Uncommon EGFR mutations in advanced non-small cell lung cancer

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ABSTRACT

Molecular profiling in advanced non-small cell lung cancer (NSCLC) has allowed for the detection of actionable mutations, which has revolutionized the treatment paradigm in this highly fatal disease. Mutations involving the epidermal growth factor receptor (EGFR) gene are most common and the ‘classical mutations’, exon 19 deletions and the point mutation L858R at exon 21, predict response to EGFR tyrosine kinase inhibitors (TKIs). The ‘uncommon’ EGFR mutations account for 10–18% of all EGFR mutations and primarily consist of exon 20 insertions, exon 18 point mutations and complex mutations. Improved detection techniques have broadened the spectrum of reported aberrations within the ‘uncommon group’ but response to TKIs is variable and not fully elucidated. This review provides an overview of the biology and incidence of uncommon EGFR mutations and summarizes reported outcomes when treated with EGFR-TKIs.

1. Introduction

Non-small cell lung cancer (NSCLC) represents over 85% of all lung cancers and is associated with a high mortality [1]. Five-year survival for all stages is approximately 17% and in stage IV disease, less than 5% of patients are alive at five years. The identification of specific somatic aberrations has led to a personalized therapeutic approach and has resulted in improved outcomes. Activating mutations in the epidermal growth factor receptor (EGFR) gene were first identified in 2004 [2–4], and can be detected in approximately 10–15% of Caucasians and up to 50% of Asian patients with NSCLC [5]

In frame deletions of amino acids LREA of exon 19 and the exon 21 L858R point mutation are considered the ‘classical’ mutations, accounting for 85% of EGFR mutations [6] and are associated generally with being female, a non-smoker and having adenocarcinoma histology [7,8]. In patients with advanced NSCLC, both first and second-generation EGFR tyrosine kinase inhibitors (TKIs) are standard first-line treatment options [9]. This evidence arises from a number of phase III trials, which demonstrated superior progression-free survival (PFS) and objective response rates (ORRs) together with improved tolerability when compared to first line platinum doublet chemotherapy [7,8,10–15]. In the pooled analysis from the Lux-Lung 3 and Lux-Lung 6 studies, afatinib has also been shown to improve overall survival in the exon 19 deletion population [16]. Most of these trials randomized patients with classical mutations and only four included patients with uncommon EGFR mutations, defined as all mutations excluding del19 and L858R. In these trials, uncommon mutations represented approximately 10% of the EGFR population [8,10,13,14]. In other studies they have accounted for up to 18% of the EGFR mutated cohort [17]. The predictive value of the uncommon mutations to TKIs in these large trials could not be determined due the small numbers enrolled. Thus, we are thus reliant on pooled analyses and small case series to evaluate EGFR-TKI response in this highly heterogeneous group.

An understanding of TKI response is imperative for physicians in treatment decision-making.

This review aims to provide an overview of the biology of the epidermal growth factor receptor and its associated driver mutations. We assess the prevalence and outcomes of EGFR mutations, excluding deletion 19 and L858R and the most common resistance mutation, T790M.

2. Literature search

PubMed was searched using the following key words: non small cell lung cancer, epidermal growth factor receptor, uncommon mutation, rare mutation, exon 20, exon 18, exon 19, exon 21, erlotinib, gefitinib, afatinib and osimertinib. Only articles in English were included. If uncommon mutations were grouped together in publications, individual exon mutations were reported where possible and grouped separately from complex mutations unless stated. T790M mutations were not included either as single or complex mutations unless stated. PFS and overall survival (OS) are only reported if calculated from the...
time of advanced disease or commencement of EGFR-TKI.

