Sultan Qaboos University Med J, May 2013, Vol. 13, Iss. 2, pp. 202-217, Epub. 9th May 13
Submitted 19th Jul 12
Revision Req. 10th Sep 12, Revision Recd. 18th Oct 12
Accepted 27th Oct 12

REVIEW

Customised, Individualised Treatment of Metastatic Non-Small-Cell Lung Carcinoma (NSCLC)

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العلاج الفردي و المخصص لسرطان الرئة المنتشر من الخلايا غير الصغيرة

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الملخص: سلسلة من دراسات المرحلة الثانية، والمرحلة الثالثة العشوائية في آسيا وأوروبا أكدت مؤخرا أن مرضى المراحل المتقدمة من سرطان الرئة من الخلايا الغير صغيرة من النوع الفرعي الغدي الآوي لطفرات محددة عندما تتعرض للعلاج المستهدف تظهر نتائج بقاء مساوية للذين عولجوا بالعلاج الكيميائي وبمنأى عن إثارة الأعراض الجانبية. ان مفهوم العلاج الكيميائي للجميع بدأ بالتلاشي، والوصول للعلاج الأمثل يبرز كنقلة نوعية في العلاج. توضح هذه المقالة بإيجاز الآليات الخلوية المساهمة في سرطنة الرئة و التي توفر أساس جزيئي للعلاج المستهدف. إن التقدم في علم الأحياء الجزيئي حسنت فهمنا للآليات التي تشارك في مقاومة الأدوية الابتدائية أو الثانوية. يورد هذا المقال بإيجاز التطورات في المؤشرات الحيوية ذات ألأهمية التكهنية والتنبؤية، وأثر البحوث المتعدية على نتائج العلاج. إن علامة ما تعتبر تكهنية إذا توقعت النتائج، بغض النظر عن العلاج، وتنبؤية إذا توقعت نتائج علاج محدد

مفتاح الكلمات: سرطان؛ سرطان الرئة من الخلايا الغير صغيرة؛ الأورام الخبيثة في الرئة؛ عامل نمو البشرة؛ عامل نمو بطانة الأوعية الدموية؛ علامات بيولوجية؛ مثبطات البروتين كينيز.

ABSTRACT: A series of phase II and randomised phase III trials in Asia and Europe have confirmed recently that advanced stage non-small-cell lung carcinoma patients with adenocarcinoma subtypes harbouring specific mutations when subjected to targeted therapy experience equivalent survival outcomes as those treated with chemotherapy and are spared from its side effects. The concept of chemotherapy for all is fading, and therapy optimisation has emerged as a paradigm shift in treatment. This article briefly describes cellular mechanisms involved in lung carcinogenesis which provide a molecular basis for targeted therapy. Advances in molecular biology have improved our understanding of mechanisms involved in primary or secondary drug resistance. Evolving biomarkers of prognostic and predictive importance, and the impact of translational research on outcomes are also covered. A marker is considered prognostic if it predicts the outcome, regardless of the treatment, and predictive if it predicts the outcome of a specific therapy.

Keywords: Carcinoma; Non-small-cell lung; Lung neoplasm; Receptor, Epidermal growth factor; Vascular endothelial growth factor; Biological markers; Protein kinase inhibitors, Bevacizumab, Erlotinib.

According to the global cancer incidence report, lung cancer is the most common malignancy and a leading cause of cancer-related death worldwide.[1] In the USA, non-small-cell lung carcinoma (NSCLC) accounts for approximately 87% of all the cases of lung cancer with adenocarcinoma as the most prevalent subtype (40%).[2] In Oman, NSCLC accounts for 88% of all lung cancers, with adenocarcinoma in 34% of cases, and squamous cell carcinoma (SCC) in 22%.[3] Despite diagnostic advances, lung cancer continues to present in advanced stages and as many as 50% of the patients have unresectable disease (stages IIIb–IV), and perhaps an even higher percentage in the developing world [Table 1].[2]

Certain clinical parameters have an impact on the outcome of the disease. Gender affects outcomes as females generally are diagnosed at a younger age and earlier stage, and perhaps have an inherent greater longevity. Different races also have different 5-year relative survival rates. In a study done between 2001–07, the 5-year survival rates were as follows: white men 13.7%; white women 18.3%; black men 11.6%; black women 14.5%.[2] Weight loss,

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Table 1: Stage, distribution, and 5-year survival rate by stage at diagnosis: 2001–07 (Adapted from SEER[2])

Stage at Diagnosis	Stage distribution (%)	5-year relative survival (%)
Localised	15	52
Regional (lymph nodes)	22	24
Distant (metastases)	56	<4
Not staged	07	08
*Stage at Diagnosis	Stage distribution (%)	5-year relative survival (%)
**NSCLC (82.7%)		
I	4.6	100
II	13	66.7
III	21.7	20
IV	60.4	10

** = Stage, distribution, and survival rate by stage of 43/52 patients with non-small-cell lung carcinoma at Sultan Qaboos University Hospital: 2002–2011 (unpublished data); **NSCLC = non-small-cell lung carcinoma.*

poor performance status, advancing age, and the presence of concomitant illness may also adversely affect patient outcomes.[4–7] Smoking has emerged as a prognostic and predictive marker. In addition, it also has certain significance, i.e. never smokers have epidermal growth factor receptor (EGFR) mutations at a rate of 37% compared to 14% in current and former smokers; *k*-ras mutations in 4% of never smokers as compared to 43% in current and former smokers, and echinoderm microtubule-associated protein-like4 anaplastic lymphoma kinase (EML4-ALK) in 12% of never smokers as compared to 2% of current and former smokers. The prevalence of *p*53 mutation was the same in never smokers and current and former smokers (26% each).[8]

Methods

Data were identified from searches of PubMed, Medscape, Google, and key cancer groups (American Society for Clinical Oncology, National Comprehensive Cancer Network, European Society for Medical Oncology) using search terms such as "chemotherapy in advanced NSCLC", and various biomarkers of prognostic and predictive markers in lung cancer, EGFR, EGFR mutation ($EGFR_{MUT}$), *k*-ras mutation, EML4-ALK mutation, MET, and T790M mutations. Reference was also made to key phase II and III trials and meta-analyses published in oncology journals including *Chest*, *Clinical Advances in Hematology & Oncology*, *Journal of Clinical Oncology*, *New England Journal of Medicine*, *The Lancet Oncology*, *Oncologist*, and *Cancer*. Information acquired from international scientific conferences was confirmed through computer searches.

