Review Article

TREATMENT APPROACHES FOR PATIENTS WITH NON-SMALL CELL LUNG CANCER THAT HAVE ACQUIRED RESISTANCE TO EGFR INHIBITORS

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Abstract

The discovery of activating mutations in EGFR and their use as a predictive biomarker to tailor patients therapy with EGFR TKI have revolutionized the treatment of patients with advanced, EGFR mutant NSCLC. Currently first line treatment with EGFR TKI (gefitinib, erlotinib and afatinib) had been approved for patients harbouring exon 19 deletions or exon 21 (L858R) substitution EGFR mutations. These agents have been shown to improve response rates, progression and overall survival. Unfortunately resistance invariably develops that limits patient benefit and poses a challenge to treating oncologists. Optimal treatment post progression is currently not clearly defined. More detailed understanding of the biology of EGFR mutant NSCLC and mechanisms of resistance to targeted therapy mean we have entered an era with treatment approaches based on rationally, developed drugs or therapeutic strategies for this patient group. Combination approaches to overcome resistance have been trialled, for example dual EGFR blockade that appeared promising but are potentially be limited by toxicity. Most recently third generation EGFR mutant selective TKI that target T790M mutant tumours, the commonest mechanism of EGFR TKI resistance, have entered clinical trials. Studies with agent's including EGFR TKI such as AZD9291 and rociletinib have reported exciting, albeit preliminary, efficacy data and are currently in further phases of clinical development. This review summarises the current literature and evidence with the aims to guide treating oncologists and to consider how further advances in outcomes might be driven for the group of patients that have progressed following EGFR TKI therapy in this rapidly advancing area.

Introduction

Lung cancer remains a leading cause of cancer-related mortality, accounting for an estimated 1·59 million deaths worldwide in the latest WHO estimate. [1] In United States, lung cancer is estimated to account for 27% of all cancer deaths. Lung cancer death is expected to cause more deaths than any other cancer in both men and women. [2] Prior to the introduction of epidermal growth factor receptor (EGFR) inhibitor therapy the majority of patients, with non-small cell lung cancer (NSCLC), despite platinum based combination chemotherapy, survived less than one year. [3]

Treatment of selected patients with advanced NSCLC, was revolutionized by the discovery and subsequent targeting of the EGFR pathway. Further advances have led to a combination of histologic- and genomic-guided, rational, therapies which have given more patients access to personalized, molecularly targeted therapy.

Mutations in *EGFR* serve as both a biomarker and rational target for treatment.[4] Activating mutations have been found in the tyrosine kinase domain of *EGFR* which drive oncogenic pathways that control cellular proliferation and survival. The two most common activating mutations are exon 19 (in-frame) deletions and point mutation of exon 21 (L858R) which collectively account for more than 90% of known activating *EGFR* mutations.[4,5] Subsequent clinical studies have led to regulatory approval, in patients with *EGFR* mutant NSCLC, for

small molecule tyrosine kinase inhibitors (TKIs) including gefitinib, erlotinib and most recently afatinib. Resistance to EGFR inhibitor therapy, either intrinsic or acquired are major clinical problems, the median progression free survival for patients is still only around 10-13 months, despite EGFR inhibitor therapy. Median life expectancy for patients with advanced NSCLC harbouring activating *EGFR* mutation has increased to around 20 - 30 months following EGFR TKI therapy.[6–8] EGFR inhibitor therapy is now standard first line treatment in this setting.[9–12] Furthermore, the definition of what constitutes optimal treatment post progression has proved elusive and led to multiple treatment strategies being employed by individual oncologists. Selected advances in developing treatment for this group of patients are summarised in Figure 1.

In order to further advance care for patients with *EGFR* mutant NSCLC a rational framework for treatment needs to be established with clinical studies built upon this scaffold. The purpose of this review is to help oncologists in the clinical decision-making by presenting the promising new and upcoming treatments for patients who had acquired resistance to EGFR TKI.

Resistance

Treatment failure from EGFR TKI can be classified broadly into intrinsic (primary) or acquired (secondary). Intrinsic resistance is upfront lack of efficacy from EGFR TKI whereas acquired resistance is progression of disease after a period of clinical benefit.

Intrinsic Resistance

Although the mechanisms of intrinsic resistance are not fully understood, several have been described in some instances of non-classical sensitizing mutations and rarely in classical mutations (exon 19 deletion and L858R).

Drug resistant EGFR mutations

Exon 20 insertion which was found in around 4-10% of *EGFR* mutations had been described to confer intrinsic resistance to EGFR TKIs. The intrinsic resistance is due to the fact that EGFR exon 20 insertion mutations did not have increased affinity for EGFR TKI, except for one insertion (EGFR-A763_Y764insFQEA) which was found to be highly sensitive to EGFR TKI in vitro.[13,14]

Pre-existing T790M mutation clones had been described in *EGFR* mutant NSCLC patients who were treatment naïve. This mutation conferred worse clinical outcomes amongst those treated with EGFR TKI and it was correlated with the frequency of the exon 20 T790M mutation. [15,16]

Molecular or Genetic Alterations with EGFR Mutations

The presence of concurrent molecular or genetic alteration could potentially decrease the sensitivity of patients with sensitizing EGFR mutations to EGFR TKIs.

Patients harbouring *BIM* deletion polymorphisms[17] or with low to intermediate level of BIM mRNA [18] had been associated with reduced clinical efficacy when treated with EGFR TKI.

