The identification that certain subtypes of non-small cell lung cancer (NSCLC) respond better than others to specific therapy has led the search to identify as many subtypes that exist in order to tailor therapy to these subtypes for maximum clinical benefit. Since the mapping of the human genome, modern molecular technology has enabled a detailed characterisation of the molecular characteristics of NSCLC, and in adenocarcinoma, new mutated genes have been recently identified beyond EGFR (epidermal growth factor receptor) that may be suitable drug targets. These alterations are often responsible for the initiation and maintenance of cancer growth and are referred to as ‘driver mutations’. This review outlines the identification of EGFR as a therapeutic target, describing its current role in treatment selection, the clinical outcomes of anti-EGFR targeted therapy and the identification of resistance to such therapies and future directions to overcome this.


EGFR

EGFR (otherwise known as ErbB1/Her1) is a receptor tyrosine kinase that belongs to a family of four membrane-bound receptors. EGFR has been shown to be over-expressed in more than 60% of NSCLC cases, and is associated with a poor prognosis. Activation of the EGFR by ligand binding results in its homo or hetero-dimerisation and intracellular tyrosine kinase activity. The downstream signalling regulated by EGFR is complex and multidimensional, involving the Ras-Raf-MEK, PI3K-Akt-mTOR, PKC and STAT pathways, and plays a critical role in cell-cycle progression and proliferation. An outline of the EGFR signalling pathway is shown in figure 1. A number of mechanisms are responsible for the aberrant activation of the EGFR pathway in cancer cells, including enhanced ligand production, increased EGFR expression and mutations in the EGFR gene.

Specific mutations in the tyrosine kinase domain of EGFR were first reported in 2004. They occur over exons 18 – 21, which encode the ATP-binding pocket of the kinase domain of EGFR and result in ligand-independent constitutive activation of the receptor. These mutations have been shown to confer sensitivity to EGFR-tyrosine kinase inhibitors (TKIs) through their preferential binding of TKI over ATP. The two most prevalent activating mutations, accounting for approximately 85% of mutations observed, are deletions within exon 19 and point mutations in exon 21. The frequency of EGFR
mutations observed depends upon the population studied, ranging from 5 – 20% in a caucasian population and up to 60% in selected asian patient populations. The clinical phenotype of a female, asian non-smoker with a tumour of adenocarcinoma histology predicts the highest likelihood of harbouring an EGFR mutation.9

There are two classes of EGFR inhibitors – TKIs that compete with ATP for binding to the intracellular kinase domain of EGFR and monoclonal antibodies (mAbs) that bind to the extracellular domain and block ligand binding. These two classes are discussed below.

**EGFR-TKIs in mutation positive patients**

The initial evidence for the role of EGFR-TKIs in lung cancer treatment came from studies evaluating the first-generation agents, gefitinib and erlotinib. In the early studies of gefitinib undertaken in unselected patients before the rate of EGFR mutations were appreciated, response rates of < 20% were observed.10 However, a subgroup of patients had dramatic and occasionally durable responses to these agents.11 The underlying molecular basis for these unprecedented responses were activating mutations in EGFR.5-7 With increasing understanding of the role of EGFR mutations in predicting EGFR-TKI sensitivity, studies have since focused on evaluating EGFR-TKIs in the first-line setting in patients with EGFR mutations. Six studies have confirmed the benefit on response rate and progression-free survival of first-generation EGFR-TKIs used in EGFR mutation positive patients over chemotherapy (table 1). Two of these studies selected patients by clinical parameters, while the remainder mandated molecular confirmation of EGFR mutations prior to study entry. Their results highlight the importance of identifying EGFR mutations prior to initiating first-line EGFR-TKIs, as worse outcomes were observed for EGFR wild-type patients receiving EGFR-TKIs compared to chemotherapy (in IPASS study12 progression free survival hazard ratio (HR) 2.85, p<0.001). Hence, selecting patients for first-line therapy on the basis of clinical characteristics can be harmful, as it can result in worse outcomes for EGFR wild-type patients who receive upfront EGFR-TKI. Furthermore, clinical selection for first-line EGFR-TKIs may miss a proportion of patients without defined clinical features who harbour an EGFR mutation that may benefit from such treatment given upfront.