Evolution of Systemic Chemotherapy in Advanced/Metastatic NSCLC

Patients with advanced or metastatic NSCLC have traditionally been treated with systemic therapy if they carry a performance status of zero to two. Untreated, these patients have a median survival time of 3–4 months, and only one in 10 patients survives 12 months on best supportive care (BSC).[9–10] Cisplatin or carboplatin is the cytotoxic backbone when considering palliative chemotherapy.[11] In 1995, a large meta-analysis revealed a 27% risk reduction in death and one year survival enhancement of 10% when comparing chemotherapy to best supportive care (BSC).[12] The Cochrane Collaboration Group upheld the advantage of platinum doublets which were associated with higher response rates (RR) and an absolute benefit of 5% improvement in one-year survival.[13]

The Eastern Cooperative Oncology Group (ECOG) E1594 study is regarded as a reference trial of advanced NSCLC comparing four different chemotherapy regimens with each other (i.e. cisplatin combined in three arms with paclitaxel, gemcitabine, and docetaxel, respectively and the fourth arm comprising carboplatin and paclitaxel). The RR improved from 10 to 19%, and the median survival improved to 9.1 months for 431 females, and 7.4 months for 726 males. The survival increased to approximately 33% in the first year and 11% in the second year. Essentially, all arms revealed similar median survival, but the regimen comprising cisplatin and gemcitabine was associated with longer time to progression (TTP), whereas carboplatin and paclitaxel was the least toxic amongst the four arms, and regarded as their reference doublet combination for future studies.[10] Other large phase III trials validated the results

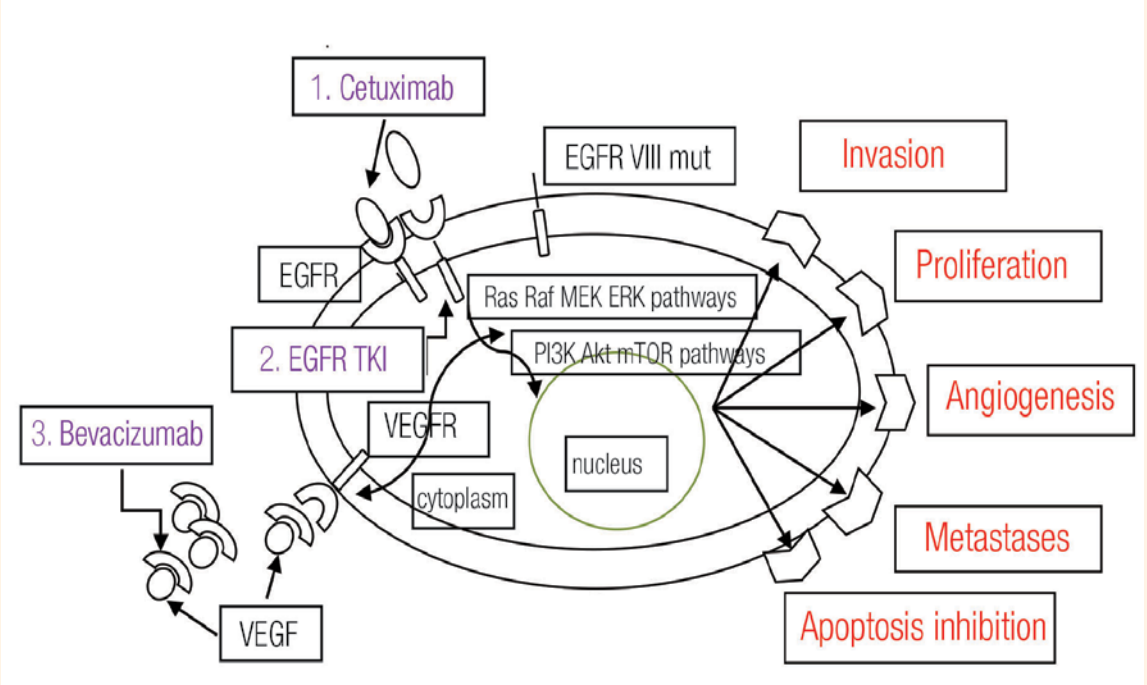

Figure 1: The EGFR and VEGFR cell signal transduction pathways and site of blockade by targeted therapies 1–3.
EGFR = epidermal growth factor receptor; TKI = tyrosine kinase inhibitor; VEGFR = vascular endothelial growth factor receptor; VEGF = vascular endothelial growth factor; mut = mutation.

of the platinum doublets used in the E1594 trial and these doublets emerged as a standard of care for patients with well-preserved organ function in performance status (PS) 0–1, and with slightly higher toxicity in selected PS 2 cases.

Towards the end of the last decade, histology emerged as a strong predictor for response and enhanced survival in non-squamous NSCLC. Data from phase II and randomised phase III trials of patients having the adenocarcinoma subtype, including large cell carcinoma and bronchioalveolar carcinoma (BAC), confirmed improvement in median survival beyond 10 months after the addition of pemetrexed. A phase III study revealed survival approaching a statistically significant 12.6 months in the pemetrexed cisplatin arm compared to 11 months in gemcitabine cisplatin arm in adenocarcinoma subtypes.[14] The survival was 10.4 *versus* 6.7 months for the experimental arm in large cell carcinoma. SCC, however, did poorly with the addition of pemetrexed where median overall survival (OS) remained at <10 months. The combination therefore emerged as an option for non-squamous subtypes, reaching a median survival time in excess of 12 months, while cisplatin plus gemcitabine or docetaxel remained the standard treatment for SCC.