3. The epidermal growth factor receptor and mechanism of TKI response

EGFR or HER1/ErbB1 is a member of the ErbB tyrosine kinase (TK) family and structurally has four domains: an extracellular ligand binding domain, an alpha-helix transmembrane domain, a cytoplasmic TK domain and a carboxy-terminal signaling domain [18]. Binding of EGFR to its ligands causes homo and heterodimerization, culminating in autophosphorylation of the TK receptor. Downstream cascade activation of pathways such as phosphatidylinositol-3-kinases (PI3K)/protein kinase B (AKT) and extracellular-signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK), results in cellular proliferation and survival [19,20]. The TK binding domain is tightly regulated so that in its inactive state, its structure is altered to prevent autoactivation. EGFR gene mutations, increased gene copy number and over-expression of EGFR proteins can however lead to constitutive TK activity and carcinogenesis [21].

In NSCLC, mutations found within exons 18–21 (93% of mutations), which code the TK domain, can lead to kinase activation in the absence of ligand binding [22]. Three types of mutation exist, Class I mutations are represented by short exon 19 in-frame deletions, class II mutations are depicted by single-nucleotide substitutions and class III mutations include insertions or duplications [6]. Complex or compound mutations, defined as more than one EGFR mutation within the same tumor specimen also are apparent in NSCLC [23–25]. More recently comprehensive profiling also has revealed rare genomic variants including duplications in the EGFR exon 18–25 domain (EGFR-KDD) and EGFR rearrangements [26,27].

The classical EGFR mutations (del19 and L858R) are known to decrease the affinity of the kinase for ATP allowing for preferential EGFR-TKI binding [28]. The first-generation TKIs reversibly bind to the ATP site and prevent phosphorylation, inhibiting both wild-type and mutated EGFR [29]. Despite this, first generation TKIs have a higher binding affinity to mutated EGFR ensuring a potent response [30]. Afatinib, a second-generation TKI, irreversibly binds to the ATP site of both EGFR, HER2 and HER4 [31]. Response rates to EGFR-TKIs in patients with classical mutations are approximately 60–80%, but acquired resistance develops in the majority of patients [8,10]. The emergence of the T790M mutation in exon 20 is the cause of resistance in over 50% of cases, remaining molecular mechanisms include MET amplification, PI3K mutations or epithelial mesenchymal transition (EMT) signaling, but are not fully known [32–35]. The T790M mutation returns preferential binding of the receptor to ATP, rendering the first generation TKIs less effective [36]. De novo T790M mutations confer resistance to first generation TKIs [37]. Afatinib initially showed promise in pre-clinical models in targeting T790M but in the clinical setting has failed. In order to achieve clinical efficacy with afatinib, much higher doses are required which is not attainable given the associated toxicity [31,38]. Third generation TKIs including osimertinib are potent inhibitors of T790M and to a large extent, spare WT EGFR [39]. Osimertinib has demonstrated an ORR of 60% in patients with the secondary T790M resistance mutation [40]. Other third generation TKIs under evaluation include nazartinib (EGF816) and ASP8273. Despite the promise of 3rd generation TKIs, mutations in EGFR – C797X can escape these therapies, highlighting the complexity of the EGFR mutational landscape [41].

4. Molecular testing for EGFR mutations

Testing for EGFR mutations is DNA based and can be performed on tissue obtained from diagnostic biopsies, surgical resections and even cytology specimens. Success rates for obtaining adequate sample for institutional analysis has ranged from 30% to 80% and is influenced by the quality of the sample, the assay used and resources within institutional molecular laboratories [42]. Initial testing utilized Sanger’s sequencing in isolated DNA from formalin-fixed paraffin embedded (FFPE) tissue but required at least 25% tumor cellularity. Direct sequencing of polymerase chain reaction-amplified genomic DNA corresponding to exons 18–22 of the EGFR gene is the minimum standard for mutation detection, however more sensitive PCR-based methods exist [43,44]. Commercial assays such as Therascreen (Qiagen Manchester, UK) and Cobas (Roche, Basel, Switzerland), which consider ‘hot spots’ thought predictive of TKI response, are widely utilized [45]. More recently, droplet digital PCR (ddPCR) and next generation sequencing (NGS) coupled with exon-capture strategies have been shown to have increased sensitivity down to 0.01% tumor cellularity. Liquid biopsies currently are being evaluated as a mechanism of detecting EGFR mutations in circulating tumor (ct) DNA and have the potential to replace or at least complement repeat tissue biopsies. The heterogeneity in detection techniques likely has resulted in inaccuracies and biases in the reported incidence of less common EGFR mutations.

