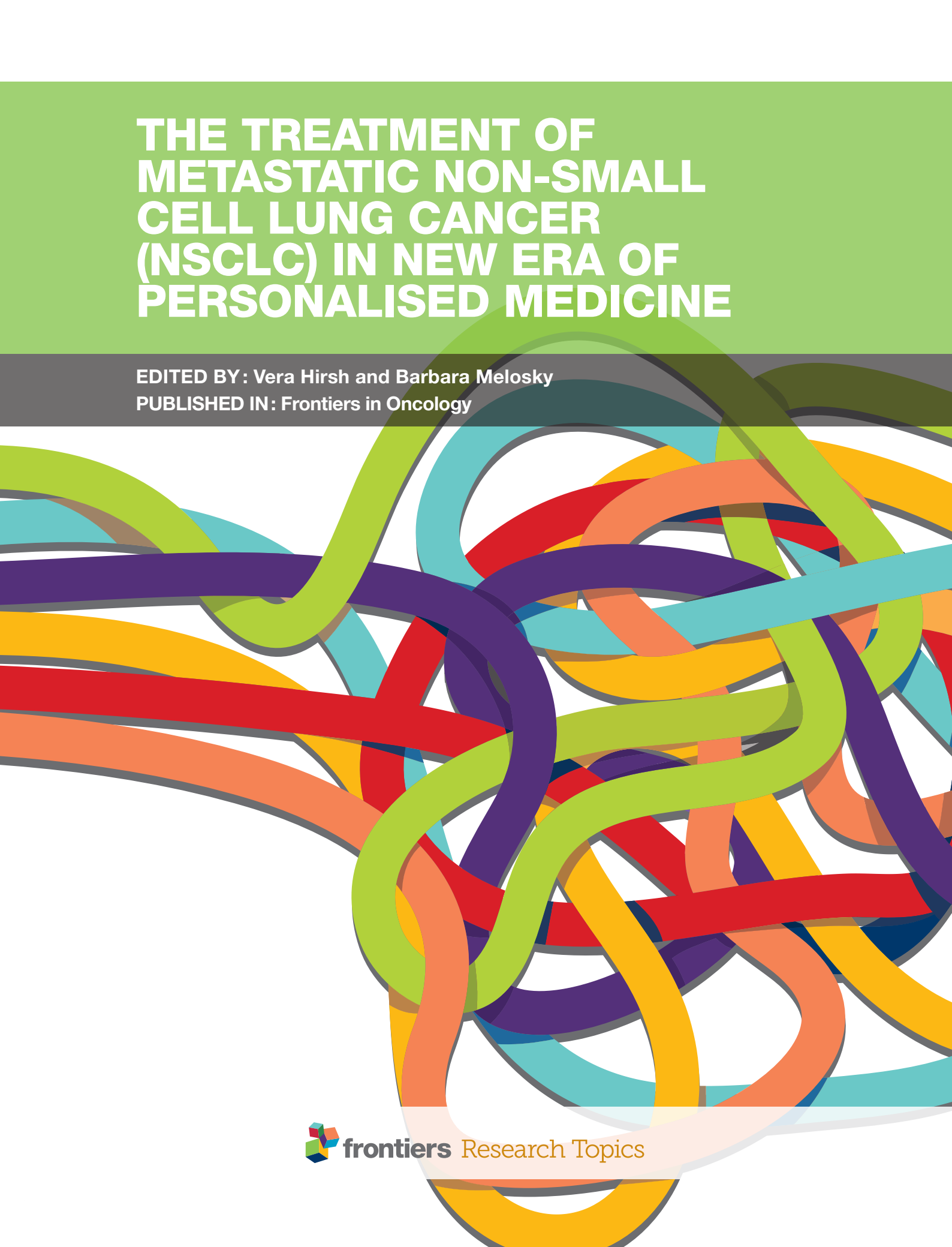
THE TREATMENT OF METASTATIC NON-SMALL CELL LUNG CANCER (NSCLC) IN NEW ERA OF PERSONALISED MEDICINE

EDITED BY: Vera Hirsh and Barbara Melosky

PUBLISHED IN: Frontiers in Oncology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88919-543-5
DOI 10.3389/978-2-88919-543-5

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: **researchtopics@frontiersin.org**

THE TREATMENT OF METASTATIC NON-SMALL CELL LUNG CANCER (NSCLC) IN NEW ERA OF PERSONALISED MEDICINE

Topic Editors:
Vera Hirsh, McGill University Health Centre, Canada
Barbara Melosky, British Columbia Cancer Agency, Canada

Lung cancer is the leading cause of cancer related mortality in Canada and USA. Majority of the patients present in advanced stage of the disease and of these only about 2% will be alive at 5 years. NSCLC is the most common form of lung cancer, accounting for approximately 87% of cases.

Systemic chemotherapies have been used to treat metastatic NSCLC for decades, but the improvements of outcomes have reached a plateau.

Recent advances in understanding signalling pathways for malignant cells, their interconections,the importance of various receptors and biomarkers and the interplay between various oncogenes have led to the development of targeted treatments that are improving both efficacy and safety of the treatments.

Knowledge about the advantages of treatments with the targeted agents in metastatic NSCLC is growing rapidly. Combining various targeted agents or sequencing them properly will be important in the era of personalised medicine and overcoming development of the resistence to various targeted agents will be challenging.

The importance of a team work,from the diagnosis through various treatments, to supportive care,from the interventional radiologists, pneumologists or surgeons, who have to obtain a satisfactory tumor tissue specimen, to pathologists, radiation and medical oncologists, to supportive care specialists, will be described in our publications. We will cover completely present and future approaches to personalised medicine in this rapidly evolving field of metastatic NSCLC.

Citation: Hirsh, V., and Melosky, B., eds. (2015). The treatment of metastatic non-small cell lung cancer (NSCLC) in new era of personalised medicine. Lausanne: Frontiers Media. doi: 10.3389/978-2-88919-543-5

Table of Contents

frontiers in
ONCOLOGY

EDITORIAL
published: 03 February 2015
doi: 10.3389/fonc.2015.00020

The treatment of metastatic non-small cell lung cancer in a new era of personalized medicine

Vera Hirsh*

Department of Medical Oncology, McGill University Health Centre, Montreal, QC, Canada
**Correspondence: vera.hirsh@muhc.mcgill.ca*

Edited and reviewed by:
Stephen V. Liu, Georgetown University, USA

Keywords: NSCLC, personalized medicine, lung cancer, NSCLC treatment, supportive care

Lung cancer is the leading cause of cancer-related mortality in Canada (1) and around the world. Non-small cell lung cancer (NSCLC) is the most frequent form of lung cancer, accounting for approximately 87% of cases and the majority of these are metastatic at the time of presentation (2, 3).

We have reached a plateau (4, 5) with different systemic chemotherapies, specifically platinum-based, which have been used to treat metastatic NSCLC for several decades; median survival improved to 8–10 months (from 4-6 months without treatment). Significant toxicities limited the number of cycles that could be administered (6).

Current recommendations for first-line treatment of advanced NSCLC use both histologic and molecular diagnostics in designing the course of treatment (7, 8). We have learned the importance of distinguishing between squamous and non-squamous histologies (9) in order to choose an appropriate chemotherapy regimen. The algorithms for first-line treatment of advanced NSCLC recommend using both histologic and molecular diagnostics in designing the treatment (7, 10, 11). This in turn requires an adequate amount of biopsied tumor tissue in order to be able to perform all the necessary testing, which is needed for right decisions (12). Tumor aspirations for the diagnosis are not acceptable anymore.

Recent advances in understanding signaling pathways for malignant cells, interconnections in those pathways, the importance of various receptors (13–15), and biomarkers, and also the interplay between various oncogenes have led to the development of targeted treatments that are improving not only the efficacy of the treatments, but also safety benefits, less toxicity (16) with improvement of patient's quality of life (17) in this palliative setting.

These treatments are aimed at specific (especially genetic) alterations in the malignant cells. Various NSCLC subtypes are associated with potentially targetable biomarkers such as mutation of the epidermal growth factor receptors (EGFR) (18–22), KRAS (23), or the presence of echinoderm microtubule-associated protein-like 4 (EML-4) and anaplastic lymphoma kinase (ALK) fusion genes, ALK rearrangements (13, 15). C-Met over-expression or amplification (24–27), are playing a role in the development of resistance to the therapies (28), i.e., with EGFR-TKIs. T790M mutation on Exon 20 in the EGFR domain is the most frequent cause of the development of this resistance (29).

Knowledge about the advantages of treatments with targeted agents in advanced NSCLC is rapidly growing, but the hope is to eventually apply this knowledge to earlier stages of NSCLC and thus to increase the cure rate of these patients. Combining various targeted agents or sequencing them properly will be of the utmost importance in the new era of personalized targeted therapy (30). Many clinical trials are ongoing to help us make the appropriate decisions how to optimally treat advanced NSCLC in future (31, 32). Immunotherapy of advanced NSCLC (33) is one of the exciting areas of research and results of phase III trials are eagerly awaited.

Contributors in this issue of Frontiers in Thoracic Oncology describe the importance of team work (34) from diagnosis through various treatments to supportive care. They explain and emphasize the importance of the treatments of brain metastases (35) and bone metastases with new bone targeted agents (36). Management of adverse events when the new targeted agents are used (16) and analysis of patients' health-related quality of life (HR QOL) (17) and the impact on patients' performance status (PS) are also discussed in this issue. It is very important to preserve a good PS of patients in order to make it possible for them to receive multiple lines of the treatments now available for advanced NSCLC.

Our review will cover the description starting with the interventional procedures (12), to treatments delivered by radiation oncologists (37), medical oncologists (10, 11, 34), including descriptions of ongoing trials to provide a glimpse of the future (31, 32). The importance of early supportive care (38), which should be an integral part of active care from the start of treatment of advanced NSCLC, will also be discussed.

We hope to provide a complete review of present and future approaches to personalized medicine in advanced NSCLC, reflecting the present views, and practices in Canada.

ACKNOWLEDGMENTS

We would like to thank the following sponsors for their support: Major sponsors: Boehringer-Ingelheim, Eli Lilly, and Roche, other sponsors: MERCK, Astra Zeneca, and Pfizer. We would also like to thank Stavroula Kalantzis for her kind secretarial support with this Research Topic, who not only helped both myself and Dr. Melosky but also many other authors in this issue.

REFERENCES

1. Canadian Cancer Society's Steering Committee. *Canadian Cancer Statistics 2010.* Toronto, ON: Canadian Cancer Society (2010).
2. United States, National Institutes of Health, National Cancer Institute (NCI). *Non-Small Cell Lung Cancer Treatment (PDQ).* Bethesda, MD: Health professional version (2010). Available from: http://www.cancer.gov/cancertopics/pdq/treatment/non-small-cell-lung/healthprofessional

3. Pisters KM, Evans WK, Azzoli CG, Kris MG, Smith CA, Desch CE, et al. Cancer care Ontario and American society of clinical oncology adjuvant chemotherapy and adjuvant radiation therapy for stages I – IIIA resectable non-small cell lung cancer guideline. *J Clin Oncol* (2007) **25**:5506–18. doi:10.1200/JCO.2007.14.1226
4. Cagle PT, Allen TC, Dacic S, Beasley MB, Borczuk AC, Chirieac LR, et al. Revolution in lung cancer: new challenges for the surgical pathologist. *Arch Pathol Lab Med* (2011) **135**:110–6. doi:10.1043/2010-0567-RA.1
5. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* (2002) **346**:92–8. doi:10.1056/NEJMoa011954
6. Cagle PT, Dacic S. Lung cancer and the future of pathology. *Arch Pathol Lab Med* (2011) **135**:293–5. doi:10.1043/2011.0037-ED.1
7. Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* (2008) **26**:3543–51. doi:10.1200/JCO.2007.15.0375
8. Brega E, Brandao G. Non-small cell lung carcinoma biomarker testing: the pathologist's perspective. *Front Oncol* (2014) **4**:182. doi:10.3389/fonc.2014.00182
9. Blais N, Hirsh V. Chemotherapy in metastatic NSCLC – new regimens (pemetrexed, nab-paclitaxel). *Front Oncol* (2014) **4**:177. doi:10.3389/fonc.2014.00177
10. Al-Farsi A, Ellis PM. Treatment paradigms for patients with metastatic non-small cell lung cancer, squamous lung cancer: first, second, and third-line. *Front Oncol* (2014) **4**:157. doi:10.3389/fonc.2014.00157
11. Melosky B. Treatment algorithms for patients with metastatic non-small cell, non-squamous lung cancer. *Front Oncol* (2014) **4**:256. doi:10.3389/fonc.2014.00256
12. Ofiara LM, Navasakulpong A, Beaudoin S, Gonzalez AV. Optimizing tissue sampling for the diagnosis, subtyping, and molecular analysis of lung cancer. *Front Oncol* (2014) **4**:253. doi:10.3389/fonc.2014.00253
13. Korpanty GJ, Graham DM, Vincent MD, Leighl NB. Biomarkers that currently affect clinical practice in lung cancer: EGFR, ALK, MET, ROS-1, and KRAS. *Front Oncol* (2014) **4**:204. doi:10.3389/fonc.2014.00204
14. Melosky B. Review of EGFR TKIs in metastatic NSCLC, including ongoing trials. *Front Oncol* (2014) **4**:244. doi:10.3389/fonc.2014.00244
15. Esfahani K, Agulnik JS, Cohen V. A systemic review of resistance mechanisms and ongoing clinical trials in ALK-rearranged non-small cell lung cancer. *Front Oncol* (2014) **4**:174. doi:10.3389/fonc.2014.00174
16. Melosky B, Hirsh V. Management of common toxicities in metastatic NSCLC related to anti-lung cancer therapies with EGFR-TKIs. *Front Oncol* (2014) **4**:238. doi:10.3389/fonc.2014.00238
17. Hirsh V. Is the evaluation of quality of life in NSCLC trials important? Are the results to be trusted? *Front Oncol* (2014) **4**:173. doi:10.3389/fonc.2014.00173
18. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* (2004) **350**:2129–39. doi:10.1056/NEJMoa040938
19. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. *EGFR* mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* (2004) **304**:1497–500. doi:10.1126/science.1099314
20. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci USA* (2004) **101**:13306–11. doi:10.1073/pnas.0405220101
21. Li AR, Chitale D, Riely GJ, Pao W, Miller VA, Zakowski MF, et al. *EGFR* mutations in lung adenocarcinomas: clinical testing experience and relationship to *EGFR* gene copy number and immunohistochemical expression. *J Mol Diagn* (2008) **10**:242–8. doi:10.2353/jmoldx.2008.070178
22. Uramoto H, Mitsudomi T. Which biomarker predicts benefit from EGFR – TKI treatment for patients with lung cancer? *Br J Cancer* (2007) **96**:857–63. doi:10.1038/sj.bjc.6603665
23. Sartori G, Cavazza A, Sgambato A, Marchioni A, Barbieri F, Longo L, et al. *EGFR* and K- *ras* mutations along the spectrum of pulmonary epithelial tumors of the lung and elaboration of a combined clinicopathologic and molecular scoring system to predict clinical responsiveness to EGFR inhibitors. *Am J Clin Pathol* (2009) **131**:478–89. doi:10.1309/AJCPH0TRMPXVZW2F
24. Ma PC, Tretiakova MS, MacKinnon AC, Ramnath N, Johnson C, Dietrich S, et al. Expression and mutational analysis of *MET* in human solid cancers. *Genes Chromosomes Cancer* (2008) **47**:1025–37. doi:10.1002/gcc.20604
25. Okuda K, Sasaki H, Yukiue H, Yano M, Fujii Y. MET gene copy number predicts the prognosis for completely resected non-small cell lung cancer. *Cancer Sci* (2008) **99**:2280–5. doi:10.1111/j.1349-7006.2008.00916.x
26. Lutterbach B, Zeng Q, Davis LJ, Hatch H, Hang G, Kohl NE, et al. Lung cancer cell lines harboring *MET* gene amplification are dependent on Met for growth and survival. *Cancer Res* (2007) **67**:2081–8. doi:10.1158/0008-5472.CAN-06-3495
27. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ErbB3 signaling. *Science* (2007) **316**:1039–43. doi:10.1126/science.1141478
28. Spaans JN, Goss GD. Trials to overcome drug resistance to EGFR and ALK targeted therapies – past, present, and future. *Front Oncol* (2014) **4**:233. doi:10.3389/fonc.2014.00233
29. Spaans JN, Goss GD. Drug resistance to molecular targeted therapy and its consequences for treatment decisions in non-small-cell lung cancer. *Front Oncol* (2014) **4**:190. doi:10.3389/fonc.2014.00190
30. Blais N, Kassouf E. Maintenance therapies for non-small cell lung cancer. *Front Oncol* (2014) **4**:213. doi:10.3389/fonc.2014.00213
31. Zer A, Leighl N. Promising targets and current clinical trials in metastatic non-squamous NSCLC. *Front Oncol* (2014) **4**:329. doi:10.3389/fonc.2014.00329
32. Vincent MD. Promising targets and current clinical trials in metastatic squamous cell lung cancer. *Front Oncol* (2014) **4**:320. doi:10.3389/fonc.2014.00320
33. Mostafa AA, Morris DG. Immunotherapy for lung cancer: has it finally arrived? *Front Oncol* (2014) **4**:288. doi:10.3389/fonc.2014.00288
34. Dawe DE, Ellis PM. The treatment of metastatic non-small cell lung cancer in the elderly: an evidence-based approach. *Front Oncol* (2014) **4**:178. doi:10.3389/fonc.2014.00178
35. Owen S, Souhami L. The management of brain metastases in non-small cell lung cancer. *Front Oncol* (2014) **4**:248. doi:10.3389/fonc.2014.00248
36. Hirsh V. Targeted treatments of bone metastases in patients with lung cancer. *Front Oncol* (2014) **4**:146. doi:10.3389/fonc.2014.00146
37. Faria SL. Role of radiotherapy in metastatic non-small cell lung cancer. *Front Oncol* (2014) **4**:229. doi:10.3389/fonc.2014.00229
38. Howe M, Burkes RL. Collaborative care in NSCLC: the role of early palliative care. *Front Oncol* (2014) **4**:192. doi:10.3389/fonc.2014.00192

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 02 December 2014; accepted: 16 January 2015; published online: 03 February 2015.

*Citation: Hirsh V (2015) The treatment of metastatic non-small cell lung cancer in a new era of personalized medicine. Front. Oncol. **5**:20. doi: 10.3389/fonc.2015.00020*

This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 22 September 2014
doi: 10.3389/fonc.2014.00253

Optimizing tissue sampling for the diagnosis, subtyping, and molecular analysis of lung cancer

***Linda Marie Ofiara*[1]*, *Asma Navasakulpong*[1,2], *Stephane Beaudoin*[1] *and Anne Valerie Gonzalez*[1]**

[1] *Respiratory Medicine Division, Department of Medicine, McGill University Health Centre, Montreal Chest Institute, Montreal, QC, Canada*
[2] *Pulmonary and Respiratory Critical Care Division, Faculty of Medicine, Prince of Songkla University, Hatyai, Thailand*

Edited by:
Barbara Melosky, British Columbia Cancer Agency, Canada

Reviewed by:
Rabab Mohamed Gaafar, Cairo University, Egypt
Jacobus A. Burgers, Antoni van Leeuwenhoek Hospital, Netherlands

***Correspondence:**
Linda Marie Ofiara, Montreal General Hospital, Room D7.201, 1650 Cedar Avenue, Montreal, QC H3G 1A4, Canada
e-mail: linda.ofiara@mcgill.ca

Lung cancer has entered the era of personalized therapy with histologic subclassification and the presence of molecular biomarkers becoming increasingly important in therapeutic algorithms. At the same time, biopsy specimens are becoming increasingly smaller as diagnostic algorithms seek to establish diagnosis and stage with the least invasive techniques. Here, we review techniques used in the diagnosis of lung cancer including bronchoscopy, ultrasound-guided bronchoscopy, transthoracic needle biopsy, and thoracoscopy. In addition to discussing indications and complications, we focus our discussion on diagnostic yields and the feasibility of testing for molecular biomarkers such as epidermal growth factor receptor and anaplastic lymphoma kinase, emphasizing the importance of a sufficient tumor biopsy.

Keywords: lung cancer, diagnosis, ultrasound bronchoscopy, diagnostic yield, transthoracic needle aspiration, molecular biomarkers, EGFR

INTRODUCTION

Lung cancer remains the leading cause of cancer death in North America. In Canada, an estimated 25,500 Canadians will be diagnosed with lung cancer in 2014 (1). The majority of these will be non-small cell lung cancer (NSCLC) and unresectable.

At diagnosis, 75% of lung cancer patients will have either locally advanced or metastatic disease (2). The goal in this group of patients is to establish the diagnosis and, ideally, confirm staging with the least invasive technique possible. As a result of this approach, biopsy specimens are becoming increasingly smaller. Up to 80% of patients receiving chemotherapy for advanced disease will have only a small biopsy and/or cytology samples available for diagnosis (3).

The adequacy of these samples has important ramifications. Lung cancer has entered an era of personalized therapy with treatment based on histologic subtypes (adenocarcinoma versus squamous) and the presence of molecular markers [epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK)]. For instance, several trials have demonstrated that response rate and overall survival is significantly better with pemetrexed in patients with non-squamous histology compared with patients with squamous histology (4). Trials using tyrosine kinase inhibitors (TKIs) have observed that patients with NSCLC tumors harboring EGFR mutations derive a greater benefit from treatment with TKIs than wild-type tumors (5). In fact, a number of trials have consistently shown a statistically significant and clinically meaningful benefit of TKIs over standard chemotherapy in mutation positive patients (5–7). The ALK inhibitor, crizotinib, is effective in patients with NSCLC harboring the ALK rearrangement (8).