By 2008, chemotherapy for NSCLC reached a plateau with median survival approaching 10–12 months, while scientific research drifted towards molecular profiling with the evolution of cancer genetics and translational work. Researchers started to study the cell signalling pathways and evaluate means to target cancer cells at the molecular level. Others started to use maintenance therapy in their effort to enhance the median survival time in this aggressive disease.

Molecular Targets and Targeted Therapy in Metastatic NSCLC

TUMOUR ANGIOGENESIS AND VASCULAR TARGETS: BEVACIZUMAB

Vascular endothelial growth factor (VEGF) was discovered by Harold Dvorak and Donald Senger in 1983, and subsequently sequenced by Napoleone Ferrara's group in 1989.[15,16] It was well-established that small tumours fail to thrive after attaining sizes as small as a few millimeters until they derive their independent vasculature. This in fact is carried out by the release of VEGF-A and other ligands that bind to the extracellular domain on the tumour cell VEGF receptors [Figure 1]. This initiates

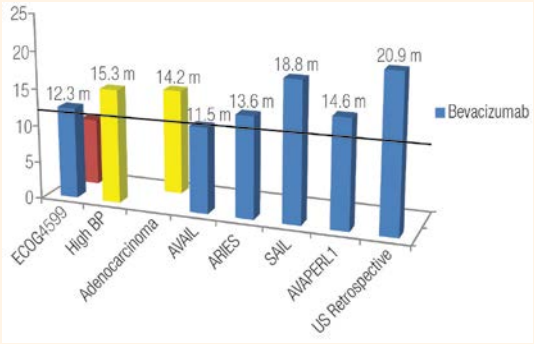

Figure 2: Continued survival benefit with bevacizumab: 2006 to 2011 showing the major trials undertaken.
-The yellow bars show the subset analysis for ECOG4599 trial.
-The red bar is the control (chemotherapy arm) of ECOG 4599 trial.

downstream cell signalling through activation of Ras/Raf/MEK/ERK or PI3K/Akt/mTOR pathways leading to cell proliferation, endothelial migration, angiogenesis, invasion, and metastases. The VEGF-A and platelet-derived growth factor (PDGF) binding to platelet-derived growth factor receptor (PDGFR) also complements the regulation of angiogenesis indirectly providing targets for dual- or multi-tyrosine kinase inhibitors (TKIs).

Bevacizumab blocks the VEGF-A and prevents binding to the VEGF receptor while the complex is recognised and eliminated by the immune system. Bevacizumab exerts its action through the following proposed mechanisms. Antivascular effects lead to the regression of the tumour vasculature and reduce tumour size. Antiangiogenesis inhibits neo-angiogenesis and recurrent blood vessel growth in the tumour. Antipermeability decreases vascular permeability, and oedema in the tumour microenvironment, reducing pleural fluid volume.

After promising results from a US phase II trial by Johnson *et al.* in 2004, a series of large randomised phase III trials of bevacizumab were initiated [Figure 2].[17] A subset analysis of a phase III trial by Sandler *et al.* revealed interesting findings: patients with an adenocarcinoma subtype had a survival advantage of 3.9 months.[18] Additionally, patients who developed hypertension on bevacizumab had a superior OS.[19] Also, elderly patients (>70 years) showed a trend of higher RR and (progression-free survival) PFS but no gains in OS. They also experienced more toxicity. The outcome of patients on maintenance bevacizumab showed promise on retrospective review and, therefore, a series of successful phase III maintenance trials were initiated to test its relevance.

The addition of bevacizumab to pemetrexed and platinum improved survival outcomes in adenocarcinoma histology in two phase II trials.[20,21] A randomised phase III maintenance trial (AVAPERL 1) in PS 0–1 advanced adenocarcinoma patients was initiated using pemetrexed-cisplatin plus bevacizumab followed by continuation bevacizumab with or without pemetrexed.[22] The interim results seem promising, as the addition of bevacizumab was well tolerated and associated with a superior PFS of 3.6 months. The median OS for the bevacizumab arm was 15.7 months, but for the pemetrexed bevacizumab arm; an OS level has not yet been reached. The Pointbreak study (900 patients) with advanced non-squamous NSCLC used pemetrexed + carboplatin + bevacizumab induction followed by pemetrexed + bevacizumab maintenance (arm A) and compared it with paclitaxel + carboplatin + bevacizumab induction followed by bevacizumab maintenance (arm B). Though it failed to reach the primary endpoint of enhancing OS, the maintenance arm A improved the survival outcomes (PFS and OS) compared to maintenance arm B.[23] Whether the gain in survival is because of pemetrexed or bevacizumab remains to be defined. The ECOG E 5508 trial is underway to address this question.[24]

In a meta-analysis of 2,252 patients from 5 randomised trials with advanced NSCLC, Andre *et al.* reported that the addition of bevacizumab to platinum doublets increased RR, enhanced PFS by 1.4 months, extended OS by 1 month, and was associated with a significant 11% reduction in death. However, the combination was associated with slightly higher toxicity and mortality and therefore warrants careful patient selection.[25]

Based on promising survival gains from a colorectal study (BRITE),[26] the ARIES trial[27] in NSCLC, and another American retrospective analysis of non-squamous NSCLC,[28] a large multi-institutional, randomised trial called Avastin in all Lung Lines (AVAll) was initiated and is currently recruiting patients to evaluate the role of post-progression bevacizumab.[29] Nearly all key trials exploring the efficacy of the addition of bevacizumab to standard platinum doublets showed a consistent improvement in RR, PFS, and median survival rates in adenocarcinoma subtypes

with PS 0–1 [Figure 2]. However, the 4,000 patients (>65 years) from the SEER database were recently analysed and the addition of bevacizumab was not found to be advantageous in the elderly population.[30]

