513-.
12. Jänne PA, Yang JC, Kim DW, Planchard D, Ohe Y, Ramalingam SS, et al. AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. N Engl J Med. 2015;372(18):1689-99.
13. Lu S, Dong X, Jian H, Chen J, Chen G, Sun Y, et al. AENEAS: A Randomized Phase III Trial of Aumolertinib Versus Gefitinib as First-Line Therapy for Locally Advanced or MetastaticNon-Small-Cell Lung Cancer With EGFR Exon 19 Deletion or L858R Mutations. J Clin Oncol. 2022;40(27):3162-71.
14. Shi Y, Zhang S, Hu X, Feng J, Ma Z, Zhou J, et al. Safety, Clinical Activity, and Pharmacokinetics of Alflutinib (AST2818) in Patients With Advanced NSCLC With EGFR T790M Mutation. J Thorac Oncol. 2020;15(6):1015-26.
15. Zhou X, Dong H, Li P, Wu C, Lu H, Xiao M, Zhou T. Short-term efficacy of furmonertinib in treatment of NSCLC patients with EGFR exon20 insertion. Journal of Clinical Oncology. 2022;40(16_suppl):e21063-e.
16. Han B, Zhou C, Wu L, Yu X, Li Q, Liu F, Shen C. 1210P Preclinical and preliminary clinical investigations of furmonertinib in NSCLC with EGFR exon 20 insertions (20ins). Annals of Oncology. 2021;32:S964.
17. Sa H, Shi Y, Ding C, Ma K. A real-world study of the efficacy and safety of furmonertinib for patients with non-small cell lung cancer with EGFR exon 20 insertion mutations. Journal of Cancer Research and Clinical Oncology. 2023;149(10):7729-42.
18. Han B, Zhou C, Zheng W, et al. A phase 1b study of furmonertinib, an oral, brain penetrant, selective EGFR inhibitor, in patients with advanced NSCLC with EGFR exon 20 insertions. Presented at: International Association for the Study of Lung Cancer 2023 World Conference on Lung Cancer; September 9-12, 2023; Singapore. Abstract OA03.04.
19. Cortot AB, Madroszyk A, Giroux-Leprieur E, Molinier O, Quoix E, Bérard H, et al. First-Line Afatinib plus Cetuximab for EGFR-Mutant Non-Small Cell Lung Cancer: Results from the Randomized Phase II IFCT-1503 ACE-Lung Study. Clin Cancer Res. 2021;27(15):4168-76.
20. Goldberg SB, Redman MW, Lilenbaum R, Politi K, Stinchcombe TE, Horn L, et al. Randomized Trial of Afatinib Plus Cetuximab Versus Afatinib Alone for First-Line Treatment of EGFR-Mutant Non-Small-Cell Lung Cancer: Final Results From SWOG S1403. J Clin Oncol. 2020;38(34):4076-85.
21. Yang CJ, Tsai MJ, Hung JY, Lee MH, Tsai YM, Tsai YC, et al. The clinical efficacy of Afatinib 30 mg daily as starting dose may not be inferior to Afatinib 40 mg daily in patients with stage IV lung Adenocarcinoma harboring exon 19 or exon 21 mutations. BMC Pharmacol Toxicol. 2017;18(1):82.
22. Hasegawa H, Yasuda H, Hamamoto J, Masuzawa K, Tani T, Nukaga S, et al. Efficacy of afatinib or osimertinib plus