Despite the impressive progression free survival results, none of the aforementioned studies, nor the subsequent meta-analyses, have demonstrated an overall survival benefit for TKIs compared to chemotherapy in EGFR mutation positive patients.16,20 This is most likely due to the confounding effect of cross-over after study treatment. Hence, it has been inferred that survival is not compromised in EGFR mutation positive patients who receive upfront chemotherapy, as long as they receive an EGFR-TKI at some point along their treatment pathway. However, given the risk of attrition rate between first and second-line therapy, the risk of this strategy is that an individual patient may miss out on potentially effective treatment, which would be considered unacceptable in light of the impressive impact of EGFR-TKIs on clinical outcomes in mutation positive patients.21 Henceforth, in clinical practice EGFR-TKIs are usually commenced as soon as sensitising mutations have been identified. Second generation EGFR-TKIs have been developed that differ from the first-generation agents in forming irreversible bonds with their target and binding to additional ErbB family members. It is hoped that these features can delay the emergence of resistance and improve upon the outcomes achieved with the first-generation EGFR-TKIs. Afatinib is one such agent that has been shown to improve progression free survival compared to platinum-pemetrexed chemotherapy in the first-line setting in EGFR mutation positive patients (11.1 v 6.9 months, HR 0.59, p 0.0004).22 The progression free survival HR with afatinib appears similar to those seen with the first-generation agents, although it was compared with pemetrexed based chemotherapy, known to be superior in adenocarcinomas.23 However, relatively high rates of class side-effects, such as skin rash and diarrhoea, were observed. Approval of afatinib in the first-line setting is currently under evaluation.

Table 1: Randomised studies comparing first-line first-generation EGFR-TKIs to chemotherapy in clinically or molecularly enriched cohorts for EGFR mutations. HR: hazard ratio.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Patient Selection</th>
<th>EGFR-TKI</th>
<th>Reference Arm</th>
<th>PFS (months)</th>
<th>HR</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPASS12</td>
<td>Clinical</td>
<td>Gefitinib</td>
<td>Carboplatin/ Paclitaxel</td>
<td>9.8 v 6.4</td>
<td>0.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>First-SIGNAL13</td>
<td>Clinical</td>
<td>Gefitinib</td>
<td>Carboplatin/ Gemcitabine</td>
<td>8.4 v 6.7</td>
<td>0.61</td>
<td>0.084</td>
</tr>
<tr>
<td>NEJ00214</td>
<td>Molecular</td>
<td>Gefitinib</td>
<td>Carboplatin/ Paclitaxel</td>
<td>10.8 v 5.4</td>
<td>0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WJTOG340515</td>
<td>Molecular</td>
<td>Gefitinib</td>
<td>Cisplatin/ Docetaxel</td>
<td>9.2 v 6.3</td>
<td>0.489</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OPTIMAL16</td>
<td>Molecular</td>
<td>Erlotinib</td>
<td>Carboplatin/ Gemcitabine</td>
<td>13.1 v 4.6</td>
<td>0.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EURTAC17</td>
<td>Molecular</td>
<td>Erlotinib</td>
<td>Platinum doublet</td>
<td>9.7 v 5.2</td>
<td>0.37</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
EGFR-TKI resistance

Resistance to EGFR-TKIs can be divided into primary and secondary forms according to the timing in relation to targeted therapy. Primary resistance refers to de novo insensitivity of a tumour to EGFR-TKI treatment. Both EGFR mutations and activation of alternate oncogenes or pathways have been identified as possible causes. Insertion mutations in exon 20 represent less than 5% of mutations in the EGFR gene and preclude the binding of first generation EGFR-TKIs to the tyrosine kinase domain, conferring resistance. Mutually exclusive mutations in KRAS, or the presence of the EML4-ALK fusion gene, also predict for primary resistance. Other less clearly validated markers include loss of PTEN, BRAF mutations and increased protein levels of IGFR1, MAPK, ABCG2 and BCL-2.

While tumours harbouring activating EGFR mutations are typically exquisitely sensitive to EGFR-TKIs, tumour progression is generally observed after a median of 10-14 months, reflecting the development of acquired resistance. A set of guidelines has been developed to standardise the clinical definition of acquired resistance and include: previous treatment with single agent EGFR-TKI and confirmed EGFR activating mutation and/or objective clinical benefit from EGFR-TKI treatment; systemic progression of disease while on continuous anti-EGFR treatment; and no intervening systemic treatment between cessation of EGFR-TKI and initiation of new therapy. Acquired resistance is associated with the development of secondary mutations in EGFR or EGFR-independent activation of growth and survival pathways, as outlined in figure 2. The most common mechanism, observed in approximately 50% of cases of acquired resistance, is the development of a second EGFR gatekeeper mutation, T790M. This mutation interrupts the binding of first-generation EGFR-TKIs and restores the receptor’s affinity for its natural substrate ATP. The second major mechanism of acquired resistance, accounting for about 10% of cases, is amplification of the MET oncogene. MET is a transmembrane receptor tyrosine kinase and its coupling to ErbB3 results in sustained activation of the PI3K/AKT signalling pathway, bypassing the inhibited EGFR. Phenotypic transformation has also been observed in the development of acquired resistance, including transformation to small cell lung cancer (SCLC) and epithelial to mesenchymal transition. At least 30% of cases of EGFR-TKI resistance remain undefined mechanistically.