5. The uncommon mutations and response to TKIs

5.1. Exon 20 insertions

Exon 20 insertions in EGFR are the most prevalent of the uncommon EGFR mutations and can be found in approximately 1.5–2.5% of all non-small cell lung cancers but account for approximately 10% (1–17%) of the mutated EGFR population [6,24,46–49]. Similar to classical mutations, exon 20 insertions are associated with female sex, Asian ethnicity and never-smoking patients [49–53]. There have been over 100 exon 20 insertions reported in the Wellcome Trust Sanger Institute catalog of somatic mutations in cancer (COSMIC), the most commonly detected mutations include: V769_D770insASV, D770_N771insNPG, D770_N771insSVD, H773_V774insH and A763_Y764insQEA.

5.1.1. Preclinical evidence

In vitro studies have shown differences in the affinity of exon 20 insertions to EGFR-TKIs, which may be due to the location of the insertion within the C-helix. Arcila et al. demonstrated through in silico modeling that insertions between codons 769–775 predict TKI resistance whereas insertions in more proximal codons may remain sensitive [52]. The A763_Y764insQEA has demonstrated sensitivity to erlotinib in cell lines at concentrations less than 0.1 μM [51]. This particular mutation is thought to cause a structural change in the ATP binding cleft similar to EGFR-L858R mutation. Hirano et al. reported higher potency for afatinib in this particular mutation with an IC50 of 8 nM where IC50 values for erlotinib and osimertinib were 45 and 40 nM, respectively. In contrast to A763_Y764insQEA the majority of exon 20 insertions differ structurally from the classical mutations and in fact do not affect ATP binding but instead induce kinase activation by forming a wedge at the end of the C-helix. Affinity to TKIs in these cases is, therefore, similar to wild-type (WT) EGFR and resulting in resistant to first generation TKIs [51]. Interestingly one study did show that osimertinib had a higher specificity than afatinib with a wide therapeutic window in the exon 20 insertions studied (Y764_V765insHH, A767V_Y769dupASV, and D770_N771insNPG).

The IC50 values were much higher than those of T790M, suggesting dosing differences may be required for efficacy. A further exon 20 variant was tested in a patient derived xenograft model (PDX) (EGFR H773_V774insNPH) and only showed partial sensitivity to afatinib and osimertinib. In contrast, EGFR816 has shown both in vitro and in vivo efficacy in variants D770_N771insSVD, V769_D770insASV, and H773_V774insNPH. Specifically in a PDX model, higher dosing (100 mg/kg) resulted in tumor regression of 81% [54]. AP32788 is a potent selective inhibitor of EGFR and Her2 which is also been shown to inhibit exon20 insertions in BA3 cell lines [55]. More recently, it has been revealed that EGFR amplification may occur in a subset of exon 20 insertions.
insertions. The dual EGFR blockade with osimertinib and cetuximab has demonstrated significant growth inhibition in in vivo models [56]. Other classes of drugs tested preclinically include Heat Shock Protein (HSP) inhibitors [57]. HSP90 is a chaperone protein integral to correct folding and stabilization of cellular proteins including mutant EGFR [58, 59].

5.1.2. Clinical evidence

Retrospective studies have evaluated patients with advanced disease, whose tumors harbor exon 20 insertions and have demonstrated

