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Targeting EGFR in lung cancer: Lessons learned and future perspectives

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Abstract

The development of individualized therapies has become the focus of current oncology research. Precision medicine has demonstrated great potential for bringing safe and effective drugs to those patients stricken with cancer, and is becoming a reality as more oncogenic drivers of malignancy are discovered. The discovery of Epidermal Growth Factor Receptor (*EGFR*) mutations as a driving mutation in non-small cell lung cancer (NSCLC) and the subsequent success of the tyrosine kinase inhibitors (TKI) have led the way for NSCLC to be at the forefront of biomarker-based drug development. However, this direction was not always so clear, and this article describes the lessons learned in targeted therapy development from *EGFR* in NSCLC.

Keywords

EGFR; NSCLC; Lung Cancer

Introduction

Advances in the understanding of the molecular pathogenesis underlying tumor development have begun to shift the field of oncology away from a “one size fits all” treatment paradigm. The discovery of oncogenic drivers has led to the development of targeted therapies that are more selective against the tumor cell. One of the first malignancies in which the biomarker-driven approach was proven successful is chronic myeloid leukemia, or CML. While the Philadelphia (Ph) chromosome had been known to be a chromosomal marker of CML for years, it was not until Druker et al. observed that specifically targeting this translocation with imatinib could cause dramatic responses.¹ This groundbreaking work was followed in the solid tumor realm by trastuzumab, which benefits patients with an activated HER2 pathway signaling.² These successes generated significant interest in researching for new targets and precision drug development. The field of non-small cell lung cancer (NSCLC) is leading the way in development of targeted therapies, many of which are now available for use in practice or clinical trials. The discovery of the role of Epidermal Growth Factor Receptor

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mutations (*EGFRm*) in NSCLC tumorigenesis and subsequent development of *EGFRm*-specific tyrosine kinase inhibitors (TKI) is an excellent example of personalized therapy. Consequently, extensive work detailing the genomic landscape of NSCLC and its effect on treatment and outcomes has been completed, such as published in The Cancer Genome Atlas (TCGA) and the Lung Cancer Mutation Consortium (LCMC).^{3, 4} However, along the path from *EGFRm* discovery to TKI approval, many lessons were learned which can help guide future biomarker research and development, not just in NSCLC, but in all malignancies.

Lesson 1: The target should be critical to the sustenance of the cancer cell

Currently, it has been proved that *EGFRm* is driving a subset of NSCLC and that targeting this oncogene with an *EGFRm* TKI such as erlotinib, gefitinib, or afatinib is the most effective treatment strategy. However, as with many groundbreaking developments, this path was not always so clear. *EGFR* is a glycoprotein that plays a complicated role in signal transduction and cellular processes. It has been shown to be important in tumorigenesis for many years.⁵ Significant research was performed examining the biological relevance of *EGFR* in NSCLC, following initial observations of receptor overexpression and a corresponding association with poor prognosis.^{6, 7} For these reasons, targeting *EGFR* became an area of interest in NSCLC drug development. The oral medications erlotinib and gefitinib were designed to target the *EGFR* pathway as reversible small molecule inhibitors of the tyrosine kinase domain of the receptor to prevent downstream signaling. Preclinical data demonstrated anti-tumor activity in cell lines and xenograft models that were dependent on *EGFR* activity.⁸

Based on promising early phase clinical trial results,^{9, 10} the *EGFR* TKI erlotinib was studied in a phase III trial of metastatic NSCLC patients who had progressed on standard-of-care chemotherapy (BR-21). The patients were randomized to erlotinib versus placebo following progression after 1 or 2 chemotherapy regimens, but were unselected in regards to *EGFR* status. The study recruited 731 patients, with 488 receiving erlotinib and 243 placebo. Erlotinib was tolerated well, with the most common adverse events being rash and diarrhea. In terms of outcomes, erlotinib had a 9% response rate and a 2.2 month median progression free survival (PFS) vs. 1.8 months for placebo ($p < 0.001$). The median overall survival was 6.7 months and 4.7 months ($p < 0.001$) for erlotinib and placebo, respectively.¹¹ Based on this significant, although clinically modest, overall survival benefit, erlotinib was approved by the FDA for NSCLC in 2004. The authors noted that Asian ethnicity, women, patients with adenocarcinoma, lifetime nonsmokers, and tumors that expressed *EGFR* in 10% of cells had improved response rates, although *EGFR* overexpression was not consistently found to be a predictive biomarker to TKI therapy.¹² It was clear from the study that while there is a survival advantage in treating with erlotinib in an unselected NSCLC population, there was clearly a subgroup that benefitted more from TKI treatment.

