EGFR MUTATED LUNG CANCER: CURRENT THERAPIES AND
POTENTIAL FUTURE TREATMENTS

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Submitted in partial fulfillment of the
requirements for the degree of
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2015
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For John Polio

For all your love and advice, solicited and otherwise.
ACKNOWLEDGEMENTS

I would like to thank Geoffrey Oxnard, MD and Gwynneth D. Offner, Ph.D for all their guidance during the writing of this thesis. Thank you for all your help and instruction.
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ABSTRACT

Lung cancer is the leading cause of cancer related deaths in the United States, with an estimated 158,040 deaths in 2015, accounting for 27% of all cancer deaths. Recent research has identified several important molecular driver oncogenes, including epidermal growth factor receptor (EGFR). EGFR is encoded by exons 18-21, each of which harbor specific mutations within the tyrosine kinase domain. These mutations can drive cell growth, proliferation, and survival, resulting in the formation of non-small cell lung cancer. The development of EGFR tyrosine kinase inhibitors, allows the targeting of these specific mutations without the toxicity normally associated with standard chemotherapy. Unfortunately, inevitably resistance to therapy manifests, requiring a change in therapy and adding complexity to treatment decision making for clinicians and patients alike. Through a comprehensive examination of current literature, this review will establish a standard for first line, targeted treatment for specific genetic mutations within the EGFR gene, as well as address treatment options once resistance to first-line therapy inevitably develops.
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LIST OF ABBREVIATIONS

ALK.......................................................... Anaplastic Lymphoma Kinase
EGFR................................................. Epidermal Growth Factor Receptor
HER2............................................. Human Epidermal Growth Receptor 2
KRAS..................................................... Kirsten Rat Sarcoma
MEK1.................................................. MAP Kinase / ERK Kinase 1
MTS.................................................. Mean Time to Survival
NRAS.................................................. Neuroblastoma Rat Sarcoma
OS.................................................. Overall Survival
PFS................................................. Progression Free Survival
PIK3CA........ Phosphatidylinositol-4,5-biphosphate 3-kinase, catalytic subunit alpha
RET.................................................. Rearranged During Transfection
RR.................................................. Response Rate
TKI.................................................. Tyrosine Kinase Inhibitor
INTRODUCTION

Lung cancer is the leading cause of cancer related deaths in the United States, with an estimated 158,040 deaths in 2015, accounting for 27% of all cancer deaths. Early detection of lung cancer is difficult with only 15% of lung cancers diagnosed at a localized stage (American Cancer Society, 2015). Once lung cancer has reached a locally advanced or metastatic stage, chemotherapy is usually prescribed. Unfortunately, chemotherapy has little success in treating this disease, with a median time to progression of 4 to 6 months and a median overall survival of 10 to 12 months (Melosky, 2014).

Despite advances in clinical therapies, the 1 and 5-year survival rates for lung cancer patients remain at a discouraging 44% and 17%, respectively (American Cancer Society, 2015). Additional research is required to establish concrete working knowledge of the molecular drivers of this disease and novel therapies to increase overall survival rates for patients.

Lung cancer is divided histologically into two groups, small cell lung cancer and non-small cell lung cancer, with NSCLC predominating with an occurrence of 83% (Ibid). NSCLC can be further largely be categorized into squamous cell carcinoma, large cell carcinoma, and adenocarcinoma (Non-Small Cell Lung Cancer Treatment, 2015). NSCLC, regardless of histological subtype, has proven to be challenging to treat and prevent. This is because NSCLC is one of the more genetically aberrant cancers (Li, 2015). Thankfully, this biologic characteristic of NSCLC also allows researchers to divide NSLC into smaller subsets based on the molecular drivers of tumor growth, providing multiple targets for novel drugs. Indeed, over the last decade, with advances in
genetic sequencing techniques and sensitivity, there has been expansive discovery of a subset of driver mutations in NSCLC (Figure 1). These subsets are divided into molecular subgroups defined by specific driver mutations occurring in several oncogenes, including AKT1, ALK, BRAF, ROS1, HER2, KRAS, MEK1, NRAS, PIK3CA, RET, and EGFR (Lovly, 2015). The discovery of EGFR mutations in patients with NSCLC has led to the development of effective therapies for this subgroup population, confirming targeted therapies as worthy of further exploration for other driver mutations for lung cancer patients.

![Figure 1: NSCLC Identified Mutations.](image)

Lung cancers that harbor EGFR activating mutations account for a significant proportion of NSCLC diagnoses, especially in Asian populations and non-smokers. The ability to offer a viable treatment course will be important in extending the overall survival of this patient population. It has been established there are several different types of EGFR mutations; with evidence showing different subtypes of this mutation should be
treated uniquely, with different treatment approaches. It is well established identifying EGFR mutations can predict which patients will benefit from treatment with TKIs (Mok et al., 2009, Douillard et al., 2009). EGFR mutational testing has, therefore, become standard procedure for this patient population. This has led to a coordinated effort to establish which EGFR mutations are sensitizing to TKI treatment, in order to better inform patients and clinicians alike as to what therapy to use when confronted with this diagnosis. While common mutations have been extensively studied, and a first-line standard has been established, there still remains debate over the correct course of treatment for these patients. A first-line standard of care has yet to be determined conclusively for patients whose tumors harbor rarer EGFR mutations. Additionally, there remains debate of how to treat patients in advanced line settings, especially once tumors have acquired resistance. To this end, there is current excitement about third-generation TKIs, currently undergoing clinical trials, and their ability to target acquired resistance mutations.

In this review, current literature will be analyzed to gain a better understanding of EGFR mutations as a whole, review current and potential future therapies for this patient population, and establish a comprehensive protocol for the treatment of these patients in order to best preserve quality of life and extend overall survival.
I. History of the EGFR Gene

In 1962, Stanley Cohen, following work on the protein, nerve growth factor (NGF), isolated another protein that stimulated proliferation of epithelial cells, and called it epidermal growth factor (EGF) (Gschwind et al., 2004). Over a decade later, Graham Carpenter confirmed the existence of specific binding receptors for EGF (EGFR) on the surface of cells, using $^{125}$I-labelled EGF and fibroblasts from various species. The development of molecular cloning in the mid-1970s eventually led to the sequencing and determination of EGF’s amino acid sequence. In 1980 researchers showed increased EGF kinase activity in A-431 human epidermoid carcinoma cells involved the phosphorylation of tyrosine residues (Ushiro, 1980). Indeed, throughout the 1980s academic and industry research indicated a role of deregulated EGFR in human cancers. Further research throughout the 1980s would characterize EGF more fully and elucidate several of its downstream signaling partners (Kamata, 1984). Throughout the 1990s, using crystallographic studies, increased detail was provided to specifically to how the dimerization is regulated through receptor binding.

Screening of cDNA libraries using EGFR probe actually showed that EGFR belongs to a family of closely related proteins. The EGFR gene belongs to a family of tyrosine kinase receptors that includes EGFR (HER1), HER2, HER3, and HER4. The binding of epidermal growth factor induces a conformational change, which leads to dimerization, phosphorylation, and activation of the receptor. Activation of the receptor triggers multiple downstream signal cascades within the cell, which include PI3K-AKT-
mTOR and the RAS-RAF-MEK-ERK pathway (Figure 2) (Zhang et al., 2008). These signal cascades lead to profuse cellular responses. These cellular responses include increased cell proliferation, motility, resistance to apoptosis, and invasion. Mutations in EGFR result in constant activation of these pathways and subsequent tumorigenesis.