cetuximab combination therapy for non-small-cell lung cancer with EGFR exon 20 insertion mutations. Lung Cancer. 2019;127:146-52.
23. Riess JW, Krailo MD, Padda SK, Groshen SG, Wakelee HA, Reckamp KL, et al. Osimertinib plus necitumumab in EGFR-mutant NSCLC: Final results from an ETCTN California Cancer Consortium phase I study. Journal of Clinical Oncology. 2022;40(16_suppl):9014-.
24. Zhao S, Zhuang W, Han B, Song Z, Guo W, Luo F, et al. Phase 1b trial of anti-EGFR antibody JMT101 and Osimertinib in EGFR exon 20 insertion-positive non-small-cell lung cancer. Nature Communications. 2023;14(1):3468.
25. Le X, Goldman J, Clarke J, Tchekmedyian N, Piotrowska Z, Chu D, et al. Poziotinib shows activity and durability of responses in subgroups of previously treated EGFR exon 20 NSCLC patients. Journal of Clinical Oncology. 2020;38:9514-.
26. Le X, Shum E, Suga JM, Brahmer JR, Dooms C, Mamdani H, et al. Abstract CT169: Poziotinib administered twice daily improves safety and tolerability in patients with EGFR or HER2 exon 20 mutant NSCLC(ZENITH20-5). Cancer Research. 2021;81(13_Supplement):CT169-CT.
27. Riely GJ, Neal JW, Camidge DR, Spira AI, Piotrowska Z, Costa DB, et al. Activity and Safety of Mobocertinib (TAK-788) in Previously Treated Non-Small Cell Lung Cancer with EGFR Exon 20 Insertion Mutations from a Phase I/II Trial. Cancer Discov. 2021;11(7):1688-99.
28. Zhou C, Ramalingam SS, Kim TM, Kim SW, Yang JC, Riely GJ, et al. Treatment Outcomes and Safety of Mobocertinib in Platinum-Pretreated Patients With EGFR Exon 20 Insertion-Positive Metastatic Non-Small Cell Lung Cancer: A Phase ½ Open-label Nonrandomized Clinical Trial. JAMA Oncol. 2021;7(12):e214761.
29. Duke ES, Stapleford L, Drezner N, Amatya AK, Mishra-Kalyani PS, Shen YL, et al. FDA Approval Summary: Mobocertinib for Metastatic Non-Small Cell Lung Cancer with EGFR Exon 20 Insertion Mutations. Clin Cancer Res. 2023;29(3):508-12.
30. Park K, Haura EB, Leighl NB, Mitchell P, Shu CA, Girard N, et al. Amivantamab in EGFR Exon 20 Insertion-Mutated Non-Small-Cell Lung Cancer Progressing on Platinum Chemotherapy: Initial Results From the CHRYSALIS Phase I Study. J Clin Oncol. 2021;39(30):3391-402.
31. Chon K, Larkins E, Chatterjee S, Mishra-Kalyani PS, Aungst S, Wearne E, et al. FDA Approval Summary: Amivantamab for the Treatment of patients with non-small cell lung cancer with EGFR exon 20 insertion mutations. Clin Cancer Res. 2023.
32. Lopez PG, Girard N, Cho BC, Sabari J, Spira A, Sanborn RE, et al. 30 Long-term efficacy, safety, and predictors of response to amivantamab among patients with post-platinum EGFR Ex20ins-mutated advanced NSCLC. Journal of Thoracic Oncology. 2023;18(4, Supplement):S36-S7.
33. Zhou C, Tang K-J, Cho BC, Liu B, Paz-Ares L, Cheng S, et al. Amivantamab plus Chemotherapy in NSCLC with EGFR Exon 20 Insertions. New England Journal of Medicine. 2023;389(22):2039-51.
34. Hasako S, Terasaka M, Abe N, Uno T, Ohsawa H, Hashimoto A, et al. TAS6417, A Novel EGFR Inhibitor Targeting Exon 20 Insertion Mutations. Mol Cancer Ther. 2018;17(8):1648-58.
35. Piotrowska Z, Tan DS, Smit EF, Spira AI, Soo RA, Nguyen D, et al. Safety, Tolerability, and Antitumor Activity of Zipalertinib Among Patients With Non-Small-Cell Lung Cancer Harboring Epidermal Growth Factor Receptor Exon 20 Insertions. J Clin Oncol. 2023:Jco2300152.
36. Wang M, Yang JC, Mitchell PL, Fang J, Camidge DR, Nian W, et al. Sunvozertinib, a Selective EGFR Inhibitor for Previously Treated Non-Small Cell Lung Cancer with EGFR Exon 20 Insertion Mutations. Cancer Discov. 2022;12(7):1676-89.
37. Wang M, Fan Y, Sun M, Wang Y, Zhao Y, Jin B, et al. Sunvozertinib for the treatment of NSCLC with EGFR Exon20 insertion mutations: The first pivotal study results. Journal of Clinical Oncology. 2023;41(16_suppl):9002-.
38. Yang J, Wang M, Luo Y, et al. Sunvozertinib as first-line treatment in NSCLC patients with EGFR Exon20 insertion mutations. Presented at: ESMO Congress 2023; October 20-24, 2023; Madrid. Abstract 1325P.
39. Rakshit S, Bansal R, Desai A, Leventakos K. 38P Brain metastases in non-small cell lung cancer in era of molecularly driven therapy. Journal of Thoracic Oncology. 2021;16(4, Supplement):S714-S5.
40. Zwierenga F, van Veggel B, Hendriks LEL, Hiltermann TJN, Hiddinga BI, Hijmering Kappelle LBM, et al. High dose osimertinib in patients with advanced stage EGFR exon 20 mutation-positive NSCLC: Results from the phase 2 multicenter POSITION20 trial. Lung Cancer. 2022;170:133-40.
41. Reungwetwattana T, Nakagawa K, Cho BC, Cobo M, Cho EK, Bertolini A, et al. CNS Response to Osimertinib Versus Standard Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Patients With Untreated EGFR-Mutated Advanced Non–Small-Cell Lung Cancer. Journal of Clinical Oncology. 2018;36(33):3290-7.
42. Nguyen D, Shum E, Baik CS, Janne PA, Patel JD, Schenk EL, et al. Emerging phase 1 data of BLU-451 in advanced NSCLC with EGFR exon 20 insertions. Journal of Clinical Oncology. 2023;41(16_suppl):9064-.
43. Kirchner M, Kluck K, Brandt R, Volckmar AL, Penzel R, Kazdal D, et al. The immune microenvironment in EGFR- and ERBB2-mutated lung adenocarcinoma. ESMO Open. 2021;6(5):100253.
44. Elamin YY, Robichaux JP, Carter BW, Altan M, Tran H, Gibbons DL, et al. Poziotinib for EGFR exon 20-mutant NSCLC: Clinical efficacy, resistance mechanisms, and impact of insertion location on drug sensitivity. Cancer Cell. 2022;40(7):754-67.e6.
45. Long JE, Kim S, Kim HY, Shin DG, Park DH, Warne R, et al. Abstract 3335: ORIC-114, an orally bioavailable, irreversible kinase inhibitor,has superior brain penetration and antitumor activity in subcutaneous and intracranial NSCLC models. Cancer Research. 2022;82(12_Supplement):3335-.
46. Siegel F, Siegel S, Graham K, Kaplan B, Petersen K, Boemer U, et al. Abstract 1470: Preclinical activity of the first reversible, potent and selective inhibitor of EGFR exon 20 insertions. Cancer Research. 2021;81(13_Supplement):1470-.
47. Duan J, Wu L, Yang K, Zhao J, Zhao Y, Dai X, et al. Safety, tolerability, pharmacokinetics, and preliminary efficacy of YK-029A in treatment-naïve patients with advanced non-small cell lung cancer harboring EGFR exon 20 insertion mutations: A phase I trial. J Thorac Oncol. 2023.
48. Hendriks LE, Kerr KM, Menis J, Mok TS, Nestle U, Passaro A, et al. Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol. 2023;34(4):339-57.
49. Chmielecki J, Mok T, Wu Y-L, Han J-Y, Ahn M-J, Ramalingam SS, et al. Analysis of acquired resistance mechanisms to osimertinib in patients with EGFR-mutated advanced non-small cell lung cancer from the AURA3 trial. Nature Communications. 2023;14(1):1071.
50. Rotow JK, Lee JK, Madison RW, Oxnard GR, Jänne PA, Schrock AB. Real-world genomic profile of EGFR second-site mutations and other osimertinib resistance mechanisms, and clinical landscape of non-small cell lung cancer (NSCLC) post-osimertinib. Journal of Thoracic Oncology. 2023.
51. Le X, Puri S, Negrao MV, Nilsson MB, Robichaux J, Boyle T, et al. Landscape of EGFR-Dependent and -Independent Resistance Mechanisms to Osimertinib and Continuation Therapy Beyond Progression in EGFR-Mutant NSCLC. Clin Cancer Res. 2018;24(24):6195-203.
52. Lim SM, Fujino T, Kim C, Lee G, Lee YH, Kim DW, et al. BBT-176, a Novel Fourth-Generation Tyrosine Kinase Inhibitor for Osimertinib-Resistant EGFR Mutations in Non-Small Cell Lung Cancer. Clin Cancer Res. 2023;29(16):3004-16.
53. Ramalingam SS, Cheng Y, Zhou C, Ohe Y, Imamura F, Cho BC, et al. LBA50 - Mechanisms of acquired resistance to first-line osimertinib: Preliminary data from the phase III FLAURA study. Annals of Oncology. 2018;29:viii740.
54. Yang Z, Yang N, Ou Q, Xiang Y, Jiang T, Wu X, et al. Investigating Novel Resistance Mechanisms to Third-Generation EGFR Tyrosine Kinase Inhibitor Osimertinib in Non-Small Cell Lung Cancer Patients. Clin Cancer Res. 2018;24(13):3097-107.
55. Hartmaier RJ, Markovets AA, Ahn MJ, Sequist LV, Han JY, Cho BC, et al. Osimertinib + Savolitinib to Overcome Acquired MET-Mediated Resistance in Epidermal Growth Factor Receptor-Mutated, MET-Amplified Non-Small Cell Lung Cancer: TATTON. Cancer Discov. 2023;13(1):98-113.
56. Yu HA, Ambrose H, Baik C, Cho BC, Cocco E, Goldberg SB, et al. 1239P ORCHARD osimertinib + savolitinib interim analysis: A biomarker-directed phase II platform study in patients (pts) with advanced non-small cell lung cancer (NSCLC) whose disease has progressed on first-line (1L) osimertinib. Annals of Oncology. 2021;32:S978-S9.
57. Cho BC, Kim D-W, Spira AI, Gomez JE, Haura EB, Kim S-W, et al. Amivantamab plus lazertinib in osimertinib-relapsed EGFR-mutant advanced non-small cell lung cancer: a phase 1 trial. Nature Medicine. 2023;29(10):2577-85.
58. Goldman JW, Horinouchi H, Cho BC, Tomasini P, Dunbar M, Hoffman D, et al. Phase 1/1b study of telisotuzumab vedotin (Teliso-V) + osimertinib (Osi), after failure on prior Osi, in patients with advanced, c-Met overexpressing, EGFR-mutated non-small cell lung cancer (NSCLC). Journal of Clinical Oncology. 2022;40(16_suppl):9013-.
59. Hayashi H, Nishio M, Takahashi M, Tsuchiya H, Kasahara-Kiritani M. Real-World Data About Treatment Outcomes for Patients with EGFR-Mutated NSCLC Resistance to Osimertinib and Platinum-Based Chemotherapy. Adv Ther. 2023;40(10):4545-60.
60. Passaro A, Wang J, Wang Y, Lee SH, Melosky B, Shih JY, et al. Amivantamab plus chemotherapy with and without lazertinib in EGFR-mutant advanced NSCLC after disease progression on osimertinib: primary results from the phase III MARIPOSA-2 study. Annals of Oncology. 2023.
61. Lee S-H, Cho BC, Han J-Y, Cho EK, Lee J-S, Lee KH, et al. Amivantamab and lazertinib in treatment-naïve EGFR-mutated advanced non–small-cell lung cancer (NSCLC): Long-term follow-up and ctDNA results from CHRYSALIS. Journal of Clinical Oncology. 2023;41(16_suppl):9134-.
62. Cho BC, et al. Amivantamab plus lazertinib vs osimertinib as first-line treatment in patients with EGFR-mutated, advanced non-small cell lung cancer (NSCLC): Primary results from MARIPOSA, a phase III, global, randomized, controlled trial. ESMO Congress 2023, LBA14.
63. Passaro A, Jänne PA, Peters S. Antibody-Drug Conjugates in Lung Cancer: Recent Advances and Implementing Strategies. J Clin Oncol. 2023;41(21):3747-61.
64. Blaquier JB, Ortiz-Cuaran S, Ricciuti B, Mezquita L, Cardona AF, Recondo G. Tackling Osimertinib Resistance in EGFR-Mutant Non–Small Cell Lung Cancer. Clinical Cancer Research. 2023;29(18):3579-91.
65. Puri S, et al. Phase Ib study to evaluate the safety and