Several other pathway activations had been implicated with decreased sensitivity of EGFR mutation cell lines to EGFR TKIs in vitro including PI3K/Akt, [19,20] insulin-like growth factor 1 receptor (IGF1R), [21] nuclear factor-kB pathways, [22] *MET* amplification [23], overexpression of hepatocyte growth factor (HGF), [24] mesenchymal-epithelial transition amplification, [25] anaplastic lymphoma kinase fusion, [25] lung cancer stem cell [26] and STAT3-IL6 pathway. [27,28] Unfortunately these alterations still require further clinical validation.

Recent clinical data reported double, de novo *EGFR* mutations of both sensitizing and resistant variants in a minority of patients. Pooled data in this heterogeneous group of patients treated with EGFR TKI showed some activity, albeit lower than in patients with a single sensitizing mutation.[29]

Acquired Resistance

Whether a patient is responding to treatment is often based on cross sectional imaging (usually combined with other important parameters such as clinical benefit). Clinical studies require a rigorous definition of response that ultimately led to the Response Evaluation Criteria in Solid Tumours (RECIST). It has been argued that criteria may not fully characterise the natural history of patients with *EGFR* mutated NSCLC. For example, new slow growing lesions may be detected following an initial response to EGFR inhibitor defined as progressive disease by RECIST. However, in clinical practice oncologists may choose to continue EGFR TKI and monitor these lesions closely, in some patients a prolonged period of disease control without clinical deterioration can be achieved. [30]

In 2010, Jackman et al. proposed a clinical definition of acquired resistance to EGFR TKI in NSCLC [31], see Table 1. These criteria aimed to help benefit both practising oncologists and research study undertaken in patients who had acquired resistance from first line EGFR TKIs but requires further clinical validation.

Gandara et al. further subdivided patients with acquired resistance to EGFR inhibitor therapy into three distinct clinical groups. Broadly the classes are 1) central nervous system (CNS) sanctuary PD, 2) oligo-progressive disease (PD) and 3) systemic PD. [32]

For patients in the first two groups (with CNS or oligo-metastatic disease) limited data have shown that it might be appropriate to consider local therapy (eg. surgery and/or radiotherapy) to the site of progression and to continue EGFR TKI thereafter. [33,34] The scope of this review and discussion is to focus on the third group of patients defined by Gandara et al.

Table 1. Criteria for Acquired Resistance to EGFR TKIs in Lung Cancer
1. Previously received treatment with a single-agent EGFR TKI (eg, gefitinib, erlotinib or afatinib)
2. Either of the following: **A. A tumor that harbors an *EGFR* mutation known to be associated with drug sensitivity (ie, G719X, exon 19 deletion, L858R, L861Q)** **B. Objective clinical benefit from treatment with an EGFR TKI as defined by either:** **i. Documented partial or complete response (RECIST or WHO), or** **ii. Significant and durable (≥ 6 months) clinical benefit (stable disease as defined by RECIST or WHO) after initiation of gefitinib, erlotinib or afatinib**
3. Systemic progression of disease (RECIST or WHO) while on continuous treatment with gefitinib, erlotinib or afatinib within the last 30 days
4. No intervening systemic therapy between cessation of gefitinib, erlotinib or afatinib and initiation of new therapy
Abbreviations: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; RECIST, Response Evaluation Criteria in Solid Tumors.

Mechanisms of resistance

Several groups have done comprehensive analysis of the resistance mechanisms in patients post progression on EGFR TKIs by undertaking intensive studies with repeat tumour biopsy at the time of disease progression. In this manner a mechanism of resistance has been defined for around 60-70% of cases, classified into the following categories:

Secondary mutations in EGFR

T790M mutation (50-60%)

The most common acquired resistance mutation (>50-60% of cases) is substitution of methionine for threonine at position 790 (T790M) at exon 20 of the EGFR that is acquired or selected for in conjunction with the original EGFR TKI-sensitive (exon 19 deletion or L858R) mutation.[35,36] The bulkier methionine side chain causes steric hindrance affecting binding of first generation EGFR TKIs, such as gefitnib and erlotinib, to the ATP-kinase-binding pocket. [37] In addition, the T790M mutation alters the affinity of EGFR to ATP such that ATP is restored as favoured substrate compared to ATP competitive EGFR TKIs. [38] Interestingly patients whose tumours harbour EGFR-T790M mutation may experience a more indolent natural history and comparatively favourable prognosis when compared to EGFR-T790M non-detected cases. [30] However even patients with acquired resistance to gefitinib, erlotinib and afatinib with EGFR-T790M potentially can have a rapid clinical decline and short survivals.

Other mutations in EGFR (< 10%)

Rarer point mutations in *EGFR* that result in resistance include D761Y [39], T854A [40] and L747S [41] The mechanism(s) underlying the resistance conferred by these are currently unclear. It was noted that patients harbouring compound EGFR TKI-sensitive mutations with these rarer genotypes in the TKI-naïve setting can respond EGFR TKI. [42]

By-pass/alternative pathway activation

MET amplification is reported in 5-10% of cases. Amplification of MET [36,43] phosphorylates ERBB3 (HER3) and activates PI3K/Akt downstream signal cascades. T790M and MET amplification can occur concurrently most commonly MET amplification occurs independent of T790M. Overexpression of HGF, which is the ligand for MET oncoprotein, also has been shown to induce resistance to EGFR TKI. [24]

Though *MET* amplification has been identified as one of the mechanisms of resistance, the threshold of resistance is less clear cut. At present, the most informative method to define MET amplification is still not clearly defined which might hinder the development of drugs targeting MET amplification.