**Figure 2: Pathways Activated by EGFR binding.** Scheme of RAS-RAF-MEK-ERK and PI3K-AKT-mTOR pathways activated by binding to EGFR receptor. “K” denotes the tyrosine kinase domain. *Adapted from Lovly et al., 2015.*
In the early 1990s it was established a higher rate of EGFR expression was found in tumor tissue than in normal, non-cancerous tissues, including NSCLC (Dittadi et al., 1991, Palazzo et al., 1993). This signified EGFR as a potential therapeutic target and initiated the clinical development of EGFR tyrosine kinase inhibitors (TKI). TKIs work by interrupting or turning off cell signaling pathways and inducing apoptosis. The initial development of TKIs culminated in the production of gefitinib (trade name Iressa), by Astrazeneca. Gefitinib inhibited cancer growth by binding selectively to the ATP-binding site of the enzyme, thus interrupting cell signaling to induce activation of the anti-apoptotic Ras signal transduction pathway. In a phase I trial, Gefitinib demonstrated a response in patients with solid cancers likely to express EGFR mutations, with a tolerable toxicity profile (Ranson et al., 2002). Additional studies showed a response in patients with NSCLC, after progression on standard chemotherapy, with both a radiographic and symptomatic response (Kris, et al., 2003). Although a response was only observed in a fraction of NSCLC patients, due to the quickness and significance of the response rates, gefitinib received accelerated approval by the FDA in May 2003 as a monotherapy for patients of patients with locally advanced or metastatic NSCLC post-progression on standard chemotherapy. At the time, while there had been established first and second chemotherapy regimens for this group of patients, the need for third therapy had not been met. Gefitinib was thus approved for use in a third line setting (Cohen, 2004). Despite its approval, it was still unknown at the time, why only a fraction of patients with NSCLC responded to gefitinib therapy. Further investigations were conducted in order to elucidate the reasoning behind this phenomenon.
In 2004, two groups independently showed EGFR mutations were potentially sensitized to treatment with the tyrosine kinase inhibitor, gefitinib (Paez et. al, 2004, Lynch et. al, 2004). Each group independently identified patients who responded to treatment with gefitinib and patients who did not respond to treatment, as defined by radiographic criteria. Within each group, patients who had pre-treatment tissue available were selected. Tumor tissue was subsequently sequenced and analyzed. An overwhelming majority of patients who responded to Gefitinib treatment harbored mutations in the tyrosine kinase domain of the EGFR gene. A majority of patients without a response, did not harbor these mutations. Furthermore, patients who exhibited EGFR mutations had matched normal tissue analyzed. This showed a lack of EGFR mutations, demonstrating conclusively these are somatic mutations. Both groups’ findings demonstrate EGFR activating mutations confer sensitivity to gefitinib therapy. Discovering this molecular correlation to a response to a TKI provided immediate and important clinical implications, as patients who harbored these activating mutations would gain significant benefit from treatment with targeted therapy. This discovery prompted additional investigation into EGFR gene mutations and potential therapies for patients who harbor these mutations.

EGFR mutations are located with exons 18 to 21, which encode a portion of the tyrosine kinase domain. Although multiple mutations have been identified, a vast majority of EGFR mutations are either exon 19 deletions or exon 21 L858R mutations, 40 and 45% respectively. Interestingly, despite one exception, mutations within exon 20 are non-sensitizing to TKI therapy (figure 3). Furthermore, these mutations have a higher
prevalence in non-smoking, Asian women, with adenocarcinoma histology (figure 4) (Mitsudomi et al., 2007). Many additional groups have confirmed the relationship between EGFR mutation status and the described clinical background. It has also been noted that EGFR mutations are exclusive with KRAS and ALK mutations (Jänne et al., 2005).

**Figure 3: Location of EGFR mutations.** This schematic represents the mutations found within exons 18-21. It also indicates if the identified mutations are sensitized to TKI therapies. *Adapted from Lovly et al., 2015*
As technologies and research continues inquiry into the molecular landscape of EGFR mutated lung cancer, the picture becomes increasingly more complex. Subsequent research has elucidated additional mutations within the EGFR gene, along with resistance mutations that thwart treatment with first and second generation TKIs. Third generation TKIs, currently undergoing clinical trials, seem to address these resistance mutations, but for how long before additional resistance develops as well as the full clinical implications of these new drugs remains to be seen. Much work is still required to elucidate a comprehensive, working knowledge of EGFR mutations and their resistance mechanisms, which will provide gateways to the development of treatment options that will procure effective and sustained responses for patients coping with this disease.
TREATMENT OF SPECIFIC EGFR MUTATIONS

As research continues investigating EGFR mutations, it has become clear there is a diverse range of specific EGFR mutations within the coding region for the tyrosine kinase domain of this gene. It has also become clear that specific mutations will dictate the treatment patients should receive. While a majority of these mutations are sensitized to treatment with TKIs, there is a documented degree of response to TKIs with each mutation (figure 5). Additionally, some mutations are not sensitized to treatment with TKIs and require alternative measures (Mitsudomi et al., 2006).

Figure 5: Response rates of specific EGFR mutations to TKI therapy. This demonstrates a gradient of response, with exon 19 deletions conferring the highest sensitivity. Adapted from Mitsudomi et al., 2006.
It is therefore important, once a patient’s mutational status is determined, to tailor treatment according the specific mutation a patient’s tumor harbors. The development of resistance or histological transformation for patients previously treated with TKIs also complicates the situation. Table 1 lists suggested treatments for TKI naïve patients as well as patients who acquire resistance to treatment. This underscores the importance of obtaining repeat biopsy samples and repeated sequencing of patient tissue, as the results can help dictate what therapy patients should receive during each course of their treatment.

**Table 1: Summary of suggested therapy for TKI naïve patients and patients with acquired resistance to TKI therapy.** Based on current literature reviewed.

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<tr>
<th>Mutation</th>
<th>Suggested Therapy</th>
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<td><strong>Tyrosine Kinase Inhibitor Treatment Naïve</strong></td>
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<td>Exon 18 Mutations</td>
<td>Irreversible TKI (afatinib, neratinib)</td>
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<td>Reversible TKI (erlotinib, gefitinib)</td>
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<td>Exon 20 A763_Y764insFQEA Insertion</td>
<td>Reversible TKI (erlotinib, gefitinib)</td>
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</tr>
<tr>
<td>T790M Mutations</td>
<td>Standard chemotherapy and germline testing</td>
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<tr>
<td><strong>Acquired Resistance to TKI Therapy</strong></td>
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<tr>
<td>T790M Mutations</td>
<td>Third-generation TKI (AZD9291, rociletinib)</td>
</tr>
<tr>
<td>MET Amplification</td>
<td>Combination therapy, EGFR inhibitor and MET inhibitor (AZD9291, savolitinib)</td>
</tr>
<tr>
<td>Transformation to SCLC</td>
<td>Platinum / etoposide chemotherapy</td>
</tr>
<tr>
<td>Other Resistance Mutations</td>
<td>Platinum-doublet chemotherapy</td>
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I. Exon 18 G719X Mutations:

Mutations within the exon 18 coding region are in the observed minority of EGFR mutations, consisting an estimated 3% of all reported cases (Mitsudomi et al., 2010). The most common mutation is a point mutation, located in the nucleotide-binding loop, replacing a glycine with another amino acid at position 719. Multiple amino acid substitutions have been described, resulting in the common notation of this gene mutation, G719X. The amino acid substitutions described in the literature are cysteine (G719C), aspartic acid (G719D), serine (G719S), valine (G719V), and alanine (G719A). G719A, G719C, and G719S are observed as most frequent, with prevalence of 0.6%, 0.3%, and 0.5% of all EGFR mutated NSCLC, respectively (Seigelin and Borczuk, 2013).

Current understanding of exon 18 mutations suggests they are sensitive to treatment to TKIs. Locatelli-Sanchez et al. collected 793 NSCLC tumor samples from 753 patients, analyzing for EGFR mutations of exon 18 to 21 by direct sequencing, identifying 133 mutations from 124 patients. This large sample retrospective study aimed to assess outcomes of patients with this mutation. Of the 133 identified EGFR mutations, 10 (7.5%) were identified in 6 patients as located within exon 18. Patients treated with TKIs experienced tumor regression, suggesting exon 18 mutations are responsive to inhibition with TKIs (Locatelli-Sanchez et al., 2013). This observation has been previously investigated and confirmed by other groups (Takano et al., 2005, Han et al., 2005).
Despite response to TKI treatment Locatelli-Sanchez et al. note patients with exon 18 mutations have a worse overall survival compared those patients exhibiting exon 19 or 21 mutations. The diminished response of these patients to targeted therapy may be due to a differential sensitivity of various EGFR mutations to TKI therapy as described by Jiang et al. The group demonstrated, although exon 21 L858R and exon 18 G719S mutations display similar ligand-independent activation of EGFR signaling, gefitinib inhibits L858R with an approximate 10-fold higher sensitivity. They postulate this is due to the location of the G719X mutation.