Several groups hypothesized that the patients that responded well to erlotinib or gefitinib had an intrinsic difference within their *EGFR* receptor. In one landmark study, the researchers obtained tumor samples from patients that had responded to TKI therapy and sequenced the *EGFR* tyrosine kinase binding domain. They found exon 19 in-frame deletions, the exon 21 point mutation L858R, and less frequently exon 18 point mutations.^{13–15} In fact, when these

studies are examined as a whole, of 31 patients that responded to erlotinib or gefitinib, 25 had one of these listed mutations.¹⁴

With increasing evidence linking *EGFRm* mutations to response to *EGFR* TKI therapy, the authors of BR-21 conducted post-hoc genomic analysis and correlated them with clinical outcomes. Unfortunately, while they found a trend towards increase responses, no increased survival was seen in patients with *EGFRm* treated with erlotinib ($p=0.65$).¹⁶ In retrospect, this negative result might have been due to small numbers of patients, suboptimal quality of specimens, and that mutation detection techniques were still evolving. While this negative analysis did delay the widespread acceptance of *EGFRm* as a predictive biomarker, the body of evidence was growing and subsequent clinical trials examined the use of *EGFR* TKI on selected populations. This shift led to the pivotal studies that confirmed the use of biomarker-driven, targeted therapy use in the treatment of NSCLC.

The first large, randomized phase 3 trial of an *EGFR* TKI in a selected population was published in 2009, entitled the IPASS study. This study randomized East Asian, never- or former light-smokers with metastatic lung adenocarcinoma that had not received prior systemic therapy to either gefitinib or standard platinum-based chemotherapy. The main goal of this study was to compare the efficacy of *EGFR* inhibition to chemotherapy in a clinically enriched subset of patients likely to benefit from the former. While the trial did not select specifically for *EGFRm* status, it was enriched for *EGFRm* because of the clinical selection factors utilized.^{14, 17} Not only did the study demonstrate non-inferiority for gefitinib relative to chemotherapy, but also showed superiority with *EGFR* inhibition with a PFS hazard ratio (HR) of 0.74, $p<0.001$.

The investigators conducted a post-hoc analysis of tumor tissue for *EGFR* mutation and analyzed outcomes based on this. Patients with *EGFRm* had response rates of 71.2% when treated with gefitinib vs. 47.3% with chemotherapy, with a HR for progression of 0.48, $P<0.001$.¹⁸ In fact, for patients with wild type *EGFR*, the outcomes were inferior with gefitinib compared to chemotherapy. This established the fact that molecular selection was superior to clinical selection with regards to utilization of *EGFR* inhibitors in advanced NSCLC.

The findings from the IPASS study led to a number of randomized studies that compared *EGFR* inhibition to chemotherapy, specifically in patients with *EGFRm* (Table 1). The EURTAC trial enrolled 172 patients with *EGFRm* NSCLC across 42 institutions in Europe and compared erlotinib to platinum-based chemotherapy as first line therapy for metastatic disease. In this selected population, erlotinib had superior outcomes to standard chemotherapy. Response rate was 64% vs. 18%, and median PFS was 9.7 months vs. 5.2 months respectively for erlotinib and chemotherapy. There was no difference in overall survival, likely due to crossover from control group to erlotinib upon disease progression.¹⁹ Similar results were seen in two studies conducted in Japan that compared gefitinib with chemotherapy in a treatment naïve, *EGFRm* NSCLC populations. In these trials, median PFS for gefitinib was 9.2–10.8 months vs. 5.4–6.3 months for chemotherapy.^{20, 21} Finally, the second generation *EGFR* TKI afatinib was also proven superior to first-line chemotherapy in patients with *EGFRm* NSCLC, with a median PFS of 13.6 months vs. 6.9

months.²² These studies led to the adoption of *EGFR* TKIs as the preferred standard of care in first-line therapy of patients with *EGFRm* NSCLC.