Other reported mechanisms, from small clinical studies, by which alternate signalling pathways are activated include:

- *PIK3CA* mutation (5%)[35]
- *HER2* amplification (12%), this mutation was mutually exclusive with T790M[44]
- *BRAF* (V600E, G469A) mutation (1%)[45]
- Increased expression of receptor tyrosine kinase *AXL* (20%) and its corresponding ligand, GAS6 (25%)[46]

Histologic and phenotypic transformation

Transformation from *EGFR* mutated adenocarcinoma to small cell lung cancer (SCLC) after an initial response to EGFR TKIs has been reported. Furthermore, in these patients, *EGFR* mutation analysis confirmed the presence of a similar activating mutation in both the original lung adenocarcinoma and the metastatic SCLC. [47,48] The prevalence of SCLC histology has been reported between 3% to 14% in patients that had acquired resistance to EGFR TKI. [35,36]

Epithelial to mesenchymal transition (EMT) was reported in 5% of patients. [35] Morphologically, the cancer cells lost their epithelial features eg. E-cadherin expression and transformed to spindle-like mesenchymal cells with gain of vimentin. [49]

Ongoing research should continue to examine the mechanism(s) of resistance in the remaining 30-40% of cases that are still unknown at the present moment

A full and comprehensive review of the biology of EGFR signalling [50], mechanisms of intrinsic [50–52] and acquired resistance [50–52] is beyond the scope of the article and has been reviewed extensively elsewhere. Further information could be obtained from reviews by Chong et al., [50] Cortot et al., [51] and Lin et al. [52]

Treatment options following progression on EGFR TKI:

Current, published international guidelines including those from NICE[9], ASCO[11] and ESMO[12] all recommend EGFR TKI as a possible first line treatment for patients with advanced NSCLC harbouring activating *EGFR* mutations. But only the National Comprehensive Cancer Network (NCCN) guideline offered a treatment algorithm for these patients who had progressed on first line EGFR TKIs, specifically that second line treatment for patients who had systemic progression could be considered for platinum doublet with or without bevacizumab.[10]

It is common practise to switch patients with EGFR mutant disease to platinum-based therapy once clinical progression has occurred on first line EGFR TKI. To date, no randomized clinical trial evidence is available to support this treatment approach. Retrospective studies report response rates (RR) of 14-18%, with median PFS around 4 months for patients that received second line chemotherapy. [53,54]

If doublet chemotherapy was to be chosen, the treatment regimen could be guided by histology. As most patients with EGFR mutation are of adenocarcinoma/non-squamous histology the optimal regimen, extrapolated from first line data, may be cisplatin and pemetrexed combination and followed by maintenance pemetrexed in patients that achieved clinical benefit.

TKI Beyond Progression

Some clinicians chose to continue EGFR TKI beyond progression, supported by reports of disease 'flare' (rapid clinical deterioration) on discontinuation of EGFR TKI. Clonal heterogeneity in progressive lesions is proposed to underlie faster regrowth of TKI-sensitive clones compared to resistant clones upon discontinuing the EGFR TKI. [55] One small study reported 23% (14/61) of patients had rapid (median < 8 days) disease 'flare' upon discontinuation of TKI. [56] Hence clinicians often continue EGFR TKI until new therapy is initiated, rather than observe a washout period.

Another specific group of patients are those who respond to a re challenge of EGFR TKI (usually after an intervening cytotoxic therapy). [57] Evolutionary cancer modelling studies support tumours with EGFR T790M mutant clones having regained TKI-sensitivity when multiply passaged in the absence of EGFR TKI selective pressure.[55]

In human tumour cell line models, EGFR TKI augment the response to cytotoxic drugs. [55] This approach has been evaluated clinically in both retrospective and prospective studies (Table 2).

A summary of results from studies investigating the role of TKI beyond progression, both retrospective and prospective studies, are summarized into Table 2. [54,58–61]

The IMPRESS trial is a placebo controlled, randomized phase III trial that investigated the efficacy of using TKI beyond progression (gefitinib plus pemetrexed/cisplatin versus placebo plus pemetrexed/cisplatin). In this trial 265 (European and Asian) patients were randomized. There was no statistical difference in the RR between the groups (31% vs 34%), median PFS was 5·4 months for both arms (HR 0·86; p = 0·273). The overall survival (OS) data were immature (33% of patients had died), although favoured placebo compared to gefitinib, 17·2 vs14·8 months respectively (HR 1·62; p = 0·029). There were slight imbalances between the two groups favouring placebo arm including: response to prior therapy CR (76% vs 68%), PR/SD (24% vs 32%), fewer brain metastasis (23% vs 33%), post progression platinum-based treatment (12·9% vs 3·8%) or other EGFR TKI (33·3% vs 22·6%) in placebo versus gefitinib arms respectively. [61]

This is the only phase III randomized trial to date that addressed the issue of continuing TKI beyond progression with addition of chemotherapy. There appeared to be no benefit of continuing gefitinib. Furthermore, there were trends towards detrimental effects impacting OS, it should be noted the OS data is still immature. This is a practice-changing trial. Unless further evidence proves otherwise, continuing TKI beyond progression in combination with chemotherapy should not be routine clinical practice.