Procurement of adequate tissue samples that allow for accurate characterization of histology and molecular testing is essential. A multidisciplinary approach is recommended. Physicians who obtain tissue samples (respirologists, interventional radiologists, and thoracic surgeons) need to be aware of the tissue yields of their procedures. Likewise, pathologists need to communicate the tissue yields and to be judicious in tissue use especially when managing small biopsy and cytology specimens. Finally, medical oncologists should be aware of when to ask for more tissue in patients in whom the treatment plan will be significantly impacted by further characterization. Medical oncologist may recommend that a patient with a known lung cancer be rebiopsied or that a metastatic site be biopsied in addition to the primary site in order to clarify the molecular status of the tumor. This can provide important information with regard to treatment options or as to why therapies fail.

In this article, techniques used in the diagnosis of lung cancer will be discussed including the expected tissue yields and the feasibility of histologic characterization and molecular testing.

DIAGNOSIS OF LUNG CANCER

RAPID ASSESSMENT CLINICS

Lung cancer guidelines recommend prompt investigation and referral for treatment (9).

Recently, rapid access clinics have been developed to reduce wait times and initiate investigations based on established algorithms to provide the most information about diagnosis and staging with the least risk to the patient. Bronchoscopy with or without lymph node sampling is frequently recommended as the initial diagnostic procedure.

FIBEROPTIC BRONCHOSCOPY

The bronchoscope is one of the primary diagnostic tools in lung cancer. Flexible bronchoscopy, usually performed under local anesthesia and with minimal sedation, provides a thorough examination of all segmental bronchi within minutes. Complications for this procedure are rare, with major complication rates

between 0.08 and 5% (10). Complications include pneumothorax, hypoxemia, and hemorrhage (11).

Endobronchial tumor may be visible as an exophytic mass or submucosal infiltration (**Figure 1A**). The diagnostic yield for endobronchial biopsy when a lesion is visible is 70–90% (12). Five biopsy specimens have been shown to be optimal for achieving a diagnostic yield in central lesions (13). Combining the results of bronchial biopsy, bronchial brushing, and bronchial washing increases tissue yields (14), and it is better to do brushing after biopsy (15).

Biopsy specimens are, in general, small averaging about 300 cells in aggregate. Bronchial lavage yields the least number of malignant cells. In biopsy specimens, the percentage (%) of tumor cells can be relatively low. Coghlin et al. found the mean % of area of tumor in an endobronchial sample to be 33%. In fewer than half of their cases (48%), tumor was found in all biopsy specimens (16). Although five specimens may be enough to establish the diagnosis of lung cancer, the number of specimens required to provide detailed sub classification and molecular analysis has not be established. In one series, EGFR testing could be performed in 100% of endobronchial biopsy specimens that established a diagnosis of lung cancer (17).

Endobronchial cryobiopsies could be one evidence-based way of achieving a higher diagnostic yield and a higher molecular analysis potential. Compared with conventional bronchoscopic biopsies, cryobiopsies result in an increase in biopsy sample size and yield (18, 19).

In the case of more peripheral lesions, when the endobronchial exam is normal, the diagnostic yield falls to 40% (20, 21). The diagnostic yield can be increased when computed tomography (CT)

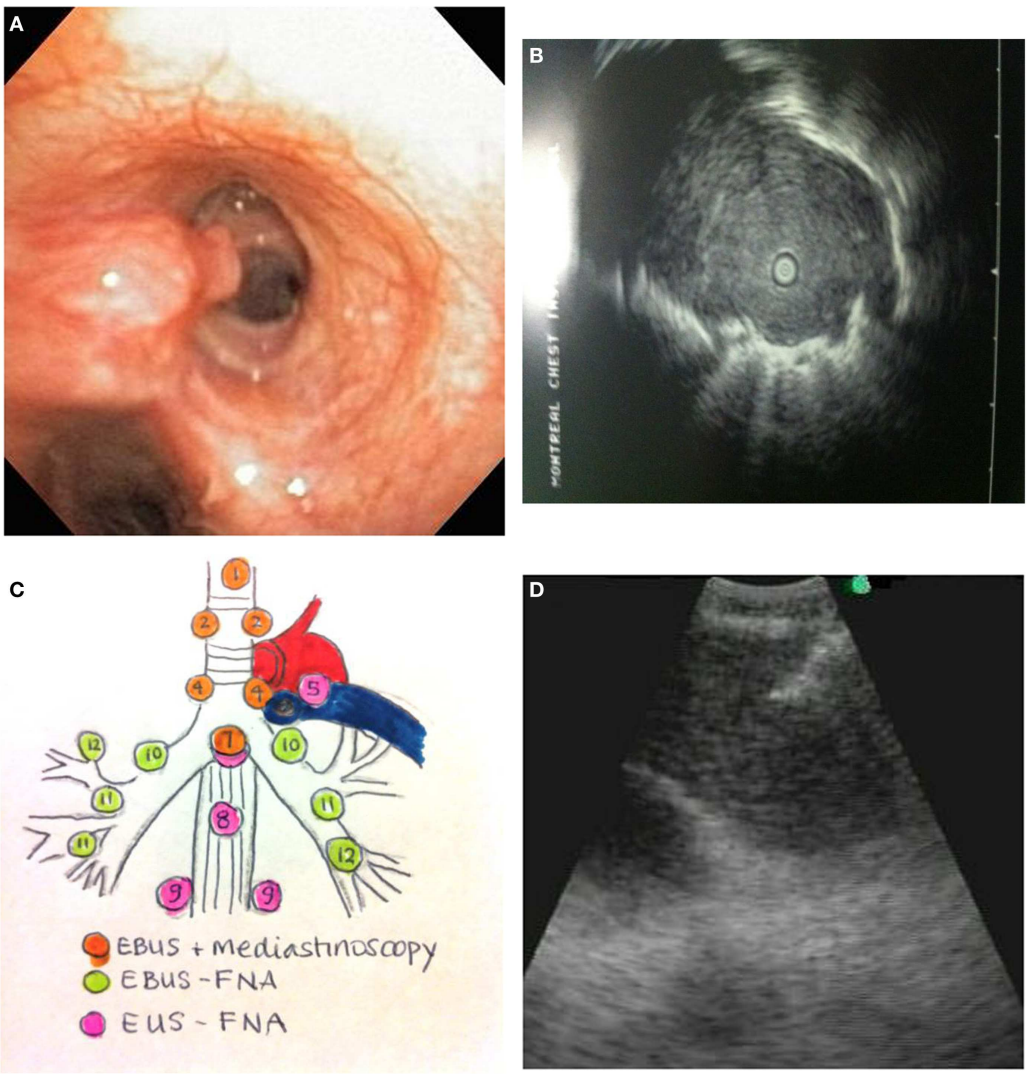


FIGURE 1 | (A) Endobronchial tumor visible in an airway. **(B)** Ultrasound image of a peripheral lung cancer as visualized by radial EBUS-GS. The clear central area is the ultrasound probe in the airway. The surrounding isoechoic shadow represents a tumor. The hyperechoic line surrounding the tumor is an ultrasound phenomenon produced by the sudden change in tissue density from tumor to aerated lung. **(C)** Mediastinal lymph node station accessibility by EBUS, mediastinoscopy, and EUS. **(D)** Real-time needle aspiration of a lymph node. The needle (hyperechoic line coming from the top left corner of the screen) is penetrating the lymph node under direct ultrasound visualization.

images are available for review prior to bronchoscopy (22). This allows the bronchoscopist to better localize the bronchial segment containing tumor. When positive, the diagnoses in these cases are usually made on the basis of cytology: bronchial brushings or washings. Molecular markers can be performed on these cytological specimens with varying degrees of success. One series, however, found that in the case of bronchial lavage, more than half of the cytological specimens that confirmed the diagnosis of lung cancer could not be used for molecular testing (23).

Ultrasonography using a guide sheath (radial EBUS-GS) and electromagnetic navigation (ENB) can provide transbronchial biopsy specimens, improving the possibility of having adequate tissue for molecular analysis. In the case of a peripheral lesion where the endobronchial exam is negative and radial EBUS-GS or ENB are not available, consideration should be given to other diagnostic procedures such as transthoracic needle aspirate.

RADIAL EBUS

Endobronchial ultrasonography using a sheath guide (EBUS-GS) can increase the diagnostic yield of peripheral lung lesions. For lesions less than 2 cm, the diagnostic yield can increase from 36% using conventional bronchoscopy to between 58 and 70% (24). This technique allows for visualization of the lesion (**Figure 1B**) and repeated access to the lesion by brush, forceps biopsy, and bronchial wash. The resulting specimens are cytological and small biopsies.

Recently, ENB and virtual bronchoscopic navigation system (VBNS) have been developed to assist the diagnosis of peripheral lung lesions in conjunction with EBUS-GS. Using ENB, yields in peripheral lesions can be further improved upon. Combining radial EBUS and ENB resulted, in one series, in a diagnostic yield approaching 90% compared with 69% for radial EBUS alone (25). No EMN complications have been reported. VBNS has also been used with EBUS-GS with an overall diagnostic yield ranging from 63.3 to 84.4%, and in lesions less than 2 cm in diameter, ranging from 44 to 75.9% (26). VBNS increases diagnostic yield and decreases procedure time (27). Presently, there is little data on the yield of molecular testing on specimens obtained by EBUS-GS or ENB/VBNS. Tsai et al. performed EBUS-guided brushings in 122 patients with peripheral lung cancer receiving flexible bronchoscopy. The yield for tumor cells was 68.9%. Genotyping of EGFR and KRAS was successfully implemented in 80 (95.2%) of the 84 cytology-proven brushing samples (28). It is probable that the yields are similar to conventional bronchoscopy as the specimens obtained are small biopsies and bronchial brushing/lavage cytology.

EBUS TRANSBRONCHIAL NEEDLE ASPIRATION

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive technique with a high diagnostic yield for mediastinal lymph node staging of lung cancer patients. Accurate staging is an essential step in the investigation of lung cancer patients. EBUS-TBNA is particularly useful as diagnosis and staging can be achieved with a single procedure.

The technique is performed using a dedicated flexible bronchoscope with an integrated ultrasound transducer. It allows for sampling of mediastinal and hilar lymph nodes under direct vision using local anesthesia and moderate sedation. The upper and lower paratracheal, prevascular, subcarinal, and hilar lymph node stations can all be sampled using this technique (**Figure 1C**).

A similar technique using a gastroscope with an integrated ultrasound probe (EUS) can also sample mediastinal lymph nodes. Nodal stations that can be accessed with EUS include aortopulmonary window, subcarinal, para-esophageal, and pulmonary ligament.

Herth et al. assessed EBUS yields in 502 patients with suspected lung cancer, comparing EBUS-TBNA results with operative findings (29). The reported sensitivity was 94% and specificity was 100%. Several studies have compared EBUS-TBNA to mediastinoscopy and found both techniques to have comparable results for mediastinal staging (30, 31). EBUS-TBNA has some advantages over mediastinoscopy, in that EBUS-TBNA can be used to restage a patient post surgery or radiation therapy, where a repeat mediastinoscopy would prove difficult because of fibrotic changes (32). Additionally, it can be performed in high-risk patients with several comorbidities such as COPD (33).

Tissue samples by EBUS-TBNA are typically small cytology samples obtained using a dedicated 22 gage needle (**Figure 1D**). Some institutions use rapid on-site evaluation (ROSE) of aspirated samples by a cytopathologist. One of the main advantages of ROSE is reduction of the number of passes and stations sampled, and avoidance of other biopsy techniques like transbronchial biopsy. Lee and colleagues have demonstrated that maximum diagnostic values for achieving a diagnosis of lung cancer are achieved with three aspirations per node when ROSE is not available (34). Molecular testing for EGFR and ALK mutations can be successfully performed on EBUS-TBNA specimens. In several series, using ROSE, molecular testing can be performed in between 70 and 90% of EBUS-TBNA samples (35–37). Yarmus et al. found that a median of four passes in the presence of ROSE provided an adequate amount of tissue for molecular analysis in 95% of patients studied (38). In the absence of ROSE, Navasakulpong and colleagues found that 93% of EBUS-TBNA specimens from a single lymph node station were adequate for EGFR testing with an average of 3.5 passes per lymph node. The minimum tumor cell count that allowed for successful EGFR testing in this series was 100 cells (39). Schmid-Bindert et al. found that EBUS-TBNA provided the highest yield for biomarker testing when compared to bronchoscopic forceps biopsy and CT-guided core biopsy (17).

Questions that remain to be answered are whether a larger needle (21 gage) results in better yields, whether mixing tissue from more than one lymph node station, once staging is established, can improve the yield of molecular testing, and finally, whether combining EBUS and EUS increases tissue yields for molecular analysis.

MEDIASTINOSCOPY

Cervical mediastinoscopy is used predominantly in the staging of lung cancer. It is performed by a thoracic surgeon under general anesthesia in an operating room. A small incision is made at the base of the neck and a mediastinoscope is introduced. The sensitivity of mediastinoscopy for detecting cancer in mediastinal lymph nodes is between 80 and 95% (32, 40). False negative rates vary between 5 and 9% and are attributed to the

inability to access para-esophageal, inferior pulmonary ligament, and aortopulmonary nodes.

Tissue samples vary from millimeters to centimeters depending on the size of the nodes biopsied. Tissue samples are sufficient for molecular testing. The complication rate is between 2 and 5% and includes hoarseness, infection, and bleeding (41).

Several series have compared EBUS to mediastinoscopy (42). Both modalities have comparable sensitivities in staging the mediastinum. Mediastinoscopy has the advantage of larger tissue samples, compared with EBUS. It is unclear if this translates into better molecular subtyping as little comparative data exist. The disadvantage of mediastinoscopy is the need for general anesthesia and OR time.

TRANSTHORACIC NEEDLE ASPIRATE

A total of 10–20% of cases of NSCLC will present as a solitary pulmonary nodule. In patients who are not candidates for surgery or in patients who have advanced disease in whom the most accessible site for biopsy is a peripheral lung nodule, transthoracic needle aspiration (TTNA) and biopsy (TTNB) are useful diagnostic procedures.

Transthoracic needle aspiration can be performed under CT or fluoroscopic guidance. CT-guided aspiration and biopsies result in a higher diagnostic yield compared to fluoroscopy (43).The most commonly used technique is a coaxial system, in which a larger gage needle is inserted into the edge of the lesion and a smaller needle is passed through the larger one. This allows for a single pleural puncture and repeat needle passes by the smaller needle reducing the risk of complications. Major complications are bleeding and pneumothorax and occur in 10% and up to 20% of cases, respectively (44). Contraindications to TTNA are previous pneumonectomy, severe chronic obstructive lung disease, especially with bullous formation, mechanical ventilation, lesions too close to vascular structures, and high risk for bleeding (45).

Transthoracic needle aspiration has a diagnostic accuracy of between 80 and 95% for lung cancer (46, 47). Specimens obtained by TTNA are cutting-needle core biopsies and needle aspirate cytology. Core-needle biopsy specimens usually contain enough cellular material for pathologic subtyping and molecular analysis. The average number of cells obtained by CT-guided needle biopsy is 500 cells per biopsy (48). Zhuang et al. showed that CT-guided TTNA/TTNB performed using an 18 or 20 gage could obtain tumor samples ranging from 0.5 to 1.5 cm in length and that these samples were 100% adequate for histological and EGFR mutation analysis (49). In addition, Fassina et al. showed that TTNA samples can be used for EGFR and KRAS mutation analysis (50). da Cunha Santos et al. found that in a review of 602 fine needle aspirates, histological subtyping agreement with resected specimens was achieved in 93% of cases (51).

PLEURAL FLUID ANALYSIS AND MEDICAL THORACOSCOPY

In rapid diagnostic clinics for the evaluation of suspected lung cancer, diagnostic procedures that allow for simultaneous staging and diagnosis are preferred. In patients with suspected lung cancer presenting with an accessible pleural effusion, thoracentesis is recommended to distinguish between a malignant versus parapneumonic effusion (21). The yield of pleural fluid cytology is 60–80% with repeat sampling (52, 53). Use of cell block methods improves the diagnostic utility of pleural cytology compared with conventional smear cytology by providing higher cellularity and better morphological features to allow for pathologic subtyping. Using cell blocks of pleural fluid, molecular testing for EGFR and KRAS has been performed with an insufficiency rate of 3.7% (1 in 27 specimens) (54).

Medical thoracoscopy is recommended when cytology specimens are non-diagnostic or insufficient for histologic classification. It offers higher yield compared with Abrams needle and CT-guided pleural biopsy in malignant pleural disease (55). In addition to being able to directly visualize and biopsy nodules on the parietal pleural surface, thoracoscopy allows for drainage of pleural fluid and talc pleurodesis in the case of malignant effusions.

Medical thoracoscopy can be performed in a dedicated sterile endoscopy suite under local anesthesia and conscious sedation. A pneumothorax is artificially induced, and a rigid thoracoscope is introduced into the pleural cavity. Under direct vision, parietal pleural nodules can be biopsied. The diagnostic yield of medical thoracoscopy for malignancy is 93–97% (56). Biopsy specimens are typically about 5 mm and multiple specimens can be obtained during the procedure. The size of these specimens is adequate for pathological subtyping, and molecular analysis was possible in 100% of specimens tested in one series (57).

Medical thoracoscopy is a relatively safe procedure with a complication rate of 1.9% (58). Persistent air leak, subcutaneous emphysema, and fever are the most common complications. Mortality is rare with 1 death reported in more than 8000 cases (53).

TISSUE STRATEGIES FOR PATHOLOGICAL SUBTYPING AND MOLECULAR ANALYSIS

Strategies have been proposed to allow for subtyping of NSCLC and testing of molecular markers in small biopsy and cytology specimens (59). With any specimen, the first approach is to establish squamous or adenocarcinoma differentiation based on morphology under light microscopy. The typical features of adenocarcinoma include glandular differentiation of cell clusters and in individual cells, the presence of basophilic cytoplasm, eccentric nuclei, and a single macronucleolus. Squamous differentiation is characterized by keratinization, intercellular bridges, and keratin pearls in small biopsies. Individual cells may have long cytoplasmic tails, central nuclei, dense chromatin, and poorly developed nucleoli.

In cases of NSCLC that cannot be subtyped based on morphology, immunohistochemistry (IHC) is used. Because of the small amounts of tissue, IHC should be used judiciously. It is recommended to use one adenocarcinoma marker (TTF1) and one squamous marker (p63 or CK 5/7) to attempt to further subtype NSCLC (60).

In the case of adenocarcinoma, molecular markers can then be performed. Currently, EGFR and ALK are performed, but other markers such as ROS1 and KRAS may also be considered. In tumors that cannot be subtyped based on morphology and IHC, a designation of NSCLC not otherwise specified (NOS) is made. Decisions can be made whether additional tissue is warranted; however, recommendations for EGFR testing include specimens designated as NSCLC-NOS (61).

Table 1 | Yields of various procedures used to diagnose lung cancer.

Diagnostic modality	Specimen types	Diagnostic yield	Adequacy for biomarker testing
Bronchoscopy	Endobronchial biopsy Brushing cytology Washing cytology	70–90% (if lesion visible) Yields improve when biopsy, brushing, and washing combined	Up to 100% in one series for endobronchial biopsy. Less than 50% in washings
Radial EBUS-GS For peripheral lesions 2 cm or less	Transbronchial biopsy Brushing cytology Washing cytology	58–70% when biopsy, brushing, and washings combined	71% in one series examining bronchial brushing
EBUS-TBNA	Needle aspirate cytology	Up to 94%	70–95%
Mediastinoscopy	Biopsy	80–95%	Not well established, but likely adequate based on size
CT-guided TTNA	Core-needle biopsy Needle aspirate	80–95%	100% in one series
Thoracentesis	Fluid cytology	60–80%	Insufficiency rate of 3.7% in one series
Medical thoracoscopy	Biopsy	93–97%	100% in one series

The minimum number of malignant tumor cells required for molecular marker testing has not been well established. In general, larger samples with at least 200–400 malignant cells are preferred (62).

Communication among the multiple physicians involved in the care of patients with lung cancer must take into consideration issues of tissue procurement strategies in order to optimize diagnostic yield and molecular characterization of tumors. Only a multidisciplinary approach can ensure that the needs of the medical oncologist for treatment planning are reflected into judicious tissue procurement, clinical staging, and thoughtful tissue analysis. Moreover, local institutional strategies must be implemented to take into consideration the local availability of different diagnostic modalities and molecular analyses. Solutions regarding issues of cost-effectiveness and quality control must be individualized for each center, and ongoing monitoring is important to ensure that safe and efficient diagnostic services are delivered. This is especially important given that many of the above-mentioned technologies have mostly been studied only in highly specialized centers.

SUMMARY AND RECOMMENDATIONS

A sufficient tumor biopsy is essential in the diagnosis of lung cancer in order to subtype NSCLC and to establish the presence molecular markers. Important therapeutic decisions are made on the basis of these specimens. In this article, we have summarized the various techniques used in the diagnosis of lung cancer and their respective yields in terms of tissue, pathological subtype, and molecular testing. **Table 1** summarizes the diagnostic yields of specimens obtained.