When a single cellular target is blocked, the cancer cell finds an alternative pathway to proliferate and hence provides a rationale for dual or multi-targeted therapy. Phase II and III trials, using dual TKI (vandetanib) after multi-line chemotherapy failure in advanced NSCLC, provided a hint of activity and efficacy in favour of the combination of chemotherapy with vandetanib.[31–34] Multi-TKI (i.e. sorafenib, which inhibits TK, VEGFR, PDGFR, and the Raf signalling pathway when compared to a placebo) failed to enhance the efficacy of the treatment.[35–36] In the MISSION trial, sorafenib failed to improve the OS, but the PFS, TTP, RR, and disease control rate (DCR) improved substantially.[37] BATTLE, a phase II study, incorporated heavily pretreated NSCLC patients with PS 0–2 (2–5 lines failures, brain metastases allowed). The study compared the effects of erlotinib in the EGFR mutation-positive group, which validated results of earlier randomised trials, *versus* vandetanib and erlotinib, which showed enhanced activity in the VEGFR2-expressing subsets *versus* erlotinib and bexarotene showing activity in cyclin D1 expressing and EGFR amplified patients with improved outcomes *versus*. sorafenib which was associated with a dismal outcome in EGFR-mutation positive subsets.[38,39] Multi-TKI roles in the metastatic setting remain under investigation. However, there is emerging data of multi-TKI use in RET translocations (1%) in adenocarcinoma subtype.

BIBF 1120, a triple angio-kinase inhibitor (active against FGFR, PDGFR, and VEGFR) in the second- or third-line setting was found to be associated with tumour shrinkage in patients with adenocarcinoma.[40] Its use is being explored in combination with docetaxel and pemetrexed.[41,42]

With advances in molecular biology and genetics, newer markers of significance are being explored to determine their prognostic or predictive value, and non-squamous NSCLC has emerged as a classical model of studying cancer genetics. Discovery of key cellular markers and their targeting has started to bring a paradigm shift in the management of advanced adenocarcinoma subtypes and will be the focus of the following discussion.

EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)

EGFR is a trans-membrane cellular protein receptor located on the cell surface. The gene that codes for EGFR protein is located in 7p11-13. It consists of 26 exons, of which the first 14 code for the extracellular ligand binding domain, exon 15 for the transmembrane domain, and the last 11 for the intracellular domain. The ATP-binding site and intrinsic TK activity is also coded by exons 16–26.[43] It is generally expressed at low levels in a wide variety of normal tissues. Excessive expression or activation of EGFR is able to induce malignant transformation. Over-expression has been observed in 40–80% of NSCLC.[44-46] A form of $EGFR_{MUT}$ (EGFRvIII) is implicated in carcinogenesis with TK activity.[47]

Receptor activation is generally initiated by the binding of EGF or related ligands, leading to receptor homodimerisation or heterodimerisation, which activates intrinsic TK activity of the receptors leading to autophosphorylation of tyrosine residues. Mutations or over-expression in the TK domain of the EGFR gene increase the activity of the intracellular signalling cascades through Ras/Raf/MAPK and PI3K/Akt/mTOR pathways [Figure 1], leading to events which result in over-proliferation, differentiation, enhanced survival, inhibition of apoptosis, neo-angiogenesis, and metastases, and is associated with a poorer prognosis.[48,49]

Determination of EGFR gene mutations has clinical relevance as it allows the optimisation of therapy and has emerged as one of the most important single predictive molecular markers in clinical oncology. Patients with certain $EGFR_{MUT}$ derive significant benefit after the addition of gefitinib or erlotinib.[50–52] These agents inhibit the TK and adversely compete with ATP for the critical ATP-binding site located in the intracellular domain inhibiting the intrinsic tyrosine kinase enzyme activity. Cetuximab is a monoclonal antibody that binds the extracellular domain of EGFR effectively and prevents the subsequent intracellular cascade of events and the inhibition of malignant transformation [Figure 1]. Certain clinical subgroups of patients derive a greater benefit from tyrosine kinase inhibitors (TKI) therapy by virtue of the presence of an $EGFR_{MUT}$. These include patients of East Asian origin, never smokers, females, and those having an adenocarcinoma.[53–54] The $EGFR_{MUT}$ are found in exons 18–21 of the EGFR gene.[43]

Table 2: Epidermal growth factor receptor mutation types, incidence, and sensitivity to tyrosine kinase inhibitors

Sr. No.	EGFR mutation type	Known mutations %		Sensitivity to TKI
1	Exon 19 $del_{(del\ 746\text{-}A750)}$ Exon 21 point $mutation_{(L858R)}$	45 45	+	Yes
2	Dual $mutations_{T790M/exon19del\ T790M/L858R/others}$	5–7	?	No, data limited
3	Exon 20 $insertion_{(T790M)}$ Exon $18_{(G719S)}$	5 5	-	No

EGFR = epidermal growth factor receptor; TKI = tyrosine kinase inhibitor.

TARGETING THE EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) LUNG

The different types of EGFR mutations provide information on whether patients with these mutations are likely to benefit from TKIs (erlotinib or gefitinib)[50–52,56] [Table 2]. An individual matching the clinical criteria above carries a 50% chance of harbouring the mutation, but this is not the sole criteria used to commence TKI therapy. $EGFR_{MUT}$ are present in 30–50% of East Asians and approximately 10% of Caucasians having NSCLC. Its exact incidence in Arabian Gulf countries or Oman is not known. Well to moderately differentiated tumours have more frequent $EGFR_{MUT}$ than their poorly differentiated counterparts.[57] Adenocarcinoma, which is thyroid transcription factor 1 (TTF1) negative, will usually harbour a wild-type (normal) EGFR ($EGFR_{WT}$).[58,59]

Around 30% of SCCs overexpress the EGFR protein (not the gene) on immune-staining, and TKI provides a similar advantage to this subtype. The evidence of adding a TKI in SCC comes from the subset analysis of the SATURN study and the exploratory retrospective analyses from the NCIC BR.21 trial according to which these agents enhance PFS and OS irrespective of histology when used as maintenance and second-line salvage therapy, respectively.[60,61] Similarly, data from the FLEX trial recommend use of cetuximab with induction chemotherapy in SCC expressing the EGFR protein. EGFR mutations rarely occur in never smoking SCC patients, and therefore are not routinely determined.