Traditionally, following the development of acquired resistance, determined according to standard response criteria, standard practice is to switch to conventional cytotoxic chemotherapy. However, a number of observations are challenging this empirical strategy. Firstly is the observation of clinically heterogeneous patterns of disease progression on EGFR-TKI therapy, including a single focus of progression, minimal multifocal progression and rapid multifocal or bulky progression. The observation of indolent cases of progression has led to some clinicians delaying the commencement of alternate systemic therapy through the continued use of first generation EGFR-TKI, local control measures involving surgery or radiotherapy or observation alone. A retrospective review of 42 patients with disease progression on erlotinib treatment found that 45% of the cohort had alternate systemic therapy delayed by more than three months through a combination of the aforementioned measures. Secondly, there is emerging evidence that tumours can retain a degree of EGFR-TKI responsiveness even after the development of resistance. This is based on the observation of accelerated disease progression on EGFR-TKI withdrawal in a subset of patients with acquired resistance, a phenomenon referred to as ‘disease flare’. To account for this observation, it is hypothesised that resistant tumours contain a mixed population of TKI resistant and latent TKI sensitive cells, which rapidly repopulate on withdrawal of the selection pressure exerted by TKI therapy. This has prompted investigation into a strategy of continued EGFR-TKI with concurrent systemic chemotherapy. While combinations of EGFR-TKI therapy and chemotherapy were not shown to be more efficacious than chemotherapy alone in a number of phase III studies in unselected patients, this approach is being re-visited in a selected cohort of EGFR mutation positive patients with acquired resistance to TKI therapy and utilising an intercalated approach in the administration of the two therapies. Results of Phase III studies evaluating the question of continuing EGFR-TKI at progression and adding chemotherapy compared with chemotherapy alone are eagerly awaited.

Further strategies designed to overcome the development of acquired resistance have focused on targeting the underlying mechanisms. This is particularly the case for the T790M mutation, which is the most common cause of acquired resistance and has emerging clinical data that suggests, paradoxically, a relatively favourable prognosis and more indolent course. While preclinical studies suggest the second-generation irreversible EGFR-TKIs are active against cells harbouring the T790M mutation, clinical trials of these agents after progression on a first generation EGFR-TKI have shown limited activity. The Lux Lung 1 trial of afatinib versus placebo in patients having previously received benefit from a first generation TKI (defined as disease control for ≥6 months) failed to demonstrate a survival advantage, although progression free survival was improved (3.3 v 1.1 months (HR 0.38, 95% CI 0.31-0.48; p<0.0001)) and a small number of responses (7% RR) were observed with afatinib. An alternate approach is to attempt vertical inhibition of the intracellular and extracellular domains of EGFR through the combination of an EGFR-TKI and mAb. While results for the combination of erlotinib and cetuximab were disappointing, more promising outcomes have been observed with the combination of cetuximab with afatinib in a phase Ib/II trial. Increased signalling through MET has been identified as a mechanism of acquired resistance, and the efficacy of MET inhibition with the use of small molecular inhibitors and mAbs is being explored in this setting. A phase III study of the oral MET inhibitor tivantinib (ARQ197) in combination with erlotinib, compared to erlotinib alone, in previously treated
advanced NSCLC patients was discontinued following an interim analysis that concluded the primary endpoint of OS would not be met.\textsuperscript{43} Detailed results are awaited. In contrast, a phase III clinical trial of the combination of MetMab with erlotinib in advanced pre-treated NSCLC with MET over-expression has been initiated, following phase II results which showed a significant benefit for the combination in patients with MET overexpression and a detrimental effect for the combination compared to erlotinib alone in patients without overexpression.\textsuperscript{44,46} Combinations of other classes of inhibitors, such as the mammalian target of rapamycin, heat shock protein-90 and SRC inhibitors, with EGFR-TKIs have been conducted, yielding disappointing results.\textsuperscript{47} This highlights the importance of identifying predictive biomarkers and elucidating molecular mechanisms underlying resistance in order to develop rational, genotype-driven therapies to overcome acquired resistance.