A multidisciplinary approach in establishing a diagnosis of lung cancer is strongly recommended to optimize tissue yields and ultimately patient outcomes. In general, the least invasive procedure should be favored and biopsy specimens favored over cytology specimens. There is, however, increasing evidence to suggest that, when handled judiciously, cytology specimens can prove to be sufficient for diagnosis and molecular analysis. Understanding the yields of diagnostic procedures is essential in diagnosing and treating lung cancer in an era of personalized therapy.

REFERENCES

1. Advisory Committee on Cancer Statistics. *Canadian Cancer Statistics*. Toronto, ON: Canadian Cancer Society (2013).
2. Pfister DG, Johnson DH, Azzoli CG, Sause W, Smith TJ, Baker S Jr, et al. American Society of Clinical Oncology. Treatment of unresectable non-small cell lung cancer guideline: update 2003. *J Clin Oncol* (2004) **22**:330–53. doi:10.1200/JCO.2004.09.053
3. Kerr KM. Personalized medicine for lung cancer: new challenges for pathology. *Histopathology* (2012) **60**:531–46. doi:10.1111/j.1365-2559.2011.03854.x
4. Scagliotti G, Hanna N, Fossella F, Sugarman K, Blatter J, Peterson P, et al. The differential efficacy of pemetrexed according to NSCLC histology: a review of two phase III studies. *Oncologist* (2009) **14**:253–63. doi:10.1634/theoncologist.2008-0232
5. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation positive non-small cell lung cancer (EURTAC) a multicentre, open-label randomized phase 3 trial. *Lancet Oncol* (2012) **13**:239–46. doi:10.1016/S1470-2045(11)70393-X
6. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, et al. Erlotinib versus chemotherapy as first line treatment for patients with advanced EGFR mutation positive non-small cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open label, randomized phase 3 trial. *Lancet Oncol* (2011) **12**:435–42. doi:10.1016/S1470-2045(11)70184-X
7. Wu YL, Zhou C, Hu C-P, Feng JF, Lu S, Huang Y, et al. LUX-Lung 6: a randomized, open label phase 3 study of Afatanib versus gemcitabine-cisplatin as first line treatment for Asian patients with EGFR mutation positive advanced adenocarcinoma. *J Clin Oncol* (2013) **31**(Suppl):490s, abstr8016.
8. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small cell lung cancer. *N Engl J Med* (2010) **363**:1693–703. doi:10.1056/NEJMoa1006448
9. Baldwin DR, White B, Schmidt-Hansen M, Champion AR, Melder AM, Guideline Development Group. Diagnosis and treatment of lung cancer: summary of updated NICE guidance. *BMJ* (2011) **342**:d2110. doi:10.1136/bmj.d2110
10. Simpson FG, Arnold AG, Purvis A, Belfield PW, Muers MF, Cooke NJ. Postal survey of bronchoscopic practice by physicians in the United Kingdom. *Thorax* (1986) **41**:311–7. doi:10.1136/thx.41.4.311
11. British Thoracic Society Bronchoscopy Guidelines Committee, a subcommittee of the Standards of Care Committee of the British Thoracic Society. British Thoracic Society guidelines on diagnostic flexible bronchoscopy. *Thorax* (2001) **56**(Suppl 1):i1–21. doi:10.1136/thx.56.suppl_1.i1

12. Mazzone P, Jain P, Arroliga AC, Matthay RA. Bronchoscopy and needle biopsy techniques for diagnosis and staging of lung cancer. *Clin Chest Med* (2002) **23**:137–58. doi:10.1016/S0272-5231(03)00065-0
13. Gellert AR, Rudd RM, Sinha G, Geddes DM. Fiberoptic bronchoscopy: effect of multiple biopsies on diagnostic yield in bronchial carcinoma. *Thorax* (1982) **37**:684–7. doi:10.1136/thx.37.9.684
14. Bodh A, Kaushal V, Kashap S, Gutali A. A cytohistological correlation in diagnosis of lung tumours by using fiberoptic bronchoscopy: study of 200 cases. *Indian J Pathol Microbiol* (2013) **56**:84–8. doi:10.4103/0377-4929.118661
15. Chen WT, Chao TY, Wu CP, Perng WC, Shen CY, Chiang CH. Comparison of the diagnostic yield of bronchial brushing cytology before and after endobronchial biopsy of flexible fiberoptic bronchoscopy – a prospective study. *J Med Sci* (1997) **18**:165–70.
16. Coghlin CL, Smith LJ, Bakar S, Stewart KN, Devereux GS, Nicolson MC, et al. Quantitative analysis of tumor in bronchial biopsy specimens. *J Thorac Oncol* (2010) **5**:448–52. doi:10.1097/JTO.0b013e3181ca12c4
17. Schmid-Bindert G, Wang Y, Jiang H, Sun H, Henzler T, Wang H, et al. EBUS-TBNA provides highest RNA yield for multiple biomarker testing from routinely obtained small biopsies in non-small cell lung cancer patients - a comparative study of three different minimal invasive sampling methods. *PLoS One* (2013) **8**(10):e77948. doi:10.1371/journal.pone.0077948
18. Hetzel J, Eberhardt R, Herth FJ, Petermann C, Reichle G, Freitag L, et al. Cryobiopsy increases the diagnostic yield of endobronchial biopsy: a multicentre trial. *Eur Respir J* (2012) **39**:685–90. doi:10.1183/09031936.00033011
19. Schumann C, Hetzel J, Babiak AJ, Merk T, Wibmer T, Möller P, et al. Cryoprobe biopsy increases the diagnostic yield in endobronchial tumor lesions. *J Thorac Cardiovasc Surg* (2010) **140**:417–21. doi:10.1016/j.jtcvs.2009.12.028
20. Baaklini WA, Reinoso MA, Gorin AB, Sharafkaneh A, Manian P. Diagnostic yield of fiberoptic bronchoscopy in evaluating solitary pulmonary nodules. *Chest* (2000) **117**:1049–54. doi:10.1378/chest.117.4.1049
21. Rivera MP, Mehta AC, American College of Chest Physicians. Initial diagnosis of lung cancer. ACCP evidence based clinical practice guidelines. *Chest* (2007) **132**(Suppl 3):131S–48S. doi:10.1378/chest.07-1357
22. Laroche C, Fairbairn I, Moss H, Pepke-Zaba J, Sharples L, Flower C, et al. Role of computed tomographic scanning of the thorax prior to bronchoscopy in the investigation of suspected lung cancer. *Thorax* (2000) **55**:359–63. doi:10.1136/thorax.55.5.359
23. Allegrini S, Antona J, Mezzapelle R, Miglio U, Paganotti A, Veggiani C, et al. Epidermal growth factor receptor gene analysis with a highly sensitive molecular assay in routine cytologic specimens of lung adenocarcinoma. *Am J Clin Pathol* (2012) **138**:377–81. doi:10.1309/AJCPVAGIUC1AHC3Y
24. Kurimoto N, Nakamura H, Miyazawa T. Overview of endobronchial ultrasonography in chest medicine. *J Med Ultrasound* (2009) **17**:31–43. doi:10.1016/S0929-6441(09)60013-8
25. Eberhardt R, Anantham D, Ernst A, Feller-Kopman D, Herth F. Multimodality bronchoscopic diagnosis of peripheral lung lesions. A randomized controlled trial. *Am J Respir Crit Care Med* (2007) **176**:37–41. doi:10.1164/rccm.200612-1866OC
26. Asahina H, Yamazaki K, Onodera Y, Kikuchi E, Shinagawa N, Asano F, et al. Transbronchial biopsy using endobronchial ultrasonography with a guide sheath and virtual bronchoscopic navigation. *Chest* (2005) **128**:1761–5. doi:10.1378/chest.128.3.1761
27. Asano F, Matsuno Y, Tsuzuku A, Anzai M, Shinagawa N, Yamazaki K, et al. Diagnosis of peripheral pulmonary lesions using a bronchoscope insertion guidance system combined with endobronchial ultrasonography with a guide sheath. *Lung Cancer* (2008) **60**:366–73. doi:10.1016/j.lungcan.2007.10.022
28. Tsai TH, Yang CY, Ho CC, Liao WY, Jan IS, Chen KY, et al. Multi-gene analyses from waste brushing specimens for patients with peripheral lung cancer receiving EBUS-assisted bronchoscopy. *Lung Cancer* (2013) **82**(3):420–5. doi:10.1016/j.lungcan.2013.10.005
29. Herth FJ, Eberhardt R, Vilmann P, Krasnik M, Ernst A. Real-time endobronchial ultrasound guided transbronchial needle aspiration for sampling mediastinal lymph nodes. *Thorax* (2006) **61**:795–8. doi:10.1136/thx.2005.047829
30. Annema JT, van Meerbeeck JP, Rintoul RC, Dooms C, Deschepper E, Dekkers OM, et al. Mediastinoscopy vs endosonography for mediastinal nodal staging of lung cancer: a randomized trial. *JAMA* (2010) **304**:2245–52. doi:10.1001/jama.2010.1705
31. Almeida FA, Uzbeck M, Jimenez C, Morice R, Rice D, Ost D, et al. Flexible bronchoscopy and endobronchial ultrasound needle aspiration (EBUS-TBNA) vs other invasive modalities in the initial diagnosis and staging of suspected or confirmed lung cancer. *Chest* (2010) **138**:423A.Abstract. doi:10.1378/chest.10655
32. Herth FJ. Nonsurgical staging of the mediastinum: EBUS and EUS. *Semin Respir Crit Care Med* (2011) **32**:62–8. doi:10.1055/s-0031-1272870
33. Nakajima T, Yasufuku K, Nakajima M, Baba M, Yoshikawa K, Kamada T, et al. Endobronchial ultrasound-guided transbronchial needle aspiration for lymph node staging in patients with non-small cell lung cancer in non- operable patients pursuing radiotherapy as a primary treatment. *J Thorac Oncol* (2010) **5**:606–11. doi:10.1097/JTO.0b013e3181d35627
34. Lee HS, Lee GK, Lee HS, Kim MS, Lee JM, Kim HY, et al. Real-time endobronchial ultrasound-guided transbronchial needle aspiration in mediastinal staging of non-small cell lung cancer: how many aspirations per target lymph node station? *Chest* (2008) **134**:368–74. doi:10.1378/chest.07-2105
35. Nakajima T, Yasufuku K, Suzuki M, Hiroshima K, Kubo R, Mohammed S, et al. Assessment of epidermal growth factor receptor mutation by endobronchial ultrasound-guided transbronchial needle aspiration. *Chest* (2007) **132**(2):597–602. doi:10.1378/chest.07-0095
36. Schuurbiers OC, Looijen-Salamon MG, Ligtenberg MJ, van derHeijden HF. A brief retrospective report on the feasibility of epidermal growth factor receptor and KRAS mutation analysis in transesophageal and endobronchial ultrasound-guided fine needle cytological aspirates. *J Thorac Oncol* (2010) **5**(10):1664–7. doi:10.1097/JTO.0b013e3181f0bd93
37. Garcia-Olive I, Monso E, Andreo F, Sanz-Santos J, Taron M, Molina-Vila MA, et al. Endobronchial ultrasound-guided transbronchial needle aspiration for identifying EGFR mutations. *Eur Respir J* (2010) **35**(2):391–5. doi:10.1183/09031936.00028109
38. Yarmus L, Akulian J, Gilbert C, Feller-Kopman D, Lee HJ, Zarogoulidis P, et al. Optimizing endobronchial ultrasound for molecular analysis. How many passes are needed? *Ann Am Thorac Soc* (2013) **10**(6):636–43. doi:10.1513/AnnalsATS.201305-130OC
39. Navasakulpong A, Jung S, Gonzalez AV. Implementation and feasibility of EGFR testing using endobronchial ultrasound guided needle aspirates (EBUS-TBNA) in patients with non-small cell lung cancer. Abstract accepted ATS May 2014 presentation.
40. Toloza EM, Harpole L, Detterbeck F, McCrory DC. Invasive staging of non-small cell lung cancer: a review of the current evidence. *Chest* (2003) **123**:157S–66S. doi:10.1378/chest.123.1_suppl.157S
41. Lemaire A, Nikolic I, Petersen T, Haney JC, Toloza EM, Harpole DH Jr, et al. Nine-year single center experience with cervical mediastinoscopy: complications and false negative rate. *Ann Thor Surg* (2006) **82**(4):1185–9. doi:10.1016/j.athoracsur.2006.05.023
42. Yasufuku K, Pierre A, Darling G, de Perrot M, Waddell T, Johnston M, et al. A prospective controlled trial of endobronchial ultrasound-guided transbronchial needle aspiration compared with mediastinoscopy for mediastinal lymph node staging of lung cancer. *J Thorac Cardiovasc Surg* (2011) **142**(1393–1400):e1391. doi:10.1016/j.jtcvs.2011.08.037
43. Schreiber G, McCrory DC. Performance characteristics of different modalities for diagnosis of suspected lung cancer: summary of published evidence. *Chest* (2003) **123**:115S–28S. doi:10.1378/chest.123.1_suppl.115S
44. Klein JS, Salomon G, Stewart EA. Transthoracic needle biopsy with a coaxially placed 20-gauge automated cutting needle: results in 122 patients. *Radiology* (1996) **198**:715–20. doi:10.1148/radiology.198.3.8628859
45. Bolliger CT, Mathur PN, Beamis JF, Becker HD, Cavaliere S, Colt H, et al. ERS/ATS statement on interventional pulmonology. European Respiratory Society/American Thoracic Society. *Eur Respir J* (2002) **19**:365–73.
46. Laurent F, Latrabe V, Vergier B, Michel P. Percutaneous CT-guided biopsy of the lung: comparison between aspiration and automated cutting needles using a coaxial technique. *Cardiovasc Interven Radiol* (2000) **23**:266–72. doi:10.1007/s002700010067
47. Levine MS, Weiss JM, Harrell JH, Cameron TJ, Moser KM. Transthoracic needle aspiration biopsy following negative fiberoptic bronchoscopy in solitary pulmonary nodules. *Chest* (1988) **93**:1152–5. doi:10.1378/chest.93.6.1152
48. Pirker R, Herth FJ, Kerr KM, Filipits M, Taron M, Gandara D, et al. Consensus for EGFR testing in non-small cell lung cancer: results from a European workshop. *J Thorac Oncol* (2010) **5**(10):1706–13. doi:10.1097/JTO.0b013e3181f1c8de

49. Zhuang YP, Wang HY, Shi MQ, Zhang J, Feng Y. Use of CT-guided fine needle aspiration biopsy in epidermal growth factor receptor mutation analysis in patients with advanced lung cancer. *Acta Radiol* (2011) **52**:1083–7. doi:10.1258/ar.2011.110150
50. Fassina A, Gazziero A, Zardo D, Corradin M, Aldighieri E, Rossi GP. Detection of EGFR and KRAS mutations on trans-thoracic needle aspiration of lung nodules by high resolution melting analysis. *J Clin Pathol* (2009) **62**:1096–102. doi:10.1136/jcp.2009.067587
51. da Cunha Santos G, Lai SW, Saieg MA, Geddie WR, Pintilie M, Tsao MS, et al. Cyto-histologic agreement in pathologic subtyping of non-small cell lung carcinoma: review of 602 fine needle aspirates with follow-up surgical specimens over a nine year period and analysis of factors underlying failure to subtype. *Lung Cancer* (2012) **77**(3):501–6. doi:10.1016/j.lungcan.2012.05.091
52. Sears D, Hajdu SI. The cytologic diagnosis of malignant neoplasms in pleural and peritoneal effusions. *Acta Cytol* (1987) **31**:85–97.
53. Boutin C, Viallat JR, Cargnino P, Farisse P. Thoracoscopy in malignant pleural effusions. *Am Rev Respir Dis* (1981) **124**:588–92.
54. Billah S, Stewart J, Staerkel G, Chen S, Gong Y, Guo M. EGFR and KRAS mutations in lung carcinoma: molecular testing by using cytology specimens. *Cancer Cytopathol* (2011) **119**:111–7. doi:10.1002/cncy.20151
55. Rahman NM, Ali NJ, Brown G, Chapman SJ, Davies RJ, Downer NJ, et al. Local anaesthetic thoracoscopy: British Thoracic Society Pleural Disease Guideline 2010. *Thorax* (2010) **65**(Suppl 2):ii54–60. doi:10.1136/thx.2010.137018
56. Roberts ME, Neville N, Berrisford RG, Antunes G, Ali NJ, et al. Management of a malignant pleural effusion. British thoracic society guidelines. *Thorax* (2010) **65**(2):ii32–40. doi:10.1136/thx.2010.136994
57. Liu D, Lu Y, Hu Z, Wu N, Nie X, Xia Y, et al. Malignant pleural effusion supernatants are substitutes for metastatic pleural tumour tissues in EGFR mutation test in patients with advanced lung adenocarcinoma. *PLoS One* (2014) **9**(2):e89946. doi:10.1371/journal.pone.0089946
58. Menzies R, Charbonneau M. Thoracoscopy for the diagnosis of pleural disease. *Ann Intern Med* (1991) **114**:271–6. doi:10.7326/0003-4819-114-4-271
59. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger K, Yatabe Y, et al. Diagnosis of lung cancer in small biopsies and cytology. Implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification. *Arch Pathol Lab Med* (2013) **137**:668–84. doi:10.5858/arpa.2012-0263-RA
60. Thunnissen E, Kerr KM, Herth FJ, Lantuejoul S, Papotti M, Rintoul RC, et al. The challenge of NSCLC diagnosis and predictive analysis on small samples. Practical approach of a working group. *Lung Cancer* (2012) **76**:1–18. doi:10.1016/j.lungcan.2011.10.017
61. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: Guideline from the College of American Pathologists (CAP), International Association for the Study of Lung Cancer(IASLC), Association of Molecular Pathologists (AMP). *Arch Pathol Lab Med* (2013) **137**(6):828–60. doi:10.5858/arpa.2012-0720-OA
62. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, et al. International Association of the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma. *J Thorac Oncol* (2011) **6**(2):244–85. doi:10.1097/JTO.0b013e318206a221

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 01 May 2014; accepted: 03 September 2014; published online: 22 September 2014.

*Citation: Ofiara LM, Navasakulpong A, Beaudoin S and Gonzalez AV (2014) Optimizing tissue sampling for the diagnosis, subtyping, and molecular analysis of lung cancer. Front. Oncol. **4**:253. doi: 10.3389/fonc.2014.00253*

This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 16 July 2014
doi: 10.3389/fonc.2014.00182

Non-small cell lung carcinoma biomarker testing: the pathologist's perspective

Elisa Brega and Guilherme Brandao*

Department of Pathology, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, Montreal, QC, Canada

Edited by:
Vera Hirsh, McGill University Health Centre, Canada

Reviewed by:
Joachim Diebold, Lucerne Cantonal Hospital, Switzerland
K. Shilo, The Ohio State University, USA

****Correspondence:***
Guilherme Brandao, Department of Pathology, Sir Mortimer B. Davis-Jewish General Hospital, Pavilion G, Suite 120.1, 3755 Côte Ste-Catherine Road, Montreal, QC H3T 1E2, Canada
e-mail: guilherme.brandao@mcgill.ca

Biomarker testing has become standard of care for patients diagnosed with non-small cell lung carcinoma (NSCLC). Although, it can be successfully performed in circulating tumor cells, at present, the vast majority of investigations are carried out using direct tumor sampling, either through aspiration methods, which render most often isolated cells, or tissue sampling, that could range from minute biopsies to large resections. Consequently, pathologists play a central role in this process. Recent evidence suggests that refining NSCLC diagnosis might be clinically significant, particularly in cases of lung adenocarcinomas (ADC), which in turn, has prompted a new proposal for the histologic classification of such pulmonary neoplasms. These changes, in conjunction with the mandatory incorporation of biomarker testing in routine NSCLC tissue processing, have directly affected the pathologist's role in lung cancer work-up. This new role pathologists must play is complex and demanding, and requires a close interaction with surgeons, oncologists, radiologists, and molecular pathologists. Pathologists often find themselves as the central figure in the coordination of a process, that involves assuring that the tumor samples are properly fixed, but without disruption of the DNA structure, obtaining the proper diagnosis with a minimum of tissue waste, providing pre-analytical evaluation of tumor samples selected for biomarker testing, which includes assessment of the proportion of tumor to normal tissues, as well as cell viability, and assuring that this entire process happens in a timely fashion. Therefore, it is part of the pathologist's responsibilities to assure that the samples received in their laboratories, be processed in a manner that allows for optimal biomarker testing. This article goal is to discuss the essential role pathologists must play in NSCLC biomarker testing, as well as to provide a summarized review of the main NSCLC biomarkers of clinical interest.

Keywords: ALK, EGFR, NSCLC, adenocarcinoma, biomarker, histology, lung

INTRODUCTION

In Canada, lung cancer represents the second most common cancer in both males and females (14 and 13%, respectively), and it is the leading cause of cancer death for both sexes (1). In fact, lung cancer, with 27.2 and 26.3% mortality rate in males and females, respectively, is responsible for more deaths among Canadians than the other two leading organ-specific cancers combined [colorectal (12.7%) and prostate (10.0%) in males, and breast (13.9%) and colorectal (11,6%) in females] (1). In the United States, approximately 84% of new lung cancer cases are classified as non-small cell lung carcinomas (NSCLC), and 15% as small cell carcinomas (SCC) (2), with the majority of patients being diagnosed at advanced-stage (56%) (3). The prognosis is poor, with the overall 5-year survival rate of 6.1% for SCC and 17.1% for NSCLC (2).