Two on-going phase II trials are investigating erlotinib continued beyond progression in combination with chemotherapy. One from the Finnish Lung Cancer Group (NCT02064491) is still recruiting, the other is by the North American group (NCT01928160) and yet to open for recruitment.

The current standard of care with platinum-doublet cytotoxic chemotherapy post progression on first line EGFR TKI continues to be a valid therapy choice but physician should actively encourage patients to participate in clinical trials whenever possible.

Table 2. TKI beyond progression

Treatment	Target Population	Number of patients	RR	PFS (month)	OS (month)	Ref
i)Chemotherapy alone ii)Erlotinib+chemotherapy	EGFR mutant, Retrospective	44 34	18% 41% (ns)	4·2 4·4 (ns)	15·0 14·2 (ns)	[54]
Gefitinib/erlotinib + pemetrexed	EGFR mutant, Prospective single arm	27	25·9%	7·0	11·4	[58]
i)Chemotherapy alone (Docetaxel or pemetrexed) ii)Erlotinib + chemotherapy	Clinical benefit from erlotinib >12 weeks, Prospective, randomized phase II	24 22	NA	5·4 4·6 (ns)	18·7 14·7 (ns)	[59]
ASPIRATION: Erlotinib	EGFR mutant, Prospective single arm, phase II	81	NA	3·7	NA	[60]
IMPRESS: i)Gefitinib + cisplatin/pemetrexed ii)cisplatin/pemetrexed	EGFR mutant, Prospective randomized phase III	133 132	31% 34% (ns)	5·4 5·4 (ns)	14·8 17·2 (p=0·029)¥	[61]

ns=not statistically significant; NA=not available; ¥ = immature data

Second Generation EGFR TKI

Acquired resistance following treatment with first generation EGFR TKIs led several groups to develop second generation EGFR TKI. Theoretical advantages postulated to overcome acquired resistance mechanisms include: (i) irreversible binding with higher affinity over EGFR/HER1 domain (ii) pan-HER inhibition, prevents dimerization with ligands ie HER2 or HER4 and (iii) *in vitro* activity against T790M mutant human tumour NSCLC cell lines. [62,63]

Despite such theoretical advantages clinical studies were beset with toxicity as a result of a narrow therapeutic window likely due to inhibition of wild-type EGFR. Common dose limiting toxicities (DLTs) were diarrhea and skin rash, which appeared to be class effects. Of

the second generation compounds, afatinib has progressed the farthest in development. (Table 3)

Afatinib (BIBW 2992; Boehringer Ingelheim; Ingelheim Germany)

At the recommended phase II dose (RP2D) of 50mg/day, DLTs were grade 3 rash, pneumonitis and mucositis during phase 1 studies. [64,65] Afatinib has currently been approved by NICE as an option for first line EGFR TKI therapy.

LUX-Lung 1 was a phase IIb/III trial comparing afatinib/BSC vs placebo/BSC in patients with NSCLC who had progressed after 1-2 lines of chemotherapy and gefitinib/erlotinib. EGFR mutation was positive in 68% of assessable patients (96/141). RR was statistically more significant in afatinib group compared to placebo at 7% vs <1% respectively, p=0·0071. In terms of PFS afatinib was shown to be superior compared to placebo at 3·3 (95% confidence interval (CI) 2·79–4·40) vs 1·1 months (95% CI 0·95–1·68, HR 0·38, 95% CI 0·31–0·48; p<0·0001). This study did not meet its primary end point of OS, where afatinib group recorded OS of 10·8 months (95% CI 10·0–12·0) vs 12·0 months (10·2–14·3) in placebo group (hazard ratio 1·08, 95% CI 0·86–1·35; p=0·74). More adverse events were noted in the afatinib arm compared to placebo. The commonest adverse events were diarrhea (87% all grade, 17% grade 3) and rash/acne (78% all grade, 14% grade 3). [66]

Exploratory sub-group analysis of data from the first line setting LUX-Lung 3 and LUX-Lung 6 studies (of afatinib compared to either pemetrexed plus cisplatin or gemcitabine plus cisplatin respectively in patients harbouring EGFR mutations) are interesting. In combined analysis of these two studies (which individually did not meet their primary endpoints), reveal patients with EGFR del19 mutations had a significant improvement in overall survival (31·7 versus 20·7 months, p=0·0001) whereas there was no improvement in OS for patients with Leu858Arg EGFR mutations. The underlying mechanism for this difference is not known at this time and cannot be explained by preclinical models. [67] The authors comment these are the first data to demonstrate improved OS for patients treated with an EGFR inhibitor. It is notable that the survival of patients with exon 19 deletion mutation on chemotherapy was significantly lower than patients with L858R mutation, which may have skewed the reported results. In day-to-day clinical practice, these results should not alter clinical decisions and it did not resolve the clinical question of the most appropriate (when considering efficacy and toxicity) EGFR TKIs that should be used. Until further data available, choice of EGFR TKI cannot be made based on the type of EGFR mutation. Prospective studies (eg. LUX-Lung 7, NCT01466660 evaluating afatinib versus gefitnib in first line setting) are recruiting to determine the most effective EGFR inhibitor in the first line setting.

Dacomitinib (PF299804; Pfizer, Ann Arbor, MI, USA)

A global phase III (NCIC CTG BR.26) trial recruited a total of 720 patients who had previous chemotherapy (1-3 lines) and EGFR TKI (erlotinib or gefitinib) and randomly assigned 2:1 to either dacomitinib or placebo. EGFR sensitizing mutation was found in 24% of dacomitinib group and 28% of placebo group. BR.26 did not meet its primary end point of improvement of OS in the overall population. [68] (Table 3).