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical outcomes in exon 20 insertions/duplications to EGFR-TKIs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong>, number in study</td>
<td><strong>No. variants reported</strong></td>
</tr>
<tr>
<td>Yang (2016) [60]</td>
<td>27</td>
</tr>
<tr>
<td>Chen (2016) [67]</td>
<td>29</td>
</tr>
<tr>
<td>Klughammer (2016) [71]</td>
<td>9</td>
</tr>
<tr>
<td>Naidoo (2015) [61]</td>
<td>46</td>
</tr>
<tr>
<td>Woo (2014) [81]</td>
<td>7</td>
</tr>
<tr>
<td>Beau-Faller (2014) [50]</td>
<td>38</td>
</tr>
<tr>
<td>Arcila (2013) [52]</td>
<td>33</td>
</tr>
<tr>
<td>Yasuda (2013) [51]</td>
<td>19</td>
</tr>
<tr>
<td>Oxnard (2013) [53]</td>
<td>27</td>
</tr>
<tr>
<td>Wu (2011) [82]</td>
<td>25</td>
</tr>
<tr>
<td>Lund-Iverson (2012) [83]</td>
<td>7</td>
</tr>
<tr>
<td>Sasaki (2007) [46]</td>
<td>7</td>
</tr>
</tbody>
</table>

NA: not applicable, NR: not reported, TTP: time to treatment progression, TTF: time to treatment failure.

* Includes 3 complex.

* Predominantly complex mutations (n = 7).

Table 2

Clinical outcomes in exon 20 point mutations and response to EGFR-TKIs.

<table>
<thead>
<tr>
<th><strong>N</strong>, number in study</th>
<th><strong>N included</strong></th>
<th><strong>Specific mutation (N)</strong></th>
<th><strong>No. evaluable TKI used</strong></th>
<th><strong>ORR (%) Mutation with response</strong></th>
<th><strong>Median PFS (months) (range)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang (2016) [60]</td>
<td>8</td>
<td>N = 8 S768I (1) S768I + G719X (7)</td>
<td>8</td>
<td>Afatinib</td>
<td>100%</td>
</tr>
<tr>
<td>Chen (2016) [67]</td>
<td>10</td>
<td>N = 10 S768I (3) S768I + del19 (3) S768I + L858R (4)</td>
<td>10</td>
<td>Erlotinib, gefitinib, icotinib</td>
<td>20%</td>
</tr>
<tr>
<td>Chiu (2015) [66]</td>
<td>17</td>
<td>N = 7 S768I (7)</td>
<td>7</td>
<td>1st gen.</td>
<td>33%</td>
</tr>
<tr>
<td>Wu (2011) [82]</td>
<td>6</td>
<td>N = 4 V774A (1) V774F (1)</td>
<td>4</td>
<td>1st gen.</td>
<td>25% V774A</td>
</tr>
<tr>
<td>Chou (2005) [68]</td>
<td>3</td>
<td>N = 3 A763V (1) V765A (1)</td>
<td>3</td>
<td>Gefitinib</td>
<td>66% V765A, T783A</td>
</tr>
</tbody>
</table>

NR: not reported.

* Predominantly complex mutations (n = 7).

* Includes 7 complex mutations.

* 10 G719X + S768I reported in Table 5.
Although there were no responses (to afatinib were only 8.7% and the median OS was 9.2 months. which revealed that among 23 patients, objective response rates (ORR) superior median OS of 31 months (9.3 months reported in a small study), the TKI resistance but favorable survival outcome associated with this variant has been corroborated by other studies [50]. The activity of third generation TKIs in preclinical models has led to clinical trials for exon 20 insertions including the phase I/II study of AP32788 (NCT02716116). HSP90 inhibitors such as AUY-922 are also promising with a median PFS of 6.1 months reported in a small study [62]. A phase II study is recruiting (NCT01854034).

In summary, exon 20 insertions, the third most common EGFR mutation, exist as a very heterogeneous group with the majority resistant to EGFR-TKIs. Newer generation TKIs may be more active in this subgroup and a concerted effort to align these patients in a trial is required.

5.2. Exon 20-point mutations

The single S768I mutation represents approximately 1% of all EGFR mutations but are the next most frequent mutation within exon 20. These mutations, however, often exist within compound mutations. A number of other point mutations within exon 20 have been reported but are rare.

5.2.1. Preclinical

NSCLC cell lines harboring mutations in S768I are not available. In vitro studies investigating TKI affinity have used Bx/F3 cell lines, which are thought, to be representative and have for the mutation S768I, demonstrated superiority with afatinib compared to erlotinib and osimertinib, with IC50 values 0.7 nM, 146 and 49 nM respectively.