Implementation of personalized targeted therapies has become a reality for a group of lung cancer patients, but this therapeutic option is usually reserved for those patients whom tumor samples have been screened for specific biomarkers. A multitude of potentially useful biomarkers have recently emerged and this list continues to grow. It has become increasingly difficult for pathologists and oncologists to define which biomarkers should be routinely tested. An expert panel in pathology and oncology, assembled by the College of American Pathologists (CAP) with representatives from the International Association for the Study of Lung Cancer (IASLC) and Association for Molecular Pathology (AMP), has recently met in an attempt to address questions regarding biomarker testing in lung cancer. The conclusions have been published in the format of testing guidelines, which presently recommends investigations of abnormalities involving only two genes: the epidermal growth factor receptor (*EGFR*) and the anaplastic lymphoma kinase (*ALK*) (4).

This review will focus on the role of the pathologist as an essential figure in the NSCLC biomarker testing process.

Abbreviations: NSCLC, non-small cell lung carcinoma; SCC, small cell carcinomas; *EGFR*, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase; ADC, adenocarcinoma; LGC, large cell carcinoma; SqCC, squamous cell carcinoma; TKI, tyrosine kinase inhibitors; TAT, turn-around-time; ALCL, anaplastic large cell lymphomas; FISH, fluorescence *in situ* hybridization.

TISSUE/CYTOLOGICAL DIAGNOSIS AND BIOMARKER TESTING

NSCLC, as a standing alone diagnosis, in either tissue or cytological samples, should be avoided whenever possible. In some

situations (when the tumor sample is restricted to a smear from a bronchial brushing of a poorly differentiated carcinoma, for example), further characterization might be impossible. However, in our experience, further characterization, particularly with the help of special histochemical stains for the detection of mucin (often with the use of PAS-D or mucicarmin), and/or immunocytochemistry, can be achieved in the majority of cases. From a practical point of view, samples containing adenocarcinoma (ADC) either pure or mixed should undergo biomarker testing. In small samples, the recommendations are less stringent, and, as long as an ADC component cannot be excluded, the tissue should undergo biomarker testing (irrespective of the main tumor component identified). In resections, however, when the pathologist has an opportunity to examine the lesion in its entirety, "pure" tumors [large cell carcinoma (LGC), squamous cell carcinoma (SqCC) or others] should not be tested (**Figure 1**).

Numerous immunomarkers are available in order to help in the sub classification of NSCLC. The most commonly used are TTF-1, Napsin-A, p63, CK 5/6, and p40 (5–13). Although, it is true that in most cases the pathologist will be reasonably at ease to sub classify NSCLC's, in some cases, sub classification might be rather difficult. It is our understanding that if the pathologist is uncertain about the specific sub classification, then the sample should be submitted for biomarker testing.

Despite the emphasis placed on focusing on ADC for biomarker testing, it is important to highlight that there are, however, isolated reports in the literature of the detection of either *EGFR* mutations or *ALK* rearrangements in tumors classified as SqCC (14–17).

Interestingly, some genetic aberrations can be generally associated with specific NSCLC subtypes and/or clinical profile (i.e., smokers versus non-smokers) (18). ADC is the predominant histologic type associated with both *EGFR*-mutated, as well as in *ALK*-rearranged cases. However, *EGFR* mutations are particularly prevalent in those cases containing non-mucinous bronchioloalveolar (lepidic) pattern (19), while in *ALK*-rearranged ADC, the most striking correlation is made with the presence of a signet-ring component (**Table 1**) (20, 21).

An important aspect that affects biomarker testing is the amount of available tumor present in a determined sample. This is a rather difficult topic to address, since the test sensitivity varies significantly according to the employed technique, particularly when searching for *EGFR* mutations, where normal DNA might interfere with test sensitivity (22). Nevertheless, the pathologist should provide an estimation of the percentage of tumor present in the sample, as well as, the viability of the tumor cells. It is recommended that testing sensitivity, as well as determination of limiting factors that might influence optimal results (fixative choice for example), should be defined locally, through proper validation methods. Of note, samples collected from aspiration biopsy methods, including direct lesion sampling (transbronchial needle aspiration biopsies), as well as the drainage of effusions, should be considered for biomarker testing (23–25).

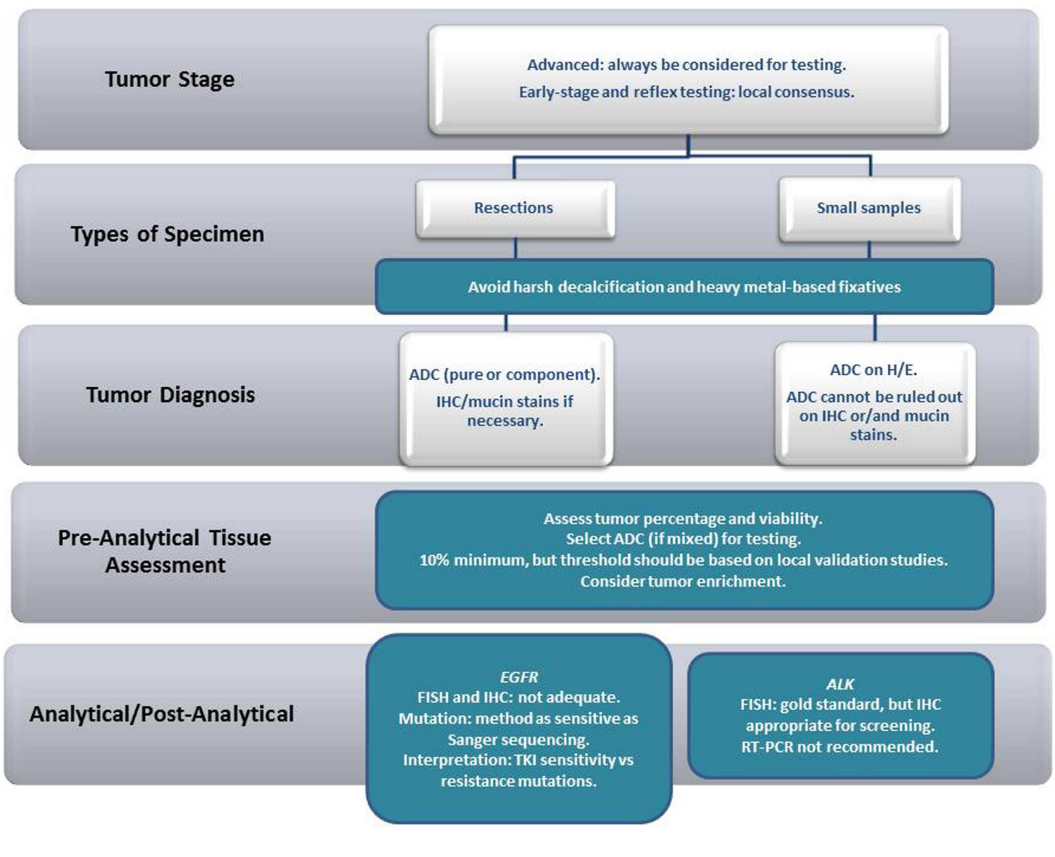


FIGURE 1 | Recommendations on specimen handling in NSCLC biomarker testing.

Table 1 | Summary of the clinical characteristics, common genetic abnormalities and respective targeting agents of the main NSCLC biomarkers.

Biomarkers	Gender and age	Prevalence	Tobacco	Ethnicity	ADC versus SqCC/distinctive histologic characteristics	Clinically relevant genetic abnormality	Examples of targeting agent (available or in development)
EGFR	Female, Younger	10-40%	Non-smokers	Asian	ADC/Non-mucinous bronchioloalveolar (lepidic)	Mutation (various, most common in-frame deletions of exon 19 and a point mutation (CTG to CGG) in exon 21)	Gefitinib, Erlotinib, Afatinib, Dacomitinib, Neratinib
ALK	Younger	2-6%	Non-smokers	Not distinctive	ADC/solid pattern, signet-ring cells	Translocation, inversion (*EML4-ALK* most common)	Crizotinib, LDK378
HER2/ERBB2	Female	1-4%	Non-smokers	Asians	ADC	In-frame insertions in exon 20	Trastuzumab Pertuzumab, Lapatinib
ROS1	Female, younger	0,5-2%	Non-smokers	Und.	ADC	Translocation *(ROS1-FIG)*	Crizotinib
RET	Younger	1-2%	Non-smokers	Not distinctive	ADC/Adenosquamous	*KIF5B–RET* and *CCDC6–RET* fusion genes	Vandetanib Cabozantinib
KRAS	Not distinctive	15-30%	Smokers	Caucasian	ADC/mucinous, particularly with lepidic (bronchioloalveolar) pattern	Mutations in codon 12 (majority) and 13	Selumetinib (via inhibition of MEK)
BRAF	Not distinctive	3% (ADC's)	Smokers	Not distinctive	ADC	Mutations in, V600E(50%), G469A(39%), D594G(11%)	Dabrafenib, Vemurafenib, XL281, Selumetinib
NRAS	Und.	0.5-1%	Smokers	Und.	ADC	Mutations in codon Q61 in exon 3 (80%) and G12 (exon 2)	Selumetinib Trametinib
FGFR1	Not distinctive	22% of SqCC	Smokers	Not distinctive	SqCC	Amplification	PD173074
PTEN	Not distinctive	4-8%	Smokers	Not distinctive	SqCC	Various mutations in exon 5-8	GSK2636771
DDR2	Und.	2.5-3.8%	Und.	Und.	SqCC	Missense mutations, several	Imatini, Dasatinib
MAP2K1/MEK1	Und.	1%	Unclear	Und.	ADC	Mutations in Q56P, K57N and D67N	AZD6244, Pimasertib, Refametinib, others
PIK3CA	Not distinctive	2-4%	Mixed reports	Not distinctive	ADC and SqCC	Mutations in E545K AND H1047R (most common), also E542K and H1047L	Everolimus, Tensirolimus, GDC-0941, XL-147, Others
AKT1	Und.	1%	Und.	Und.	ADC and SqCC	Mutation in E17K	MK-2206
MET	Not distinctive	1-5%	Not distinctive	Und.	ADC	Amplification, protein overexpression and mutation	Vandetanib, Cabozantinib

Und: undetermined.

EPIDERMAL GROWTH FACTOR RECEPTOR

Epidermal growth factor receptor (also known as *HER-1 or Erb1*) is a member of the ErbB receptor tyrosine kinase family, which also includes *HER-2/neu (ErbB2), HER-3 (ErbB3)*, and *HER-4 (ErbB4)*. *EGFR* activation is associated with cancer cell growth, invasion, proliferation, apoptosis, tumor angiogenesis, and metastatic spread. Therefore, it plays an important role in carcinogenesis and tumor progression by activation mechanisms, including overexpression, mutation, and autocrine ligand production. These actions are accomplished through activation of the RAS/RAF/MEK/MAPK and the PI3K/AKT/mTOR pathways (26).

The two most common *EGFR* activating mutations that confer sensitivity to tyrosine kinase inhibitors (TKI) are short in-frame deletions of exon 19, and a point mutation (CTG to CGG) in exon 21 at nucleotide 2573, that results in substitution of leucine by arginine at codon 858 (L858R) (27). Despite the fact that these two mutations might represent approximately 90% of all known clinically significant *EGFR* mutations, the consensus recommendations are that all *EGFR* mutations that account for at least 1% should be tested (4). It is important to emphasize that among the tested mutations, exon 20 T790M, as well as most exon 20 insertions are associated with resistance to first-generation TKI's (28).

Epidermal growth factor receptor mutations occur at a higher frequency in tumors from East Asians than from non-Asians (30 versus 8%), from women than from men (59 versus 26%), from never smokers than from ever smokers (66 versus 22%), and in ADC's compared with other NSCLC histologies (49 versus 2%) (29). In the United States, it is estimated that activating *EGFR* mutations are found in 15% of patients with primary lung ADC (**Table 1**) (30).

Turn-around-time (TAT) might be a very important factor for advanced-stage patients, whom might benefit from early institution of targeted therapy. The consensus recommends a maximum of 10 working days as an acceptable TAT from the date the laboratory receives the sample to be tested (4).

ANAPLASTIC LYMPHOMA KINASE

Translocations involving *ALK* have previously been identified in anaplastic large cell lymphomas (ALCL), and in a rare mesenchymal neoplasm known as inflammatory myofibroblastic tumor or inflammatory pseudotumor (31, 32). In lung carcinomas, *ALK* rearrangement was first demonstrated in 2007 by Soda et al. (33) when *ALK* fusion transcripts were found in 6.7% (5 out of 75) of NSCLC samples. However, the prevalence of *ALK* rearrangement in lung carcinomas varies significantly (34–36).

ALK rearrangements tend to be mutually exclusive with other known driver mutations in NSCLC (18). However, it has rarely been described together with *EGFR* and *PI3K* mutations (36–38).

ALK-rearranged NSCLC patients, when compared to *ALK*-non-rearranged, are more frequently non- or light-smokers, younger, and present with advanced clinical stage. Histologically, the tumors demonstrate most frequently ADC with solid pattern and signet-ring cells (20, 21, 39).

Although fluorescence *in situ* hybridization (FISH) is currently the gold standard method for detecting *ALK* rearrangements according to the United States Food and Drug Administration (FDA) (40), the CAP consensus accepts that, if carefully validated, immunohistochemistry can be considered as a screening method (4). This proposition is in concert with the literature, which has shown in several different articles that immunohistochemistry can be very effective in the detection of *ALK* rearrangement in lung carcinomas (41–43).

OTHER BIOMARKERS

Currently, in over 50% of NSCLC's, a driver oncogene can be identified (18). In addition to the previously discussed *ALK* and *EGFR* genes, several other potential targets have been uncovered in NSCLC's, including the V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*), the human epidermal growth factor receptor 2 (*HER2*), reactive oxygen species 1 (*ROS1*), v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*), phosphoinositide-3-kinase catalytic alpha polypeptide (PIK3CA), c-mesenchymal-epithelial transition mitogen (*c-MET*), activated protein kinase (*MAP2K1*), fibroblast growth factor receptor (FGFR), discoidin domain receptor 2 (DDR2), phosphatase and tensin homolog (PTEN), protein kinase B (AKT), rearranged during transfection (RET), and the neuroblastoma RAS viral oncogene homolog (*NRAS*). It is beyond the scope of this review to discuss each in detail. Current general knowledge of the characteristics of lung cancers carrying abnormalities in these genes has been summarized in **Table 1** (18, 20, 21, 28, 31, 33, 36, 37, 41, 44–72).

In conclusion, targeted therapy is already a reality for many patients and it is certain that several other components will soon follow to become valid options in the therapeutic arsenal of oncologists. In view of the overwhelming amount of information being constantly generated into the molecular derangements associated with the development of lung cancer, it is not far-fetched to expect that the current consensus guidelines will soon become obsolete. As a pathologist, I witness on a daily basis a continuous and inexorable change in our practice: our job no longer ends with the histological diagnosis. In fact, molecular profiling has become an integral part of the surgical pathology report. It is crucial that us pathologists embrace this new format of oncologic surgical pathology practice, and question ourselves, after each new malignant diagnosis: "what should I do now that might translate into a potential treatment alternative for this patient?"

REFERENCES

1. Canadian Cancer Society's Advisory Committee on Cancer Statistics. *Canadian Cancer Statistics 2013.* Toronto, ON: CCS (2013).
2. Siegel R, DeSantis C, Virgo K, Stein K, Mariotto A, Smith T, et al. Cancer treatment and survivorship statistics, 2012. *CA Cancer J Clin* (2012) **62**(4):220–41. doi:10.3322/caac.21149
3. Howlader N, Noone AM, Krapcho M, Garshell J, Neyman N, Altekruse SF, et al. *SEER Cancer Statistics Review, 1975-2010.* Bethesda, MD: National Cancer Institute (2013).
4. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *Arch Pathol Lab Med* (2013) **137**(6):828–60. doi:10.5858/arpa.2012-0720-OA
5. Bishop JA, Teruya-Feldstein J, Westra WH, Pelosi G, Travis WD, Rekhtman N. p40 (DeltaNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. *Mod Pathol* (2012) **25**(3):405–15. doi:10.1038/modpathol.2011.173