Pelitinib, Canertinib & Neratinib

Three other compounds were investigated but unfortunately due to disappointing clinical results, they are no longer in development for NSCLC patients. Their results are summarized in Table 3.

Table 3: Summary of trials with irreversible second generation EGFR TKIs

Compound/Trial	Phase	Target population	Number of patients	RR (%)	DCR (%)	PFS (mth)	OS (mth)	Ref
Afatinib (BIBW2992)	II (LL-4)	Jackman criteria	62	8·2	65·6	4·4	19·0	[69]
	IIb/III (LL-1)	Unselected for EGFR status, Patient progressed on 1-2 lines of chemotherapy and erlotinib/gefitinib	390 (afatinib) 195 (placebo)	7 <1	58 18	3·3 1·1	10·8 12·0 (ns)	[66]
Afatinib + cetuximab	Ib	EGFR mutation or Jackman criteria	126 71 # 53 ^	29 32 # 25 ^ (ns)	71 76 # 62 ^ (ns)	4·7 4·6 # 4·8 ^ (ns)	NA	[70]
Dacomitinib (PF-00299804)	I/II	Unselected for EGFR status, Adenocarcinoma NSCLC, KRAS WT, received previous platinum-based therapy and gefitinib/erlotinib, Korean patients	55 (43[∞])	17·1[∞]	29·3[∞]	3·5[∞]	10·6[∞]	[71]
	II	EGFR mutant or KRAS WT, Prev erlotinib and 1 or 2 chemo	66 26 [µ]	5·2 8 [µ]	22·4 28 [µ]	2·8 4·1 [µ]	8·5 13·1 [µ]	[72]
	III (BR.26)	Unselected for EGFR status, NSCLC who had previously received at least 1	480 (dacomitinib) 240 (placebo) 114 [µ]	7 1***	NA	2·66 1·38 *** 3·52 [µ]	6·83 6·31 (ns)	[68]

		line of chemotherapy and EGFR TKI	(dacomitinib) 68 $^{\mu}$ (Placebo)			0·95 $^{\mu}$ * 	7·23 $^{\mu}$ 7·52 $^{\mu}$ (ns)	
Pelitinib (EKB-569)	I	Unselected for EGFR status, advanced solid malignancies	15 (10 NSCLC)	NA	NA	NA	NA	[73]
Canertinib (CI-1033)	II	Unselected for EGFR status, ≥2nd line after platinum-based therapy IHC + for ERBB	60 (50mg) 60 (150mg) 46 (450mg)	2 2 2	18 25 22	1·9 1·9 1·9	6·5 6·6 6·0	[74]
Neratinib (HKI-272)	I	Unselected for EGFR status, advanced solid malignancies	72 (14 NSCLC)	NA	43	NA	NA	[75]
	II	EGFR mutant in arm A A) Prior EGFR TKI – EGFR mutant B) Prior EGFR TKI – EGFR WT C) EGFR TKI naïve	 91 48 28	 3·4 0 0	 53·4 64 32	 3·5 3·7 2·1	 NA NA NA	[76]

WT = Wild type; ∞ = phase II patient cohort; μ = EGFR mutant cohort; ns = not statistically significant, NA = not available; # T790M-positive; ^ T790M-negative; * <0.05; *** < 0.001; DCR was defined differently in various trials, please refer to individual trials for further details.

Third Generation EGFR TKI

Understanding the biological mechanism for resistance to EGFR TKI has led to an exciting era and the development of the third class of EGFR TKI. One of the first reports on the discovery of these agents identified the anilopyrimidine core that gave these agents their unique property. [77] These agents target the main cause of acquired resistance, T790M, relatively sparing wild-type (WT) EGFR. In this manner toxicity due to wild-type EGFR inhibition is ameliorated (Table 4). At the present moment, both AZD9291 and rociletinib have progressed the farthest in terms of clinical development in AURA and TIGER series respectively.

<u>AZD9291</u>

AZD9291 (AstraZeneca, Macclesfield, UK) is a potent irreversible inhibitor of both sensitizing EGFR and T790M mutations. In pre-clinical data, AZD9291 was around 200 times more potent against L858R/T790M than wild type EGFR. [78]

An open label, multicentre, phase 1 study enrolled both Asian and Western patients with advanced, EGFR mutation positive patients. Patients had to be EGFR mutation positive or have derived prior clinical benefit as per Jackman criteria and developed resistance to EGFR TKI. Prior EGFR TKI or systemic therapies were not limited. Prospective mandatory central testing of T790M was required in expansion cohort but optional in escalation cohorts. The primary study objectives were assessment of the safety, tolerability and efficacy (objective response rate).

This trial had recruited 253 patients to date, 31 in the dose escalation (20-240mg oral, daily) and 222 in the expansion phases. Patients demographic included: female 61%, Asian (60%) vs Caucasian (37%). In the expansion stage 79% of patients tested positive for sensitizing EGFR mutation. No dose-limiting toxicities reported during the dose escalation stage. At the recommended phase II dose of 80mg once daily, grade 3 toxicities were infrequent, eg diarrhoea 1% (all grades 33%) and no patients reported grade 3 rash (Grade 1 or 2, 32%). All grade hyperglycemia was reported in 2% of patients. No mechanism identified to date. Pneumonitis appears to be a rare, potentially serious side effect with frequency estimated at 2·09% (13 events from 620 patients) including 1 fatality.