Table 4
Clinical outcomes in exon 21-L861Q treated with EGFR-TKIs.

<table>
<thead>
<tr>
<th>N, number in study</th>
<th>N included Specific mutation (N)</th>
<th>No. evaluable TKI TKI exposure</th>
<th>ORR (%) Mutation with response</th>
<th>Median PFS (months) (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al. (2016) [60]</td>
<td>N = 16 L861Q (12)</td>
<td>Afatinib</td>
<td>56.8%*</td>
<td>8.2 months (4.5–16.6)</td>
</tr>
<tr>
<td>Wu (2011) [82]</td>
<td>N = 7 L861Q (6)</td>
<td>Gefitinib</td>
<td>71%</td>
<td>8.7 months (2.8–37.5)</td>
</tr>
<tr>
<td>Chiu (2015) [66]</td>
<td>N = 3 L861Q (3)</td>
<td>Gefitinib</td>
<td>50%</td>
<td>6.4 + 10.6 months</td>
</tr>
<tr>
<td>Watanabe (NEJ002) (2014) [85]</td>
<td>N = 3 L861Q (3)</td>
<td>Gefitinib</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

* 4 complex mutations included.

primary resistance to TKIs (Table 1). The only prospective data arises from the post hoc analysis of LUX-Lung 2, LUX-Lung 3 and LUX-Lung 6 which revealed that among 23 patients, objective response rates (ORR) to afatinib were only 8.7% and the median OS was 9.2 months. Although there were no responses (n = 4) to platinum-based chemotherapy, exon 20 insertions treated with platinum doublets had a superior median OS of 31 months (9.3–42.3 months) [60]. Higher response rates to chemotherapy in exon 20 insertions have been revealed in other retrospective studies. Naidoo et al. reported an ORR of 63% (22 of 35) to platinum doublet chemotherapy compared to an ORR of 27% (3 of 11) in patients treated with erlotinib. Overall survival was 19.5 months in all patients treated. Another small study reported RRs of 58% (5 of 12 pts.) when chemotherapy was used vs. 0% when patients were treated with EGFR-TKIs and also showed an improved time to treatment failure (TTF) of 5.9 vs. 2.4 months [53].

The A763_Y764insFQEA appears to be predictive of TKI response both preclinically and clinically. In the recent study by Klughammer et al., one patient with an A763_Y764insFQEA mutation treated with second-line erlotinib had a partial response (PR) lasting 17.5 months and an OS of 24 months. Others report PFS of 3.2 months [61] and 9 months [50] despite favorable survival of 25 and 17.5 months, respectively. Other insertions with evidence of TKI response include EGFR V769_D770insASV (TTP = 19.8 months, OS = 24 months) [61], and EGFR-N771delinsKPP (TTP = 8 months and OS 10 months). Two patients with EGFR-D770_N771insSVD enrolled in the SATURN study and treated with erlotinib had short PFS intervals of 2.8 and 4.2 months but an extended OS of 19.7 and 29 months. The TKI resistance but favorable survival outcome associated with this variant has been corroborated by other studies [50]. The activity of third generation TKIs in preclinical models has led to clinical trials for exon 20 insertions including the phase I/II study of AP32788 (NCT02716116). HSP90 inhibitors such as AUY-922 are also promising with a median PFS of 6.1 months reported in a small study [62]. A phase II study is recruiting (NCT01854034).

In summary, exon 20 insertions, the third most common EGFR mutation, exist as a very heterogeneous group with the majority resistant to EGFR-TKIs. Newer generation TKIs may be more active in this subgroup and a concerted effort to align these patients in a trial is required.
patients had complex mutations including patients with S768I included, responded to afatinib, seven of eight LUX-Lung 2, LUX-Lung 3 and LUX-Lung 6 although 100% of the included complex mutations. In the Yang et al. response to first generation TKIs [65]. It is notable that most retrospective studies reporting outcomes [66]. In contrast, in the study by Chen et al., despite the presence of a classical mutation in seven of 10 patients with S768I, the response rate to first generation TKIs overall was only 20% [67]. Single mutations may have inferior outcomes compared to tumors with complex S768I mutations. Afatinib could be considered as the choice of EGFR-TKI in this cohort. Other point mutations in exon 20 with reported sensitivity include V765A, V774A and T783A although of G. M. O’Kane et al. Lung Cancer 109 (2017) 137–144

Table 5
Clinical outcomes in complex mutations excluding co-occurring T790M mutations, treated with EGFR-TKIs.