In cases of phenotypic transformation, there is anecdotal evidence of the persistence of the original EGFR mutation and efficacy of standard SCLC chemotherapy in cases of acquired resistance mediated by conversion to SCLC.\textsuperscript{27,47} In preclinical models, the emergence of epithelial to mesenchymal transition features is associated with a loss of dependence upon the EGFR signalling pathway, although sensitivity to EGFR-TKIs may be restored with histone deacetylase inhibitors, an approach undergoing further evaluation.\textsuperscript{48}

**EGFR targeted therapy in wild-type patients**

While the greatest clinical benefits of EGFR-TKIs are observed in mutation positive patients, they also have a place in the management of wild-type patients. A number of studies were initiated in a variety of settings in unselected patients prior to the discovery of EGFR mutations.

In patients with wild-type tumours or unknown EGFR mutation status, there is no role for the use of EGFR-TKIs in the first-line setting. The IPASS and First-SIGNAL studies clearly demonstrated inferior outcomes for EGFR wild-type patients who received first-line gefitinib compared to chemotherapy.\textsuperscript{2,3} Four trials administering EGFR-TKIs concurrently with chemotherapy in unselected patients were also negative.\textsuperscript{33-36} However, in patients with stable disease following platinum-based therapy, erlotinib is an option for switch maintenance therapy based on the results of the SATURN trial, which demonstrated a progression free survival and overall survival advantage to erlotinib over placebo in this setting. However, there have been conflicting results regarding the clinical efficacy of EGFR-TKIs compared to chemotherapy in the second-line setting, including the non-inferiority of gefitinib to docetaxel, a progression free survival advantage to docetaxel compared to erlotinib and an underpowered trial suggesting comparable efficacy between erlotinib and pemetrexed or docetaxel.\textsuperscript{45-51}

In patients with refractory disease, the BR.21 trial showed erlotinib to confer an overall survival benefit (6.7 versus 4.7 months, p= 0.001) and delayed time to symptom worsening compared to placebo in the second and third-line settings.\textsuperscript{52} A similar trial conducted using gefitinib (ISEL) did not find a significant overall survival improvement.\textsuperscript{53} Hence, only erlotinib is approved by the US Food and Drug Administration, Australian Therapeutic Goods Administration and Pharmaceutical Benefit Scheme for use in unselected patients as a second or subsequent line of therapy. Reasons proposed for the discrepancy in outcomes between gefitinib and erlotinib in this setting include a poorer prognostic group of patients evaluated in the ISEL study and the dosing of gefitinib, which is given at well below its maximum tolerated dose (250mg/day v 800mg/day).

A number of EGFR-directed mAbs have been evaluated in NSCLC, with results for cetuximab from the FLEX trial having gathered the most interest to date. A statistically, but not clinically significant overall survival advantage, was observed with the addition of cetuximab to cisplatin/vinorelbine in patients with EGFR-expressing tumours as determined by immunohistochemistry (11.3 v 10.1 months, HR 0.87, p 0.04).\textsuperscript{54} EGFR mutation status did not predict for clinical outcome.\textsuperscript{55} Subsequent analysis of this study has found a semi-quantitative assessment of EGFR protein expression based on a histo-score predicted treatment outcomes, however this remains to be validated prospectively.\textsuperscript{56} At present, cetuximab is not reimbursed and is not recommended as part of clinical lung cancer care outside of a clinical trial setting.

In summary, EGFR targeted therapy can offer a modest clinical benefit in certain settings in patients with EGFR wild-type tumours, which reflects the importance of the EGFR pathway on the malignant phenotype. However, further research is needed to identify predictive biomarkers for response in this heterogeneous patient group.

The recognition of the importance of the EGFR pathway in the malignant phenotype and development of therapies targeting EGFR has revolutionised the management of NSCLC. This is particularly true for the subset of patients identified to harbour EGFR mutations, where improved outcomes have been achieved with the personalisation of therapy using EGFR-TKIs compared to empirically-selected chemotherapy. To improve upon the survival gains achieved with EGFR targeted therapies, further research is required into the mechanisms of resistance in order to develop genotype-driven treatment options.

**References**