Data (summarised in Table 4) including ORR 51% (123/239; 95% CI 45-58) [T790M positive > T790M negative group, 61% (78/127; 95% CI 52-70) vs 21% (13/61; 95% CI 12-34) respectively] and with the preliminary PFS of T790M positive being 9·6 months (95% CI 8·3-not calculable) compared to 2·8 months (95% CI 2·1-4·3) in T790M negative group. [79]

AZD9291 is currently in further phases of studies and they are summarized in Table 5.

Rociletinib (CO-1686)

Rociletinib (CO-1686; Clovis Oncology, Boulder, Colo) is a potent irreversible inhibitor of both sensitizing EGFR mutation and T790M resistance mutations.[80] TIGER-X (NCT01526928) is a phase I/II trial of rociletinib for patients who are EGFR mutation positive and received prior EGFR TKIs. Having completed the phase 1 stage, 625mg BID of optimized oral formulation is the recommended phase II dose.

In the phase II expansion cohort, T790M positive, patients are stratified to two groups, patients that have either progressed after 1 line of EGFR TKI, or progressed following ≥2 TKIs or chemotherapy. Interim results for from 56 patients were presented at 26th EORTC-NCI-AACR 2014 Symposium (18-21 November 2014, Barcelona, Spain). 70% patients were female, only 11% were of Asian ethnicity.

The ORR for phase 1/II patients (n=179) was 46% as compared to 67% in T790M positive cohort (n=56) and 36% in T790M negative cohort. Median PFS T790M positive cohort was 10.4 months as compared to 7·5 months in the T790M negative cohort.

Hyperglycemia was the commonest adverse event affecting 32% (all grade) of patients, 14% were grade 3/4 and related to the accumulation of a rociletinib metabolite, M502 (an IGF1R inhibitor). All grade diarrhea was reported in 25% of patients. Less commonly, 2 patients were noted to have a transient skin rash. Reversible pneumonitis was observed in 4 patients. [81]

Rociletinib is currently in further phases of studies and they are summarized in Table 5.

HM61713

HM61713 (Hanmi Pharmaceutical Company Ltd, Seoul, South Korea) is another irreversible, potent mutant selective and EGFR WT sparing compound. [82] A phase I study of HM61713 was conducted in 7 centres in Korea for EGFR mutation positive patients who had progressed on prior EGFR TKI and chemotherapy. MTD was not reached during dose escalation to 800mg. Patients in the expansion cohort (n=83) were treated with 300mg, daily, underwent mandatory biopsy testing for T790M mutation which was positive in 48 patients (57·8%) and 62·7% of patients were female . Adverse events were nausea 32·2% (grade 1/2), diarrhea 21·1% (grade 1/2) and rash 23·7% (grade 1/2). The ORR was 21·7% in the expansion cohort at 300mg and T790M positive group had higher ORR compared to T790M negative group, 29·2% vs 11.8% respectively. The median PFS in the T790M positive group was 4·3 months vs 2·3 months in the T790M negative group, respectively. An expansion cohort at higher dose level is planned. [83]

ASP8273

ASP8273 (Astellas Pharma Inc, Tokyo, Japan) preclinical data confirms mutant selectivity and EGFR WT sparing. [84] Preliminary reports are that DLTs were observed using 600mg daily and included diarrhea, colitis and cholangitis. Adverse events were diarrhea (52%, all grades, 12·9% grade 3),vomiting (32·3%), nausea (29·0%) and rash (7%) of patients respectively. Tumor responses were reported at doses above 100mg. [85]

EGF816

EGF816 (Novartis Pharmaceuticals, Basel, Switzerland) is currently being investigated in several phase I/II trials (NCT02108964, NCT02335944, NCT02323126).

In summary, rationally designed, third generation EGFR TKIs that inhibit both sensitising EGFR and T790M mutations but relatively spare wild type EGFR are an important step forward in the treatment of patients with acquired resistance EGFR TKI. This appears to have increased the therapeutic window so that toxicity such as diarrhea or rash are less frequent or severe.

Interestingly, third generation TKI appear to have activity in patients with T790M negative disease, response rates range from 10-30%. The reasons behind this observed clinical activity are unclear. Hypotheses include: a retreatment effect from previous EGFR TKI, heterogeneity between the tumour sites, active metabolites targeting bypass signalling pathways, false negative testing of T790M mutation, genuine treatment response, a combination of these factors or other as yet unknown reasons. Further study is certainly warranted in this patient group.

As data mature on these compounds it might be that a superior drug emerges as the most effective therapy. Equally which third generation EGFR TKI to use for an individual patient might be decided based on the differing side effect profiles. Whatever the outcome, these are exciting times with multiple, active new drugs in development for patients with EGFR mutant lung cancer.