<table>
<thead>
<tr>
<th>Authors (Year)</th>
<th>N, number in study</th>
<th>N included Specific mutation (N)</th>
<th>No. evaluable TKI exposure</th>
<th>ORR (%) Mutation with response</th>
<th>Median PFS (months) (range)</th>
</tr>
</thead>
</table>
| Chiu (2015)   | 19                | N = 19
G719X + L861Q (9)
G719X + S768I (10) | 19 1st gen.
G719X + L861Q (8) | 88.9% | 11.9 months |
| Cheng (2015)  | 5                 | N = 5
G719X + L819Q (1)
E709X + other (2)
V668L + L858R (1)
G724S + S768I (1) | 5 1st gen.
G719S + L861Q | 90% | RFS |
| Faller (2014) | 8                 | N = 5
G719X + S768I (2)
G719X + L861Q (1) | 5 1st gen.
G719X + other (3) | 60% | TTP |
| Back (2015)   | 23                | N = 23
G719X + Other (12)
G719X + other or other (11) | 23 1st gen.
Classical + other (9) | 78% | 7.4 months |
| Hata (2010)   | 21, 4            | N = 16
Del19 + L858R (8)
G719S + L858R (6)
Del 19 + L861Q (1)
Del 19 + L819Q (1) | 12 Gefitinib
Del19 + L858R (6)
Del19 + L861Q (1)
L833Y + L858R (1) | 67% | 9.7 months |
| Keam (2014)   | 19, 4            | N = 18
G719X + other (1)
Del 19 + other (6)
L858R + del 19 (1)
L858R + del 19 + other (9)
Other/UK (1) | 18 1st gen.
Del19 + other (4) | 67% | 7.6 months |
| Wu (2011)     | 42               | N = 32
E709X + other (5)
G719X + other (5)
S768I + L858R (2)
Other (20) | 32 1st gen.
Q701L + I706T + G719S (1)
E709X + L858R (2)
G719X + Other (3)
G719X + L861Q (2)
L861Q + del19 (2) | 56% | 3.5 months |
| Kobayashi (2013) | 11            | N = 7
G719X + other (3)
L858R + other (3)
Del747, T751 + R776S(1) | 7 Erlotinib
G719X + other (3)
Del747, T751 + R776S(1)
L858R + other (2) | 86% | 6 + months |
| Peng (2014)   | 22               | N = 9
G719X + other (3)
E709X + other (1)
Del 19 + other (3)
S768I + other (1)
Other (1) | 9 Gefitinib
G719A, L833V, V834C (1)
Del19 + L858R (1) | 22% | 8 months |

NR; not reported, RFS; relapse-free survival, TTP; time to treatment progression.

* Two patients with T790M and L858R were excluded from analysis, 1 not treated with EGFR-TKI.
  1 The classical group includes 2 co-occurring T790M and the non-classical group includes 2 single L861Q mutations.
  2 5 patients with no available information excluded, 16 remaining and included in survival, 12 available for response.
  3 1 T790M complex mutation excluded.
  4 2 T790M complex mutations excluded.