TARGETING THE EGFR INTRACELLULAR DOMAIN: TYROSINE KINASE INHIBITORS (TKIs)

After successful preclinical studies, evidence that the $EGFR_{MUT}$ correlated with response to TKI in clinical practice first emerged in 2004 from phase II trials in previously treated NSCLC patients.

Trials of salvage TKIs have been undertaken. In the IDEAL 1 trial, gefitinib was used in patients pretreated with two or fewer previous chemotherapy regimens, while in IDEAL 2 the same agent was used in pretreated NSCLC patients who received more than two regimens.[62,63] In 210 patients, the RR were 18–19% and there were median survival gains of 7.6–8 months in the IDEAL 1 trial. The RR was 11.8% and median OS was 6.5 months in the IDEAL 2 trial. The subset analyses revealed that Asian, never smoking females having adenocarcinoma (especially BAC) reacted with a predictable response to gefitinib. The ISEL, a phase III study, used gefitinib as first salvage in NSCLC after one or more prior chemotherapy failures. Survival was increased in patients who were Asians or never smokers.[53] INTEREST, a phase III trial, utilised gefitinib *versus* docetaxel in a second-line salvage setting. The TKI was associated with a superior quality of life (QoL) and fewer side effects.[64] Erlotinib was also used as a monotherapy in patients with NSCLC who progressed on 1–2 previous chemotherapies in the phase III BR.21 trial against BSC, and was associated with improvement in disease-related symptoms, superior TTP, and median OS enhancement by 2 months.[61]

Phase III trials of concurrent use of TKIs with chemotherapy have also been conducted. TALENT was a negative trial (>1,172 patients) with two arms that used carboplatin and paclitaxel with or without erlotinib.[65] The RR, PFS, and OS remained unchanged when the TKI was used concurrent with chemotherapy as compared to chemotherapy alone. The TRIBUTE study utilised cisplatin and gemcitabine alone or concurrent with erlotinib and validated the results of the TALENT trial.[66] However, subset analyses revealed increased survival in never smokers. INTACT 1 and INTACT 2 also used carboplatin and paclitaxel alone or concurrent

erlotinib without improvement in efficacy.[67,68] CALGB 30406, presented in ASCO 2012, reveals erlotinib efficacy as singlet or concurrent with paclitaxel carboplatin in never or light smokers, harboring adenocarcinoma. Erlotinib was found to have similar efficacy in $EGFR_{MUT}$ in this phase II setting whether used as a singlet, and with higher toxicity or when used in combination with chemotherapy.[69] There is a hint of TKI continuation concurrent with chemotherapy in some NSCLC cases associated with flare-up of the disease on discontinuation of the TKI. TKI use concurrent with chemotherapy remains investigational and necessitates further study in the context of a phase III trials.

Trials comparing TKIs to chemotherapy as initial therapy have also been carried out. When TKI was used as frontline therapy in adenocarcinoma harbouring the $EGFR_{MUT}$, the RR and PFS improved substantially but with similar OS gains to those seen with chemotherapy.[50–52,56,70–74] Similar efficacy, superior tolerability, and less toxicity made TKI a new therapeutic option for adenocarcinoma subtypes, as well as its potential use in PS 3 and selected PS 4 cases. Certain clinical subgroups of patients with an $EGFR_{MUT}$ had a substantial enhancement in median survival, including those having an adenocarcinoma, never smokers, females, ethnic Asians, and those who developed a high-grade, acne-like rash with TKI.[64–68,70–74]

In the Iressa Pan-Asia Study (IPASS), a phase III, multicentre, randomised trial of advanced NSCLC, 1,217 patients from East Asia were randomly assigned to receive daily TKI orally *versus* a platinum doublet every 3 weeks for 6 cycles.[50] Crossover was allowed. One-third of the patients had an $EGFR_{MUT}$. The objective RR was statistically and numerically superior in the gefitinib arm in all clinical subgroups, with marked improvement of RR in the $EGFR_{MUT}$-positive subgroup. However, the median OS remained similar, and mutation-negative patients benefited from chemotherapy but did extremely poorly with gefitinib (RR = 1%). The TKI are therefore not indicated in $EGFR_{WT}$. The OS was similar in the $EGFR_{MUT}$ positive or negative groups and is possibly due to crossover in the two arms. The survival was also superior in patients with a high EGFR gene copy number, however, at the expense of mild toxicity.

The OPTIMAL trial was conducted by the Chinese Thoracic Oncology Group, and involved 23 centres using erlotinib.[51,52] The trial also validated results from the IPASS study. In subgroup analyses, PFS was 15.3 months in exon 19 del and 12.5 months in L585R mutation. Table 3 shows the key phase III, prospective randomised clinical trials of upfront TKIs in non-squamous NSCLC with almost similar outcomes.[50–52,55,72–74] At disease progression on TKIs, patients may still be treated with salvage platinum doublets.

The TORCH trial revealed that in unselected advanced NSCLC patients, first-line erlotinib (followed by cisplatin gemcitabine at progression) was inferior in terms of OS compared with the standard sequence of first-line chemotherapy followed by erlotinib at progression.[75] TAILOR is another phase III trial, where 218 evaluable patients ($EGFR_{WT}$) were subjected to second-line docetaxel or erlotinib. The PFS was superior in the docetaxel arm with an absolute difference in 6 months PFS of 12% and remains a negative trial for TKI in wild type EGFR.[76]