Gefitinib acts competitively with ATP for binding within the ATP binding loop, where the G719X mutation is located. The substitution of glycine, a small amino acid, with a larger amino acid, such as serine or aspartic acid, could drastically disrupt the structure or the binding domain, inducing a less favorable confirmation for gefitinib binding (Jiang et al., 2005). Watanabe et al. confirmed the lesser response of G719X mutations to targeted therapy after analyzing 225 patients who had received gefitinib at any treatment line (Watanabe et al., 2014). The group’s findings suggest for patient’s harboring rare EGFR mutations, first line chemotherapy over targeted therapy may be an effective treatment strategy. Of further note, a trial investigating the pan-ErbB inhibitor TKI, neratinib, showed activity against the G719X mutation. Sequist et al. investigated the activity of neratinib against T790M mutations, a resistance mutation that develops in some patients after treatment with targeted therapy. The group showed neratinib has low clinical activity against TKI naïve patients and patients who had a history of TKI treatment. However, the group notes of the 4 patients harboring G719X mutations, 3
experienced partial responses, with the 4th patient experiencing stable disease lasting 40 weeks (figure 6)(Sequist et al., 2010). This finding concurs with previous preclinical models, which note neratinib may be a more potent inhibitor of point mutations, such as G719X, versus deletions (Yuza et al., 2007). Similar to neratinib, is afatinib, another irreversible covalent inhibitor of EGFR and HER2. Afatinib has demonstrated efficacy against EGFR sensitizing mutations and may also be effective therapy for patients harboring these mutations (Joshi et al., 2015). It has been recently demonstrated cells transfected with G719X mutations respond at a substantially higher rate to afatinib and neratinib than to first generation TKIs (Kobayashi, 2015). The results published by Sequist et al. demonstrate the importance of complete genetic testing to be performed in indicated patient populations, especially in the context of clinical trial testing.
Figure 6: Plot of best response for patients previously exposed to TKI treatment on neratinib. The best response of patients shows the efficacy of neratinib on patients harboring G719X mutations. It underscores the finding of Yuza et al., as most patients with deletion mutations did not receive benefit from treatment with neratinib. Adapted from Sequist et al., 2010.

The paucity of G719X mutations in exon 18 occurring in patients with NSCLC, make it difficult to generate enough data to form concrete discoveries about the best treatment for these patients. Most published information of these mutations occurs in extremely small numbers or as case reports, the results of which are many times controversial. Current research shows G719X mutations are sensitizing to treatment with TKIs, despite evidence that the response is diminished in relation to other EGFR mutations. Targeted therapies, therefore, may prove beneficial to this patient group. However, it should be noted, it has been postulated by some groups, TKIs may not be the
most effective therapy for this patient population and until more robust data is published, they argue chemotherapy is the adequate therapy. With the current lack of data available for this subset of patients, it has been suggested these patients be assessed individually, rather than a group (Karachaliou et al., 2015). Compilation of data from multiple databases and various institutions experiences with this rare mutation will further elucidate the clinical course necessary for the treatment of this mutation. Until then, the question of how to most effectively treat these patients will go unanswered. This further investigation will enable researchers to further define a standard of treatment that will provide a better quality of life and extended overall survival.

II. Exon 19 Mutations:

Mutations occurring in exon 19 of the EGFR gene constitute one of the most common somatic mutations within EGFR. Two major types of exon 19 mutations have been identified to date: exon 19 deletions and exon 19 insertions. Exon 19 deletions comprise the majority of the mutations originating from this exon, with an occurrence rate of approximately 48%, while exon 19 insertions are much more rare, with an occurrence of only approximately 1% (Lovly et al., 2015, He et al., 2012). Comprising of almost half of all reported EGFR mutations, exon 19 deletions therefore have been extensively investigated.

IIa. Exon 19 Deletions:

Deletion mutations occurring in exon 19 are some of the more frequent noted in patients with NSCLC. Exon 19 deletions are located in the gene region responsible for
encoding the C-helix portion of the tyrosine kinase domain. Multiple deletions have been described, with the majority encompassing codons L747 to E749, also known as the LRE fragment, located between strand β3 and the αC helix (Chung et al., 2012)(figure 7). This fragment contributes to hydrophobic residues, which stabilize the inactive state of the protein. Mutations to this area can disrupt this stabilization, thus leading to an ever-active confirmation.
Figure 7: Structure of the EGFR tyrosine kinase domain, shown here bound to erlotinib. The domain consists of a C-lobe and N-lobe. The ATP binding site is adjacent to the P-loop, shown here bound to erlotinib. Various EGFR mutation locations are highlighted with black arrows. Adapted from Yasuda et al., 2012
The most frequent mutations to this area are delE746-A750, delL747-P753insS, delL747-A750insP, and delL747-T751, with reported rates of occurrence of 66.1%, 56.8%, 4.0%, and 3.7%, respectively (Ibid). Deletion mutations have also been discovered that do not contain the region L747-E749, most notably, delS752-I759.

Exon 19 deletions have been extensively described in the literature due to their high occurrence in patients with EGFR mutant tumors. Various groups have demonstrated, in vitro, exon 19 deletions are sensitive to inhibition by TKIs, which inhibit autophosphorylation at 10-100 fold lower concentrations of gefitinib needed to inactivate wild type EGFR (Ono et al., 2004, Paez et al., 2004). Exposing mutant cell lines to therapeutic doses of gefinitib showed inhibition of EGFR and provided validation for further clinical exploration. This led to a plethora of studies exploring the efficacy of TKIs as suitable treatment for patients with exon 19 deletions.

It has now been well-established patients exhibiting exon 19 deletions should receive TKI as first-line therapy. One such study to establish this is the OPTIMAL study. A phase III open label randomized study, it compared the efficacy and tolerability of first-line erlotinib treatment versus carboplatin plus gemcitabine in patients with stage IIIB or IV cancer harboring exon 19 deletion or exon 21 L858R activating mutations. This study demonstrated this patient population benefits from erlotinib therapy in the first-line setting with a median progression free survival significantly longer than that of the chemotherapy alone arm (13.1 [95% CI 10.58-16.53] versus 4.6 [4.21-5.42] months, p<0.0001)(figure 8)(Zhou et al., 2011). Additionally, the study showed erlotinib to be
substantially more tolerable, with a lower association to grade 3 and 4 toxicities than standard chemotherapy.

Figure 8: Progression free survival in both treatment groups. Patients who received erlotinib in the first-line setting fared significantly better than their counterparts who received chemotherapy alone. Additionally, they experienced far less toxicity. Adapted from Zhou et al., 2011.

While this study’s participants comprised of solely Asian patients, various other groups have confirmed this findings in other patient populations (Rosell et al., 2012, Jänne et al., 2012). The EURTAC study, a pivotal open-label, randomized phase III trial, is the basis for the FDA approval of erlotinib in the first-line setting for patient with sensitizing EGFR mutations. Rosell et al., evaluated 173 patients, identified as chemotherapy naïve, exhibiting sensitizing EGFR mutations, stratified by EGFR mutation type and ECOG
performance status. Patients were randomized in a 1:1 ratio to treatment with erlotinib or a chemotherapy regimen and followed for PFS. Median PFS was found to be 9.7 months in the erlotinib group versus 5.2 months in the standard chemotherapy group (hazard ratio 0.37, 95% CI 0.25–0.54; p<0.0001)(figure 9)(Rosell et al., 2012). These results were pivotal in determining FDA approval for erlotinib in the first line setting for patients with sensitizing mutations.

Figure 9: Progression free survival for each treatment group. This Kaplan-Meier plot of progression free survival based on radiological evidence demonstrates erlotinib as a more efficacious treatment for this patient population. *Adapted from Rosell, 2012.*

The results of these two studies has been confirmed by numerous trials and analyses conducted by various groups (Gao et al., 2012, Lee et al., 2013, Mok et al., 2009, Mitsudomi et al., 2010, Maemondo et al., 2010). Interestingly, afatinib has also proven to be a viable treatment option in the first-line setting.
The LUX-Lung 3 trial, a large, open-label randomized phase III study investigated afatinib versus pemetrexed and cisplatin combination therapy as a first-line treatment option for patients with advanced adenocarcinoma, with EGFR activating mutations. 345 patients were randomized in a 2:1 (afatinib:chemotherapy) ratio and followed for PFS. 308 patients harbored common mutations and experienced a median PFS of 13.6 months compared to 6.9 months (HR=0.47, p<0.0001)(Yang et al., 2012). Similar activity was demonstrated in the LUX-Lung 2 study (Yang et al., 2012). In response to the results of the LUX-Lung 3 trial, in 2013, afatinib was approved for use in the first line setting for patients with exon 19 deletion and exon 21 mutations.