6. Mukhopadhyay S, Katzenstein AL. Subclassification of non-small cell lung carcinomas lacking morphologic differentiation on biopsy specimens: utility of an immunohistochemical panel containing TTF-1, napsin A, p63, and CK5/6. *Am J Surg Pathol* (2011) **35**(1):15–25. doi:10.1097/PAS.0b013e3182036d05
7. Nobre AR, Albergaria A, Schmitt F. p40: a p63 isoform useful for lung cancer diagnosis - a review of the physiological and pathological role of p63. *Acta Cytol* (2013) **57**(1):1–8. doi:10.1159/000345245
8. Nonaka D. A study of DeltaNp63 expression in lung non-small cell carcinomas. *Am J Surg Pathol* (2012) **36**(6):895–9. doi:10.1097/PAS.0b013e3182498f2b
9. Pelosi G, Fabbri A, Bianchi F, Maisonneuve P, Rossi G, Barbareschi M, et al. DeltaNp63 (p40) and thyroid transcription factor-1 immunoreactivity on small biopsies or cellblocks for typing non-small cell lung cancer: a novel two-hit, sparing-material approach. *J Thorac Oncol* (2012) **7**(2):281–90. doi:10.1097/JTO.0b013e31823815d3
10. Rekhtman N, Ang DC, Sima CS, Travis WD, Moreira AL. Immunohistochemical algorithm for differentiation of lung adenocarcinoma and squamous cell carcinoma based on large series of whole-tissue sections with validation in small specimens. *Mod Pathol* (2011) **24**(10):1348–59. doi:10.1038/modpathol.2011.92
11. Righi L, Graziano P, Fornari A, Rossi G, Barbareschi M, Cavazza A, et al. Immunohistochemical subtyping of nonsmall cell lung cancer not otherwise specified in fine-needle aspiration cytology: a retrospective study of 103 cases with surgical correlation. *Cancer* (2011) **117**(15):3416–23. doi:10.1002/cncr.25830
12. Ring BZ, Seitz RS, Beck RA, Shasteen WJ, Soltermann A, Arbogast S, et al. A novel five-antibody immunohistochemical test for subclassification of lung carcinoma. *Mod Pathol* (2009) **22**(8):1032–43. doi:10.1038/modpathol.2009.60
13. Stoll LM, Johnson MW, Gabrielson E, Askin F, Clark DP, Li QK. The utility of napsin-A in the identification of primary and metastatic lung adenocarcinoma among cytologically poorly differentiated carcinomas. *Cancer Cytopathol* (2010) **118**(6):441–9. doi:10.1002/cncy.20108
14. Caliò A, Nottegar A, Gilioli E, Bria E, Pilotto S, Peretti U, et al. ALK/EML4 Fusion Gene May Be Found in Pure Squamous Carcinoma of the Lung. *J Thorac Oncol* (2014) **9**(5):729–32. doi:10.1097/JTO.0000000000000109
15. Miyamae Y, Shimizu K, Hirato J, Araki T, Tanaka K, Ogawa H, et al. Significance of epidermal growth factor receptor gene mutations in squamous cell lung carcinoma. *Oncol Rep* (2011) **25**(4):921–8. doi:10.3892/or.2011.1182
16. Park SH, Ha SY, Lee JI, Lee H, Sim H, Kim YS, et al. Epidermal growth factor receptor mutations and the clinical outcome in male smokers with squamous cell carcinoma of lung. *J Korean Med Sci* (2009) **24**(3):448–52. doi:10.3346/jkms.2009.24.3.448
17. Tanaka K, Hata A, Kida Y, Kaji R, Fujita S, Katakami N, et al. Gefitinib for a poor performance status patient with squamous cell carcinoma of the lung harboring EGFR mutation. *Intern Med* (2012) **51**(6):659–61. doi:10.2169/internalmedicine.51.6386
18. Sequist LV, Heist RS, Shaw AT, Fidias P, Rosovsky R, Temel JS, et al. Implementing multiplexed genotyping of non-small-cell lung cancers into routine clinical practice. *Ann Oncol* (2011) **22**(12):2616–24. doi:10.1093/annonc/mdr489
19. Blons H, Côté JF, Le CorreD, Riquet M, Fabre-Guilevin E, Laurent-Puig P, et al. Epidermal growth factor receptor mutation in lung cancer are linked to bronchioloalveolar differentiation. *Am J Surg Pathol* (2006) **30**(10):1309–15. doi:10.1097/01.pas.0000213285.65907.31
20. Rodig SJ, Mino-Kenudson M, Dacic S, Yeap BY, Shaw A, Barletta JA, et al. Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the western population. *Clin Cancer Res* (2009) **15**(16):5216–23. doi:10.1158/1078-0432.CCR-09-0802
21. Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. *J Clin Oncol* (2009) **27**(26):4247–53. doi:10.1200/JCO.2009.22.6993
22. Pao W, Ladanyi M. Epidermal growth factor receptor mutation testing in lung cancer: searching for the ideal method. *Clin Cancer Res* (2007) **13**(17):4954–5. doi:10.1158/1078-0432.CCR-07-1387
23. Aisner DL, Deshpande C, Baloch Z, Watt CD, Litzky LA, Malhotra B, et al. Evaluation of EGFR mutation status in cytology specimens: an institutional experience. *Diagn Cytopathol* (2013) **41**(4):316–23. doi:10.1002/dc.21851
24. Billah S, Stewart J, Staerkel G, Chen S, Gong Y, Guo M. EGFR and KRAS mutations in lung carcinoma: molecular testing by using cytology specimens. *Cancer Cytopathol* (2011) **119**(2):111–7. doi:10.1002/cncy.20151
25. Malapelle U, Bellevicine C, Zeppa P, Palombini L, Troncone G. Cytology-based gene mutation tests to predict response to anti-epidermal growth factor receptor therapy: a review. *Diagn Cytopathol* (2011) **39**(9):703–10. doi:10.1002/dc.21512
26. Salomon DS, Brandt R, Ciardiello F, Normanno N, et al. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit Rev Oncol Hematol* (1995) **19**(3):183–232. doi:10.1016/1040-8428(94)00144-I
27. Ladanyi M, Pao W. Lung adenocarcinoma: guiding EGFR-targeted therapy and beyond. *Mod Pathol* (2008) **21**(Suppl 2):S16–22. doi:10.1038/modpathol.3801018
28. Ludovini V, Bianconi F, Pistola L, Pistola V, Chiari R, Colella R, et al. Optimization of patient selection for EGFR-TKIs in advanced non-small cell lung cancer by combined analysis of KRAS, PIK3CA, MET, and non-sensitizing EGFR mutations. *Cancer Chemother Pharmacol* (2012) **69**(5):1289–99. doi:10.1007/s00280-012-1829-7
29. Bell DW, Brannigan BW, Matsuo K, Finkelstein DM, Sordella R, Settleman J, et al. Increased prevalence of EGFR-mutant lung cancer in women and in East Asian populations: analysis of estrogen-related polymorphisms. *Clin Cancer Res* (2008) **14**(13):4079–84. doi:10.1158/1078-0432.CCR-07-5030
30. Keedy VL, Temin S, Somerfield MR, Beasley MB, Johnson DH, McShane LM, et al. American Society of Clinical Oncology provisional clinical opinion: epidermal growth factor receptor (EGFR) Mutation testing for patients with advanced non-small-cell lung cancer considering first-line EGFR tyrosine kinase inhibitor therapy. *J Clin Oncol* (2011) **29**(15):2121–7. doi:10.1200/JCO.2010.31.8923
31. Lawrence B, Perez-Atayde A, Hibbard MK, Rubin BP, Dal CinP, Pinkus JL, et al. TPM3-ALK and TPM4-ALK oncogenes in inflammatory myofibroblastic tumors. *Am J Pathol* (2000) **157**(2):377–84. doi:10.1016/S0002-9440(10)64550-6
32. Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Saltman DL, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science* (1994) **263**(5151):1281–4. doi:10.1126/science.8122112
33. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* (2007) **448**(7153):561–6. doi:10.1038/nature05945
34. Boland JM, Erdogan S, Vasmatzis G, Yang P, Tillmans LS, Johnson MR, et al. Anaplastic lymphoma kinase immunoreactivity correlates with ALK gene rearrangement and transcriptional up-regulation in non-small cell lung carcinomas. *Hum Pathol* (2009) **40**(8):1152–8. doi:10.1016/j.humpath.2009.01.012
35. Camidge DR, Kono SA, Flacco A, Tan AC, Doebele RC, Zhou Q, et al. Optimizing the detection of lung cancer patients harboring anaplastic lymphoma kinase (ALK) gene rearrangements potentially suitable for ALK inhibitor treatment. *Clin Cancer Res* (2010) **16**(22):5581–90. doi:10.1158/1078-0432.CCR-10-0851
36. Wang Z, Zhang X, Bai H, Zhao J, Zhuo M, An T, et al. EML4-ALK rearrangement and its clinical significance in Chinese patients with advanced non-small cell lung cancer. *Oncology* (2012) **83**(5):248–56. doi:10.1159/000341381
37. Chaft JE, Arcila ME, Paik PK, Lau C, Riely GJ, Pietanza MC, et al. Coexistence of PIK3CA and other oncogene mutations in lung adenocarcinoma-rationale for comprehensive mutation profiling. *Mol Cancer Ther* (2012) **11**(2):485–91. doi:10.1158/1535-7163.MCT-11-0692
38. Wallander ML, Geiersbach KB, Tripp SR, Layfield LJ. Comparison of reverse transcription-polymerase chain reaction, immunohistochemistry, and fluorescence in situ hybridization methodologies for detection of echinoderm microtubule-associated proteinlike 4-anaplastic lymphoma kinase fusion-positive non-small cell lung carcinoma: implications for optimal clinical testing. *Arch Pathol Lab Med* (2012) **136**(7):796–803. doi:10.5858/arpa.2011-0321-OA
39. Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, Holmes AJ, et al. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. *Clin Cancer Res* (2008) **14**(13):4275–83. doi:10.1158/1078-0432.CCR-08-0168
40. FDA. *CRIZOTINIB* (2011). Available from: http://www.fda.gov/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm270058.htm
41. McLeer-Florin A, Moro-Sibilot D, Melis A, Salameire D, Lefebvre C, Ceccaldi F, et al. Dual IHC and FISH testing for ALK gene rearrangement in lung adenocarcinomas in a routine practice: a French study. *J Thorac Oncol* (2012) **7**(2):348–54. doi:10.1097/JTO.0b013e3182381535
42. Paik JH, Choe G, Kim H, Choe JY, Lee HJ, Lee CT, et al. Screening of anaplastic lymphoma kinase rearrangement by immunohistochemistry in non-small cell lung cancer: correlation with fluorescence in situ hybridization. *J Thorac Oncol* (2011) **6**(3):466–72. doi:10.1097/JTO.0b013e31820b82e8

43. Selinger CI, Rogers TM, Russell PA, O'Toole S, Yip P, Wright GM, et al. Testing for ALK rearrangement in lung adenocarcinoma: a multicenter comparison of immunohistochemistry and fluorescent in situ hybridization. *Mod Pathol* (2013) **26**(12):1545–53. doi:10.1038/modpathol.2013.87
44. Bergethon K, Shaw AT, Ou SH, Katayama R, Lovly CM, McDonald NT, et al. ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol* (2012) **30**(8):863–70. doi:10.1200/JCO.2011.35.6345
45. Cappuzzo F, Marchetti A, Skokan M, Rossi E, Gajapathy S, Felicioni L, et al. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. *J Clin Oncol* (2009) **27**(10):1667–74. doi:10.1200/JCO.2008.19.1635
46. Dent P. Met in lung cancer. *Cancer Biol Ther* (2014) **15**:6. doi:10.4161/cbt.28504
47. Drilon A, Wang L, Hasanovic A, Suehara Y, Lipson D, Stephens P, et al. Response to Cabozantinib in patients with RET fusion-positive lung adenocarcinomas. *Cancer Discov* (2013) **3**(6):630–5. doi:10.1158/2159-8290.CD-13-0035
48. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* (2007) **316**(5827):1039–43. doi:10.1126/science.1141478
49. Go H, Kim DW, Kim D, Keam B, Kim TM, Lee SH, et al. Clinicopathologic analysis of ROS1-rearranged non-small-cell lung cancer and proposal of a diagnostic algorithm. *J Thorac Oncol* (2013) **8**(11):1445–50. doi:10.1097/JTO.0b013e3182a4dd6e
50. Hammerman PS, Sos ML, Ramos AH, Xu C, Dutt A, Zhou W, et al. Mutations in the DDR2 kinase gene identify a novel therapeutic target in squamous cell lung cancer. *Cancer Discov* (2011) **1**(1):78–89. doi:10.1158/2159-8274.CD-11-0005
51. Huang L, An SJ, Chen ZH, Su J, Yan HH, Wu YL. MET expression plays differing roles in non-small-cell lung cancer patients with or without EGFR mutation. *J Thorac Oncol* (2014) **9**(5):725–8. doi:10.1097/JTO.0000000000000105
52. Iida M, Brand TM, Campbell DA, Starr MM, Luthar N, Traynor AM, et al. Targeting AKT with the allosteric AKT inhibitor MK-2206 in non-small cell lung cancer cells with acquired resistance to cetuximab. *Cancer Biol Ther* (2013) **14**(6):481–91. doi:10.4161/cbt.24342
53. Jänne PA, Shaw AT, Pereira JR, Jeannin G, Vansteenkiste J, Barrios C, et al. Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. *Lancet Oncol* (2013) **14**(1):38–47. doi:10.1016/S1470-2045(12)70489-8
54. Jin G, Kim MJ, Jeon HS, Choi JE, Kim DS, Lee EB, et al. PTEN mutations and relationship to EGFR, ERBB2, KRAS, and TP53 mutations in non-small cell lung cancers. *Lung Cancer* (2010) **69**(3):279–83. doi:10.1016/j.lungcan.2009.11.012
55. Kawano O, Sasaki H, Endo K, Suzuki E, Haneda H, Yukiue H, et al. PIK3CA mutation status in Japanese lung cancer patients. *Lung Cancer* (2006) **54**(2):209–15. doi:10.1016/j.lungcan.2006.07.006
56. Kohno T, Tsuta K, Tsuchihara K, Nakaoku T, Yoh K, Goto K. RET fusion gene: translation to personalized lung cancer therapy. *Cancer Sci* (2013) **104**(11):1396–400. doi:10.1111/cas.12275
57. Li C, Sun Y, Fang R, Han X, Luo X, Wang R, et al. Lung adenocarcinomas with HER2-activating mutations are associated with distinct clinical features and HER2/EGFR copy number gains. *J Thorac Oncol* (2012) **7**(1):85–9. doi:10.1097/JTO.0b013e318234f0a2
58. Malanga D, Scrima M, De Marco C, Fabiani F, De Rosa N, De Gisi S, et al. Activating E17K mutation in the gene encoding the protein kinase AKT1 in a subset of squamous cell carcinoma of the lung. *Cell Cycle* (2008) **7**(5):665–9. doi:10.4161/cc.7.5.5485
59. Marks JL, Gong Y, Chitale D, Golas B, McLellan MD, Kasai Y, et al. Novel MEK1 mutation identified by mutational analysis of epidermal growth factor receptor signaling pathway genes in lung adenocarcinoma. *Cancer Res* (2008) **68**(14):5524–8. doi:10.1158/0008-5472.CAN-08-0099
60. Ohashi K, Sequist LV, Arcila ME, Lovly CM, Chen X, Rudin CM, et al. Characteristics of lung cancers harboring NRAS mutations. *Clin Cancer Res* (2013) **19**(9):2584–91. doi:10.1158/1078-0432.CCR-12-3173
61. Onozato R, Kosaka T, Kuwano H, Sekido Y, Yatabe Y, Mitsudomi T. Activation of MET by gene amplification or by splice mutations deleting the juxtamembrane domain in primary resected lung cancers. *J Thorac Oncol* (2009) **4**(1):5–11. doi:10.1097/JTO.0b013e3181913e0e
62. Paik PK, Arcila ME, Fara M, Sima CS, Miller VA, Kris MG, et al. Clinical characteristics of patients with lung adenocarcinomas harboring BRAF mutations. *J Clin Oncol* (2011) **29**(15):2046–51. doi:10.1200/JCO.2010.33.1280
63. Pan Y, Zhang Y, Li Y, Hu H, Wang L, Li H, et al. ALK, ROS1 and RET fusions in 1139 lung adenocarcinomas: a comprehensive study of common and fusion pattern-specific clinicopathologic, histologic and cytologic features. *Lung Cancer* (2014) **84**(2):121–6. doi:10.1016/j.lungcan.2014.02.007
64. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. *Lancet Oncol* (2011) **12**(2):175–80. doi:10.1016/S1470-2045(10)70087-5
65. Reungwetwattana T, Dy GK. Targeted therapies in development for non-small cell lung cancer. *J Carcinog* (2013) **12**:22. doi:10.4103/1477-3163.123972
66. Reungwetwattana T, Weroha SJ, Molina JR. Oncogenic pathways, molecularly targeted therapies, and highlighted clinical trials in non-small-cell lung cancer (NSCLC). *Clin Lung Cancer* (2012) **13**(4):252–66. doi:10.1016/j.cllc.2011.09.004
67. Serizawa M, Koh Y, Kenmotsu H, Isaka M, Murakami H, Akamatsu H, et al. Assessment of mutational profile of Japanese lung adenocarcinoma patients by multitarget assays: a prospective, single-institute study. *Cancer* (2014) **120**(10):1471–81. doi:10.1002/cncr.28604
68. Shigematsu H, Takahashi T, Nomura M, Majmudar K, Suzuki M, Lee H, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. *Cancer Res* (2005) **65**(5):1642–6. doi:10.1158/0008-5472.CAN-04-4235
69. Tsuta K, Kohno T, Yoshida A, Shimada Y, Asamura H, Furuta K, et al. RET-rearranged non-small-cell lung carcinoma: a clinicopathological and molecular analysis. *Br J Cancer* (2014) **110**(6):1571–8. doi:10.1038/bjc.2014.36
70. Villaruz LC, Socinski MA, Cunningham DE, Chiosea SI, Burns TF, Siegfried JM, et al. The prognostic and predictive value of KRAS oncogene substitutions in lung adenocarcinoma. *Cancer* (2013) **119**(12):2268–74. doi:10.1002/cncr.28039
71. Warth A, Muley T, Dienemann H, Goeppert B, Stenzinger A, Schnabel PA, et al. ROS1 expression and translocations in non-small-cell lung cancer: clinicopathological analysis of 1478 cases. *Histopathology* (2014). doi:10.1111/his.12379
72. Weiss J, Sos ML, Seidel D, Peifer M, Zander T, Heuckmann JM, et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. *Sci Transl Med* (2010) **2**(62):62ra93. doi:10.1126/scitranslmed.3001451

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 01 May 2014; accepted: 27 June 2014; published online: 16 July 2014.
*Citation: Brega E and Brandao G (2014) Non-small cell lung carcinoma biomarker testing: the pathologist's perspective. Front. Oncol. **4**:182. doi: 10.3389/fonc.2014.00182*
This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 29 September 2014
doi: 10.3389/fonc.2014.00256

Treatment algorithms for patients with metastatic non-small cell, non-squamous lung cancer

Barbara Melosky*

Medical Oncology, British Columbia Cancer Agency – Vancouver Centre, Vancouver, BC, Canada

Edited by:
Barbara Jennifer Gitlitz, University of Southern California Keck School of Medicine, USA

Reviewed by:
Vera Hirsh, McGill University Health Centre, Canada
Shahab Babakoohi, Medstar Good Samaritan Hospital, USA

Correspondence:
Barbara Melosky, Medical Oncology, British Columbia Cancer Agency – Vancouver Centre, 600 West 10th Avenue, Vancouver, BC V5Z 4E6, Canada
e-mail: bmelosky@bccancer.bc.ca

A number of developments have altered the treatment paradigm for metastatic non-small cell, non-squamous lung cancer. These include increasing knowledge of molecular signal pathways, as well as the outcomes of several large-scale trials. As a result, treatments are becoming more efficacious and more personalized, and are changing the management and prognosis of non-small cell lung cancer patients. This is resulting in increased survival in select patient groups. In this paper, a simplified algorithm for treating patients with metastatic non-small cell, non-squamous lung cancer is presented.

Keywords: metatstatic non-squamous NSCLC, systemic therapy, chemotherapy, targeted therapy

TREATMENT PARADIGMS

The previous standard of care in metastatic non-small cell lung cancer (NSCLC) was to treat patients with a platinum doublet for four to six cycles and to offer second-line therapy upon progression (1). The emergence of molecular testing, specifically for the epidermal growth factor receptor (EGFR) and for anaplastic lymphoma kinase (ALK), enables us to better tailor treatment strategies. The results from many recent large-scale clinical trials have validated these new treatment approaches.

Chemotherapy is still one of our most important weapons. Patients are now surviving longer. All patients should get three lines of therapy. With more treatment options becoming available, algorithms must be strategically designed to balance the need to give the best drugs first while ensuring that there are many more options available for later.

The treatment algorithm discussed in this chapter is based on Canadian recommendations. Although other health authorities may have different therapeutics available, basic principles still apply.

FIRST TREATMENT DECISION POINT: HISTOLOGY AND MUTATION TESTING

HISTOLOGY

In the past, the only histological criterion for therapeutic decision making was whether the lung cancer was small cell or non-small cell. The distinction between squamous or non-squamous cell histology became important and with the evolution of immunostaining, this distinction has become more evident. The reported incidence of squamous cell lung cancer has decreased over the last several decades (2), which may be due to natural phenomena or to the development of better immunostaining. For this same reason, the reported incidence of large cell, squamous, and non-small cell (otherwise unspecified) cancer is decreasing and the incidence adenocarcinoma is increasing. The emergence of more molecular tests is unlikely to lessen the importance of histology.

MUTATIONAL TESTING

Mutation status influences the selection of first-line therapies. At this time, testing for EGFR mutations and for rearrangements in the ALK gene is recommended for patients with non-squamous histology. A number of initiatives are underway to help ensure that all advanced lung cancer patients will have mutation and biomarker testing available. Cooperation of all specialties is required, including respirologists, interventional radiologists, surgeons, and pathologists (3, 4).

Mutation profiles of cancer continue to rapidly evolve, especially for adenocarcinomas. As we better understand how other gene mutations influence lung cancer, mutation testing for other targets including MET, RET, and KRAS (5, 6) will become more likely and treatment algorithms will become even more complex.

TREATMENT OPTIONS FOR NON-SQUAMOUS NSCLC

Histological analysis determines if patients have tumors with squamous or non-squamous histology. This chapter discusses non-squamous histology only. With mutation testing, patients can be divided into three groups: those whose tumors are positive for the EGFR mutation, which is 10–30% (6) (group A); those whose tumors are positive for the ALK mutation, approximately 5–7% (6) (group B); and those whose tumors do not have mutations in either EGFR or ALK or their mutation status is unknown, approximately

Abbreviations: ATP, adenosine triphosphate; ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; HER 2, human epidermal growth factor receptor 2; NCI, National Cancer Institute; NSCLC, non-small cell lung cancer; RCT, randomized clinical trial; OS, overall survival; PFS, progression free survival; TKI, tyrosine kinase inhibitor.

63–85% (group C). Therapy is selected based on these distinctions (**Figure 1**).

GROUP A: EPIDERMAL GROWTH FACTOR RECEPTOR MUTATION POSITIVE

FIRST LINE

Activity of EGFR is inhibited by tyrosine kinase inhibitors (TKIs), a unique class of orally administered, small molecule therapeutics that have found their way into the standard of care treatment in almost all types of malignancy. Several trials have demonstrated that TKIs, including erlotinib (7), gefitinib (8), and afatinib (9, 10), are efficacious first-line treatments for this patient population.

The efficacy of gefitinib was demonstrated in the IPASS trial, which compared first-line gefitinib with a carboplatin/paclitaxel doublet in an EGFR-unselected population. Although the gefitinib-treated patients demonstrated no increase in overall survival (OS), the time to progression (9.5 versus 6.3 months, respectively, HR 0.48; 95% CI 0.36 versus 0.64; $P < 0.0001$), overall response rate (71.2 versus 47.3%), and quality of life was improved in a subset of patients with EGFR-mutated tumors (8).

Erlotinib was shown to be advantageous in the first-line setting in the phase III EURTAC trial, where erlotinib-treated patients with EGFR mutation-positive tumors experienced progression free survival (PFS) of 9.7 months as compared to 5.2 months (HR = 0.37; $P < 0.0001$) in those patients treated with a platinum-based doublet such as docetaxel or gemcitabine (11). Response rate was 58% in the erlotinib arm versus 15% in the chemotherapy arm ($P < 0.0001$).

Afatinib has been shown to be superior to chemotherapy in the first line, in both the LUX-LUNG 3 (12) and the LUX-LUNG 6 (10) trials. LUX-LUNG3 was a phase III trial comparing afatinib versus chemotherapy (cisplatin/pemetrexed) as first-line treatments in chemo-naïve, NSCLC patients with EGFR mutation-positive tumors. LUX-LUNG 3 demonstrated that in the overall study population, median PFS was significantly longer with afatinib as compared to chemotherapy (11.1 versus 6.9 months; HR 0.58, 95% CI 0.43–0.78; $P = 0.0004$) (12). In patients with common EGFR mutation-positive tumors, median PFS was 13.6 versus 6.9 months on chemotherapy.

LUX-LUNG 6, a trial comparing afatinib with cisplatin/gemcitabine, confirmed that afatinib significantly improves PFS with a tolerable and manageable safety profile in Asian patients with advanced NSCLC who had tumors with EGFR mutations. In the overall study population, median PFS was significantly longer with afatinib as compared to chemotherapy (11.0 versus 5.6 months; HR 0.28, 95% CI 0.20–0.39; $P < 0.0001$) (10).

Selecting the TKI in this situation depends on many factors and is discussed in great detail elsewhere (13). Erlotinib and gefitinib are first generation TKIs, while afatinib and dacomitinib are second generation TKIs. Second generation TKIs differ from first generation. They block more ligands of the HER family. Perhaps more importantly, they are non-competitive inhibitors at the kinase site, so theoretically should prove to be more effective or confer a longer period to resistance than the first generation TKIs. We await the results and publication of several pivotal dacomitinib trials. Patient performance status, comorbidities, and age will all come into play in the decision making, as well as the availability of each therapeutic in a particular health authority. Unlike chemotherapy, TKIs can often be continued past progression in the lung cancer context, as long as there is a clinical benefit to the patient.