TARGETING THE EGFR EXTRACELLULAR DOMAIN: CETUXIMAB

After encouraging results from phase II trials of the addition of cetuximab to induction chemotherapy in metastatic NSCLC, phase III trials were initiated. The monoclonal antibody binds to the extracellular ligand binding domain of the EGFR, blocking the intracellular cell signalling [Figure 1]. FLEX (First Line Erbitux in Lung Cancer trial) is a key phase III, randomised trial comparing the addition of cetuximab to cisplatin and vinorelbine, given for 6 cycles and cetuximab continued as maintenance therapy until progression of disease or unacceptable toxicity *versus* chemotherapy alone. The median OS was superior.[77] Moreover, a high EGFR expression was associated with a better survival with the addition of cetuximab. Survival figures were also superior for females, those of Asian ethnicity, in those who developed acne, and in those having adenocarcinoma. The addition of cetuximab to chemotherapy was found to be as effective in SCC with a prolongation of one year survival by 19%.[78] Another USA-based trial looked at unselected NSCLC patients treated with taxane plus cetuximab, which enhanced median OS.[79] The addition of cetuximab to platinum doublets remains a reasonable option in distinct PS 0–1 patients, as

Table 3: Phase III, prospective, randomised clinical trials of upfront tyrosine kinase inhibitor- erlotinib (E), and gefitinib (G)

Study	No. of Patients	ORR %	DCR %	Median PFS (months)	Median OS (months)	HR/*P* value for PFS
TKI trials	**$EGFR_{MUT}$**				***P* value NS**	
IPASS[50](G) China	261; 132	71.2	91.7	9.8 vs. 6.4	21.3 vs. 23.3	0.48
OPTIMAL[51,52](E) Asia	154; 82	82.9	96.3	13.7 vs. 4.6	18.8 vs. 17.4	0.16
EURTAC[56](E) Europe	1275; 174	54.5	10.5	10.4 vs. 5.1	22.9 vs. 18.8	0.34
First-SIGNAL[70](G) Korea	42; 26	84.6	88.5	8.4 vs. 6.7	21.3 vs. 23.3	0.613
NEJSG 002[71](G) Japan	228; 114	73.7	89.5	10.8 vs. 5.4	30.5 vs. 23.6	0.30
WJTOG 3405[72](G) Japan	172; 86	62.1	93.1	9.2 vs. 6.3	36.3 vs. 39	0.489
SLCG[73](E) Spain	217; 217	70.6	89.8	14.0	27	--
CALGB 30406[69](E) US	181; 164	71	NR	14.1 vs. 2.6	OS 31 vs.18	

ORR = objective response rate; DCR = disease control rate; PFS = progression free survival; OS = overall survival; HR = hazard ratio; TKI = tyrosine kinase inhibitor; $EGFR_{MUT}$ = epidermal growth factor mutation; NS = not significant; NR = not reported.

shown in the algorithm in Figure 3. It is reasonable to consider adding cetuximab to platinum doublets in patients with SCC (30% of these express the EGFR protein) and then use it as maintenance in those stablising after induction therapy, where it adds 1 month to OS.

Molecular Markers of Significance in Targeted Therapy

With advances in molecular biology and translational research, molecular targets are evolving which are potential targets of newer drugs [Tables 3 and 4]. Some key markers of prognostic and predictive value are described below.

MET AMPLIFICATION

MET is a cell surface receptor with TK activity expressed by normal cells as well as malignant cells.[80] It is amplified in 10–20% of NSCLC. Ligand-like hepatocyte growth factor/scatter factor binds to MET, activating downstream cell signalling and leading to cell proliferation, angiogenesis, invasion and metastases. High expression is defined on immunostaining, when >50% cells stain strongly (>3+) or, by an increased gene copy number (>5 copies/cell) as detected by fluorescent *in situ* hybridisation (FISH). MET and EGFR coamplification occurs rarely—in only 1% of cases. The incidence of MET mutation was highest in Asians (13%), 0% in blacks, and N375S was the commonest mutation.[81] MET amplification may lead to acquired resistance to TKI therapy through activation of alternate cell signalling pathways like PI3K in up to 20% of patients.[82,83] It is also associated with reduced TTP. The PFS varies inversely with MET overexpression. Patients with high MET had superior survival with chemotherapy (*P*0.25). Several MET inhibitors, with or without dual inhibition of EGFR and MET as a potential mechanism to overcome resistance, are currently undergoing trials. MET inhibitors like METMab plus erlotinib *versus* placebo plus erlotinib were compared in a phase II trial and showed 50% enhancement in PFS and OS in patients with high MET expression.[84] Patients with low MET expression fared worse with the same combination. ARQ 197 is another MEK inhibitor that showed superior PFS when combined with erlotinib in a phase II trial with a trend towards increased OS.[85] The MetLung trial is a global phase III randomised trial recruiting patients with metastatic or recurrent disease after one previous chemotherapy failure in MET-positive NSCLC. Patients are being randomised to erlotinib plus onartuzumab (MetMab) *versus* erlotinib plus placebo.[86]

K-RAS MUTATION

Mutations in the Kirsten rat sarcoma virus oncogene homologue gene (*k*-ras) occurs in approximately 20–30% of NSCLC cases. They are common in mucinous adenocarcinoma (but not in large-cell carcinoma or BAC), in the elderly, in heavy smokers, in stage I and tumour grade I but frequently fall with stage and grade progression, while the