Comparison of gefitinib, erlotinib, and afatinib in a meta-analysis published in 2014, yielded no statistically significant differences (Haaland et al., 2014). Despite these results, erlotinib remains the standard treatment course for patients with exon 19 deletions in the first-line setting. This may be due to its already widespread use in this setting or its toxicity profile, which, when compared to afatinib, tends to be more tolerable. Most influential perhaps, however, is the fact that afatinib is an irreversible pan-HER inhibitor. This characteristic enables afatinib some activity against T790M resistance mutations, which develops post-progression in a significant percentage of patients. It was thought this would enable afatinib to be a potent second-line therapy for patients who progress on first-line targeted therapies. It has since been demonstrated, however, response to afatinib in the second-line is modest at best, with a substantial toxicity profile (Joshi et al., 2015). Therefore, afatinib is not recommended in the second-line setting as a monotherapy.
While exon 19 deletions that encompass the LRE fragment are responsive to TKI therapy, non-LRE deletions have been shown to have a worse response rate to targeted therapies. Chung et al., enrolled patients with exon 19 deletions and evaluated patients treated with TKIs for RR, PFS, and OS. They stratified patients based on the specific deletion their tumor harbored. Their analysis shows varying RR based on the specific deletion mutation (Table 1). Of all the deletions analyzed, non-LRE deletions demonstrated the lowest RR, with a response rate of 42.9% compared to 76.9% for delL747-P753insS (Chung et al., 2012).

Table 1: Best overall response to TKI therapy for tumors exhibiting varying exon 19 deletions. Non-LRE deletions show the worst response to targeted therapy. Adapted from Chung et al., 2012.

<table>
<thead>
<tr>
<th>Deletion</th>
<th>N</th>
<th>PR</th>
<th>SD</th>
<th>PD</th>
<th>RR (%)</th>
<th>Adjusted OR a (95% CI)</th>
<th>P a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>204</td>
<td>143</td>
<td>37</td>
<td>24</td>
<td>70.1</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
| Patient groups categorized by deletions in exon 19 of EGFR
| ΔL747            | 49 | 39 | 3  | 7  | 79.6   | 1                      | 0.019|
| delL747-P753insS| 13 | 10 | 1  | 2  | 76.9   |                        |      |
| delL747-T751     | 10 | 7  | 0  | 3  | 70.0   |                        |      |
| delL747-S752     | 9  | 8  | 1  | 0  | 88.9   |                        |      |
| ΔE746            | 148| 101| 30 | 17 | 68.2   | 0.265 (0.095–0.736)    | 0.011|
| delE746-A750     | 137| 93 | 29 | 15 | 67.9   | 0.120 (0.020–0.737)    | 0.022|
| Non-LRE          | 7  | 3  | 4  | 0  | 42.9   |                        |      |

However, despite the lower RR of non-LRE deletions, there was no statistically significant difference in PFS or OS between different deletion mutations (figure 10). This study shows that while different deletion mutations may affect response rates to TKI therapy, overall survival and PFS remains similar for all deletions. It is therefore prudent
to treat exon 19 deletions with targeted therapy, namely erlotinib, in the first-line setting. It is also important to note, non-LRE deletions are uncommon. Further investigation into this rare mutation class should be conducted in order to validate and confirm these results.

Figure 10: Kaplan-Meier curves demonstrating (A) PFS and (B) OS for exon 19 deletions. No significant difference is noted between varying exon 19 deletion mutations. Adapted from Chung et al., 2012
Interestingly, it appears tumors harboring exon 19 deletions are more responsive to targeted therapy than the other EGFR mutations currently known, including the L585R mutation. Mitsudomi et al., noted this in their literature analysis investigating the biological and clinical implications of EGFR mutations in lung cancer. In a compilation of 224 patient response rates, from various literature reports, to TKI therapy, patients with exon 19 deletions had an 84% response rate, as compared to 71% for L858R mutations. Furthermore, patients with L858R mutations experienced a much shorter mean survival time after TKI treatment than patients with exon 19 deletions (8 vs. 34 months, p<0.001)(Mitsudomi et al., 2006). Zhang et al. recently mirrored these results in their literature review.

Zhang et al. compiled data from a total of 13 studies and compared hazard ratios for PFS by indirect comparison for patients with exon 19 deletions or L858R mutations after first-line TKI therapy. They found patients with exon 19 deletions had a lower hazard ratio and an association with a longer PFS than patients with an L858R mutation (Zhang et al., 2014). Therefore, patients with exon 19 deletions may experience superior results with TKI treatment. However, the low number of studies used for their meta-analysis is a significant limitation of this study. Additional studies with larger data sets and more convincing evidence will be needed to confirm this group’s hypothesis.

**IIb. Exon 19 Insertion:**

Exon 19 insertions are a relatively newly discovered mutation within the EGFR tyrosine kinase domain. It is estimated they comprise 2% of all exon 19 mutations and 1% of all EGFR mutations (He et al., 2012). Structural studies show this mutation occurs
in the end of the β3 strand, in the N-terminus of the kinase domain, which lies adjacent to the αC helix. This mutation results in the addition of 6 amino acid residues to this area, extending the loop that connects to the αC helix (figure 7). Importantly, this results in the altered identity of Glu746 and Leu747. The alteration to Glue746 is varied and structurally of little significance, while Leu747 is consistently altered to proline and has structurally consequence. The change to proline alters the stabilizing hydrophobic interactions of this area and leads to an altered structural confirmation of the protein that favors activation (Ibid). This mechanism is a similar to the proposed mechanism as the L858R mutation, which occurs in a similar area of the protein (Yun et al., 2007). Until recently, exon 19 insertions sensitivity to TKI therapy was poorly described.

He et al., addressed this question by studying lung cancers harboring this mutation by examining structural effects, assessing in vitro sensitivity to TKIs, and assessed radiological response of patients receiving TKIs. In vitro, Ba/F3 cells transfected with exon 19 insertion mutations were found to be sensitive to both gefitinib and afatinib. Using Western blotting, the group assessed EHFR phosphorlyation, finding both gefitinib and afatinib inhibited EGFR phosphorylation in cells harboring exon 19 deletions. Of interesting note, the sensitivity of exon 19 insertions was less than that of exon 19 deletions. The group identified 12 patients with exon 19 insertions. Of the 12, 4 received EGFR TKI in a palliative setting. Of the 4, 3 patients experienced a partial response by RECIST criteria, and 1 had a minor response, although to a novel EGFR TKI (He et al., 2012). These results have been noted elsewhere in the literature, although reports have limited, mainly due to the rare occurrence of this mutation (Agbarya et al., 2014, Chan et
al., 2013, Otto et al., 2012). In addition to establishing exon 19 insertions as TKI sensitive, these studies also underscores the importance of using multi-platform assays in genomic testing in order to better characterize complex mutations in this patient population (Politi et al., 2012). Better characterization of these tumor’s genomics will allow clinicians to make better-informed decisions with regards to patient care.

These results demonstrate exon 19 insertions as responsive to TKI therapy, much like exon 19 deletions, with durable responses in the first-line setting. TKI therapy for these patients should therefore be prioritized as a first-line treatment.

III. Exon 20 Mutations:

Exon 20 of EGFR translates amino acid positions 762 to 823, containing part of the αC helix (figure 11)(Yasuda et al., 2012). Exon 20 mutations are the third most common EGFR mutation described in the literature, comprising an estimated 3-10% of all reported cases (Mitsudomi et al., 2009, Oxnard et al., 2013). Despite their prevalence, relatively little is known about this type of EGFR mutation.

IIIa. Exon 20 Insertions:

Exon 20 insertions are highly varied, and commonly are reported to occur after the αC helix and up to Cys775, although a small number (4%) have been discovered to affect residues within the αC helix (Yasuda et al., 2012). These insertions add 1-4 residues to the end of the αC helix (Eck et al., 2009). In addition to being important for the ATP phosphotransfer, the αC helix’s position is important in determining an active or inactive confirmation (Walker et al., 1998). As seen with exon 19 deletions, which alter
the LRE fragment prior to the αC helix, exon 20 insertions are thought to be able to push the αC helix into an inward confirmation by forming a “wedge” at the base of the C helix (Yasuda et al., 2013). This change of confirmation renders the protein constitutively active.

Figure 11: Schematic of Exon 20 with indications of all reported exon 20 insertion locations. Reported prevalence of each insertion is listed in parentheses next to the insertion. Adapted from Yasuda, 2012.

The frequency of exon 20 insertions is reported over a range, spanning 1 to 10%, although with a general consensus of 4-5% (Wu et al., 2008, Yasuda et al., 2012). This number may be inaccurate, as Oxnard et al., reports in their characterization of their
single-center experience with patients harboring exon 20 insertion mutations. They report a prevalence of 9.2%, which although may be referral bias, is likely due to the genotyping technique employed by prior studies (Oxnard et al., 2013). Using a non-specific mutational assay, they argue, will be essential in order to not miss mutations usually overlooked by mutation specific assays.