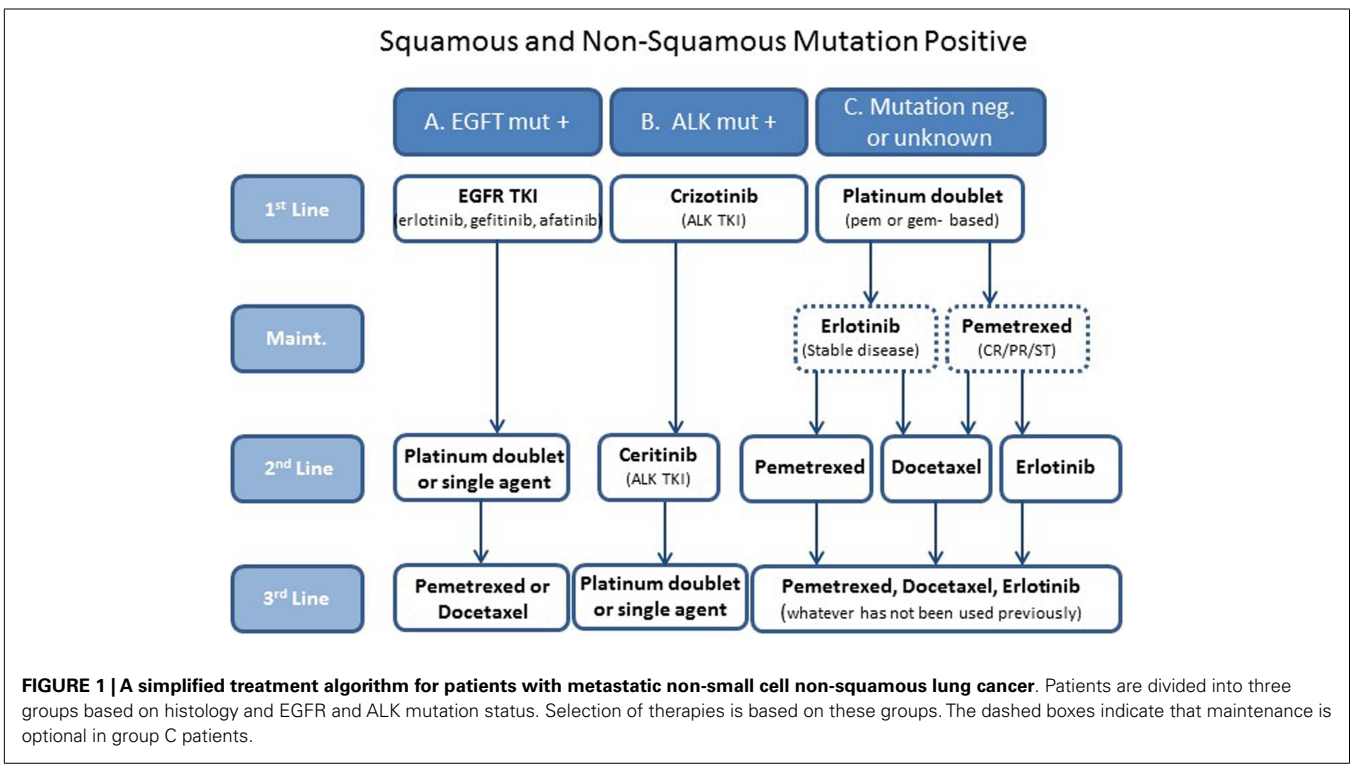


FIGURE 1 | A simplified treatment algorithm for patients with metastatic non-small cell non-squamous lung cancer. Patients are divided into three groups based on histology and EGFR and ALK mutation status. Selection of therapies is based on these groups. The dashed boxes indicate that maintenance is optional in group C patients.

SECOND-LINE THERAPY (FIRST-LINE SYSTEMIC THERAPY)

For all mutation-positive patients, the second-line therapy is the standard chemotherapy: a platinum doublet such as platinum/pemetrexed for four to six cycles (1). A single agent, such as pemetrexed, is an option for patients who are elderly or who may have a poor performance status and are not candidates for a platinum doublet. After second-line therapy, the patient is observed until progression.

THIRD LINE THERAPY

Selection of third line therapy in these patients is straightforward; the single agent that has not been used so far. In most cases, this will be either docetaxel or pemetrexed to be continued until disease progression. After disease progression, patients with adequate performance status may be considered for clinical trials.

GROUP B: ANAPLASTIC LYMPHOMA KINASE MUTATION POSITIVE

FIRST-LINE THERAPY

Anaplastic lymphoma kinase gene rearrangements are found more commonly in adenocarcinomas than other types of lung cancers, and also found more commonly in light smokers or non-smokers. ALK gene rearrangements are thought to be exclusive of EGFR and KRAS mutations and occur in approximately 4–7% of lung cancers (6).

Patients with chromosomal rearrangements of the ALK gene have shown to have a stronger clinical response to crizotinib, an ALK-targeted TKI. A phase I trial in patients with advanced ALK-positive NSCLC demonstrated that crizotinib is associated with higher response rates and improved survival compared to that of crizotinib-naive controls (14), and as a result, received approval from FDA in the US and Health Canada in 2011 for use in this patient population.

Crizotinib was shown to be superior to standard chemotherapy in ALK mutation-positive pre-treated patients with advanced NSCLC (median PFS 7.7 months in the crizotinib group versus 3.0 months in the chemotherapy group (HR, 0.49; 95% CI, 0.37–0.64; $P < 0.001$); response rates 65% (95% CI, 58–72) for crizotinib versus 20% (95% CI, 14–26) with chemotherapy ($P < 0.001$) (15). Although this trial was conducted in pre-treated patients, using a drug that specifically inhibits the ALK pathway is perfect rationale to provide this treatment in the first-line. NCCN guidelines have recommended a first-line approach. Newly released results from the PROFILE 1014 phase III trial showed that crizotinib significantly prolonged PFS as compared to platinum-based chemotherapy in a first-line setting. (Available at: http://www.marketwatch.com/story/pfizer-reports-positive-phase-3-study-outcome-of-xalkori-crizotinib-compared-to-chemotherapy-in-previously-untreated-patients-with-alk-positive-advanced-non-small-cell-lung-cancer-nsclc-2014-03-25?reflink=MW_news_stmp. Accessed on April 4, 2014).

As with the other TKIs, crizotinib is often continued past progression as long as there is a clinical benefit to the patient.

SECOND AND THIRD LINE THERAPY

Advanced NSCLC patients positive for the ALK mutation now have a new second-line agent (16). In April 2014, the FDA approved ceritinib for patients with ALK-positive NSCLC following treatment with crizotinib. The addition of this new ALK-targeted TKI into the ALK mutation-positive treatment paradigm pushes the use of a platinum doublet or single agent into the third line. As the treatment of ALK-positive patients evolves, we can expect treatment paradigms to continue to shift.

GROUP C: MUTATION STATUS NEGATIVE OR UNKNOWN

FIRST LINE

Patients with advanced NSCLC who have no known mutations in the EGFR or ALK genes or whose mutation status is unknown, receive the standard of care: a platinum doublet featuring pemetrexed or gemcitabine for four to six cycles. While there are many doublets to choose from in the first line including cisplatin/paclitaxel, cisplatin/gemcitabine, cisplatin/docetaxel, carboplatin/paclitaxel (1), the pivotal Scagliotti trial (17) demonstrated that patients with adenocarcinoma fare better with cisplatin/pemetrexed than cisplatin/gemcitabine in the first line (OS 12.6 versus 10.9 months; HR, 0.84; 95% CI, 0.71–0.99; superiority $P = 0.033$).

ANOTHER DECISION POINT: MAINTENANCE AFTER FIRST LINE

Maintenance therapy is the focus of another article in this journal (18). This therapeutic approach is important enough that it will be addressed in this article as well, albeit briefly.

Maintenance therapy in NSCLC is defined as a therapeutic agent that is administered after completion of the first line, but before the disease progresses. Results suggest that NSCLC patients may be more likely to receive additional therapy if maintenance is offered immediately after front-line therapy, before progression occurs (19–21). A recent meta-analysis of 13 maintenance chemotherapy trials demonstrated an improvement in PFS and in OS in patients who had experienced maintenance therapy (22). The most promising strategies involved administering an approved second-line NSCLC therapeutic for maintenance therapy (23, 24).

There are two types of maintenance therapy to consider, continuous and switch maintenance. Continuous maintenance is when patients are offered one of the agents in the induction doublet to be continued after first-line therapy until progression. This is an option for patients who have not progressed on first line. The PARAMOUNT trial demonstrated that pemetrexed maintenance given to NSCLC patients with tumors having non-squamous histology after first-line platinum/pemetrexed had a significantly reduced risk of disease progression over placebo (20). Switch maintenance, also referred to as "early second line," is when a new agent is given after the completion of four cycles of first-line-doublet. Studies have shown that both pemetrexed (19) and erlotinib (21) improve both PFS and OS when administered as maintenance therapy after first-line chemotherapy is completed.

To ask our patients to take maintenance therapy requires careful discussion and consideration. Residual nausea, fatigue, and alopecia from chemotherapy can take time to resolve, and many patients may choose to have drug holiday after 3–4 months of a platinum regimen. Many may refuse maintenance therapy as it requires monitoring visits in addition to treatment. Patients who decline maintenance therapy should be observed closely until progression so that they may receive another line of therapy.

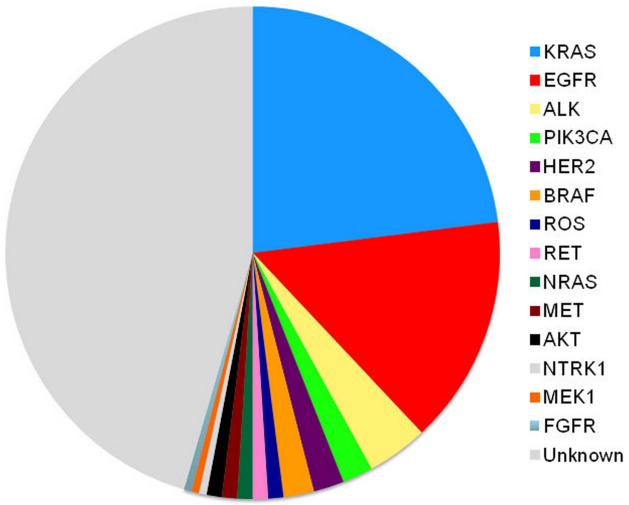


FIGURE 2 | Oncogenic drivers in lung adenocarcinoma (27–29).

SECOND LINE

Proven second-line options for patients whose tumors are mutation negative or mutation unknown, include docetaxel (23), erlotinib (25), and pemetrexed (26). Pemetrexed can only be offered if it was not used in first-line or maintenance therapy. If a pemetrexed platinum doublet was selected in the first line or for maintenance, docetaxel or erlotinib is selected for the second line.

The BR-21 trial demonstrated that erlotinib prolongs survival in patients with NCSLC following the failure of first-line or second-line chemotherapy (25). This multicenter, randomized, controlled, Phase III study randomized patients who had failed first- or second-line chemotherapy to either erlotinib or to placebo. Patient selection was not based on EGFR status, gender, smoking history, or type of NSCLC. The study met its primary endpoint of improving OS (median OS of 6.7 versus 4.7 months (HR, 0.70; 95 CI, 0.58–0.85; $P < 0.001$), and demonstrated statistically significant effects in secondary endpoints including PFS, time to symptom deterioration, and response rate. Overall, 8.9% of patients achieved an objective response to erlotinib ($P < 0.001$); the median duration of response was 34.2 weeks. This trial demonstrated a survival benefit in all patients regardless of EGFR mutation status or histology (25). Although still controversial, BR-21 led to an EGFR TKI to become standard of care in second and third line in unselected patients with NSCLC.

THIRD LINE

Third line therapies for mutation negative or mutation unknown patients may include whatever agents were not given in previous lines. This may include docetaxel (23), erlotinib (25), and pemetrexed (26). A significant limitation of therapy selection is that few trials have tested these different agents in later therapy, and sequences and combinations of these therapies have not been tested. Third line therapy is continued until disease progression or undue toxicity. After disease progression patients with adequate performance status may be considered for clinical trials.

CONCLUSION

Although we test for EGFR and ALK mutations and have treatments for those patients, therapy is still palliative in nature. Chemotherapy still remains our therapeutic backbone. However, the treatment algorithm will always be changing. As we continue to define the drivers of thoracic malignancy (**Figure 2**), our discovery and understanding of mutations in non-squamous, NSCLC will evolve. We will combine different targeted agents to overcome the development of resistance and will learn about the best ways to sequence these agents. Physicians should aim to provide three lines of therapy to patients. The discovery of new molecular targets and the development of targeted therapy ultimately benefit the patients with NSCLC.

REFERENCES

1. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* (2002) **346**(2):92–8. doi:10.1056/NEJMoa011954
2. Gazdar AF, Linnoila RI. The pathology of lung cancer – changing concepts and newer diagnostic techniques. *Semin Oncol* (1988) **15**(3):215–25.
3. Dubinski W, Leighl NB, Tsao M-S, Hwang DM. Ancillary testing in lung cancer diagnosis. *Pulm Med* (2012) **2012**:1–8. doi:10.1155/2012/249082
4. Ellis PM, Blais N, Soulieres D, Ionescu DN, Kashyap M, Liu G, et al. A systematic review and Canadian consensus recommendations on the use of biomarkers in the treatment of non-small cell lung cancer. *J Thorac Oncol* (2011) **6**(8):1379–91. doi:10.1097/JTO.0b013e318220cb8e
5. Vincent MD, Kuruvilla MS, Leighl NB, Kamel-Reid S. Biomarkers that currently affect clinical practice: EGFR, ALK, MET, KRAS. *Curr Oncol* (2012) **19**(Suppl):1. doi:10.3747/co.19.1149
6. Janku F, Garrido-Laguna I, Petruzelka LB, Stewart DJ, Kurzrock R. Novel therapeutic targets in non-small cell lung cancer. *J Thorac Oncol* (2011) **6**(9):1601–12. doi:10.1097/JTO.0b013e31822944b3
7. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* (2012) **13**(3):239–46. doi:10.1016/S1470-2045(11)70393-X
8. Mok TSWY, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, et al. Gefitinib or carboplatin–paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* (2009) **361**(10):947–57. doi:10.1056/NEJMoa0810699
9. Yang JC, Hirsh V, Schuler M, Yamamoto N, O'Byrne KJ, Mok TS, et al. Symptom control and quality of life in LUX-Lung 3: a phase III study of afatinib or cisplatin/pemetrexed in patients with advanced lung adenocarcinoma with EGFR mutations. *J Clin Oncol* (2013) **31**(27):3342–50. doi:10.1200/JCO.2012.46.1764
10. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. *Lancet Oncol* (2014) **15**(2):213–22. doi:10.1016/S1470-2045(13)70604-1
11. Costa C, Molina-Vila MA, Drozdowskyj A, Gimenez-Capitan A, Bertran-Alamillo J, Karachaliou N, et al. The impact of EGFR T790M mutations and BIM mRNA expression on outcome in patients with EGFR-mutant NSCLC treated with erlotinib or chemotherapy in the randomized phase III EURTAC trial. *Clin Cancer Res* (2014) **3**:3. doi:10.1158/1078-0432.CCR-13-2233
12. Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol* (2013) **31**(27):3327–34. doi:10.1200/JCO.2012.44.2806
13. Melosky B. Review of EGFR TKIs in metastatic NSCLC, including ongoing trials. *Front Oncol* (2014) **4**:244. doi:10.3389/fonc.2014.00244
14. Shaw AT, Yeap BY, Solomon BJ, Riely GJ, Gainor J, Engelman JA, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. *Lancet Oncol* (2011) **12**(11):1004–12. doi:10.1016/S1470-2045(11)70232-7

15. Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, Ahn MJ, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med* (2013) **368**(25):2385–94. doi:10.1056/NEJMoa1214886
16. Shaw AT, Kim DW, Mehra R, Tan DS, Felip E, Chow LQ, et al. Ceritinib in ALK-rearranged non-small-cell lung cancer. *N Engl J Med* (2014) **370**(13):1189–97. doi:10.1056/NEJMoa1311107
17. Scagliotti G, Hanna N, Fossella F, Sugarman K, Blatter J, Peterson P, et al. The differential efficacy of pemetrexed according to NSCLC histology: a review of two phase III studies. *Oncologist* (2009) **14**(3):253–63. doi:10.1634/theoncologist.2008-0232
18. Blais N, Kassouf E. Maintenance therapies for non-small cell lung cancer. *Front. Oncol.* (2014) **4**:213. doi:10.3389/fonc.2014.00213
19. Ciuleanu TBT, Zielinski C, Kim JH, Krzakowski M, Laack E, Wu YL, et al. Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomised, double-blind, phase 3 study. *Lancet* (2009) **374**(9699):1432–40. doi:10.1016/S0140-6736(09)61497-5
20. Paz-Ares LG, de Marinis F, Dediu M, Thomas M, Pujol JL, Bidoli P, et al. PARAMOUNT: final overall survival results of the phase III study of maintenance pemetrexed versus placebo immediately after induction treatment with pemetrexed plus cisplatin for advanced nonsquamous non-small-cell lung cancer. *J Clin Oncol* (2013) **31**(23):2895–902. doi:10.1200/JCO.2012.47.1102
21. Cappuzzo FCT, Stelmakh L, Cicenas S, Szczésna A, Juhász E, Esteban E, et al. SATURN investigators. Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomised, placebo-controlled phase 3 study. *Lancet Oncol* (2010) **11**(6):521–9. doi:10.1016/S1470-2045(10)70112-1
22. Soon YY, Stockler MR, Askie LM, Boyer MJ. Duration of chemotherapy for advanced non-small-cell lung cancer: a systematic review and meta-analysis of randomized trials. *J Clin Oncol* (2009) **27**(20):3277–83. doi:10.1200/JCO.2008.19.4522
23. Shepherd FA, Dancey J, Ramlau R, Mattson K, Gralla R, O'Rourke M, et al. Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. *J Clin Oncol* (2000) **18**(10):2095–103.
24. Gentzler RD, Patel JD. Maintenance treatment after induction therapy in non-small cell lung cancer: latest evidence and clinical implications. *Ther Adv Med Oncol* (2014) **6**(1):4–15. doi:10.1177/1758834013510589
25. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* (2005) **353**(2):123–32. doi:10.1056/NEJMoa050753
26. Hanna N. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *J Clin Oncol* (2004) **22**(9):1589–97. doi:10.1200/JCO.2004.08.163
27. Sequist LV, Heist RS, Shaw AT, Fidias P, Rosovsky R, Temel JS, et al. Implementing multiplexed genotyping of non-small-cell lung cancers into routine clinical practice. *Ann Oncol* (2011) **22**(12):2616–24. doi:10.1093/annonc/mdr489
28. Bergethon K, Shaw AT, Ou SH, Katayama R, Lovly CM, McDonald NT, et al. ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol* (2012) **30**(8):863–70. doi:10.1200/JCO.2011.35.6345
29. Kris MG, Johnson BE, Kwiatkowski DJ, Iafrate AJ, Wistuba II, Aronson SL, et al. Identification of driver mutations in tumor specimens from 1,000 patients with lung adenocarcinoma: the NCI's Lung Cancer Mutation Consortium (LCMC). *J Clin Oncol* (2011) **29**: suppl; abstr CRA7506.

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The Review Editor Vera Hirsh declares that, despite having collaborated with author Barbara Melosky, the review process was handled objectively and no conflict of interest exists.

Received: 28 April 2014; accepted: 05 September 2014; published online: 29 September 2014.

*Citation: Melosky B (2014) Treatment algorithms for patients with metastatic non-small cell, non-squamous lung cancer. Front. Oncol. **4**:256. doi: 10.3389/fonc.2014.00256*

This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 27 June 2014
doi: 10.3389/fonc.2014.00157

Treatment paradigms for patients with metastatic non-small cell lung cancer, squamous lung cancer: first, second, and third-line

Abdulaziz Al-Farsi and Peter Michael Ellis*

Department of Oncology, Juravinski Cancer Centre, McMaster University, Hamilton, ON, Canada

Edited by:
Vera Hirsh, McGill University Health Centre, Canada

Reviewed by:
Sacha I. Rothschild, University Hospital Basel, Switzerland
Rachel E. Sanborn, Providence Cancer Center, USA

****Correspondence:***
Peter Michael Ellis, Department of Oncology, Juravinski Cancer Centre, McMaster University, 699 Concession Street, Hamilton, ON L8V 5C2, Canada
e-mail: peter.ellis@jcc.hhsc.ca

Historically, the treatment algorithm applied to non-small cell lung cancer (NSCLC) was the same for all histologic subtypes. However, recent advances in our understanding of the molecular profiles of squamous and non-squamous NSCLC have changed this perspective. Histologic subtype and the presence of specific molecular abnormalities have predictive value for response to and toxicity from therapy, as well as overall survival. For patients with squamous NSCLC, a platinum agent plus gemcitabine, or paclitaxel is recommended as first-line therapy. The role of epidermal growth factor receptor monoclonal antibodies is uncertain. Maintenance therapy is not widely recommended, although data exist for the use of erlotinib. The standard recommendation for second-line therapy is docetaxel and erlotinib should be considered as second or third-line therapy. There is ongoing research identifying molecular targets in squamous NSCLC and many agents are in early phase clinical trials. Immunotherapeutic approaches targeting programed death-1 receptor and its ligand (PD-L1) appear promising.