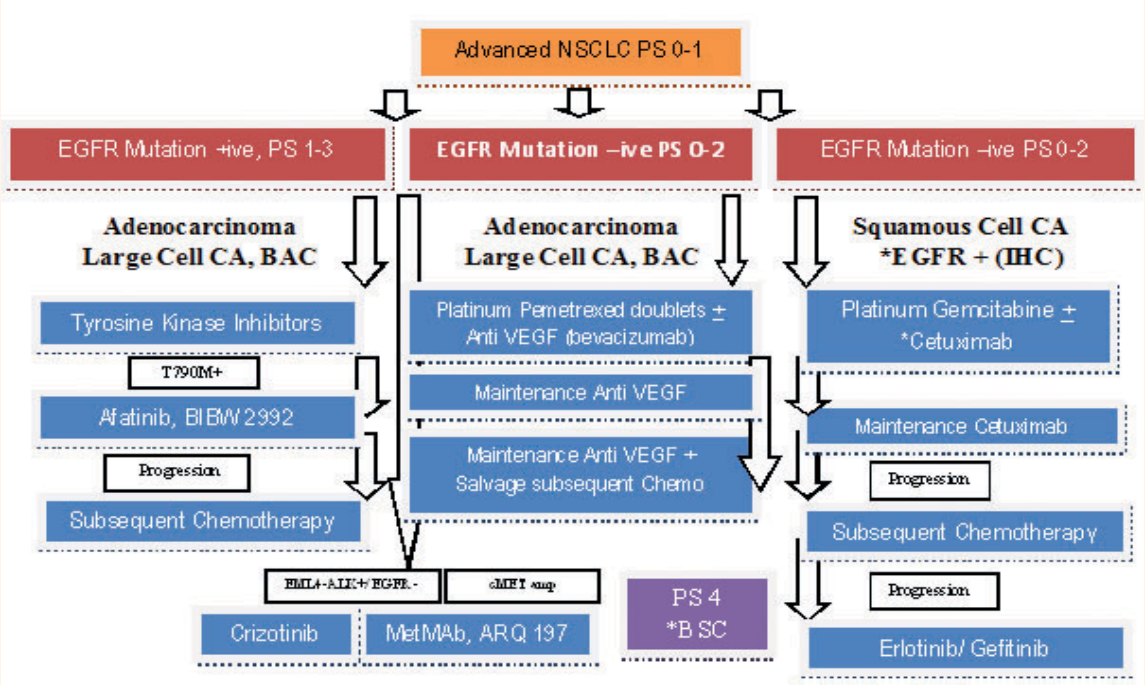

Figure 3: Evolving treatment algorithm of non-small-cell lung carcinoma in 2012.

*NSCLC = non-small-cell lung carcinoma; EGFR = epidermal growth factor receptor; CA = carcinoma; BAC = bronchioalveolar carcinoma; IHC = immunohistochemistry; VEGF = vascular endothelial growth factor; EML4-ALK = echinoderm microtubule-associated protein-like4 anaplastic lymphoma kinase; PS = performance status; *BSC = best supportive care.*

occurrence in non-smokers is low. The majority of mutations occur in codon 12 (>90%—guanine to thymine transversion) and 13, and the gene leads to impaired GTPase activity, located towards the inner surface of the cell membrane. This leads to subsequent constitutive activation of RAS signalling, which is downstream of the EGFR, leading to activation of proliferative and anti-apoptotic pathways such as ERK. In the early 1990s, it was considered a negative prognostic marker, and perhaps a negative predictive marker responsible for chemo-resistance. However, data from recent trials like TRIBUTE and FLEX negates it.[66,77] Unlike colorectal cancers, where *k*-ras mutation is a strong predictor of response to cetuximab, its status in NSCLC could not confirm it to be so on retrospective analyses of FLEX and BMS 099 trials.[77,79]

In the NCIC BR.21 study, 28% of 731 patients had *k*-ras genotype.[61] The majority was wild type that responded well to TKI, while 15% had a mutation that conferred primary resistance to TKI therapy. Data from TRIBUTE reveals that EGFR and *k*-ras mutations rarely occur together, and that survival was inferior in the group of patients with *k*-ras mutation who were treated with chemotherapy plus TKI.[66] Hence, the presence of *k*-ras mutation not only rules out EGFR mutation, but is also a marker of TKI inactivity. To date, patients harbouring the *k*-ras mutation are best treated with chemotherapy. In a recent trial comparing docetaxel with or without selumetinib (MEK 1 and 2 inhibitor downstream of *k*-ras), the combination resulted in superior RR, PFS, and OS in patients with the *k*-ras mutation.[87]

Echinoderm Microtubule-Associated Protein-Like4 Anaplastic Lymphoma Kinase (EML4-ALK) Mutation

EML4-ALK was isolated from a surgically resected lung adenocarcioma specimen, but originally identified in anaplastic large-cell lymphoma (ALCL).[88,89] It occurs in 4–11% of NSCLC. EML4-ALK exhibits activating mutations or translocations of the anaplastic lymphoma kinase aberrant fusion gene (result of a small inversion within short arm of chromosome 2p, in which the EML4 becomes fused to the intracellular kinase domain of ALK). It leads to the activation of downstream cell signalling through PI3K and STAT pathways. The gene encodes a cytoplasmic chimeric protein with kinase activity, and typically tested positive in younger subjects, having adenocarcinoma of a predominantly signet ring subtype, and in never or

Table 4: Personalised therapy in 2012 for non-small cell lung carcinoma

Factors	Featurers/ Molecular Targets	Targeted Therapy/Chemotherapy Options
Clinical	Female, Never smoker, Asian, Adenocarcinoma Treated CNS metastases, No hemoptysis, Controlled hypertension, No tumor near great vessels, No VTE, No therapeutic anticoagulant therapy	TKI Bevacizumab
Histologic	Non mucinous Adenocarcinoma$_{(EGFR\ Mutation+)}$ Mucinous adenocarcinoma$_{(k\ ras\ mutation/\ EGFR\ wild)}$ Mucinous adenocarcinoma$_{(EML4\ ALK+)}$ Nonsquamous subtypes Squamous$_{(30\%\ EGFR\ protein\ expression)}$	TKI Platinum doublets (pemetrexed) Crizotinib 1st line or after induc chemo Pemetrexed/ Cisplatin/ or both Bevacizumab, Pemetrexed + Platinum Gemcitabine or taxanes + Platinum + Cetuximab No pemetrexed/ No bevacizumab
Molecular (markers in targeted therapy)	EGFR Mutation + (FISH) *k* ras +ive , EGFR wild type EGFR overexpression on (for SCC) cMET mutation T790M mutation EML4 ALK mutation/ ROS 1 mutation RET translocation	TKI Chemotherapy, suitable combination? Cetuximab Chemotherapy, (?ARQ 197, METMab) Afatinib, (?AEE788+Everolimus) Crizotinib Pemetrexed/ Cisplatin/ or both ?Multi-TKI (Sorafenib/ Sunitinib)
Unanswered	Triple negative lung cancer*$_{(EGFR-,\ k\ ras\ -,\ EML4\ ALK-)}$ Primary vs Metastases	Do poorly with standard chemotherapy, Best regimen? Do these harbour same mutations?
Biomarkers in Chemotherapy**	ERCC1 low ERCC1 high RRM1 high β Tubulin	Platinum sensitive disease Gemcitabine Docetaxel (cisplatin resistance) Non-gemcitabine doublets (gemcitabine resist) Non taxane combination (taxane resistance)