tolerability of osimertinib with ipilimumab in EGFRm NSCLC. ESMO Congress 2023, 1335P.
66. Creelan BC, Wang C, Teer JK, Toloza EM, Yao J, Kim S, et al. Tumor-infiltrating lymphocyte treatment for anti-PD-1-resistant metastatic lung cancer: a phase 1 trial. Nat Med. 2021;27(8):1410-8.
67. Stockhammer P, Grant M, Wurtz A, Foggetti G, Expósito F, Gu J, et al. Co-occurring Alterations in Multiple Tumor Suppressor Genes are Associated with Worse Outcomes in Patients with EGFR-mutant Lung Cancer. Journal of Thoracic Oncology. 2023.
68. Skoulidis F, Heymach JV. Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy. Nat Rev Cancer. 2019;19(9):495-509.

Summary

In the Netherlands, approximately 10% of NSCLC patients harbor a mutation in the epidermal growth factor receptor (*EGFR*). This mutation is predominantly seen in non-smokers, women and younger patients. This thesis focuses on patients with metastatic non-small cell lung cancer harboring an *EGFR* mutation.

The *EGFR* gene is located on chromosome 7 and consists of four domains: an extracellular domain, a transmembrane domain and two intracellular domains, the tyrosine kinase and autopholphorylation regions. *EGFR* mutations in NSCLC are usually clustered in the tyrosine kinase domain between exon 18 and 21, around the ATP binding pocket. The most common *EGFR* mutations are the *EGFR* exon 19 in-frame deletions and *EGFR* L858R point mutations. These are also referred to as the classical *EGFR* mutations and account for approximately 85% of all *EGFR* mutations. These mutations cause the kinase activity of EGFR to become ligand-independent, leading to uninhibited cell growth. EGFR tyrosine kinase inhibitors (TKIs) are effective in blocking EGFR autophosphorylation by binding to the receptor, however, eventually resistance occurs.

The third most common *EGFR* mutation concerns the *EGFR* exon 20 insertion (ex20ins) mutation. This mutation affects 4 – 12% of all *EGFR* mutations. It is a heterogeneous group consisting of more than 60 variants of in-frame insertions or duplications within the tyrosine kinase domain. This regions of exon 20 contains two major regions: the regulatory C-helix and the adjacent loop that follows it. Unfortunately, early generation EGFR TKIs cannot effectively target *EGFR* ex20ins mutations because the C-helix is pushed inward towards the drug-binding pocket, which prevents binding of EGFR TKIs. In addition, ex20ins mutations activate EGFR without diminishing ATP affinity, which makes it difficult to selectively target the ex20ins mutant over wild-type *EGFR*. At the start of this thesis, no targeted treatment options were available for *EGFR* ex20ins mutations. **Chapter 1** provides a general introduction to the epidemiology, molecular alterations and possible treatment options of *EGFR* mutated NSCLC, including *EGFR* ex20ins mutations.