Overall, this pathway to a biomarker-driven treatment has helped teach the importance of choosing the right biomarker and the appropriate population in the era of targeted therapy. In fact, a recent meta-analysis that compared *EGFR* TKIs to chemotherapy in patients with *EGFR* wt lung tumors demonstrated superiority for chemotherapy. A total 11 trials with 1605 patients were included in the analysis. For this population, chemotherapy was associated with an improved PFS over *EGFR* TKIs (HR for TKI, 1.41; 95% CI, 1.10–1.81).²³

Lesson 2: Resistance inevitably develops

Despite response rates of over 70%, patients with *EGFR* driving mutations on TKI therapy eventually progress. The median time to progression has ranged from approximately 10–13 months.^{18, 19, 22} Unfortunately, resistance to targeted therapy is not a problem unique to this population. Similar patterns of resistance have been seen with imatinib use in CML and crizotinib in ALK+ NSCLC.^{24, 25} However, secondary to the dedication of researchers and the generosity of patients in consenting to biopsies post-TKI progression, research into the resistance to *EGFRm* TKIs has provided valuable insights. The most common resistance mechanism seen in *EGFRm* patients is the development of a secondary point mutation in the *EGFR* active domain, substituting a bulky methionine amino acid for threonine (T790M) and inhibiting *EGFR* TKI binding, similar to that of the T315I mutation seen in CML.²⁵ It was first described as a case report in 2005 in a 71 yo former smoker with an *EGFR* exon 19 deletion positive advanced NSCLC. The patient achieved a complete response on gefitinib, but progressed after 2 years. A repeat tumor biopsy was performed and the *EGFR* exons 18–21 were sequenced. While the original del19 mutation was still present, a new exon 20 T790M mutation was also found. Introduction of the T790M into *EGFRm* cell lines was found to confer resistance to gefitinib.²⁶ A subsequent study found that in 2 of 5 patients with *EGFRm* NSCLC that had progressed on erlotinib or gefitinib harbored the T790M mutation, which again was confirmed to be resistant to *EGFR* TKI in in-vitro studies. However, 3 of the patients did not have the T790M mutation, indicating that other forms of resistant were likely to be present.²⁷ Currently, the T790M mutation estimated to represent 50–60% of resistance to the first and second generation *EGFRm* TKIs.²⁸ Signaling pathways that bypass the *EGFRm* TKI blockade have also been shown to play an integral part in resistance.

In recent years, c-MET has been shown to be a potential oncogenic driver of NSCLC, and efforts are currently underway to target c-Met amplification in a targeted fashion in NSCLC.²⁹ c-MET, coded for by the oncogene MET, is a receptor tyrosine kinase that play an important role in a variety of cell processes, including cell invasion, growth and angiogenesis.³⁰ c-MET has also been shown to play a role in evading TKI inhibition of *EGFRm* NSCLC. Engelman et al. demonstrated that one mechanism by which *EGFRm* leads to NSCLC is by activation of the phosphoinositide 3-kinase (PI3K)/Akt signaling through ERBB3 (HER3).³¹ They hypothesized that in *EGFRm* TKI resistant NSCLC, a secondary pathway caused activation of ERBB3 and continued tumorigenesis. In gefitinib-

resistant cell lines, this was due to amplification of *MET*, leading to *EGFRm* independent ERBB3 signaling. When *MET* amplification was studied in TKI-resistant NSCLC biopsies, it was observed in 4/18 (22%) samples.³² This finding was soon confirmed with *MET* amplification in 9/43 (21%) of resistant patients, although interestingly was commonly seen in conjunction with the T790M mutation.³²