Table 4. Summary of trials with third generation mutant-selective, EGFR wild-type sparing TKI

Compound/ Trial	Phase	Target population	Number of patients	RR (%)	DCR (%)	PFS (mth)	OS (mth)	Ref
AZD9291	I/II (AURA) (NCT01 802632)	EGFR mutant or Jackman criteria, Progressed on previous EGFR TKI or systemic treatment	253 138[#]	51 61[#] 21^	84 95[#] 61^	NA 9·6[#] 2·8^	NA	[79]

Rociletinib (CO-1686)	1/II (TIGER -X) (NCT01 526928)	EGFR mutant, Received previous EGFR TKI	179 56[#]	46 67[#] 36^	84 89[#] NA	NA 10·4[#] 7·5^	NA	[81]
HM61713	I (NCT01 588145)	EGFR mutant, Progressed on chemotherapy and EGFR TKI	118 48[#]	21·7 29·2[#] 11·8^	67.5 75[#] 55·9^	NA 4·3[#] 2·3^	NA	[83]
ASP8273	I (NCT02 113813)	EGFR mutant, received prior EGFR TKI	31 13[#]	42 78[#]	NA	NA	NA	[85]

#T790M mutation positive; ^T790M mutation negative; NA = not available; NA = not available; DCR was defined differently in various trials, please refer to individual trials for further details.

Table 5. Ongoing clinical studies, further development of third generation TKIs

Compound	Trial Name	Phase	Primary end-point	Status	T790M status	Key features
AZD9291	AURA-2 (NCT02094261)	II	ORR	On-going but not recruiting	Positive	-failed EGFR TKI -EGFR mutant
	AURA-3 (NCT02151981)	III	PFS	Recruiting	Positive	-failed first line EGFR TKI -EGFR mutant -standard arm: platinum-based doublet chemotherapy
	FLAURA (NCT02296125)	III	PFS	Recruiting	Positive/negative	-First line -EGFR mutant -standard arm: gefitinib/erlotinib
	NCT02143466	I	Safety and tolerability	Recruiting	Positive/negative	-failed EGFR TKI -EGFR mutant -AZD9291 in combination with either MEDI4736 or AZD6094 or selumetinib
Rociletinib (CO-1686)	TIGER-1 (NCT02186301)	II	PFS	Recruiting	Positive/negative	-first line, randomized -EGFR mutant -standard arm: erlotinib
	TIGER-2 (NCT02147990)	II	ORR	Recruiting	Positive	-single arm -EGFR mutant -failed first line EGFR TKI
	TIGER-3 (NCT02322281)	III	PFS	Not yet recruiting	Positive/negative	-failed EGFR TKI and platinum doublet chemotherapy -EGFR mutant -standard arm: single agent chemotherapy

Combination approaches

An alternate strategy to overcome acquire resistance in EGFR TKI would be combination treatment. This strategy is meant to reverse the drug resistance due to 'bypass' signalling mechanism(s) by targeting horizontal (multiple, parallel signalling pathways) and/or vertical (multiple levels within a single signal pathway). A review of the rational and challenges in developing combination therapeutic approaches are beyond the scope of this article, further information could be obtained from recent reviews including those by Yap et al. [86] or Harrington et al.[87]

Vertical Pathway

Combined EGFR blockade

Vertical EGFR signalling pathway blockade was investigated using afatinib combined with cetuximab (EGFR targeting antibody) in patients with acquired resistance. Preclinical data of afatinib with cetuximab combination , but not the individual drugs, resulted in tumor response in erlotinib-resistant tumor (L858R/T790M) in human tumour xenografts. [88]

126 patients were enrolled into the phase I combination study of which 124 patients were assessable for T790M status. 71 patients were T790M-positive. For all patients, the RR was 29% and median PFS was 4·7 months. Further analysis between the T790m positive vs negative groups did not reveal any statistical differences in terms of RR or PFS. This seemed to suggest the clinical benefits from the combination were irrespective of the T790M mutation status. The two commonest adverse events were rash (90% all grade, 20% grade 3) and diarrhea (71% all grade, 6% grade 3).[70] (Table 2). There is a plan to conduct a phase II/III trial (S1403) investigating the efficacy of afatinib/cetuximab combination vs afatinib alone in treatment naïve EGFR mutant NSCLC patients by the SWOG group. [89] (Table 3)

Another combination of erlotinib with cetuximab was explored in a phase 1/II study but the results were disappointing with RR of zero [90]

Horizontal Pathway

One combination strategy would be to maintain potent inhibition of EGFR pathway signalling while adding inhibitors for the 'bypass' signalling pathway that is proposed to mediateresistance.

Several horizontal combination strategies are currently being investigated and their results are still preliminary and immature. They included combination (of EGFR TKI) with:

- MET inhibitors: cabozantinib (NCT00596648), tivantinib (NCT01580735) or INC280 (NCT01610336);
- PI3K inhibitor: buparlisib (BKM120) (NCT01570296, NCT01487265)
- Heat shock protein 90 (HSP90) inhibitor, AUY922 (NCT01259089, NCT01646125); JAK inhibitor: ruxolitinib (NCT02155465)

In general, combination strategies have been hampered by increased toxicities with a lack of patient selection criteria that did not take into account of patients' genotype. For example, some of the early phase combination trials included patients who had progressed after EGFR TKI without confirming that the patients possessed the acquired resistance mechanism that was being investigated.

As in other therapeutic areas, there is an urgent need to improve the design, patient population (ideally biomarker based) included and execution of early phase trials of drug combinations to define the optimal dose and schedule especially considering there will be a need to develop combination trials with third generation TKIs. [87]

Immunotherapy

Preclinical data had shown that activation of PD-1 pathway contributed to the immune evasion in EGFR-driven lung cancers. [91] There are on-going phase I trials looking at combining EGFR TKI with immunotherapies: anti-PD-1 human monoclonal antibody Nivolumab (NCT01454102), anti-PD-1 monoclonal antibody Pembrolizumab (NCT02039674), anti PDL-1 monoclonal antibody MPDL3280A (NCT02013219).