Unlike classic EGFR mutations, G719X, exon 19 deletions, and L858R, exon 20 insertions are not predictive of TKI sensitivity. In both preclinical and clinical models, resistance to TKI therapy was observed, despite occurring in patient populations with similar characteristics as other mutations (Wu et al., 2008, Greulich et al., 2005, Sasaki et al., 2007, Yasuda et al., 2013). Oxnard et al. support this last finding in their review of patients at their center. The group reviewed cases of 1086 patients who had undergone conclusive EGFR genotyping. Of the 1086, 294 were identified with EGFR mutations, 27 of which harbored EGFR exon 20 insertion mutations. Survival analysis performed on 839 patients showed patients harboring exon 20 insertions had a median survival of 16.5 months (95% CI: 10.4-NA); poorer than patients with common mutations, who experienced median survival rates of 33.0 months (95% CI: 28.7-40.6). Interestingly, patients with exon 20 insertions fared similarly to patients with WT EGFR (figure 12)(Oxnard et al., 2013).
Figure 12: Survival of patients from date of advanced disease. The survival curves show exon 20 insertions have a poorer median survival than common EGFR mutations, but similar survival rates to patients with WT EGFR mutational status. Adapted from Oxnard et al., 2013.

The results from this review are supported by several other studies, all which show exon 20 insertions confer decreased sensitivity to TKI treatment of both first generation reversible and second generation, irreversible inhibitors (Yasuda et al., 2012, Sasaki et al., 2007). Due to this, it has become common practice to restrict patients with exon 20 insertions from clinical trials exploring the activity of novel TKIs. It has therefore become necessary to develop mutation specific targeted therapies for this subgroup of patients.

One such drug that has shown moderate promise is AUY922, a heat shock protein 90 (HSP90) inhibitor, which shows clinical activity in patients harboring the exon 20 mutation. AUY922 works by disrupting HSP90, which helps stabilize other proteins required in cell survival processes. AUY922 was first noted to have anti-cancer activity in a prior trial, NCT01124864, with one patient experiencing a durable partial response by RECIST criteria. Investigators designed a phase II trial, currently ongoing, to further
explore AUY922’s clinical activity (Piotrowska et al., 2015). So far, the study has enrolled 15 patients in the second stage. Of those 15 patients, 3 have achieved a confirmed partial response by RECIST criteria and an additional 7 have experienced durable stable disease, with an overall PFS of 6.1 months (95% CI, 1.2 to NR)(figure 13)(Piotrowska et al., 2015). Importantly, the drug is well tolerated with reversible grade 1 toxicities.

![Graph showing median PFS for AUY922 treatment]

**Figure 13: Overall PFS for patients under AUY922 treatment.** Median PFS has been established as 6.1 months. *Adapted from Piotrowska et al., 2015.*

This is the first study designed and executed that specifically targets patients with EGFR exon 20 insertions. Initial data suggests AUY922 may be a viable treatment option for patients with this mutation, with patients currently experiencing a median PFS of 6.1 months and low toxicity. Further study of AUY922 is therefore warranted in this population to determine if it should replace current standard therapies for these patients.
Until the story of AUY922 becomes more developed, treatment with standard chemotherapy is the best available option for these patients. A recent retrospective study supports this clinical decision. Naidoo et al. identified 1,882 patients, 46 of which had EGFR exon 20 insertions. The majority of these patients did not respond to EGFR TKI therapy, but did experience moderately high response rates to chemotherapy, most effectively platinum based doublet therapy (Naidoo et al., 2015). Similar results were noted in previous studies (Oxnard et al., 2013). The conclusion should therefore be made standard chemotherapy, most likely platinum-based doublet chemotherapy, should be used to treat patients who present with metastatic EGFR exon 20 insertion mutations in the first-line setting.

IIIb. Exon 20 A763_Y764insFQEA Insertion:

One variant of an exon 20 insertion, specifically the A763_Y764insFQEA, an in-frame insertion, occurring with a frequency of approximately <1%, has been shown to confer increased sensitivity to TKI treatment (Costa, 2014). Yasuda et al., unexpectedly found this mutation is highly sensitive to TKI in vitro and in patients whose tumor harbors this specific insertion. The group postulates this specific insertion, located within the C helix, shifts the C helix toward its N-terminus, altering the length of the β3-αC loop, and causing a I759A replacement. These changes are at the site of exon 19 deletions and L858R and L861 mutations, respectively. Therefore, this specific insertion may resemble exon 19 deletions and L858R mutations more closely than its fellow exon 20 insertions. In their experience, three patients with an EGFR A763_Y764insFQEA insertion, had clinical and radiological regressions or stable disease while on TKI therapy.
(Yasuda et al., 2013). In vitro and clinical data, therefore suggests, while patients with EGFR exon 20 insertions be treated with standard platinum-doublet chemotherapy, patients with the specific EGFR A763_Y764insFQEA insertion should be treated preferentially with EGFR TKI therapy.

IV. Exon 21 Mutations:

Exon 21 point mutations are the other most common somatic EGFR mutations, comprising, along with exon 19 deletions, approximately 86% of all EGFR mutations reported. The two most commonly reported exon 21 mutations are L858R mutations and L861Q mutations, with an occurrence of 43% and 2%, respectively (Lovly, 2015). Both of these mutations increased phosphorlyation of EGFR in the absence of ligand stimulation (Mitsudomi et al., 2010). Due to the high rate of occurrence, exon 21 point mutations, specifically L858R mutations, have been extensively studied.

IVa. Exon 21 L858R Mutation:

The exon 21 L858R mutation is a thymine to guanine transversion point mutation that results in the replacement of leucine at position 858 with an arginine residue (Riley et al., 2006). The mutation occurs at the N-terminus of the helical turn in the activation loop in the inactive confirmation of the protein (figure 7)(Eck et al., 2010)(Yun et al., 2007). This area of the activation loop is integrated with a hydrophobic grouping of residues, which hold the C helix in an inactive confirmation. The positively charged arginine residue destabilizes this confirmation, forcing the protein into its active confirmation. Interestingly, it appears the arginine residue also forms a hydrogen bond.
with the arginine residue at position 836, further stabilizing the protein in an active confirmation (Yun et al., 2007). This structural analysis provides valuable insight in how L858R mutations affect the confirmation of the EGFR protein.

Exon 21 L858R mutations have a reported occurrence of 43%, making them one of the most common EGFR mutations (Mitsudomi et al., 2010). Like exon 19 deletions and exon 18 G719X mutations, they exhibit sensitivity to inhibition by TKI therapy, both in vivo and in vitro (Paez et al., 2004, Rosell et al., 2012). These results have been confirmed by multiple studies within the last few years.

As noted above, the OPTIMAL study conducted by Zhou et al., demonstrated superior results for patients harboring activating mutations (exon 19 deletions or L858R mutations) treated with targeted therapy versus standard chemotherapy (PFS 13.1 versus 4.6 months, CI 95%, HR = 0.16)(Zhou et al., 2011). Wu et al., reported similar results with the LUX-Lung 6 trial, an open label, phase III trial comparing afatinib with cisplatin plus gemcitabine in first-line, EGFR mutant positive Asian patients. The majority of patients harbored exon 19 deletion (51%) or L858R mutations (37%). 364 patients were randomized in a 2:1 ratio to the afatinib arm or standard chemotherapy arm, respectively. By independent-central review, patients who received targeted therapy experienced increased PFS over patients treated with standard chemotherapy (11.0 versus 5.6 months, CI 95%, HR = 0.28)(figure 14)(Wu et al., 2012). Additionally, a greater percentage of patients receiving afatinib reached objective response (66.9% versus 23.0%). They also reached an objective response quicker than patients receiving standard chemotherapy (By 6 weeks, 49.2% versus 13.1%).
Figure 14: Independent-central review of overall PFS for patients receiving afatinib versus standard chemotherapy. Patients randomized to the afatinib arm experienced a significantly longer PFS compared to those patients on standard chemotherapy. Adapted from Wu et al., 2012.

Importantly, tolerable toxicity was also noted in the experimental treatment group. The main toxicities noted in the experimental group were rash (14.6%), diarrhea (5.4%), and stomatitis (5.4), while in the control arm neutropenia (26.5%), vomiting (19.5%), and leukopenia (15.0%) were most common. These results demonstrate targeted therapy in the first-line setting, significantly improves PFS with tolerable toxicity profile for Asian patients whose tumor is EGFR mutant positive.