Keywords: non-small cell lung cancer, squamous cancer, molecular abnormalities, chemotherapy, EGFR inhibitors, immunotherapy, novel agents

INTRODUCTION

Historically, one simplified management algorithm was applied to all patients with non-small cell lung cancer (NSCLC). A platinum agent combined with paclitaxel, docetaxel, vinorelbine, or gemcitabine was recommended for first-line chemotherapy (1, 2). Second-line chemotherapy at the time of disease progression could include docetaxel (3, 4), or pemetrexed (5) and erlotinib was recommended as second/third-line therapy (6). There was no evidence that histologic subtype of NSCLC impacted on the response to, or survival gained from chemotherapy (7).

However, important differences exist between the major subtypes of NSCLC, which have prognostic and predictive value. There is evidence from some trials, that patients with squamous histology have worse overall survival (OS) than patients with adenocarcinoma (8). Additionally, qualitative interactions exist between histology and the efficacy of some treatments (9). Differences also exist in the molecular profile of squamous and non-squamous NSCLC (10). Mutations of the *Epidermal Growth Factor Receptor* (*EGFR*), or *Kirsten Rat Sarcoma* (*K-RAS*) genes are rare in squamous cancers (10). However, EGFR protein overexpression, or increased gene copy number occur commonly. Differences exist in the expression of thymidylate synthase (TS) (11). Higher messenger RNA (mRNA) and protein levels for TS are seen in squamous cancers compared to non-squamous cancers, although there is no direct clinical correlation between levels of TS mRNA and measures of response to treatment. Nevertheless, separate treatment algorithms for squamous cancers have evolved. In this review, we summarize the approach to the management of squamous NSCLC.

FIRST-LINE THERAPY FOR SQUAMOUS CANCERS

Randomized trials in the 1980s demonstrated that chemotherapy improved OS as well as quality of life (12). These initial studies of platinum-based chemotherapy did not observe any differential response, or survival, based on histologic subtype. Multiple subsequent studies comparing various platinum-based chemotherapy regimens demonstrated similar response rates and OS (1, 2, 13). A retrospective review by the Southwest Oncology Group (SWOG), of systemic therapy trials of anti-microtubule agents, also showed no differential effect in outcomes according to histologic subtype (7).

More recent data demonstrate that histologic subtype is predictive of a differential response rate, OS, or toxicity profile from certain systemic therapies (9, 14, 15). The JMDB trial randomized 1725 patients with NSCLC (all histologic subtypes), to six cycles of first-line chemotherapy with cisplatin and gemcitabine, or cisplatin and pemetrexed (16). The primary outcome for the trial showed that cisplatin and pemetrexed was non-inferior to cisplatin and gemcitabine (median OS 10.3 months for both arms, HR 0.94, 95% CI 0.84–1.05). A planned sub-group analysis demonstrated evidence of a qualitative interaction between treatment effect and histology (interaction $p = 0.0011$). OS was significantly improved for patients with non-squamous histology randomized to cisplatin and pemetrexed, versus cisplatin and gemcitabine (11.8 vs. 10.4 months, HR 0.81, 95% CI 0.70–0.94). However, in patients with squamous cancer, OS was significantly worse for patients randomized to cisplatin and pemetrexed (9.4 vs. 10.8 months, HR 1.23, 95% CI 1.0–1.53).

Similar evidence of an interaction between histology and treatment efficacy for pemetrexed was observed in the JMEN trial evaluating maintenance therapy with pemetrexed, following four cycles of platinum-based chemotherapy (17). The improvement in progression free survival (PFS) from maintenance pemetrexed was significantly greater in patients with non-squamous histology than squamous histology (HR 0.44, 95% CI 0.36–0.55 vs. HR 0.69, 95% CI 0.49–0.98; interaction $p = 0.036$). Similarly the observed improvement in OS was limited to patients with non-squamous histology (HR 0.70, 95% CI 0.56–0.88 vs. HR 1.07, 95% CI 0.77–1.50, interaction $p = 0.033$). A second large randomized trial of maintenance therapy following first-line platinum-based chemotherapy evaluated erlotinib (18). The SATURN trial, which included approximately 40% of patients with squamous cancers, showed a modest improvement in OS for patients randomized to maintenance erlotinib compared with placebo (12 vs. 11 months, HR 0.81, 95% CI 0.70–0.95). There was no evidence of any interaction between treatment and histology (squamous HR 0.86, 95% CI 0.68–1.10, adenocarcinoma HR 0.77, 95% CI 0.61–0.97).

Several trials have evaluated gemcitabine as maintenance therapy (19–21). Brodowicz et al. (20) randomized patients to maintenance gemcitabine or placebo, after four cycles of cisplatin and gemcitabine. There was a significant improvement in TTP, but no significant improvement in OS. In contrast, Belani et al. (19) found no improvement in PFS or OS, for continuation maintenance gemcitabine. Data were not analyzed according to histology. Lastly, the IFCT-0502 trial randomized patients to gemcitabine, erlotinib, or observation, after first-line cisplatin and gemcitabine (21). Improvements in PFS were observed for patients randomized to both gemcitabine and erlotinib, compared with observation, but no differences were observed in OS. For patients randomized to gemcitabine, the improvement in PFS seemed less for patients with adenocarcinoma than other histologies (HR 0.98 vs. 0.79). There is insufficient data to recommend maintenance gemcitabine in this setting.

The selection of first-line therapy for NSCLC is also influenced by differing toxicity profiles between the histologic subtypes. Major hemoptysis was observed in the randomized phase II trial evaluating the addition of bevacizumab to first-line carboplatin and paclitaxel (14). There was an excess risk of life threatening hemoptysis in patients with squamous cancers and these patients were excluded from subsequent trials of bevacizumab in NSCLC. More recently, an open-label single-arm phase II study, the BRIDGE trial, evaluated delaying bevacizumab until after the third cycle of carboplatin and paclitaxel, in 31 patients with squamous NSCLC (22). Severe pulmonary hemorrhage was observed in 1 patient and grade 3 toxicities occurred in 9 of 31 patients overall. The authors conclude that bevacizumab remains experimental therapy in patients with squamous NSCLC.

Additional trials evaluating other targeted agents have also shown worse outcomes for patients with squamous NSCLC. The ESCAPE trial randomized 926 patients with advanced NSCLC (all histologies) to carboplatin, paclitaxel with or without sorafenib (15). The study was discontinued early following an interim analysis demonstrating futility. In the final analysis of this trial, patients with squamous histology ($n = 223$) randomized to chemotherapy plus sorafenib had worse OS than patients randomized to chemotherapy alone (8.9 vs. 13.6 months, 95% CI 1.22–2.81), as well as lower PFS (4.3 vs. 5.8 months, HR 1.31 95% CI 0.94–1.83).

However, for some agents, squamous histology appears to predict better outcomes from treatment. The BMS-099 phase III trial randomized patients with advanced NSCLC to carboplatin and paclitaxel or docetaxel, with or without cetuximab (23). Although the response rate was better for patients randomized to chemotherapy plus cetuximab (25.7 vs. 17.2%, $p = 0.007$), there was no difference in PFS, or OS. There was a trend to greater benefit from the addition of cetuximab to chemotherapy, in patients with squamous NSCLC (HR 0.7, 95% CI 0.47–1.05) compared to patients with adenocarcinoma (HR 0.9, 95% CI 0.71–1.14). A second phase III trial, the FLEX trial, evaluated the addition of cetuximab to cisplatin and vinorelbine chemotherapy in patients with advanced NSCLC demonstrating EGFR expression on immunohistochemistry (IHC) (24). There was a modest improvement in OS for patients randomized to chemotherapy plus cetuximab compared with chemotherapy alone (11.3 vs. 10.1 months, HR 0.871, 95% CI 0.762–0.996). There was a trend for greater benefit in patients with squamous histology (HR 0.8, 95% CI 0.64–1.0) compared to patients with adenocarcinoma (HR 0.94, 95% CI 0.77–1.15). However, a meta-analysis of four trials of first-line cetuximab plus chemotherapy in advanced NSCLC did not find clear evidence for a differential effect of treatment according to histology (25).

There does appear to be a differential effect of treatment according to histology for necitumumab, a second EGFR monoclonal antibody. The INSPIRE trial evaluating the addition of necitumumab to cisplatin and pemetrexed in non-squamous NSCLC patients, was stopped early because of an increased risk of thromboembolic complications in the necitumumab arm (26). However, a press release from Eli Lilly earlier in 2014, announced that a similar trial in patients with squamous NSCLC, had demonstrated improved OS for patients receiving cisplatin plus gemcitabine in combination with necitumumab, compared with cisplatin and gemcitabine alone. These data should be available during 2014.

The available data support adopting a different algorithm for first-line therapy of patients with squamous NSCLC compared with non-squamous histology. While cisplatin and pemetrexed appears to be the preferred chemotherapy in patients with non-squamous NSCLC, pemetrexed appears to be an ineffective drug in patients with squamous cancers. The JMBD trial did show that cisplatin and gemcitabine was superior chemotherapy in patients with squamous cancers (16). However, other trials, such as ECOG 1594, have demonstrated similar survival to cisplatin and gemcitabine from alternate chemotherapy regimens such as carboplatin and paclitaxel (2). Either one of these regimens would be an appropriate choice for first-line chemotherapy in patients with advanced squamous NSCLC. The role of EGFR monoclonal antibodies is somewhat uncertain. The benefit from cetuximab is very modest and there are additional toxicities. Therefore, it has not been widely implemented into first-line treatments. Data on necitumumab will need to be examined more closely once it is presented, in order to determine whether it should be incorporated into routine management of patients with squamous NSCLC. Finally, maintenance options in squamous NSCLC are limited. Pemetrexed is ineffective as maintenance therapy for patients with squamous cancers and

the benefit of erlotinib is modest. Therefore, maintenance therapy in this population is not routinely employed.

SECOND-LINE CHEMOTHERAPY FOR SQUAMOUS CANCERS

Second-line chemotherapy for NSCLC has been widely adopted since publication of two randomized trials in 2000 (3, 4). The TAX 317 trial randomized patients to docetaxel or best supportive care following first-line platinum chemotherapy (4), whereas the TAX 320 trial randomized patients to docetaxel vs. either vinorelbine, or ifosfamide (3). Both trials showed modest, but significant improvements in OS and the TAX 317 trial also demonstrated improvement in lung cancer related symptoms (27). A further trial compared second-line therapy with docetaxel to pemetrexed (JMEI) (5). This trial demonstrated that pemetrexed was non-inferior to docetaxel (median OS 8.3 vs. 7.9 months, HR 0.99, 95% CI 0.83–1.2). Secondary outcomes including response rate, time to progression, and duration of response were also similar between the two groups. Therefore, pemetrexed and docetaxel were both established as options for second-line chemotherapy for NSCLC.

A retrospective analysis was subsequently undertaken of the JMEI trial to look for an interaction between treatment effect and histology (8, 9). Similar to data in the first-line setting, there was a qualitative interaction between histology and treatment effect. Patients with non-squamous histology treated with pemetrexed, had significantly longer survival (9.3 vs. 8.0 m, HR 0.78 95% CI 0.60–1.02, interaction $p = 0.04$). On the other hand, patients with squamous histology who received pemetrexed had inferior survival (6.2 vs. 7.4 months, HR 1.56 95% CI 1.08–2.26). This analysis primarily showed that patients with squamous NSCLC treated with pemetrexed had an inferior outcome.

These data support a differential approach to second-line chemotherapy according to histology. Docetaxel is recommended as second-line chemotherapy in patients with squamous type NSCLC, whereas pemetrexed is recommended for patients with non-squamous NSCLC. However, due to the adoption of pemetrexed in the first-line setting, docetaxel remains an option for second-line chemotherapy, in patients with non-squamous histology.

ERLOTINIB THERAPY IN PATIENTS WITH SQUAMOUS CANCERS

Many patients with advanced NSCLC are still candidates for further systemic therapy, at the time of progression on second-line chemotherapy. There is little evidence though, to support the use of a third-line of chemotherapy. However, the results of the BR21 trial of erlotinib vs. best supportive care support the use of erlotinib as second or third-line therapy (6). This trial enrolled 731 patients, including patients with poor performance status (ECOG 2 and 3), who had previously received one or two lines of chemotherapy (49% received two prior lines of chemotherapy). The trial demonstrated that erlotinib significantly improved PFS (2.2 vs. 1.8 months, HR 0.61, 95% CI 0.51–0.74) and OS (6.7 vs. 4.7 months, HR 0.70, 95% CI 0.58–0.85). In addition, time to deterioration in cough, dyspnea, and pain were all significantly improved for patients randomized to erlotinib. The benefit of erlotinib was similar in both second and third-line settings.

Since publication of the BR21 trial, there has been a considerable body of evidence demonstrating that the presence of activating mutations of the *EGFR* gene are strongly predictive of benefit from an EGFR tyrosine kinase inhibitor (TKI) (28). It has been argued that the benefit of an EGFR TKI is limited to such patients with an *EGFR* mutation. Given that *EGFR* mutations are rare in patients with squamous cancers, it has also been argued that EGFR TKIs are ineffective in this group of patients as well. However, the data from both BR21 and the SATURN trial of maintenance erlotinib (18), demonstrate that patients who are *EGFR* wild type also benefit from EGFR TKIs, although the magnitude of benefit may be smaller. Similarly, available data do not support the contention that patients with squamous cancers fail to benefit from an EGFR TKI. In the BR21 trial, 31% of patients had squamous histology. The magnitude of benefit from erlotinib appeared similar in patients with squamous cancers (HR 0.8, 95% CI 0.6–1.0) and patients with adenocarcinoma (HR 0.7, 95% CI 0.6–0.9). Therefore, erlotinib should be considered as a second or third-line option for treatment in patients with squamous NSCLC. To date no other agents have be shown to improve survival in this setting.

FUTURE DIRECTIONS

The molecular profile of adenocarcinomas of the lung has been well described (29). Common mutations in lung adenocarcinoma include *K-RAS*, *EGFR*, as well as translocations of the *Anaplastic Lymphoma Kinase (ALK)* gene. However, there has been less research characterizing molecular abnormalities in lung cancer patients with squamous tumors. The cancer genome atlas (TCGA) network published data on genomic and epigenetic analysis of 178 squamous NSCLC patients from across the world (30). The data suggest squamous NSCLC's are genetically complex tumors. A high proportion of squamous cancers (171 of 178) contain one or more mutations in tyrosine kinases, serine/threonine kinases, PI3K catalytic and regulatory subunits, nuclear hormone receptors, G protein-coupled receptors, proteases, and tyrosine phosphatases. Common molecular abnormalities include mutations and amplification of *fibroblast growth factor receptor gene* (*FGFR*), mutations of *phosphatidylinositol 3-kinase, catalytic subunit alpha gene (PIK3CA)*, *phosphatase and tensin homolog gene (PTEN)*, *discoid domain receptor 2 gene* (*DDR2*), *BRAF*, as well as *EGFR* amplification (31). This would suggest that squamous cancers have a different molecular phenotype, hence the need for a separate treatment algorithm. Multiple clinical trials are ongoing evaluating agents targeting these molecular abnormalities, which will further refine the treatment algorithm in squamous NSCLC.

Immune based therapies also appear to be gaining momentum in NSCLC. There is considerable interest in immune checkpoint regulation in NSCLC. In particular, therapy directed toward the programed death-1 receptor (PD-1), or its ligand PD-L1 has shown considerable promise in early phase clinical trials. Phase I trials of several PD-1 receptor monoclonal antibodies, including nivolumab (32), MK-3475 (33), as well as the PD-L1 antibody MPDL-3280A (34) have all shown evidence of anti-tumor activity in heavily treated NSCLC patients. Approximately 20% of patients have shown objective tumor responses and many of these responses have been durable beyond 1 year. Response rates of 60–70% were observed in patients with tumors expressing PD-L1,

although there is a need to develop valid and reliable methods of assessment of this. Of note, the response rates among patients with squamous cancers appear to be higher than response rates in patients with adenocarcinoma, although it is unclear whether histology or some underlying molecular difference may account for these findings. Data also exist for the CTLA-4 antibody, ipilimumab. A randomized phase II trial of chemotherapy with six cycles of carboplatin and paclitaxel, chemotherapy plus ipilimumab in cycles one to four (concurrent) and chemotherapy plus ipilimumab in cycles three to six (phased). Patients randomized to carboplatin, paclitaxel, and phased ipilimumab had improved PFS compared with the control arm (HR 0.72, 95% CI 0.50–1.06, $p = 0.05$). These data require confirmation in phase III trials. The data suggest patients with squamous cancers may have more benefit from the addition of ipilimumab (HR 0.55, 95% CI 0.27–1.12) than patients with non-squamous cancers (HR 0.81, 95% CI 0.53–1.26).

CONCLUSION

It is clear that a different treatment algorithm has emerged for patients with squamous NSCLC. In the first-line setting platinum doublets involving gemcitabine, or potentially paclitaxel should be considered. The role of EGFR monoclonal antibodies such as cetuximab and necitumumab, require further clarification. Treatment options beyond progression of first-line therapy include docetaxel and erlotinib. Multiple trials are currently examining new therapies targeting the common molecular abnormalities observed in squamous lung cancers.

REFERENCES

1. Scagliotti GV, de Marinis F, Rinaldi M, Crinò L, Gridelli C, Ricci S, et al. Phase III randomized trial comparing three platinum-based doublets in advanced non-small-cell lung cancer. *J Clin Oncol* (2002) **20**:4285–91. doi:10.1200/JCO.2002.02.068
2. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* (2002) **346**:92–8. doi:10.1056/NEJMoa011954
3. Fossella FV, DeVore R, Kerr RN, Crawford J, Natale RR, Dunphy F, et al. Randomized phase III trial of docetaxel versus vinorelbine or ifosfamide in patients with advanced non-small-cell lung cancer previously treated with platinum-containing chemotherapy regimens. The TAX 320 non-small cell lung cancer study group. *J Clin Oncol* (2000) **18**:2354–62.
4. Shepherd FA, Dancey J, Ramlau R, Mattson K, Gralla R, O'Rourke M, et al. Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. *J Clin Oncol* (2000) **18**:2095–103.
5. Hanna N, Shepherd FA, Fossella FV, Pereira JR, De Marinis F, von Pawel J, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *J Clin Oncol* (2004) **22**:1589–97. doi:10.1200/JCO.2004.08.163
6. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Huat Tan E, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* (2005) **353**:123–32. doi:10.1056/NEJMoa050753
7. Kelly K, Chansky K, Mack PC, Lara PNJr, Hirsch FR, Franklin WA, et al. Chemotherapy outcomes by histologic subtype of non-small cell lung cancer (NSCLC): analysis of the SWOG database for antimicrotubule-platinum therapy. *J Thorac Oncol* (2009) **4**:B2.7. doi:10.1016/j.cllc.2013.06.010
8. Peterson P, Park K, Fossella F, Gatzemeier U, John W, Scagliotti GV, et al. Is pemetrexed more effective in adenocarcinoma and large cell lung cancer than in squamous cell carcinoma? A retrospective analysis of a phase III trial of pemetrexed vs docetaxel in previously treated patients with advanced non-small cell lung cancer (NSCLC). *J Thorac Oncol* (2007) **2**:2–328. doi:10.1097/01.JTO.0000284677.33344.62
9. Scagliotti G, Hanna N, Fossella F, Sugarman K, Blatter J, Peterson P, et al. The differential efficacy of pemetrexed according to NSCLC histology: a review of two phase III studies. *Oncologist* (2009) **14**:253–63. doi:10.1634/theoncologist.2008-0232
10. Rekhtman N, Paik PK, Arcila ME, Tafe LJ, Oxnard GR, Moreira AL, et al. Clarifying the spectrum of driver oncogene mutations in biomarker-verified squamous carcinoma of lung: lack of EGFR/KRAS and presence of PIK3CA/AKT1 mutations. *Clin Cancer Res* (2012) **18**:1167–76. doi:10.1158/1078-0432.CCR-11-2109
11. Ceppi P, Volante M, Saviozzi S, Rapa I, Novello S, Cambieri A, et al. Squamous cell carcinoma of the lung compared with other histotypes shows higher messenger RNA and protein levels for thymidylate synthase. *Cancer* (2006) **107**:1589–96. doi:10.1002/cncr.22208
12. Rapp E, Pater JL, Willan A, Cormier Y, Murray N, Evans WK, et al. Chemotherapy can prolong survival in patients with advanced non-small-cell lung cancer – report of a Canadian multicenter randomized trial. *J Clin Oncol* (1988) **6**:633–41.
13. Kelly K, Crowley J, Bunn PA Jr, Presant CA, Grevstad PK, Moinpour CM, et al. Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non-small-cell lung cancer: a Southwest Oncology Group trial. *J Clin Oncol* (2001) **19**:3210–8.
14. Johnson DH, Fehrenbacher L, Novotny WF, Herbst RS, Nemunaitis JJ, Jablons DM, et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol* (2004) **22**:2184–91. doi:10.1200/JCO.2004.11.022
15. Scagliotti G, Novello S, von Pawel J, Reck M, Pereira JR, Thomas M, et al. Phase III study of carboplatin and paclitaxel alone or with sorafenib in advanced non-small-cell lung cancer. *J Clin Oncol* (2010) **28**:1835–42. doi:10.1200/JCO.2009.26.1321
16. Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* (2008) **26**:3543–51. doi:10.1200/JCO.2007.15.0375
17. Ciuleanu T, Brodowicz T, Zielinski C, Hang Kim J, Krzakowski M, Laack E, et al. Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomised, double-blind, phase 3 study. *Lancet* (2009) **374**:1432–40. doi:10.1016/S0140-6736(09)61497-5
18. Cappuzzo F, Ciuleanu T, Stelmakh L, Cicenas S, Szczésna A, Juhász E, et al. Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomised, placebo-controlled phase 3 study. *Lancet Oncol* (2010) **11**:521–9. doi:10.1016/S1470-2045(10)70112-1
19. Belani CP, Waterhouse DM, Ghazal H, Ramalingam SS, Bordoni R, Greenberg R, et al. Phase III study of maintenance gemcitabine (G) and best supportive care (BSC) versus BSC, following standard combination therapy with gemcitabine-carboplatin (G-Cb) for patients with advanced non-small cell lung cancer (NSCLC). *ASCO Meet Abs* (2010) **28**:7506.
20. Brodowicz T, Krzakowski M, Zwitter M, Tzekova V, Ramlau R, Ghilezan N, et al. Cisplatin and gemcitabine first-line chemotherapy followed by maintenance gemcitabine or best supportive care in advanced non-small cell lung cancer: a phase III trial. *Lung Cancer* (2006) **52**:155–63. doi:10.1016/j.lungcan.2006.01.006
21. Pérol M, Chouaid C, Pérol D, Barlési F, Gervais R, Westeel V, et al. Randomized, phase III study of gemcitabine or erlotinib maintenance therapy versus observation, with predefined second-line treatment, after cisplatin-gemcitabine induction chemotherapy in advanced non-small-cell lung cancer. *J Clin Oncol* (2012) **30**:3516–24. doi:10.1200/JCO.2011.39.9782
22. Hainsworth JD, Fang L, Huang JE, Karlin D, Russell K, Faoro L, et al. BRIDGE: an open-label phase II trial evaluating the safety of bevacizumab + carboplatin/paclitaxel as first-line treatment for patients with advanced, previously untreated, squamous non-small cell lung cancer. *J Thorac Oncol* (2011) **6**(109–14):doi:10.1097/JTO.0b013e3181f94ad4
23. Lynch TJ, Patel T, Dreisbach L, McCleod M, Heim WJ, Hermann RC, et al. Cetuximab and first-line taxane/carboplatin chemotherapy in advanced non-small-cell lung cancer: results of the randomized multicenter phase III trial BMS099. *J Clin Oncol* (2010) **28**:911–7. doi:10.1200/JCO.2009.21.9618