PART I. Targeted treatment of *EGFR* exon 20 insertion mutations

This part of the thesis focuses on finding new targeted treatment options for patients harboring an *EGFR* ex20ins mutation. It was already known that this mutation was not sensitive to first-generation EGFR TKIs and barely sensitive to second-generation EGFR TKIs. However, preclinical data showed that *EGFR* ex20ins mutations might be sensitive to third-generation EGFR TKIs. In **chapter 2**, we retrospectively collected data from 21 patients with an *EGFR* ex20ins mutation who were treated with osimertinib. Almost all patients were treated with the standard dose of 80 mg osimertinib once daily. This proved not effective with a response rate (ORR) of only 5%, also confirmed in following studies with response rates of 0% to 6.5%. Despite this low response rate, a small reduction in tumor lesions was seen in 67% of patients and in some of the patients (38%), the disease stabilized for five months or longer.

In preclinical studies, there were indications that a higher dose of osimertinib might be more effective with acceptable toxicity. We then started a prospective phase II study in which patients with an *EGFR* exon 20 mutation were treated with a high dose of osimertinib, namely 160 mg once daily. Previous treatment with chemotherapy was permitted but nog mandatory. Patients with asymptomatic brain metastases could also be included, as well

as patients with point mutations in *EGFR* exon 20. **Chapter 3** describes the results of this study in which 25 patients with an *EGFR* ex20ins mutation were treated with osimertinib 160 mg once daily. High-dose osimertinib resulted in an ORR of 28% and 80% of patients showed a reduction in tumor lesions with a median duration of response of 5.3 months.

Although this study did not meet its primary endpoint, a quarter of patients benefited from this treatment, with limited toxicity. Only 4% of patients reported grade ≥ 3 side effects. A reduction in brain metastases was also seen in the three patients with asymptomatic brain metastases at the start of study treatment. Responses were seen across the spectrum of *EGFR* exon 20 mutations and three out of four patients with a point mutation showed a response. There appears to be a dose-response relationship regarding efficacy of osimertinib in patients harboring an *EGFR* exon 20 mutation, but with limited gain in effectiveness.

There are currently other third-generation EGFR TKIs in development that have been specially designed to improve binding to *EGFR* ex20ins mutations through a slightly modified structure. Furmonertinib is such a modified third-generation TKI and has strong similarities with osimertinib in terms of dosage, effectiveness and toxicity. It is also currently being tested in high doses, even up to 240 mg per day. The ORR of furmonertinib 240 mg per day in treatment-naïve patients is 78.6% and is therefore promising. The long-term results are not yet reported. Toxicity at the highest dose level is still acceptable with 13 – 29% grade ≥ 3 side effects.

Another strategy to treat *EGFR* ex20ins mutations is to combine an EGFR TKI with a monoclonal antibody (mAb) directed against EGFR. In this way, an attempt is made to achieve double EGFR blockade from both the extracellular domain and intracellularly. In **chapter 4** we described a case series about the results of four *EGFR* ex20ins positive NSCLC patients treated with afatinib (a second generation EGFR TKI) in combination with cetuximab (a monoclonal antibody directed against EGFR). Three of four patients showed a partial response with a median progression-free survival (PFS) of 5.4 months. These results led to a single-arm phase II study, in which 37 stage IV NSCLC patients with an EGFR ex20ins mutation were treated with afatinib and cetuximab. Previous treatment with chemo(immuno) therapy and/or EGFR TKIs was permitted but not mandatory. Patients with asymptomatic brain metastases could also be included. The results are shown in **chapter 5**.

The primary endpoint was met as 54% of patients had disease control at 18 weeks. The ORR was 43% with a median PFS of 5.5 months. The combination of afatinib and cetuximab proved to be ineffective in brain metastases. Patients previously treated with osimertinib also had worse outcomes. Afatinib in combination with cetuximab appeared to be more effective as a first-line treatment compared to previously treated patients. Responses were seen across the spectrum of *EGFR* ex20ins mutations. Although this is a positive study and there appears to be synergy between EGFR TKIs and EGFR-targeted mAbs, the role of this combination will be limited given its toxicity profile. 54% of patients experienced ≥ grade 3 adverse events, 68% required a dose reduction and 16% discontinued treatment due to adverse events. Other combinations of EGFR TKIs with mAbs such as lazertinib plus amivantamab are also currently being tested.

Meanwhile, the field of EGFR TKIs, specially designed to treat *EGFR* ex20ins mutations, is in full development. Mobocertinib, a selective TKI targeting *EGFR* and *HER2* showed a modest ORR of 28% in patients already treated with platinum-based chemotherapy. However, this treatment turned out to be quite toxic and was therefore not registered in Europe, unlike in America. In addition, a bispecific mAb directed against both EGFR and cMET has also been registered and is currently available in second line after failure of platinum-based chemotherapy. Responses are slightly higher with an ORR of 37% and a median PFS of 6.9 months. However, toxicity is also a concern here, with in addition to EGFR-related toxicity also transfusion reactions. Also, this treatment requires biweekly infusions which is quite burdensome for patients. In addition to the aforementioned furmonertinib, there are other EGFR TKIs in development such as sunvozertinib that are much more promising with higher response rates, a more favorable toxicity profile and good intracerebral activity.

Although all *EGFR* ex20ins mutations are seen as one group when testing new targeted treatment options, it is actually a very heterogeneous group with more than 60 different variants. For some targeted treatment options, it has been shown that the effect differs depending on the position of the *EGFR* mutation, but with most TKIs no difference in effectiveness is seen within the entire spectrum of *EGFR* ex20ins. Yet it does not seem right to consider this group as an identical entity. In **chapter 6** we present an overview of all available preclinical and clinical results of current treatment options seen from the different insertion positions. The limitations of this overview were in particular the fact that in most studies only the response for the entire group was reported and not specified by mutation type. In addition, the varying use of nomenclature made it difficult to interpret and compare such data. Perhaps modeling the structure and function of a specific *EGFR* ex20ins variant can also contribute to predicting responses, but this technique needs further investigation.