Following this work, Sequist et al. published a case series examining resistance to the *EGFR* inhibitors. Biopsies were obtained from 37 patients with *EGFRm* NSCLC post-progression on TKI therapy. The tumor samples were examined by multiplex SNaPshot genomic sequencing, fluorescent in-situ hybridization (FISH) testing, and pathological evaluation. All patients retained their original *EGFRm*. Of the 37 patients, 18 (49%) were found to have the T790M mutation, which included 3 patients who concomitantly developed *EGFR* gene amplification, and 2 (5%) had *MET* amplification by FISH. For the first time, *PIK3CA* mutations were seen as a resistance mechanism in 2 (5%) of patients. Additionally, phenotypically, 2 (5%) patients were noted to have an epithelial-to-mesenchymal transition (EMT). Perhaps most unexpected, in 5 (14%) patients, a full histological change was noted from adenocarcinoma to small cell lung cancer (SCLC) while retaining their original *EGFRm*.²⁸ This finding was seen in another cohort of 106 biopsies of TKI resistant tumors, 2 of which were found to be of a SCLC phenotype, and 1 was a high-grade neuroendocrine tumor.³³

Lesson 3. Resistance can be overcome by mechanism-based drug discovery

The best approach to treating *EGFRm* NSCLC patients that have progressed on first or second line *EGFR TKI* therapy remains under active research. Nonetheless, significant progress has been made and lessons learned along the path. For example, in the treatment of *EGFR* wt NSCLC, when progression is noted on radiographic imaging, typically the treatment is discontinued and a subsequent line of therapy is adopted. However, this paradigm is not as clearly applicable in *EGFRm* targeted therapy. Currently, *EGFRm* NSCLC patients who have asymptomatic, mild radiographic progression are often continued on their TKI therapy. Additionally, if there is progression in the CNS (of which the TKIs have relative low penetration) or with oligometastatic disease, local therapy such as radiation is often utilized and the TKI is continued for systemic control. In one study, patients were enrolled upon progression on either gefitinib or erlotinib, and then had their TKI discontinued. Of 10 patients that had their TKI held, 7 developed worsening of symptoms such as increased dyspnea, fatigue and pleural effusions. The patients then had their TKI restarted and all had clinical improvement or stabilization.³⁴ In a separate retrospective study, 18 patients who progressed with non-CNS oligometastatic disease on TKI therapy and were treated with local therapy were included. Of these patients, 85% restarted TKI therapy within one month of local therapy and the median PFS after treatment re-initiation was 10 months, with the average time to a change in systemic therapy of 22 months.³⁵ In patients that develop CNS progression, again retrospective studies support the use of local therapy to the brain followed by continuation of TKI therapy.^{36, 37} While local therapy and continued

TKI for asymptomatic, oligoprogressive disease has become an accepted treatment standard, these non-randomized retrospective reports have to be interpreted with caution.

The recently reported IMPRESS trial has clearly demonstrated that the previously used practice of continuing TKI after progression along with subsequent chemotherapy is not beneficial. This study enrolled an Asian and European population with advanced *EGFRm* NSCLC that were chemotherapy naïve, initially achieved a response to first-line gefitinib and then progressed within 4 weeks prior to accrual. Patients were then randomized to cisplatin and pemetrexed with gefitinib, or the same chemotherapy regimen with placebo. From 71 centers, 265 patients were randomized and the cohorts were well balanced for baseline characteristics. They found no difference for gefitinib vs. placebo in terms of ORR (31.6% vs. 34.1%, $p=0.76$), disease control rate (84.2% vs. 78.8%, $p=0.308$), or PFS (5.4m vs. 5.4m, $p=0.273$), respectively. In fact, the investigators found that the OS was worse in patients who continued gefitinib (14.8m vs. 17.2m, $p=0.029$).³⁸ Based on this definitive study, it is no longer necessary to continue a TKI with subsequent lines of therapy beyond progression. Additionally, the unsuccessful attempts to use various drugs, such as HDAC, c-MET, and IGF-1R inhibitors, in combination with *EGFR* TKIs to improve efficacy in an unselected population highlights the importance of utilizing predictive biomarkers in trial design.^{39–41} Interestingly, however, the combination of erlotinib with bevacizumab in an *EGFRm* frontline population appeared efficacious in phase II randomized trial vs. erlotinib alone, with a median PFS of 16 vs. 9.7 months, respectively.⁴²