5.2.2. Clinical
Point mutations in S768I have been reported as partially sensitive to EGFR-TKIs [65]. It is notable that most retrospective studies reporting response to first generation TKIs in patients harboring S768I also included complex mutations. In the Yang et al. post hoc analysis of LUX-Lung 2, LUX-Lung 3 and LUX-Lung 6 although 100% of the patients with S768I included, responded to afatinib, seven of eight patients had complex mutations including five co-occurring G719X and two L858R [60]. In the small retrospective study by Chiu et al. superior responses were seen in patients harboring the complex mutation G719X, S768I (N = 10, 50%) compared to 33% in single S768I mutations [66]. In contrast, in the study by Chen et al., despite the presence of a classical mutation in seven of 10 patients with S768I, the response rate to first generation TKIs overall was only 20% [67]. Single mutations may have inferior outcomes compared to tumors with complex S768I mutations. Afatinib could be considered as the first choice of EGFR-TKI in this cohort. Other point mutations in exon 20 with reported sensitivity include V765A, V774A and T783A although the PFS described was short [68,69].

5.3. Exon 18 point mutations
Exon 18 point mutations are thought to represent 3–4% of all EGFR mutations [70,50]. The most common involve a Gly719 change to Ser, Ala or Cys together with the E709A mutation. Most of the literature on exon 18 mutations comes from small retrospective studies; it appears that these may be associated with current or former smokers with no

[63]. Relative resistance to first generation TKIs has also been demonstrated in a further in vitro study [64].
sex predilection [50,71].

5.3.1. Preclinical evidence

The sensitivity of G719X mutations to TKIs is much less than that of classical mutations in vitro [36,72]. This may be due to the conformational change induced by the switch from G to A, C or S, which challenges binding of gefitinib [73]. Afatinib and other second-generation TKIs in vitro have demonstrated much lower IC50 values, suggesting an upfront role in these mutations [74]. It has been further shown that E709X mutations are less sensitive than G719X [64].

5.3.2. Clinical evidence of response to TKIs

Studies reporting outcomes in exon 18 mutations often have included complex mutations. Table 2 primarily reports outcomes in single exon 18 mutations unless stated otherwise. The largest study of TKI response in this population, was published by Chiu et al., who described an ORR of 37% and a disease control rate of 72.4% [66]. The median PFS was shorter in patients with exon 18 mutations compared to patients with classical mutations (6.3 months vs. 11.1 months). Notably 10 patients with a complex mutation of G719X + S768I and nine patients harboring complex G719X + L819Q mutations had ORRs of 50% and 89%, respectively. In the post hoc analysis of the LUX-Lung studies afatinib was reported to provide a median PFS of 13.8 months and an OS of 26.9 months in the 18 patients with G719X mutations, however half of patients had tumors harboring complex exon 18 mutations [60]. The ORR of single exon 18 point mutations in this study is unknown. Strikingly a phase II study of the second generation TKI neratinib, included four patients with G719X mutations of which three responded with a median PFS of 12.1 months. The multicenter observational study by Beau-Fallier provides extensive epidemiological data on patients with exon 18 mutations and of the 15 patients treated with EGFR-TKIs, only one responded. This patient was treated with erlotinib in the second line setting and time to progression was only 3 months (G719S) [50]. Klughammer et al. reviewed participants from the MERIT, SATURN, TITAN, TRUST, ATLAS, BeTa and FASTACT-2 trials. Most patients with exon 18 mutations were treated in first or second line. Again only one patient with a G719C mutation had a response, which lasted 16 months. Many of the patients included were treated with erlotinib and bevacizumab. Interestingly, two patients with G719X mutations treated with carboplatin/gemcitabine had a PFS of 4.3 and 7 months but OS of 33 and 36 months. The number of patients in the literature treated with chemotherapy is small; however, favorable outcomes are noted with a median OS of 40.8 months vs. 26.9 months (afatinib) in the study by Yang et al. In a similar study by Cheng et al., which also focused on exon 18 mutations, those with complex mutations had a significantly longer relapse free survival and OS than patients with single exon 18 mutations. Remarkably, one patient with a double point mutation G719S + L861Q achieved a relapse free survival (RFS) of 65.0 months [80]. The post hoc analysis by Yang et al. did not report differences in response to afatinib in complex mutations vs. single mutations. However, as stated previously, the S768I EGFR mutation cohort contained 8 patients in whom seven had complex mutations co-occurring with either L858R or G719X; the RR was 100% and median PFS 14.7 months [60].