** = Retrospective subgroup analyse; ** = Markers undergoing research; CNS = central nervous system; VTE = venous thromboembolism; TKI = tyrosine-kinase; EGFR = epidermal growth factor reactor; FISH = fluorescence in situ hybridization; SCC = squamous cell carcinoma; EML4 ALK = echinoderm microtubule-associated protein-like4 anaplastic lymphoma kinase; ERCC1 = excision repair cross-complementation group 1; RRM1 = ribonucleotide reductase M1 gene inhibitor.*

light smokers who did not harbour EGFR mutations. Immunohistochemistry (IHC) could detect the protein, and reverse transcription polymerase chain reaction (RT-PCR), gene sequencing, and FISH could detect the break-apart rearrangement.[90] The break removes the inhibitory effect of EML4 and ALK initiates carcinogenesis due to its inherent oncogenic activity. EML4-ALK positivity mutually rules out EGFR and *k*-ras mutations.

Currently, patients with this distinct expression have a similar RR and OS to platinum-based chemotherapy compared to the wild type patients. Crizotinib, an oral ALK inhibitor which produced a 90% clinical benefit (57% objective response rate [ORR]) when used as a second-line compared favourably to salvage second-line chemotherapy which produced a RR of 10% in a phase II trial.[91] Another phase II trial (PROFILE 1005) is assessing salvage crizotinib in adenocarcinoma patients treated with one previous platinum, with the finding that the ORRs were 54% and the DCRs were 74%, which was considered safe and tolerable.[92] Recently, PROFILE 1007 data were presented to the European Society for Medical Oncology. ALK-positive patients who failed one previous platinum doublet were treated with crizotinib compared with chemotherapy (docetaxel or pemetrexed). Crizotinib enhanced PFS (7.7 *versus* 3 months; P <0.0001), RR (60% *versus* 20%; P <0.001), and QoL.[93] The exact sequencing of the drug still needs to be defined. Currently all patients harbouring wild type EGFR are treated with chemotherapy doublets and crizotinib is reserved as salvage in those who have the EML4-ALK mutation. The new agent is being tested in metastatic setting as frontline treatment. Crizotinib has also been found effective against ROS1 (~1%) mutation in NSCLC.

Resistance to Epidermal Growth Factor Receptor (EGFR) Tyrosine Kinase Inhibitors (TKIs)

At some point during the course of disease, NSCLC progresses, despite therapy, because of the emergence of resistance due to underlying

genetic alterations. Primary resistance affects patients who are refractory to TKI therapy at the time of initiation. Molecular factors identified as predictive of an EGFR TKI response are often EGFR-related due to an increased EGFR gene copy number; activating mutations within the EGFR TKI domain; or resistant EGFR mutants. For example, the presence of insertions in EGFR's exon 20 (5% of all known mutations) precludes the binding of gefitinib or erlotinib to the EGFR TKI domain, conferring resistance.[94] Exon 18 (G719S) or exon 21 mutations (T854A), the presence of the *k*-ras mutation, and the loss of PTEN, B Raf mutations, or overexpression of MAPK and bcl2 also confer primary resistance.

Secondary or acquired resistance generally affects patients who initially respond to TKI therapy but experience a loss of response after 6–12 months. Acquired resistance generally occurs with changes in MET over-expression, which accounts for 20% of resistance to the TKI.[82,83] A T790M mutation (substitution of methionine for threonine at position 790) in progressing tumours accounts for 50% of resistance, and interferes with the binding of TKIs to the ATP-kinase binding pocket leading to continued activation of downstream signalling.[95–97] This mutation is responsible for acquired drug resistance in patients receiving TKI therapy over a period of time. Patients harbouring this mutation have an indolent course as the disease is not rapidly fatal and metastasises late.

The LUX1 trial used irreversible TKI (afatinib) that inhibits T790M after failure of first-line TKI with a DCR of 58% *versus* 19% and a 2.2 month improvement in PFS in favour of the agent.[98] The agent was subsequently used in chemo-naive metastatic NSCLC patients in the LUX Lung 3 trial, revealing a superior RR and enhanced PFS of 11.1 months in patients with an exon 19 del and 13.6 months in the L585R mutation when the agent was compared to pemetrexed and cisplatin.[99] The LUX Lung 7 and 8 trials compare afatinib *versus* TKIs (gefitinib and erlotinib) respectively.

AEE788 is a multiple RTK inhibitor that blocks EGFR, VEGFR, and human epidermal growth factor receptor 2 (HER2) pathways and, when combined with a mTOR inhibitor (everolimus), was found to be effective against the T790M mutation.[100] Numerous unknown mechanisms (e.g. rare D761Y, L747S, T854A mutations, IGF-1R, etc.) account for 30% of acquired resistance.[101] It has also been shown that patients who have developed resistance to TKI, when subjected to chemotherapy, may respond again to the re-introduction of TKI.[102]

Conclusion

Bevacizumab and cetuximab have emerged as targeted agents to be used with cisplatin doublets in metastatic adenocarcinoma in patients with PS 0–1 and well preserved organ function. Those harbouring the EGFRMUT in PS 0–3 and highly selected PS 4 are now candidates for oral TKI therapy which offers equal efficacy, superior tolerability, and a lesser and different side effect profile, bringing a paradigm shift in the management of these cancers. There are compelling data for using more or prolonged therapy, and optimising therapy based on histology and genetic mutations, while resistance patterns continue to be recognised. The era of one chemotherapy that fits all is fading as patients' median survival is improving gradually under customised individualised therapy.

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