PART II. Analysis and treatment of resistance mechanisms after third generation EGFR TKI treatment.

In the second part of this thesis we tried to gain new insights into acquired resistance mechanisms that occur after treatment with a third generation EGFR TKI in NSCLC patients with a classical *EGFR* mutation. Third-generation EGFR TKIs are very effective and currently the standard first-line treatment option for *EGFR*-mutated NSCLC, but eventually almost everyone develops resistance. At the start of this thesis, only limited literature was available on these mechanisms and was mostly based on circulating tumor (ct) DNA.
In **chapter 7**, we analyzed serial biopsies of patients with an *EGFR* T790M resistance mutation who were subsequently treated with a third generation TKI to gain more insight into the innate and acquired resistance mechanisms. Tumor heterogeneity turned out to be one of the most important factors. After loss of *EGFR* T790M, *cMET* amplification was found to be the most common resistance mechanism in 29% of patients.
In **chapter 8**, we present a retrospective case series in which eight patients with acquired *cMET* amplification after EGFR-directed treatment with a third-generation EGFR TKI were treated with crizotinib. Crizotinib (a cMET inhibitor) was added to treatment with a third-generation EGFR TKI. This proved effective with an ORR of 50%; however, responses were short-lasting and often heterogeneous with mixed responses.

Future perspectives

Chapter 9 provides a general discussion of the previously described findings in this thesis and expectations for the future. With regard to *EGFR* ex20ins mutations, the developments of furmonertinib and sunvozertinib are currently the most promising. In addition, various new TKIs are currently being tested, mainly of which only pre-clinical data is available. It remains a challenge to find effective compounds with intracerebral activity and a favorable toxicity profile. Although platinum-based chemotherapy is currently the first-line treatment of choice, this is expected to change in the short term.

The role of immunotherapy in this group of patients needs to be further investigated. It seems less effective, but there is not yet enough data available. The role of combination treatments is also not yet clear. For example, studies are already underway in which amivantamab or other EGFR TKIs are combined with chemotherapy in first-line. The balance between toxicity and effectiveness will be important to determine the first-line treatment of choice. In order to identify the best next-line treatment after failure of targeted treatment, more knowledge is needed regarding resistance mechanisms after *EGFR* ex20ins-directed therapy.

Also for classical *EGFR* mutations, more research is needed regarding the best treatment options after failure of first-line treatment with osimertinib. Due to tumor heterogeneity, treatments aimed at one resistance mechanism often only work briefly or partially. That is why more research is being done into combination treatments in which chemotherapy is added to a TKI in the case of acquired resistance. However, such treatments are associated with significantly increased toxicity.

Preventing or delaying resistance by starting in first-line with combination treatments of an EGFR TKI plus chemotherapy and possibly also a cMET inhibitor appears to be effective with regard to progression-free survival, but whether this ultimately leads to a survival advantage is not yet known. Toxicity and burden for the patient are major limiting factors here. It will be necessary to look for subgroups that could benefit more from such an aggressive treatment, like patients with brain metastases or co-mutations such as TP53 that are associated with worse outcomes. The development of antibody-drug conjugates may also be interesting in the resistance setting and developments in the field of cell therapy and immunotherapy.

Samenvatting

In Nederland komt bij ongeveer 10% van de patiënten met een niet-kleincellig longcarcinoom (NSCLC) een mutatie in de epidermal growth factor receptor (*EGFR*). Deze mutatie wordt overwegend gezien bij niet-rokers, vrouwen en jongere patiënten. Dit proefschrift focust zich op patiënten met gemetastaseerd niet-kleincellig longcarcinoom die gekenmerkt wordt door een moleculaire alteratie in *EGFR*.

Het *EGFR* gen is gelokaliseerd op chromosoom zeven en bestaat uit vier domeinen: een extracellulair domein, een transmembraan domein en twee intracellulaire domeinen, de tyrosine kinase en autofosforylatie regio's. De *EGFR* -mutaties bij NSCLC zijn vrijwel altijd geclusterd in het tyrosine kinase domein tussen exon 18 en 21, rondom de ATP binding pocket.

De meest voorkomende *EGFR* -mutaties zijn de *EGFR* exon 19 in-frame deleties en *EGFR* L858R puntmutaties. Deze worden ook wel de klassieke *EGFR* -mutaties genoemd en bedragen ongeveer 85% van alle *EGFR* -mutaties. Deze mutaties zorgen ervoor dat de kinase activiteit van EGFR ligand-onafhankelijk wordt wat leidt tot ongeremde celgroei. EGFR tyrosine kinase remmers (TKI's) zijn zeer effectief in het remmen van de EGFR autofosforylering door te binden aan de receptor, echter uiteindelijk treedt er resistentie op.

De derde meest voorkomende *EGFR* mutatie betreft de *EGFR* exon 20 insertie (ex20ins) mutatie. Deze mutatie betreft 4 – 12% van alle *EGFR* mutaties. Het is een heterogene groep die bestaat uit meer dan 60 varianten van in-frame inserties of duplicaties binnen het tyrosine kinase domein. De EGFR exon 20 regio bevat twee belangrijke gebieden: de C-helix en de aangrenzende lus. De vroege generatie EGFR TKI's kunnen helaas niet goed binden aan *EGFR* ex20ins mutaties doordat de C-helix naar binnen geduwd wordt richting de plaats waar TKI's kunnen binden. Daarnaast activeren ex20ins mutaties EGFR zonder afname van ATP affiniteit ten opzichte van wild-type EGFR. Hierdoor is het moeilijk om selectief de mutatie te blokkeren. Hoe meer wild-type EGFR ook geblokkeerd wordt, hoe meer bijwerkingen zullen optreden. Bij de start van dit proefschrift waren er nog geen doelgerichte behandelopties beschikbaar voor *EGFR* ex20ins mutaties. In **hoofdstuk 1** wordt een algemene inleiding gegeven over de epidemiologie, de moleculaire veranderingen en eventuele behandelopties van *EGFR* gemuteerd NSCLC, inclusief EGFR ex20ins mutaties.