These results have also been replicated in non-Asian populations. Rosell et al. noted the superior response to targeted therapy in their prospective trial of Spanish patients (Rosell et al., 2009). They screened 2105 patients with NSCLC for EGFR
mutations. Of the 2105 patients screened, 350 tested positive for EGFR mutations. Of the 350 patients identified, 217 received erlotinib, of which 197 were evaluated for a response. A majority of patients had clinical characteristics concurrent with those previously reported (adenocarcinoma, women, never-smokers), although the group notes unintentional selection bias may be responsible. Patients receiving erlotinib in the first line setting experienced a median PFS of 14 months, an increase compared to previously reported PFS of patients on standard chemotherapy (Schiller et al., 2002). These results prompted the group to undertake the aforementioned phase III EURTAC study, which demonstrated targeted therapy as superior to standard chemotherapy for non-Asian patients with exon 19 deletions or L858R mutations in the first line setting (Rosell et al., 2012). The results from these studies established firmly, targeted therapy should preferentially be utilized in the first-line setting for both Asian and non-Asian patients whose tumors harbor exon 21 L858R mutations.

While both exon 19 deletions and L858R mutations confer sensitivity to TKI therapy, as noted previously, L858R mutations have lower response rates to therapy. This has been shown by multiple groups (Jackman et al., 2009, 2012, Goto et al., 2013, Mitsudomi et al., 2005, Riley et al., 2006). In a recent pooled analysis of the LUX-Lung 3 and LUX-Lung 6 trials, both examining the affect of afatinib versus standard chemotherapy for EGFR mutant positive patients, Yang et al. demonstrated patients with exon 19 deletions experienced a statistically significant increase in overall survival compared to patients on standard chemotherapy (Yang et al., 2015). This survival advantage was not extended to patients with exon 21 L858R mutations, however. The
reasons for this are still unclear. It may be due to the specific structural changes each mutation inflicts upon the kinase region of the EGFR protein. Exon 19 deletions may alter the confirmation of the ATP binding region in a way that makes the area more conducive to binding with TKIs. Additionally, L858R mutations may be more commonly associated with other mutations, which may affect the way in which TKIs bind to EGFR (Mitsudomi et al., 2007). Additional research is needed to further elucidate why exon 19 deletions have better response to TKI therapy than exon 21 L858R mutations.

While these reasons may explain the differential in drug sensitivity conferred by exon 19 deletions or exon 21 L858R mutations, patients whose tumors harbor exon 21 L858R mutations benefited significantly from treatment with TKI compared to standard chemotherapy, thus establishing TKI therapy as the preferred treatment choice for this patient population in the first-line setting.

**IVb. Exon 21 L861Q Mutation:**

The exon 21 L861Q mutation is an amino acid substitution of glutamine for leucine at position 861 in EGFR. It is considered a rare mutation, with a reported occurrence of only 2% (Lovly, 2015). Exon 21 L861Q mutations also occur in the activation loop of the EGFR tyrosine kinase and, similarly to exon 21 L858R mutations, may disrupt the hydrophobic interactions of that area, thus forcing the protein into an ever-active confirmation.

Despite the paucity of this mutation, it has been determined exon 21 L861Q mutations confer sensitivity to TKI therapy (Lynch et al., 2004). Additional research confirms this finding by Lynch et al., but also illuminates the effect of TKI on this
specific mutation further. It has been noted by various groups that while this mutation is sensitive to TKI treatment, as is the case for other rare EGFR mutations, it is less responsive than the “classic” EGFR mutations of exon 19 deletions and exon 21 L858R mutations (Lohinai et al., 2015, Chiu et al., 2015, Watanabe et al., 2014, Wu et al., 2011). Lohinai et al. compiled 814 lung adenocarcinoma patients with KRAS and/or EGFR mutations, analyzing 419 for clinical outcome. They identified 49, or 6%, as rare mutations, including L861Q mutations. Interestingly, a majority of rare mutations identified were significantly correlated to smoking history. The group also noted patients with previously identified sensitizing rare mutations (G719X and L861Q) experienced higher response rates to TKI than patients with other rare mutations. Perhaps unsurprisingly, the group noted patients harboring rare mutations experienced a significantly shorter TKI response rate compared to patients with defined classic EGFR mutations (71% versus 37%, p = 0.039). These results were mirrored by the work of Chiu et al. The results from these studies indicate patients with these mutations should still be considered for TKI therapy, and highlight the importance of comprehensive genomic testing for patients in this setting.

Watanabe et al. performed a post hoc analysis of the NEJ002 trial, a randomized phase III trial comparing gefitinib to standard chemotherapy in chemo naïve patients, demonstrating results similar to Lohinai and Chiu. They show patients with rare EGFR mutations fare worse with TKI therapy compared to patients with common EGFR mutations in terms of overall survival (figure 15). The group also noted a similar relationship with regards to PFS.
Figure 15: Kaplan-Meier plot of overall survival for patients with rare or common mutations after treatment with gefitinib. The plot highlights significantly lower overall survival for patients with rare mutations treated with targeted therapy, compared to patients harboring common mutations. The low number of patients with rare mutations (5) should be noted as a limitation of this analysis. Adapted from Watanabe, 2014.

Due to the rarity of this mutation, it has been hard to establish a first line therapy for patients with this mutation. In vitro and in vivo studies have shown sensitivity to treatment with TKI, albeit with reduced response rates compared to more common EGFR mutations. However, there remains uncertainty about the role of TKI therapy in the clinical course of these patients, with some groups arguing first-line chemotherapy may be most beneficial for this patient population (Watanabe et al., 2014). Other groups report promising response rates with treatment of irreversible TKI, such as afatinib (Yang et al., 2013). Yang et al. presented their analysis of data from the LUX-Lung 2, 3 and 6 trials,
focusing on patients who were identified as harboring rare mutations. The group noted promising PFS and OS for patients with L861Q mutations. Based on these results, treatment of exon 21 L861Q mutations, and rare mutations in general, with afatinib may be an appropriate clinical decision.

At this time, there is still insufficient data to define a first-line treatment for patients with exon 21 L861Q mutations. Most published information on this mutation is reports of small sample size or case reports, the results of which are often controversial or contradictory. Current understanding indicates this mutation confers sensitivity to treatment with TKI, and there has been reports of promising response using irreversible inhibitors like afatinib. Despite this, due to the limited information currently available, it is advised patients with this mutation be assessed individually, although treatment with TKIs should be considered, as they have shown to be beneficial in the past. Additional research, and compilation of data from various centers will help to further understanding of this mutation and elucidate a more concrete treatment course for this subset of patients.

V. Resistance Mutations:

Patients who present with NSCLC with activating EGFR mutations, particularly exon 19 deletions and exon 21 L858R mutations, show remarkable responses to TKI therapy, especially compared to standard chemotherapy (Mok et al., 2009, Mitsudomi et al., 2010). Unfortunately, despite marked response to these drugs, patients almost unequivocally develop acquired resistance and subsequent tumor progression, limiting the long-term potential of TKI therapy (Tartarone et al., 2015). Resistance occurs approximately 1 to 2 years after initiation of TKI therapy, with the majority of resistance
mutations revealing themselves as T790M mutations, with an occurrence of 50 to 60% at re-biopsy (Acria et al., 2011). Other mechanisms of resistance include MET amplification, phenotypic and other point mutations, and interestingly, histological transformation to SCLC (Politi et al., 2015, Rosell et al., 2012). There is a great clinical need to address these emerging resistance mutations in order to provide better, more effective treatments for patients once these mutations are acquired. Due to its high prevalence in this patient population, extensive work has been done to understand and treat T790M mutations.

**Va. T790M Mutations:**

The T790M mutation is located in exon 20 and is resultant of a base change from cytosine to thymine, which codes for the substitution of methionine for threonine at position 790 in the catalytic cleft of the EGFR tyrosine kinase domain (figure 8)(Kobayashi et al., 2005). Structural modeling shows this substitution affects erlotinib binding in the ATP domain by, one, introducing a bulkier amino acid into the region possibly causing steric inference and, two, removing a hydrogen bond interaction between erlotinib and threonine which likely contributed to erlotinib’s previous high affinity to the binding site (Pao et al., 2005). T790M have similar interfering affects on gefitinib as well. While T790M mutations affect the ATP binding site of the EGFR tyrosine kinase domain, it is not expected to alter the ability of ATP binding to the domain, and therefore the activity of the kinase in general, meaning EGFR inhibition may still be a viable therapeutic option (Kobayashi et al., 2005, Ohashi et al., 2013).
Clinically, T790M mutations in exon 20 have been suggested to be associated with indolent growth characteristics and a longer PFS. The prognosis for patients with T790M mutations is better than patients without the mutation, although this may be reflective of other resistance mutations present in T790M negative tumors. Interestingly, T790M mutations are also more likely to occur in an existing site of disease (Chmielecki et al., 2011, Oxnard et al, 2011). While most T790M mutations are noted post-progression, a small subset of approximately 1 to 2% of cases is detected de novo. These cases usually display decreased sensitivity to EGFR TKI therapy and occur in conjunction with other sensitizing EGFR mutations (Wu, 2011). Of this group of patients, approximately 50% of them harbor germline T790M mutations (Oxnard et al, 2013). This is of high importance, as patients with germline T790M mutations should receive familial testing so to establish appropriate screening measures for early detection of disease. The issue of resistance by the T790M mechanism, both pre and post treatment, provides an interesting challenge to the treatment and management of disease in this patient population.