Currently, 3rd generation *EGFR* TKIs are in clinical trials and have demonstrated significant activity in patients that have progressed on erlotinib, gefitinib, or afatinib. The unique aspect of these next generation agents' development is that they were designed to target both the activating *EGFRm*, as well as the most common resistance mechanism, the T790M mutation, while relatively sparing the *EGFR* wt receptor. The drugs that are currently furthest along in development are AZD9291 (AstraZeneca, Macclesfield, United Kingdom) and Rociletinib (CO-1686, Clovis Oncology, Boulder, CO). Both targeted therapies have shown impressive results in preclinical models.^{43, 44} The results of the completed phase I dose escalation and expansion cohort AZD9291 have recently been updated. The patients were heavily pretreated, 38% male and mostly Asian race. A total of 253 *EGFRm* patients that progressed on 1st or 2nd generation TKIs patients were recruited to the study, out of whom 222 were included in the expansion cohort. The presence of T790M mutation was confirmed in 138 patients. Outcomes with AZD9291 were impressive, but were clearly better in the patients with the T790M resistance mutation. For T790M positive patients, RR was 61% with a clinical benefit rate (CBR) of 95% and, a preliminary median PFS of 9.6 months, while for T790M negative patients, RR was 21%, CBR was 61% and median PFS 2.8 months.⁴⁵ Adverse events were similar, although less frequent, than the 1st generation TKIs.

Rociletinib was also developed to target T790M and has shown promise. The early efficacy data from the expansion cohort of a phase I/II study of *EGFRm* patients that had progressed on first line TKIs were reported recently. For the expansion cohort, the patients had to be T790M positive. The response rate for the forty patients that were T790M positive was 58%. The median PFS had not been reached at the time of the report. Interestingly, hyperglycemia

was the most common adverse event, presumably related to inhibition of the IGF-1R pathway.⁴⁶ Clinical trials are progressing rapidly for these agents, as both drugs are being compared to chemotherapy in patients with acquired resistance. Other 3rd generation *EGFRm* TKIs are being examined, but are early in development (Table 2). It will be important moving forward to obtain biopsies from patients on a 3rd generation TKI upon progression in order to determine the resistance mechanisms. Furthermore, given the efficacy of these 3rd generation TKIs, they are currently being compared to the 1st generation *EGFR* TKIs in randomized trials of a treatment-naïve population.^{47, 48}

These results hold promise for *EGFRm* NSCLC patients who progress on *EGFR* TKIs. However, there is significant room for improvement in patients who are T790M negative. Additionally, there is evidence that tumor heterogeneity for *EGFR* resistance exists within *EGFRm* NSCLC. In one study, 42 patients that had multiple biopsies post-TKI progression were reviewed. The mechanism of resistance changed between individual patient samples in 20/42 patients, with 10 patients either gaining or losing the T790M mutation while the original driver *EGFRm* was preserved.⁴⁹

One active area of research to improve the treatment after resistance formation is combination therapy, which holds promise for the T790M negative population. Rizvi et al. recently reported data examining the anti-PD1 antibody nivolumab in combination with erlotinib in patients with advanced *EGFRm* NSCLC. Twenty out of 21 patients had progressed on prior therapy with erlotinib. The ORR was 19%, and 45% had stable disease as best response. The median PFS was 29.4 weeks and median overall survival had not been reached.⁵⁰ While early, PD-1/PDL-1 inhibition in addition to *EGFR TKI* might hold promise for patients who progress on *EGFR* TKIs. Similarly, afatinib and cetuximab were studied in the *EGFR* resistant setting in a phase Ib clinical trial of 126 patients. There was a modest response to treatment with an ORR of 29% and median PFS of 4.7 on the combination; however, there was a significant skin toxicity, with 46% grade 3/4, making the clinical use of this regimen unclear.⁵¹ However, the work that has been done in exploring the best ways to approach *EGFR* TKI resistance should provide guidance to the development of future targeted therapies across tumor types.

Lesson 4: Achieving cure with *EGFR* TKI is still elusive

Finally, a key issue relates to the use of targeted therapies in early stage disease to improve the cure rate. Despite their impressive efficacy in advanced stage patients, targeted therapies to date are not curative treatments.