DEEL 1. Doelgerichte behandeling van *EGFR* exon 20 insertie mutaties

Dit deel van het proefschrift richt zich op het vinden van nieuwe doelgerichte behandelopties voor patiënten met een *EGFR* ex20ins mutatie. Er was reeds bekend dat deze mutatie niet gevoelig was voor eerste generatie EGFR TKI's en slechts zeer beperkt gevoelig voor tweede generatie EGFR TKI's. Wel was er preklinische data beschikbaar waaruit bleek dat *EGFR* ex20ins mutaties mogelijk gevoelig zouden kunnen zijn voor derde generatie EGFR TKI's.
In **hoofdstuk 2** hebben we retrospectief data verzameld van 21 patiënten met een *EGFR* ex20ins mutatie die behandeld werden met osimertinib. Vrijwel alle patiënten werden behandeld met 80 mg osimertinib per dag. Dit bleek nauwelijks effectief met een respons percentage (ORR) van slechts 5%. Ondanks dit lage respons percentage werd wel bij 67% van de patiënten een kleine afname van de tumoren gezien en bij een deel van de patiënten

(38%) stabiliseerde de ziekte voor vijf maanden of langer. Maar concluderend bleek osimertinib in deze dosering onvoldoende effectief en dit werd ook bevestigd in andere studies met responspercentages van 0% tot 6,5%.

In preklinische studies waren er aanwijzingen dat een hogere dosering osimertinib mogelijk effectiever zou kunnen zijn met tevens acceptabele toxiciteit. Vervolgens zijn we een prospectieve fase II studie gestart waarbij patiënten met een *EGFR* exon 20 mutatie behandeld werden met een hoge dosering osimertinib, namelijk 160 mg per dag. Een eerdere behandeling met chemotherapie was toegestaan maar niet verplicht. Patiënten met asymptomatische hersenmetastasen mochten ook geïncludeerd worden, evenals patiënten met puntmutaties in *EGFR* exon 20. **Hoofdstuk 3** beschrijft de resultaten van deze studie waarin 25 patiënten met een EGFR ex20ins mutatie behandeld werden met osimertinib 160 mg per dag. Behandeling met hoge dosis osimertinib gaf een ORR van 28% en 80% van de patiënten hadden een afname van tumor laesies met een mediane responsduur van 5,3 maanden. Hoewel deze studie niet aan haar primaire eindpunt voldeed, heeft toch een kwart van de patiënten baat bij deze behandeling, waarbij ook nauwelijks ernstige bijwerkingen gezien werden, slechts 4% ervaart graad ≥ 3 bijwerkingen. Ook werd er afname van hersenmetastasen gezien bij de drie patiënten met asymptomatische hersenmetastasen bij start van de studiebehandeling. Responses werden gezien over het gehele spectrum van *EGFR* exon 20 mutaties en drie van de vier patiënten met een puntmutatie toonden een respons. Er lijkt dus inderdaad sprake van een dosis-respons relatie bij osimertinib, echter met nog beperkte winst in effectiviteit.

Er zijn momenteel ook andere derde generatie EGFR TKI's in ontwikkeling die speciaal ontwikkeld zijn om wel beter te kunnen binden aan *EGFR* ex20ins mutaties door een iets aangepaste structuur. Furmonertinib is zo'n aangepaste derde generatie TKI en heeft sterke overeenkomsten met osimertinib qua dosering, effectiviteit en toxiciteit. Het wordt momenteel ook getest in hoge dosis, zelfs tot 240 mg per dag. De ORR van furmonertinib 240 mg per dag in behandel-naive patienten bedraagt 78,6% en is daarmee veelbelovend. De lange termijn resultaten zijn nog niet bekend. De toxiciteit in de hoogste dosering is nog beperkt met 13 – 29% graad ≥ 3 bijwerkingen.

Een andere strategie om EGFR ex20ins mutaties te behandelen is het combineren van een EGFR TKI met een monoclonaal antilichaam (mAb) gericht tegen EGFR. Zo wordt gepoogd dubbele EGFR blokkade te bereiken vanaf zowel het extracellulaire domein als intracellulair. In **hoofdstuk 4** tonen we in een case series de resultaten van vier *EGFR* ex20ins positieve NSCLC patiënten die behandeld zijn met afatinib (een tweede generatie EGFR TKI) in combinatie met cetuximab (een monoclonaal antilichaam gericht tegen EGFR). Drie van de vier patiënten toonden een partiële respons met een mediane progressievrije overleving (PFS) van 5,4 maanden. Deze resultaten hebben geleid tot een single-arm fase II studie, waarbij 37 stadium IV NSCLC patiënten met een *EGFR* ex20ins mutatie behandeld werden met afatinib en cetuximab. Eerdere behandeling met chemo(immuno)therapie en/of EGFR TKI's was toegestaan maar niet verplicht. Patiënten met asymptomatische hersenmetastasen mochten ook geïncludeerd worden. De resultaten worden getoond in **hoofdstuk 5**. Het primaire eindpunt werd behaald aangezien 54% van de patiënten ziektecontrole had na 18 weken. De ORR betrof 43% met een mediane PFS van 5,5 maanden. De combinatie van afatinib en cetuximab bleek niet effectief bij hersenmetastasen. Patiënten die eerder behandeld werden met osimertinib, hadden ook slechtere uitkomsten. Afatinib in combinatie met cetuximab bleek effectiever als eerstelijnsbehandeling in vergelijking met eerder behandelde patiënten. Responses werden gezien over het gehele spectrum van *EGFR* ex20ins mutaties. Hoewel dit een positieve studie betreft en er dus synergie lijkt te zijn tussen EGFR TKI's en EGFR-gerichte mAb's, zal de rol van deze combinatie beperkt zijn gezien het bijwerkingenprofiel. 54% van de patiënten ervaarden ≥ graad 3 bijwerkingen, 68% had een dosisaanpassing nodig en 16% staakte de behandeling vanwege bijwerkingen. Andere combinaties van EGFR TKI's met mAb's zoals lazertinib plus amivantamab worden momenteel ook getest.

Ondertussen is het veld van EGFR TKI's, speciaal ontwikkeld om *EGFR* ex20ins mutaties te behandelen, volop in ontwikkeling. Mobocertinib, een selectieve TKI gericht tegen *EGFR* en *HER2* toonde een ORR van 28% in patiënten die reeds behandeld waren met chemotherapie. Deze behandeling bleek echter erg toxisch en heeft daardoor in Europa geen registratie verkregen in tegenstelling tot in Amerika. Daarnaast is er ook een bispecifieke mAb gericht tegen zowel EGFR als cMET geregistreerd en beschikbaar in tweede lijn na platinum-bevattende chemotherapie. De responses zijn iets hoger met een ORR van 37% en een mediane PFS van 6,9 maanden. Echter ook hier is toxiciteit een punt van zorg met naast EGFR gerelateerde toxiciteit ook transfusiereacties en betreft het een intraveneuze behandeling met tweewekelijkse infusen wat voor patiënten behoorlijk belastend is. Er zijn naast furmonertinib nog andere EGFR TKI's in ontwikkeling zoals sunvozertinib die veel meer veelbelovend zijn met hogere respons percentages, een gunstiger toxiciteitsprofiel en goede intracerebrale activiteit.