Afatinib, as a potent irreversible TKI, has shown preclinical and clinical activity against T790M mutations (Miller et al., 2012, Katakami et al., 2013, Soca et al., 2012, Li et al., 2008). Despite its demonstrated activity against T790M, it has a high frequency of gastrointestinal and skin toxicity that limits its usefulness in clinical settings. However, preclinical findings indicate the combination of afatinib and cetuximab lead to dramatic response of tumors harboring T790M mutations (Regales et al., 2009). Based on these findings, Janjigian et al. conducted a study to explore the efficacy and safety of afatinib
and cetuximab in combination for patients who met criteria for acquired resistance as proposed by Jackman et al. (Jackman et al., 2010, Janjigian et al., 2014). Patients were allowed to continue EGFR TKI therapy past progression to limit risk of disease flare (Chaft et al., 2013). The dual blockade of EGFR by afatinib and cetuximab, in this study, demonstrated a strong and durable response, with an objective response rate of 29%. Gomes and Cruz reported two cases in which patients with T790M mutated NSCLC reached partial responses with treatment of afatinib and cetuximab in combination further extending the results reported by Janjigian et al. (Ribeiro Gomes and Cruz, 2015). Interestingly, response rates were similar for both T790M positive and negative patients. Additionally important, the combination demonstrated a somewhat manageable toxicity profile with rates of grade 3 and 4 therapy related adverse events of 44% and 2% respectively. This combination represents a viable treatment option for heavily pretreatment patients who have developed resistance after prior TKI therapy.

More recently, third generation TKI drugs have entered clinical trial testing, emerging as potentially effective treatments for patients whose tumors have developed T790M resistance. Two of these third generation TKIs are AZD9291 and rociletinib (CO-1686).

AZD9291 is an oral, irreversible EGFR TKI that is selective for common sensitizing mutations as well as the T90M resistance mutation. In preclinical models it showed significantly lower activity against wild-type EGFR and higher activity against T790M tumors than previously demonstrated by afatinib (Cross et al., 2014). In a phase I study to determine the efficacy and safety of AZD9291 in patients with advanced EGFR
mutated NSCLC with acquired resistance to EGFR TKI therapy, Jänne et al. found AZD9291 to have potent activity against T790M mutated NSCLC. 138 patients harbored a centrally detected T790M mutation, of which 127 could be evaluated for a response. Of these patients, there was a 61% rate of achieved objective response and a 95% disease control rate (figure 16). Disease control is defined as the percentage of patients who experience, as defined by RECIST criteria, stable disease, partial response, or complete response. Of the patients with a detected T790M mutation, the median PFS was 9.6 months (figure 17)(Jänne et al., 2015). Interestingly, although not surprising based on AZD9291’s preclinical activity against wild-type EGFR, AZD9291 has a very manageable toxicity profile, with limited skin and gastrointestinal side effects, especially in comparison to afatinib and cetuximab combination therapy.

Figure 16: Waterfall plot of EGFR T790M positive response to AZD9291. Overall 61% of patients in the T790M positive sub group achieved objective response. Of note, patients from all dosing level cohorts experienced objective response. Adapted from Jänne et al, 2015.
Figure 17: Kaplan-Meier plot of PFS of T790M positive patients versus T790M negative patients. T790M positive patients demonstrated a median PFS of 9.6 months, compared to median 2.8 months for patients with no detectable T790M mutation. Adapted from Jänne et al, 2015.

With a response rate and PFS that nearly doubles that of afatinib and cetuximab in a T790M mutated NSCLC patient population, with a much more manageable toxicity profile, AZD9291 appears to be a potent therapy for patients with this mutation.

While the above results were achieved with patients who had previous exposure to EGFR TKI, AZD9291 was also utilized in treatment naïve patients. Presented by Ramalingam et al, results from the treatment naïve cohort of the AZD9291 phase I study show positive results from treatment with AZD9291 in a treatment naïve patient population (Ramalingam et al., 2015). FLAURA, a trial comparing AZD9291 to erlotinib...
or gefitinib in EGFR mutant treatment naïve patients, is currently underway and will explore if AZD9291 is superior to first generation TKIs in a first-line setting.

Rociletinib (CO-1686) is another third generation EGFR TKI, also with impressive results in current clinical trials. Rociletinib is an EGFR selective, oral, irreversible inhibitor that has shown preclinical activity against common EGFR mutations as well as T790M resistance mutations (Walter et al., 2014). Structural models show rociletinib covalently binds to the cysteine at position 797, modifying the ATP binding site, resulting in inhibition of the tyrosine kinase domain (figure 18). It, like AZD9291, spares EGFR wild-type.

![Figure 18: Structural model of rociletinib bound to EGFR T790M.](https://example.com/figure18.png)

Covalently binding to Cys797 places rociletinib in position to inhibit the mutated tyrosine kinase. Adapted from Walter, 2014

In a clinical setting, rociletinib exhibited strong efficacy in EGFR T790M positive tumors with a response rate of 59% and an estimated median PFS of 13.1 months. 93% of
patients experienced disease control (figure 19) (Sequist et al., 2015). As seen with AZD9291, again explained by the selective nature of the inhibitor, there was infrequent and controllable skin and gastrointestinal adverse events. The most common grade 3 adverse event experienced was hyperglycemia, which occurred at a rate of 22%. No patients discontinued treatment due to this adverse event and were treated with success by dose reduction or a hypoglycemic agent, usually metformin.

![Figure 19: Waterfall plot of rociletinib’s activity against EGFR T790M positive tumors.](image)

Overall objective response rate of patients in this subgroup was 59%. Adapted from Sequist et al., 2015.

Preclinical studies show a metabolite of rociletinib is what causes hyperglycemia through inhibition of type I IGF-IR. Interestingly, activation of this pathway has been
proposed as a resistance mechanism to EGFR inhibition. It is currently unknown if rociletinib’s inhibitory nature on this pathway contributes to the drug’s efficacy in EGFR TKI resistance NSCLC.

A major limitation of this study is the limited number of patients to receive study drug, a result of the design of the study. Larger studies are currently underway and are essential to confirm the results of this striking, yet small study.

Additional studies will further elucidate the activity of AZD9291 and rociletinib, and are needed to verify the results discussed above. Both drugs display selective, potent activity against EGFR T790M mutated NSCLC. However, with rociletinib’s tendency to induce hyperglycemia in patients, patients who have a comorbidity of diabetes, or who are pre-diabetic, perhaps should be preferentially steered toward treatment with AZD9291 in this setting. Regardless, both AZD9291 and rociletinib exemplify major steps forward in the treatment of resistance acquired, EGFR mutated NSCLC. Both therapies will extend overall survival of this patient population, and do so with less toxicity than previous treatments. The presence of T790M mutations in patient’s tumors therefore present a targetable biomarker, reinforcing the importance of obtaining tumor samples post progression on EGFR TKI therapy.

Vb. MET Amplification:

MET amplification is an alternative resistance mechanism that occurs in approximately 20% of patients in the acquired resistance setting. The MET is a proto-oncogene that encodes a heterodimeric transmembrane receptor kinase. Binding of the receptor to its ligand activates dimerization leading to increased tyrosine kinase activity.
This can activate multiple downstream pathways, including those responsible for cell growth and survival (Bean et al., 2007). MET amplification, therefore, can bypass EGFR signaling, to activate secondary messengers downstream of EGFR, rendering EGFR TKIs ineffectual. In studies conducted by Bean et al., they identified MET amplification in 21% of samples of EGFR mutant NSCLC that had acquired resistance to EGFR TKI therapy. Interestingly, 40% of these samples also harbored the EGFR T790M mutation, demonstrating these mutations can occur within the same cells. Engleman et al. noted similar results, identifying MET amplification in 22% of lung cancer samples that had developed resistance to EGFR TKIs (Engleman et al, 2007). It has been established, EGFR TKI resistance can be overcome by treatment of MET amplified tumors with EGFR and MET inhibitors (Turke et al., 2011). This has set the stage for combination therapies, specifically with third generation TKIs. With their well-tolerated toxicity profiles, agents like AZD9291 and rociletinib are attractive agents to use in combination therapy with MET inhibitors to address EGFR TKI resistance by MET amplification mechanisms. Trials are currently underway to explore this as a treatment option for these patients.

