Anti-tumour Treatment

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 and 7 amino acids (3 and 21 base pairs). Most EGFRex20+ mutations (90 %) affect the loop following the C-helix (amino acids 767–775). 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 V769D_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 harboring EGFRex20+ mutations are similar to
patients with common EGFR mutations, i.e. being more common in women 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-ac 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.

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. 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.

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 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. In general, mutations targeting the loop following the C-helix were resistant to 1st and 2nd generation TKIs, but more sensitive to 3rd generation TKIs. However, the 2nd generation TKI poziotinib shows an increased sensitivity in Ba/F3 cells carrying mutations in the far-loop. TAS6417 (CLN-081) is a 3rd generation TKI with a unique core unit that effectively targets all exon20 insertions.

### 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_Y769dup (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 produg tarloxotinib. Additional, mobocertinib also had a clear inhibitory potency on CUTO14 cells carrying the A767_Y769dupASV 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%).

### 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 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) [18,24,25]. 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

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**Fig. 5.** 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. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
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). The most common treatment-related Grade ≥ 3 AEs were rash (28–34 %) and diarrhea (22–26 %) [26,27]. 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 [28].

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 (80 mg daily), the clinical evidence regarding efficacy in EGFRex20+ NSCLC patients is disappointing [29,30]. Four retrospective cohort studies have mentioned tumor response to osimertinib 80 mg in relation to different EGFRex20+ mutations for a total of 109 patients [30-33] (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 160 mg. 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) [34]. 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) [35]. 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 80 mg [35].

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 1/2 trial investigated a daily dose of 160 mg in 28 EGFRex20+ NSCLC patients [36] (Fig. 6). Overall, Riely et al observed a confirmed ORR of 43 % and a mPFS of 7 months in patients with different EGFRex20+ mutations [36]. 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 [37]. 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 [38]. 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...
Fig. 5. Clinical benefit with 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} generation TKIs based on tumor responses and mPFS in NSCLC patients across different EGFRex20\textsuperscript{+} 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\textsuperscript{+} 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\textsuperscript{+} 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. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
after platinum-based chemotherapy ([39] “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 [40].

The hsp90 inhibitors luminespib and onalespib (tested in combination with erlotinib), showed limited responses in patients with EGFRex20+ NSCLC ([38] Fig. 6). Overall, the ORR of luminespib was 17 %, and mPFS was 2.9 months (95 % CI, 1.4–5.6) [41]. The trial testing the combination onalespib/erlotinib was closed early due to dose limiting toxicities, mainly diarrhea, and a disappointing clinical activity ([Fig. 6] [42].

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 [43] (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 [44].

Discussion

EGFRex20+ NSCLC patients are currently treated as a single disease entity, irrespective of the nature and location of the mutation. We have

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**Table:**

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**Fig. 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. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
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_Y767insFQEA 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 (160 mg). 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 a 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 to poziotinib, osimertinib, CLN-081 and mobocertinib. Consistent with these findings, patient-derived cell lines and patients carrying these mutations also were sensitive to osimertinib (160 mg in patients) and mobocertinib. Thus, osimertinib at a dose of 160 mg 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 (Sup. 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_Y767insFQEA) are structurally different from other exon 20 mutations. This mutation was reported to shift the β3-σ 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_Y767insFQEA 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_Y767insFQEA 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 (180 mg).

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 [45]. 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 [46]. 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) [47]. 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 [48]. Also, treatment with immune checkpoint inhibitors (ICI) as single agents have limited efficacy in oncogene-addicted NSCLC [49]. 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) [50,51]. 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:NCT04366882) evaluating CLN-081 reported 6 confirmed PR and 2 unconfirmed PR among 25 evaluable patients [52]. 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 [53]. In the cohort of 59 patients harbouring an EGFRex20+ mutation, 31 patients were treated with the recommended phase 2 dose of 300 mg once daily. The ORR was 48.4 % (15/31), and disease control rate (DCR) was 90.3 % (28/31) across different EGFRex20+ mutations [54]. 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 [55]. 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 unspecific 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 [56]. 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 included in the reviewed studies. Moreover, in case reports 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 (160 mg). The EGFRex20+ mutations should not be analyzed as an exoned-based group, but a structural understanding of the impact of mutations and patients-derived cell lines is warranted.

CRediT authorship contribution statement

Fenneke Zwieonga: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Project administration. Bianca A.M.H. van Veggel: Formal analysis, Investigation, Writing – review & editing. Anke van den Berg: Investigation, Writing – review & editing. Harry J.M. Groen: Writing – review & editing. Lili Zhang: Investigation, Writing – review & editing. Matthew R. Groves: Writing – review & editing. K. Koel: Data curation, Writing – review & editing. E.F. Smit: Investigation, Writing – review & editing. T. Jeroen N. Hiltemann: Investigation, Writing – review & editing. Adrianus J. de Langen: Writing – review & editing. Anthonie J. van der Wekken: Conceptualization, Methodology, Investigation, Writing – review & editing. Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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References