Hoewel *EGFR* ex20ins mutaties gezien worden als één groep bij het testen van nieuwe doelgerichte behandelopties, betreft het eigenlijk een zeer heterogene groep met meer dan 60 verschillende varianten. Voor sommige doelgerichte behandelopties is aangetoond dat de werking verschillend is afhankelijk van de positie van de *EGFR* -mutatie echter bij de meeste TKI's wordt geen verschil in effectiviteit gezien binnen het gehele spectrum van *EGFR* ex20ins mutaties. Toch lijkt het niet juist om deze groep als een identieke entiteit te beschouwen. In **hoofdstuk 6** hebben we gepoogd een overzicht te presenteren van alle beschikbare preklinische en klinische resultaten van de huidige behandelopties (al dan niet nog enkel in studieverband) gezien vanuit de verschillende insertie posities. De beperkingen van dit overzicht waren met name het feit dat regelmatig enkel de respons voor de gehele groep vermeld werd en niet uitgesplitst per mutatietype. Daarnaast maakt het wisselend gebruik van de nomenclatuur het moeilijk om dergelijke gegevens te interpreteren en vergelijken. Wellicht kan modellering van de structuur en functie van een specifieke *EGFR* ex20ins variant in de toekomst ook nog een bijdrage leveren in het voorspellen van responses, echter deze techniek dient nog verder onderzocht te worden.

DEEL 2. Analyse en behandeling van resistentie mechanismen na behandeling met een derde generatie EGFR TKI

In het tweede deel van dit proefschrift probeerden we nieuwe inzichten te verkrijgen in de verworden resistentiemechanismen die optreden na behandeling met een derde generatie EGFR TKI in NSCLC-patiënten met een klassieke *EGFR* mutatie. Derde generatie EGFR TKI's zijn erg effectief en momenteel de standaard eerstelijns behandeling bij *EGFR* gemuteerd NSCLC, echter uiteindelijk ontwikkelt vrijwel iedereen resistentie. Over deze mechanismen was ten tijde van dit proefschrift slechts beperkte literatuur beschikbaar en meestal gebaseerd op circulerend tumor (ct) DNA.

In **hoofdstuk 7** hebben we seriële biopten geanalyseerd van patiënten met een *EGFR* T790M resistentiemutatie die vervolgens behandeld werden met een derde generatie TKI om meer inzicht te verkrijgen in de primaire en verworven resistentiemechanismen. Tumor heterogeniteit bleek één van de belangrijkste factoren. Na het verlies van *EGFR* T790M,

bleek *cMET* amplificatie het meest voorkomende resistentiemechanisme in 29% van de patiënten.

In **hoofdstuk 8** presenteren we een retrospectieve case series waarbij acht patiënten met verworven *cMET* amplificatie na EGFR gerichte behandeling met een derde generatie EGFR TKI, behandeld werden met crizotinib. Crizotinib (een cMET remmer) werd toegevoegd aan de behandeling met een derde generatie EGFR TKI. Dit bleek effectief met een ORR van 50%, echter de responses hielden kort aan en waren vaak heterogeen waarbij gemengde responses gezien werden met gedeeltelijke afname en toename van tumoren.

Toekomstperspectieven

Hoofdstuk 9 geeft een algemene discussie over de eerder beschreven bevindingen in dit proefschrift en de verwachtingen voor de toekomst. Ten aanzien van *EGFR* ex20ins mutaties zijn de ontwikkelingen van furmonertinib en sunvozertinib momenteel het meest veelbelovend. Daarnaast worden diverse nieuwe TKI's getest momenteel waarvan overwegend nog pre-klinische data bekend is. Het blijft een uitdaging om effectieve compounds te vinden met goede intracerebrale werking en een gunstig bijwerkingen profiel. Hoewel platinum-bevattende chemotherapie nu nog de eerstelijns behandeling van keuze is, is het de verwachting dat dat op korte termijn gaat veranderen.

De rol van immunotherapie in deze groep patiënten dient nog verder uitgezocht te worden. Het lijkt minder effectief maar over de effectiviteit is nog onvoldoende data beschikbaar om hier harde uitspraken over te doen. Verder zal meer onderzoek nodig zijn om te beoordelen wat de beste eerstelijns behandeling zou zijn en eventueel in welke combinatie. Er zijn bijvoorbeeld al onderzoeken gaande waarbij amivantamab of andere EGFR TKI's gecombineerd worden met chemotherapie. Om een beste vervolgbehandeling te kunnen aanwijzen zal meer kennis verkregen moeten worden ten aanzien van resistentie mechanismen na *EGFR* ex20ins gerichte therapie.

Ook bij de klassieke *EGFR* mutaties wordt momenteel veel onderzoek verricht naar de beste behandeling na behandeling met derde generatie EGFR TKI's. Wegens tumorheterogeniteit werken behandelingen gericht op één mechanisme vaak maar kortdurend of gedeeltelijk. Daarom worden steeds meer combinaties getest bijvoorbeeld met de toevoeging van chemotherapie. Echter dergelijke behandelingen gaan wel gepaard met significante toxiciteit.

Het voorkomen of uitstellen van resistentie door al in de eerste lijn te starten met combinatie behandelingen van een EGFR TKI plus chemotherapie en eventueel ook nog een cMET remmer blijkt effectief ten aanzien van progressie vrije overleving, maar of dat uiteindelijk leidt tot een overlevingsvoordeel is nog niet bekend. Ook hierbij is toxiciteit een limiterende factor. Er zal gezocht moeten worden naar subgroepen die meer gebaat zijn bij een dergelijke behandeling zoals patiënten met hersenmetastasen of co-mutaties zoals TP53 die geassocieerd zijn met slechtere uitkomsten.

De ontwikkeling van antibody-drug conjugaten kan tevens interessant zijn in de resistentie setting en de ontwikkelingen op het gebied van cel therapie en immunotherapie.