One such trial is the TATTON trial, a multi-arm, phase 1b trial of AZD9291 combined with AZD6094, selumetinib (both MET inhibitors) or MEDI4736, an anti-PD-L1 monoclonal antibody. Preliminary results suggest the toxicity profile of combination therapy with EGFR and MET inhibitors is tolerable in patients. Furthermore, in the selumetinib and AZD6094 treatment arms, there is a reported partial response in 9 of 23 and 6 of 11 patients, respectively (Oxnard et al., 2015). These preliminary results suggest
combination therapy is tolerated and has a high efficacy in patients with MET amplification resistance. A final analysis of this study once it is completed will allow greater insight into the role of combination EGFR/MET inhibitors in treating EGFR TKI resistance in this patient population. Further studies will also be needed to expand upon and verify the results of this trial.
CONCLUSIONS AND FUTURE DIRECTIONS

With the discovery and implementation of targeted therapies, especially in the setting of EGFR mutated NSCLC introduced a paradigm shift in how these cancers were approached and treated by physicians. The influx of investigation into these mutations have greatly increased the medical communities understanding of the underlying mechanisms of EGFR mutated driven NSCLC and has led to the development of first, second, and third generation EGFR TKI therapies that have improved patient lives and extended overall survival. Unfortunately, these cancers continue to develop resistance to targeted therapies designed to curtail their affect on cell growth and proliferation. Even with third generation inhibitors, like AZD9291, resistance has started to appear in the form of a novel EGFR C797S mutation, adding further to the complexity of the situation and presenting yet additional challenges to researchers and clinicians alike (Thress et al., 2015).

As the story of EGFR mutated NSCLC continues to evolve, it continues to highlight the importance of understanding the molecular mechanisms of cell signaling in this setting, obtaining and sequencing patient tissue post progression to determine any targetable mutations that may have developed, and the ever growing complexity researchers and clinicians face in their efforts to provide safe and efficacious therapies to patients afflicted with this disease.
APPENDIX

Timeline of Important EGFR related Discoveries (Chong, Jänne, 2013)

- Cohen and colleagues demonstrate EGFR binding to EGFR stimulates phosphorylation.
- Monoclonal antibodies to EGFR noted to inhibit tumor cell proliferation.
- Identification of ERBB2 (HER2) and similarity to EGFR noted.
- EGFR overexpression observed in malignant gliomas and is later found to correlate with poor prognosis in head and neck, ovarian, cervical, bladder and esophageal cancers.
- Gefitinib identified as a potent inhibitor of EGFR.
- Identification of EGFR mutations in patients responsive to erlotinib or gefitinib.
- FDA approval of erlotinib and cetuximab for treatment of metastatic lung and colorectal cancers, respectively.
- Cetuximab approved by FDA to treat head and neck cancer.
- EGFR T790M mutations detected in lung cancer CTCs.
- Jackman criteria for acquired resistance published.
- Histologic transformation to SCLC reported in a patient with an EGFR mutation.
- Erlotinib and gefitinib FDA approved for first-line treatment of metastatic EGFR-mutant lung cancer.
- Clinical trials of EGFR T790M mutant-specific inhibitor AZD9291 begin.
- EGFR T790M resistance mutation reported.
- Second-line erlotinib shown to improve OS in patients with metastatic lung cancer, regardless of EGFR mutation status.
- Alitretinib approved by FDA in combination with gemcitabine for treatment of metastatic pancreatic cancer.
- KRAS mutation associated with intrinsic resistance in EGFR wild-type tumors and found to be mutually exclusive with EGFR mutations.
- BIM reported as a mechanism of resistance to EGFR TKIs in lung cancer.
- MET amplification identified as a mechanism of resistance to gefitinib.
- EGFR T790M mutant-specific irreversible inhibitors reported.
- Dual targeting of EGFR with cetuximab and alitretinib found to overcome resistance in EGFR T790M.
- IPI065 study shows gefitinib is superior to carboplatin-paclitaxel in east-Asian light-smoking or nonsmoking patients with lung cancer.
- AXL kinase activation reported as a potential mediator of BIM in cells and patients resistant to EGFR TKIs.
- Mutations in the extracellular domain of EGFR found to confer resistance to cetuximab but not panitumumab.
- Exome sequencing of circulating oncogenic DNA in plasma reported.
- Clinical trials of the EGFR T790M mutant-specific irreversible inhibitor CD-1638 begin.
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EDUCATION

University of Connecticut School of Medicine – Farmington, CT
Doctor of Medicine, 2019 (anticipated)

Boston University School of Medicine – Boston, MA
Master of Science in Medical Sciences, 2015

College of the Holy Cross – Worcester, MA
Bachelor of Arts in Chemistry, Health Professions Program, May 2012

PROFESSIONAL EXPERIENCE

Dana-Farber Cancer Institute
Clinical Research Coordinator, September 2012 – Present

• Responsible for the primary data collection and management of patient clinical information as it pertains to clinical trials. Maintain regulatory binders and ensures study compliance with all state, federal and IRB requirements. Prepares and submits protocol application packets, amendments to protocols, continuing review forms and adverse event reports to IRB and Sponsor.

Hartford Hospital
Summer Student Research Fellow, Summer 2011

• Completed a clinical research project on combined liver-kidney transplants. Observed hospital staff, clinical rounds, and surgical procedures.

College of the Holy Cross
Laboratory Researcher, Summer 2010

• Chemical research on the total synthesis of natural products. Performed chemical reactions using standard practices, purified products by column chromatography, and characterized by NMR

Connecticut Gastroenterology Associates
Clinical Researcher, Winter 2009
• Organized and analyzed treatment data for previous and present Hepatitis C patients

Simsbury Farms Golf Course
Groundskeeper, Summer 2008, 2009
• Maintained golf grounds and clubhouse; in charge of maintaining all the sand traps on the course.

RELATED EXPERIENCE

Pine Street Inn, Boston, MA
Volunteer, February 2014 – Present
• Plan and execute a monthly game night. Includes planning, cooking, and serving dinner to houseguests. Requires planning and execution of a night activity, with prizes, for houseguests.

College of the Holy Cross Research Program,
Student Researcher, June 2010 – May 2012
• Chemical research on the total synthesis of natural products. Perform chemical reactions using standard practices, purify products by column chromatography, and characterize products by NMR.

Student Advisory Committee – Chemistry Department
President, Spring 2010-May 2012
• Assess new faculty hiring and assist with faculty tenure decisions; review teacher evaluation forms. Organize and execute department sponsored events for chemistry students.

Organic Chemistry Peer Assisted Learning Tutoring Program
• Observe organic chemistry classes, plan study sessions and problem sets and material for students to complete. Attend weekly meetings with other tutors to assess weekly progress of students.

Nativity Tutoring Program
Tutor, Fall 2008 – Spring 2012
• Volunteer position, tutor 5th to 8th grade under-privileged boys in a variety of academic subjects.

College of the Holy Cross Admissions
Tour Guide Fall 2008, 2009
• Led tours of the college campus to groups of prospective students and parents. Acted as ambassador to the College of the Holy Cross at former high school.
AWARDS

2010 • Sherman Fairchild Foundation Summer Research Fellowship, $3,850

Spring 2009, Fall 2011, Spring 2012 • Dean’s List

PUBLICATIONS AND PRESENTATIONS


A.J. Polio, K.J. Quinn
*Efforts Toward the Total Synthesis of (+)-Sorangicin A*
Academic Conference Presentations, College of the Holy Cross, May 2012


A.J. Polio, D. Hull
*Combined Liver-Kidney Transplantation: Indications, Outcome Analysis, and the Hartford Hospital Transplant Program Experience*
Fall Research Symposium, College of the Holy Cross, September 2011

A.J. Polio, D. Hull
*Combined Liver-Kidney Transplantation: Indications, Outcome Analysis, and the Hartford Hospital Transplant Program Experience*
Student Research Fellowship Poster Presentation, Hartford Hospital, August 2011

A.J. Polio, K.J. Quinn
*Efforts Toward the Total Synthesis of (+) Boronolide*
Spring Research Symposium, College of the Holy Cross, April 2011

A.J. Polio, K.J. Quinn
*Efforts Toward the Total Synthesis of (+) Boronolide*
American Chemistry Society (ACS) Meeting, Anaheim, CA, March 2011

A.J. Polio, K.J. Quinn
*Efforts Toward the Total Synthesis of (+) Boronolide*
Fall Research Symposium, College of the Holy Cross, September 2010