5.4. Exon 21 L861Q

The exon 21 point mutation, L861Q, is thought to represent 2% of EGFR mutations.

5.4.1. Preclinical and clinical evidence

In in vitro preclinical models resistance to first generation TKIs has been found, however sensitivity to afatinib and osimertinib has been demonstrated [63,64]. By far the largest cohort of patients with NSCLC harboring EGFR-L861Q mutations was reported by Chiu et al. in a retrospective analysis [66]. Fifty-four patients were eligible for assessment of response to first generation TKIs erlotinib or gefitinib. The ORR was 40% and PFS 8.1 months. The reported RR to afatinib was slightly greater (56%) in a post hoc analysis by Yang; however, four of 16 patients (25%) had complex mutations. The PFS was similar and it is likely that L861Q is somewhat sensitive to TKIs but to a lesser extent than classical mutations. Afatinib could be considered first line in these patients (Table 4).

6. Complex mutations

The variation in platforms used to detect EGFR mutations previously may have underestimated complex mutations and these have been reported in up to 14% of the EGFR mutated population [76]. It has also been observed that the tissue quantity may be important for the detection of complex mutations [77].

6.1. Preclinical evidence

In vitro, where cell lines have represented complex mutations with co-occurring classical mutations (del19 + L858R), inhibition by TKIs was more potent than either of the single mutations [23]. On the other hand other complex mutations in vitro despite the presence of a single L858R mutation have demonstrated inferior sensitivity to gefitinib compared to single classical missense mutations [78].

6.2. Clinical evidence

Where possible if a complex mutation included a T790M exon 20 mutation these were excluded from the individual reports in Table 5. In available studies of complex mutations, ORRs generally were higher than individual uncommon single mutations and as expected, compound mutations with co-occurring with classical mutations had the best survival outcomes [79] (Table 5). Wu et al. reported the largest cohort of complex mutations from which 32 patients were evaluable for TKI response (first generation); the ORR was 56%. Of 10 patients with a PFS greater than 10 months, seven harbored one classical mutation. The median PFS was 3.5 months and overall survival 8.5 months. In six patients within this group with EGFR mutation combinations of G719X, S768I, L861Q and L858R the ORR was 67%, median PFS was 9 months and median OS 12.4 months. The analysis by Chiu et al. included the largest cohort of single exon 18 mutations but also reported 19 exon 18 complex mutations. The response rate differed according to the second co-occurring mutation. Those with G719X and S768I co-mutations had an ORR of 50% compared to 89% in those with co-occurring G719X and L861Q. Notably the PFS for the complex 18 mutations was significantly longer than the single point mutations (11.5 vs. 6.3 months, p = 0.01) [66]. In a similar study by Cheng et al., which also focused on exon 18 mutations, those with complex mutations had a significantly longer relapse free survival and OS than patients with single exon 18 mutations. Remarkably, one patient with a double point mutation G719S + L861Q achieved a relapse free survival (RFS) of 65.0 months [80]. The post hoc analysis by Yang et al. did not report differences in response to afatinib in complex mutations vs. single mutations. However, as stated previously, the S768I EGFR mutation cohort contained 8 patients in whom seven had complex mutations co-occurring with either L858R or G719X; the RR was 100% and median PFS 14.7 months [60].

7. Conclusion

Uncommon EGFR mutations including complex EGFR mutations are increasingly reported, given the use of more comprehensive profiling. The studies we have summarized are variable in the line of treatment TKI therapy was used and frequently group uncommon mutations together. Complex mutations are often included with single mutation groupings but appear more sensitive to TKIs. Exon 20 insertions generally should be treated with upfront chemotherapy, however a minority may respond to first line TKIs and we would consider afatinib the most appropriate option. Afatinib may be considered for EGFR mutations G719X, L861Q and S768I but does not consistently provide good response rates. Expanding genomic profiling panels in NSCLC to include less common mutations may further help determine patient...
responses to targeted therapies. It is imperative that we collaborate internationally to collect outcomes in this heterogeneous group of patients.

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Conflict of interest

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References


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