Part IV

Appendices

List of publications

This thesis

van Veggel B, Madeira R Santos JFV, Hashemi SMS, Paats MS, Monkhorst K, Heideman DAM, Groves M, Radonic T, Smit EF, Schuuring E, van der Wekken AJ, de Langen AJ. Osimertinib treatment for patients with EGFR exon 20 mutation positive non-small cell lung cancer.
Lung Cancer. 2020 Mar;141:9-13.

Zwierenga F, **van Veggel B**, Hendriks LEL, Hiltermann TJN, Hiddinga BI, Hijmering Kappelle LBM, Ter Elst A, Hashemi SMS, Dingemans AC, van der Leest C, de Langen AJ, van den Heuvel MM, van der Wekken AJ. High dose osimertinib in patients with advanced stage EGFR exon 20 mutation-positive NSCLC: Results from the phase 2 multicenter POSITION20 trial.
Lung Cancer. 2022 Aug;170:133-140.

van Veggel B, de Langen AJ, Hashemi SMS, Monkhorst K, Heideman DAM, Thunnissen E, Smit EF. Afatinib and Cetuximab in Four Patients With EGFR Exon 20 Insertion-Positive Advanced NSCLC.
J Thorac Oncol. 2018 Aug;13(8):1222-1226.

van Veggel BAMH, van der Wekken AJ, Paats MS, Hendriks LEL, Hashemi SMS, Daletzakis A, van den Broek D, Bosch LJW, Monkhorst K, Smit EF, de Langen AJ. A phase 2 trial combining afatinib with cetuximab in patients with EGFR exon 20 insertion-positive non-small cell lung cancer.
Cancer. 2023 Oct 31; Epub ahead of print.

Zwierenga F, **van Veggel BAMH**, van den Berg A, Groen HJM, Zhang L, Groves MR, Kok K, Smit EF, Hiltermann TJN, de Langen AJ, van der Wekken AJ. A comprehensive overview of the heterogeneity of EGFR exon 20 variants in NSCLC and (pre)clinical activity to currently available treatments.
Cancer Treat Rev. 2023 Nov;120:102628.

Michels S, Heydt C, **van Veggel B,** Deschler-Baier B, Pardo N, Monkhorst K, Rüsseler V, Stratmann J, Griesinger F, Steinhauser S, Kostenko A, Diebold J, Fassunke J, Fischer R, Engel-Riedel W, Gautschi O, Geissinger E, Haneder S, Ihle MA, Kopp HG, de Langen AJ, Martinez-Marti A, Nogova L, Persigehl T, Plenker D, Puesken M, Rodermann E, Rosenwald A, Scheel AH, Scheffler M, Spengler W, Seggewiss-Bernhardt R, Brägelmann J, Sebastian M, Vrugt B, Hellmich M, Sos ML, Heukamp LC, Felip E, Merkelbach-Bruse S, Smit EF, Büttner R, Wolf J. Genomic Profiling Identifies Outcome-Relevant Mechanisms of Innate and Acquired Resistance to Third-Generation Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor Therapy in Lung Cancer.
JCO Precis Oncol. 2019 Mar 27;3:PO.18.00210.

van Veggel B, de Langen AJ, Hashemi S, Monkhorst K, Rosenberg EH, Heideman DAM, Radonic T, Smit EF. Crizotinib treatment for patients with EGFR mutation positive NSCLC that acquire cMET amplification after EGFR TKI therapy results in short-lived and heterogeneous responses.
Lung Cancer. 2018 Oct;124:130-134.

Other publications

van Veggel BAMH, van Oort IM, Witjes JA, Kiemeney LA, Hulsbergen-van de Kaa CA. Quantification of extraprostatic extension in prostate cancer: different parameters correlated to biochemical recurrence after radical prostatectomy.
Histopathology. 2011 Oct;59(4):692-702.

van Veggel BAMH, Biesma B, Smit EF. Pharmacotherapy for treatment of lung cancer in the elderly.
Expert Opin Pharmacother. 2015 May;16(7):1021-34.

van Veggel B, de Langen AJ. A reply to “Comment on: ‘Osimertinib treatment for patients with EGFR exon 20 mutation positive non-small cell lung cancer”.
Lung Cancer. 2020 Oct;148:181.

Boosman RJ, Jebbink M, Veldhuis WB, Groenland SL, **van Veggel BAMH,** Moeskops P, de Langen AJ, Beijnen JH, Smit EF, Huitema ADR, Steeghs N. Exposure-Response Analysis of Osimertinib in EGFR Mutation Positive Non-Small Cell Lung Cancer Patients in a Real-Life Setting.
Pharm Res. 2022 Oct;39(10):2507-2514.

F. Zwierenga, L. Zhang, J. Melcr, E. Schuuring, **B.A.M.H. van Veggel**, A.J. de Langen, H.J.M. groen, M.R. Groves, A.J. van der Wekken. The prediction of treatment outcome in NSCLC patients harboring an EGFR exon 20 mutation using molecular modeling.
Lung Cancer. 2024 Sept; article in press.

Curriculum vitae

Bianca van Veggel werd op 31 augustus 1984 geboren in Deurne. In 2003 behaalde zij haar gymnasium diploma aan het Udens Collega in Uden. In hetzelfde jaar startte ze met haar studie geneeskunde aan de Radboud Universiteit in Nijmegen. In 2011 begon zij met haar opleiding tot longarts in het Jeroen Bosch Ziekenhuis in 's-Hertogenbosch onder begeleiding van Dr. A.J.M. Schreurs. In 2017 startte zij tijdens haar opleiding met een promotieonderzoek, onder leiding van prof. dr. E.F. Smit en dr. A.J. de Langen. In 2019 studeerde zij af als longarts en was nadien werkzaam als chef de clinique in hetzelfde ziekenhuis. In 2020 maakte zij de overstap naar het Antoni van Leeuwenhoek waarbij ze klinische taken combineerde met promotieonderzoek binnen een fellowship longoncologie. Sinds 2022 is zij weer werkzaam in het Jeroen Bosch Ziekenhuis als algemeen longarts met als aandachtgebied longoncologie. Bianca woont samen met haar partner Jasper en hun drie kinderen Norah, Romée en Amy.

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Dit proefschrift heeft niet tot stand kunnen komen zonder de medewerking van alle patiënten die belangeloos hebben deelgenomen hebben aan de onderzoeken.
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