Challenges for personalising therapy

In order to individualise treatment detailed understanding of resistance mechanism(s) are necessary. However there are significant obstacles to obtain new/repeat tissue biopsies. Furthermore, intra-patient tumour heterogeneity confounds the genomic analyses. [36,92] Prospective re biopsy studies report success rates ranging from 75% to 95%, with serious complications experience in around 1% of cases. [36,93] While these data imply re biopsy is feasible in routine clinical practice it is challenging to achieve such high compliance rates due to patient factors (eg safety or tolerability), physicians' preference or resource limitation. Repeat biopsy upon disease progression is currently not standard clinical practise unlike other clinical scenarios.

Many groups are working to circumvent such issues with technologies to allow sampling of patients' blood for analysis of either circulating tumor cells (CTC) or plasma for circulating cell-free tumour DNA (ctDNA), often termed "liquid biopsy". [94]

As peripheral blood contains aggregate of cells or DNA contents from tumours at different sites this may better represent the natural history of disease progression and therapeutic response.

Murtaza et al. established the proof of principle that exome-wide analysis of ctDNA could identify mutations associated with acquired resistance in several solid advanced tumours, including a patient with NSCLC specifically who developed emergence of, and an increase in allele frequency of T790M mutation following treatment with gefitinib. [94] ctDNA can also been used to detect both activating *EGFR* and T790M mutation the ratio of these alleles could potentially be used to monitor disease status during EGFR TKI treatment. The proportion of T790M allele would rise eventually will reach a threshold to acquire resistance.[95,96] Furthermore the on-going ctDNA-tumor concordance testing of matched tumour biopsies and plasma samples study appeared promising. [97]

It is possible to detect EGFR mutation from CTCs and they were concordant with matched biopsy sample. [98] CTC have also been investigated to detect T790M resistance clone development.[99] As CTC are intact, viable tumour cells DNA, RNA and proteins analyses can be analysed.

Information from ctDNA and CTC analyses are potentially complementary and the emerging data from clinical studies in this patient population should be monitored with interest.

It is tempting to speculate that patients therapy might be guided by such non-invasive assays, acquired more readily than repeat tumour biopsy as increasingly the role of EGFR mutation sub-type in response to therapeutic agent is dissected, for example recent data supporting EGFR del19 or Leu858Arg mutation status being relevant to the choice of EGFR inhibitor.

Take Home Messages:

Patients that experience progressive disease on EGFR TKI are a heterogeneous group, with multiple underlying mechanisms of which T790M mutation is the most common occurring in 50-60% of patients. Empiric treatment with cytotoxic chemotherapy post progression remains the default choice however, this situation is rapidly evolving. It could possibly be foreseen that, in the near future, the community are entering an era where patients are treated with multiple targeted agents either sequentially or concurrently. It is unlikely cytotoxic chemotherapy will be removed from the pathway completely and, for selected patients, will most likely continue to provide palliative benefit.

Combination therapy such as dual EGFR blockade (afatinib and cetuximab) had promising clinical efficacy but the toxicity of the regimen limited its potential. Efforts to define effective combination regimens of molecularly targeted drugs are ongoing.

Exciting data from studies with third generation EGFR TKI that are EGFR mutant selective and WT EGFR sparing have emerged recently. These agents overcome toxicity liabilities of earlier EGFR inhibitors. Interestingly, comparing the agents in development reveals slightly different side effect profiles which might influence future clinical use.

An urgent objective is to personalise treatment, given the challenges of (repeated) tumour biopsies we envisage non-invasive testing methods, such as those based on CTC or ctDNA, are critical to drive advances in patient outcomes. These assays remain in development and require further clinical validation prior to widespread use. However, their impact could be transformative to tailor individual patient therapy given the increasing options for patients with EGFR mutant NSCLC.

In summary, treatment for patients whom acquired resistance to EGFR TKI has entered a new exciting phase driven by better understanding of the mechanisms of resistance and treatment strategies that are rationally tailored to them.

Search Strategy:

We searched PubMed for articles published in English using the terms “lung cancer”, “non-small cell lung cancer”, “NSCLC”, “*EGFR*”, “*EGFR* mutation”, “acquired resistance mechanism”, “T790M”, “mesenchymal-epithelial transition factor”, “Hepatocyte growth factor”, “HSP90 inhibitor”, “targeted therapy”, “chemotherapy”, biologic therapy”, “post-EGFR”, “salvage therapy”, “tyrosine kinase inhibitors”, “TKIs”, “irreversible ”, “TKI

beyond progression", "combined EGFR blockade", "mutant-selective EGFR inhibitor", " third generation EGFR TKI", "combination therapy"

Our search was not limited by date and was conducted from September 2014 through March 2015. We also reviewed relevant articles cited by other papers. Additional search was also done on abstracts at major medical oncology conferences including American Society of Clinical Oncology (ASCO), European Society of Medical Oncology (ESMO), American Association for Cancer Research (AACR) and EORTC-NCI-AACR (ENA).

Contributors:

CST and SP conceived the idea for the review, searched the scientific literature, wrote the manuscript and prepared the figure and tables.

DG participated equally in writing and revising the manuscript.

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CST has received honoraria from Eli Lilly

DG has received honoraria and fee to attend conferences from Astra Zeneca, Pfizer, Eli Lilly, Boehringer Ingelheim, Merck and Bristol-Myers Squib.

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