This was established by the results of the RADIANT trial, a large, randomized phase III trial that compared adjuvant erlotinib to placebo for early stage (stage IB-IIIa) disease. Patients that were *EGFR* positive by IHC/FISH were included following surgical resection of their primary tumor. It is important to note that *EGFRm* was not used as the biomarker. For the 973 patients randomized, there was no difference in disease free survival or overall survival between erlotinib and placebo. However, 161 patients were *EGFRm* positive, and in subset analysis, median disease-free survival was 46.4 months for erlotinib vs. 28.5 months for placebo in this patient population. The overall survival results are not mature, but the

preliminary results do not demonstrate a robust difference.⁵² The ongoing ALCHEMIST trial is seeking to definitively answer this question, enrolling early stage *EGFRm NSCLC* patients who underwent surgery to either receive erlotinib or placebo for up to a two-year period with overall survival as the definitive endpoint (Figure 1).⁵³

Conclusions

The field of oncology has entered an age of biomarker-driven, targeted therapy, and *EGFR* mutated NSCLC has been at the forefront. Innovative research and drug design have followed the discovery of this driving mutation. However, lessons learned along the way will help guide future biomarker development. The developmental history of EGFR inhibitors has not only influenced subsequent research in lung cancer, but has extended to all solid organ malignancies. Evaluation of predictive biomarkers is now an essential part of even early phase studies. Many clinical trials are utilizing extension cohorts of phase 1 studies to evaluate efficacy in patient subsets based on putative biomarkers. It also changed the perception of lung cancer as one disease; in fact, there are many ongoing studies for evaluating agents in various subsets based on driver mutations. We have also learned that even the most targeted agent does not result in complete control/cure of the disease, since resistance develops in all patients. The use of novel combination regimens to avoid resistance might pave the way to achieve durable benefits for majority of the patients with driver mutations. The promising results with immune checkpoint inhibitors provide another avenue to improve the efficacy of targeted agents in NSCLC. It is also important to conduct tumor biopsy at the time of clinical resistance to understand the specific mechanism, since this knowledge could guide the next therapeutic choice. Thankfully, the rapid emergence of technology to conduct molecular studies in cell-free DNA obtained in peripheral blood (liquid biopsy) may obviate the need for multiple tumor biopsies. In conclusion, understanding the molecular characteristics of the tumor at every step of the way is likely to achieve the best possible therapeutic outcomes for patients with driver mutations.

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Figure 1.
ALCHEMIST study schema

Table 1

Randomized phase III trials comparing chemotherapy with 1st and 2nd generation *EGFR* TKIs

Study	Year	Regimen		ORR		Median PFS		Median OS	
		TKI (N)	Chemo (N)	TKI (%)	Chemo (%)	TKI (months)	Chemo (months)	TKI (months)	Chemo (months)
Mok et al. ^{18,54}	2009	Gefitinib (132)	Carbo-Taxol (129)	71.2	47.3	9.5	6.3	21.6	21.9
Mitsudomi et al. ^{21,55}	2010	Gefitinib (86)	Cis-Taxotere (86)	62.1	32.2	9.2	6.3	34.8	37.3
Maemondo et al. ²⁰	2010	Gefitinib (114)	Carbo-Taxol (114)	73.7	30.7	10.8	5.4	30.5	23.6
Zhou et al. ^{56,57}	2011	Erlotinib (83)	Carbo-Gem (82)	83	36	13.1	4.6	22.7	28.9
Rosell et al. ¹⁹	2012	Erlotinib (86)	Platinum Doublet (87)	64	18	9.7	5.2	19.3	19.5
Sequist et al. ^{22,58}	2013	Afatinib (230)	Cis-Pem (115)	56	23	11.1	6.9	28.2	28.2
Wu et al. ^{58,59}	2014	Afatinib (242)	Cis-Gem (122)	66.9	23	11	5.6	23.1	23.5

Table 23rd generation *EGFR* TKIs in development

T790M Specific <i>EGFR</i> m TKI	Current Clinical Status	Comments
Rociletinib	Phase I and II, III soon	Breakthrough Therapy designation
AZD 9291	Phase II and III	Breakthrough Therapy designation
EGF 816	Phase I/II	
Astellas 8273	Phase I/II	
AZD 3759	Phase I	
HM 61713	Phase I	