24. Pirker R, Pereira JR, Szczesna A, von Pawel J, Krzakowski M, Ramlau R, et al. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. *Lancet* (2009) **373**:1525–31. doi:10.1016/S0140-6736(09)60569-9
25. Pujol JL, Pirker R, Lynch TJ, Butts CA, Rosell R, Shepherd FA, et al. Chemotherapy (CT) plus cetuximab as 1st-line treatment for advanced non small cell lung cancer (NSCLC): meta-analysis of individual patients data. *Ann Oncol* (2010) **21**:436. doi:10.1016/j.lungcan.2013.11.006
26. Paz-Ares L, Mezger J, Ciuleanu T, Fischer JR, Von Pawel J, Provencio M, et al. Randomized phase-3 trial (INSPIRE) of necitumumab plus cisplatin-pemetrexed versus cisplatin-pemetrexed alone as first-line therapy in stage IV non-squamous NSCLC. *J Thorac Oncol* (2013) **8**(Suppl 2):O03.2.
27. Dancey J, Shepherd FA, Gralla RJ, Kim YS. Quality of life assessment of second-line docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy: results of a prospective, randomized phase III trial. *Lung Cancer* (2004) **43**:183–94. doi:10.1016/j.lungcan.2003.09.001
28. Ellis PM, Blais N, Soulieres D, Ionescu DN, Kashyap M, Liu G, et al. A systematic review and Canadian consensus recommendations on the use of biomarkers in the treatment of non-small cell lung cancer. *J Thorac Oncol* (2011) **6**:1379–91. doi:10.1097/JTO.0b013e318220cb8e
29. Kris MG, Johnson BE, Kwiatkowski DJ, Iafrate AJ, Wistuba II, Aronson SL, et al. Identification of driver mutations in tumor specimens from 1,000 patients with lung adenocarcinoma: the NCI's lung cancer mutation consortium (LCMC). *ASCO Meet Abs* (2011) **29**:CRA7506.
30. The Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. *Nature* (2012) **489**:519–25. doi:10.1038/nature11404
31. Hammerman P, Sivachenko A, Pho N, Cherniak A, Ramos A, Getz G, et al. Genomic characterization and targeted therapeutics in squamous cell lung cancer. *J Thorac Oncol* (2011) **6**(Suppl 2):PRS.1.
32. Brahmer JR, Horn L, Antonia SJ, Spigel DR, Gandhi L, Sequist LV, et al. Nivolumab (anti-PD-1; BMS-936558; ONO-4538) in patients with non-small cell lung cancer (NSCLC): overall survival and long-term safety in a phase 1 trial. *J Thorac Oncol* (2013) **8**(Suppl 2):MO18.03.
33. Garon EB, Balmanoukian A, Hamid O, Hui R Gandhi L, Leighl N, et al. Preliminary clinical safety and activity of MK-3475 monotherapy for the treatment of previously treated patients with non-small cell lung cancer (NSCLC). *J Thorac Oncol* (2013) **8**(Suppl 2):MO18.02.
34. Horn L, Herbst RS, Spigel D, Gettinger SN, Gordon MS, Hollebecque A, et al. An analysis of the relationship of clinical activity to baseline EGFR status, PDL1 expression and prior treatment history in patients with non-small cell lung cancer (NSCLC) following PD-L1 blockade with MPDL3280A (anti-PDL1). *J Thorac Oncol* (2013) **8**(Suppl 2):MO18.01.

Conflict of Interest Statement: Peter Michael Ellis has received honoraria from Eli Lilly and Roche for advisory board meetings. The other co-author reports no conflicts of interest.

Received: 25 April 2014; accepted: 04 June 2014; published online: 27 June 2014.
*Citation: Al-Farsi A and Ellis PM (2014) Treatment paradigms for patients with metastatic non-small cell lung cancer, squamous lung cancer: first, second, and third-line. Front. Oncol. **4**:157. doi: 10.3389/fonc.2014.00157*
This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 21 July 2014
doi: 10.3389/fonc.2014.00177

Chemotherapy in metastatic NSCLC – new regimens (pemetrexed, nab-paclitaxel)

Normand Blais*[1] *and Vera Hirsh*[2]*

[1] *Centre hospitalier de l'Université de Montréal (CHUM), Hôpital Notre-Dame, Montreal, QC, Canada*
[2] *McGill University Health Centre (MUHC), Royal Victoria Hospital, Montreal, QC, Canada*

Edited by:
Barbara Melosky, British Columbia Cancer Agency, Canada

Reviewed by:
Michael Thomas Mark, Kantonsspital Graubünden, Switzerland
Bo H. Chao, The Ohio State University, USA

****Correspondence:***
Vera Hirsh, McGill University Health Centre (MUHC), Royal Victoria Hospital, 687 Pine Avenue West, Montreal, QC H3A 1A1, Canada
e-mail: vera.hirsh@muhc.mcgill.ca

Platinum-based chemotherapy doublets have been the standard approach to first-line therapy for more than a decade. Many randomized trials testing new combinations have not been able to produce significant gains in patient outcomes when these studies have looked at an unselected patient population. The recognition of the biologic importance of histology and molecular features of lung cancer has dramatically impacted on patient care, as can be easily recognized by the advent of targeted therapy for molecularly defined lung cancers. Similarly, for lung cancers without recognized driver mutations, subgroup evaluations of trials-based histology has identified that some chemotherapy regimens offer greater benefit in the squamous cell or the non-squamous cell groups. Two such examples are nab-paclitaxel and pemetrexed. These have shown improved anti-tumor activity and a decreased toxicity profile compared to standard combinations. Preferential activity in histologic divided patient subgroups can allow the clinician to personalize his approach to care. The role of these two agents in the management of NSCLC will be described in this article.

Keywords: metastatic, non-small cell lung carcinoma, solvent-based paclitaxel, nab-paclitaxel, pemetrexed, histology, clinical trials

INTRODUCTION

The standard of care for the first-line treatment of advanced NSCLC without epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) mutations (about 80% of advanced NSCLC) remains a platinum-based doublet in patients with good performance status (PS) and no significant comorbidities. This includes third-generation cytotoxic agents (i.e., cisplatin plus gemcitabine or carboplatin plus paclitaxel) (1, 2). Until recently no platinum doublet has demonstrated superiority over another in the treatment of advanced NSCLC (3–10). Lately, histology has been shown to affect the treatment outcomes (1). Patients with non-squamous cell cancer (NSCC) currently have a variety of first-line treatment options (1). Testing for EGFR and ALK mutations and tailoring therapy accordingly are now accepted as a standard practice in patients with NSCLC (1). For these patients, current guidelines recommend targeted therapies as first-line treatment (1). However, about 60–90%, varying largely according to ethnicity and smoking status, of patients with NSCLC have wild-type EGFR. Several studies have demonstrated that EGFR mutations occur infrequently in patients with squamous cell carcinoma (11–14). ALK mutations/fusions have been observed in only 1–7% of patients (15–20). Because EGFR and ALK mutations occur infrequently in patients with squamous cell NSCLC, mutation testing is not recommended routinely with the exception of never smokers and mixed histologies (1, 21, 22). Overall, for patients with EGFR and Alk unmutated NSCLC, platinum-based chemotherapy regimens remain the standard of care in first-line therapy (1). Key phase III studies reporting outcomes with platinum-doublet regimens for patients with squamous histology have demonstrated a median overall survival (OS) ranging from 8.9 to 13.7 months (5, 23–26) whereas the NSCC population fared slightly better with an OS of 10.4–14.9 months. Similarly, recently reported trials have renewed the interest in adapting treatment based on histology. Two key examples of potential application of personalized therapy based on histology are nab-paclitaxel, which has shown improved results in the squamous cell cancer (SCC) population and pemetrexed, which has on the contrary, shown clear benefit in the NSCC patients. This mini-review will highlight these two unique drugs and how they can be incorporated into practice to improve outcomes in NSCLC therapy.

PEMETREXED

Pemetrexed is a folic acid derivative that inhibits both purine and pyrimidine synthesis by blocking three key metabolic enzymes involved in DNA synthesis: thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT) (27). Building on the efficacy of methotrexate in many human cancers, research focusing on the identification of more potent inhibitors of purine synthesis has led to the identification of the promising activity of pemetrexed in lung cancer in the late 1990s (27). Although several hundred trials have been conducted to evaluate the efficacy of pemetrexed in many cancer settings, the first pivotal study was reported by Hanna in 2004 (28). It is interesting to note that at this time in pemetrexed development, studies were conducted in unselected patient populations with NSCLC. Emerging science since 2003 (29) and in particular the study published by Ceppi et al. (30) in 2006, identified that chemonaïve patients with squamous carcinoma had tumors expressing higher levels of TS

than patients with adenocarcinoma. These seminal studies led to the re-evaluation of previously published as well as ongoing randomized trials to evaluate the interaction of histology with clinical efficacy in NSCLC. The demonstration of a consistent effect in NSCC and the lack of effect in SCC (**Table 1**) has eventually directed the registration process for pemetrexed use and dictated its current clinical use, which is now restricted to non-squamous histology. Interestingly, knowledge of anti-folate metabolism has also been able to decrease the incidence of pemetrexed toxicity by the addition of folic acid and vitamin B12 supplementation (31).

SECOND-LINE STUDIES

Initially published in 2004 (28) as a study showing similar efficacy between pemetrexed and docetaxel in unselected patients with NSCLC, Hanna et al. presented a subgroup analysis based on histology at the 12th WCLC in Seoul, Korea in 2007. The reported median OS in the NSCC population was 9.2 vs. 8.2 months (pemetrexed vs. docetaxel) and this was respectively 6.2 vs. 7.4 months in the SCC cohort. This translates to an adjusted HR for OS of 0.78 in favor of pemetrexed in the NSCC group and a HR of 1.56 in the SCC group. Results were similar for PFS, with the HR being 0.82 in the NSCC group and 1.40 in the SCC group. The statistical test demonstrating a quantitative interaction was positive for OS ($p = 0.001$) and PFS ($p = 0.004$) (32).

MAINTENANCE STUDIES

Three large randomized, placebo-controlled trials address the issue of pemetrexed maintenance and are discussed in the article of this issue of Frontiers related to maintenance therapy. As the PARAMOUNT (33) and AVAPERL (34) study are composed of an exclusively NSCC population, only JMEN is informative as to the histological interaction of pemetrexed therapy in this setting. The JMEN study, published in 2009 by Ciuleanu (35) was reported as a positive trial in an unselected population. The PFS after first-line therapy was 4.3 months in the pemetrexed group and 2.6 months in the placebo group (HR 0.50, $p < 0.001$). The corresponding OS values were 13.4 and 10.6 months (HR 0.79, $p = 0.012$). To validate, the biologic relevance of the histologic interaction previously observed in the Hanna study, Belani et al. reported this subgroup analysis at ASCO in 2009. This analysis convincingly supported the previously observed finding of a histological effect. Whereas PFS was indeed favorably impacted in the NSCC population (HR 0.47, $p < 0.0001$), this was not the case for SCC (HR 1.03, $p = 0.90$). Similar findings were found for OS (HR 0.70, $p = 0.002$ and HR 1.07, $p = 0.68$). From this moment on, pemetrexed use in the setting of SCC decreased substantially. The histologic effect of pemetrexed was major contributors to the major revolution in lung cancer care. A diagnosis of NSCLC (non-otherwise specified) is now considered suboptimal for patient care.

FIRST-LINE STUDIES

The JMDB study compared pemetrexed plus cisplatin with gemcitabine plus cisplatin in patients with advanced NSCLC (5). Similarly with other previously conducted trials evaluating pemetrexed, patients of all histologies were accrued to this trial that was published before the widespread acceptance of a clear histologic effect. Whereas, the overall study results did not appear to favor the pemetrexed plus cisplatin combination (OS HR 0.94, p = NS), subgroup analysis based on histology did show once again a significant interaction. The gemcitabine regimen produced a significantly longer median OS compared with pemetrexed regimen in patients with SCC (10.8 vs. 9.4 months, HR 1.23, $p = 0.05$). The opposite was seen in patients with NSCC (10.4 vs. 11.8 months, HR 0.84, $p = 0.011$). Similarly, PFS was impacted in a similar manner in the SCC group (HR 1.36, $p = 0.002$) and the NSCC group (HR 0.95, $p = 0.35$). The treatment-by-histology interaction test was positive for PFS and for OS.

One outlier in the pemetrexed–histology interaction story is the Gronberg trial published in 2009 (36). This smaller study

Table 1 | Pemetrexed-histology interaction in randomized clinical trials of NSCLC.

	First-line therapy ($n = 1725$)		Maintenance therapy ($N = 663$)		Second-line therapy ($N = 571$)	
	Squamous ($N = 473$)	Non-squamous ($N = 1252$)	Squamous ($N = 182$)	Non-squamous ($N = 481$)	Squamous ($N = 172$)	Non-squamous ($N = 399$)
Overall survival						
Adjusted HR	1.23	0.84	1.07	0.70	1.56	0.78
Superiority *p*	0.050	0.011	0.678	0.002	0.018	0.048
Interaction test *p*	0.002		0.033		0.001	
Progression-free survival						
Adjusted HR	1.36	0.95	1.03	0.47	1.40	0.82
Superiority *p*	0.002	0.349	0.896	<0.001	0.046	0.076
Interaction test *p*	0.002		0.036		0.004	

Adapted from Scagliotti et al. (32).

Hazard ratio <1 favors pemetrexed in non-squamous subgroups of all three studies.

Hazard ratio (HR) > 1.0 favors comparator arm in squamous subgroups of all three studies.

included 436 patients, including 248 patients with NSCC histology. Median OS was 7.3 months in the pemetrexed with carboplatin arm compared to 7.0 months in the gemcitabine with carboplatin arm ($p = 0.63$). Subgroup analysis was respectively 7.8 vs. 7.5 in patients with NSCC ($p = 0.77$) and not reported for the SCC subgroup. This trial was not designed to evaluate RR or PFS as radiological assessment was not mandatory. These seemingly inferior results may be due to the patient population, which included 22% of patients with PS 2 status, to the upfront 25% decrease in pemetrexed dose in the 18% of the population that was >75 years of age, to the use of carboplatin instead of cisplatin, or to the small sample size of this study.

Although the combination of carboplatin with pemetrexed and bevacizumab has never been formally compared with standard cisplatin and pemetrexed, the PointBreak (37) study compared another standard regimen – carboplatin plus paclitaxel and bevacizumab compared with carboplatin with pemetrexed and bevacizumab. This extensive, 939-patient, exclusively-NSCC trial failed to show a clearly superior regimen in terms of efficacy. The HR for OS was 1.00 and for PFS was 0.83 ($p = 0.012$) slightly favoring the pemetrexed triplet. The contribution of bevacizumab to a platinum-pemetrexed doublet has never been studied in a randomized trial.

SAFETY AND TOLERABILITY

The study by Hanna et al. (28) provides a good opportunity to compare pemetrexed and docetaxel's toxicity profile. In this study, Grade 3–4 hematologic toxicities were significantly worse with docetaxel: neutropenia (40.2 vs. 5.3%), febrile neutropenia (12.7 vs. 1.9%), compared to pemetrexed.

Non-hematologic toxicities (all grades) were relatively similar except for alopecia (37.7 vs. 6.4%) and diarrhea (24.3 vs. 12.8%) more frequently observed with docetaxel and ALT elevations (1.4 vs. 7.9%) more frequent with pemetrexed.

Relevant to second-line therapy decisions, there have been no large randomized trial comparing pemetrexed alone and an EGFR inhibitor in the second-line setting. A small phase II randomized trial did compare pemetrexed to erlotinib in EGFR mutation-negative but EGFR–FISH positive NSCLC (38). Although efficacy differences could not be demonstrated between both regimens, toxicity analysis did provide interesting observations. Few Grade 3–4 toxicities were described. For erlotinib and pemetrexed, respectively, rash was present in 3.3 and 0%; diarrhea in 1.6 and 0%; anorexia in 1.6 and 0%, and nausea in 0 and 3.2% (38). Compared to placebo in the Ciuleanu maintenance trial, patients having received four courses of a first-line platinum containing doublet rarely developed Grade 3–4 toxicities (all <5%) while on pemetrexed alone. The most frequent toxicities (mostly Grade 1–2) were fatigue (24 vs. 10%), anorexia (19 vs. 2%), and nausea (19 vs. 5%); all compared to placebo.

The first difference between cisplatin with pemetrexed and cisplatin with gemcitabine is its administration schedule in that the latter regimen requires a second visit to the chemotherapy suite on day 8 whereas the former is a day 1 infusion alone. The JMDB trial also showed some differences in the Grade 3–4 toxicity profile of both combinations. Compared with the pemetrexed regimen, the gemcitabine doublet was associated with more neutropenia (26.7 vs. 15.1%), febrile neutropenia (3.7 vs. 1.3%), anemia (7.6 vs. 4.8%) and thrombocytopenia (12.7 vs. 4.1%), and less anorexia (0.7 vs. 2.4%) and nausea (3.9 vs. 7.2%).

In the Gronberg trial, the use of pemetrexed and carboplatin was associated with less granulocytopenia (40 vs. 51%) and thrombocytopenia (24 vs. 56%) compared to carboplatin and gemcitabine. This was associated with a decrease in the transfusion of red blood cell (29 vs. 43%) and platelet (3 vs. 9%) transfusions with pemetrexed use (5).

The PointBreak trial showed clinically relevant differences in the Grade 3–4 toxicities of the studied regimens. The pemetrexed arm was associated with more anemia (14.5 vs. 2.7%), thrombocytopenia (23.3 vs. 5.6%), and fatigue (10.9 vs. 5.0%) whereas neutropenia (40.6 vs. 25.8%), febrile neutropenia (4.1 vs. 1.4%), sensory neuropathy (4.1 vs. 0%), and alopecia (36.8 vs. 6.6%) occurred more frequently in the paclitaxel triplet (37).

CONCLUSION

Pemetrexed use has shown consistent effects in favor of its use in the setting of non-squamous cell lung cancer. Compared with paclitaxel, gemcitabine, or docetaxel, its favorable efficacy, toxicity profile, and convenient schedule of administration makes this an agent of choice in this setting. As discussed in the article of this journal on maintenance therapy, data from the PARAMOUNT and AVAPERL trial lends increasing support for the first-line use of pemetrexed and its consideration for maintenance in non-progressing patients after induction therapy.

NAB-PACLITAXEL

Nab-paclitaxel is a 130 nm, albumin-bound formulation of the microtubule inhibitor paclitaxel (39), and is a solvent-free option for the first-line treatment of advanced NSCLC (39).

Solvents such as Cremophor EL and polysorbate 80 require specialized tubing for administration, which is not required for the administration of nab-paclitaxel (39–42). These solvents have intrinsic toxicities, i.e., hypersensitivity reactions requiring steroid/antihistamine pre-treatments, neuropathy, and excessive fluid retention (40, 43, 44). In a pre-clinical study, nab-paclitaxel demonstrated both enhanced endothelial cell binding and transport, and improved delivery of paclitaxel to tumors compared with solvent-based paclitaxel (45).

A phase III trial demonstrated overall response rates (ORRs) superior for nab-paclitaxel plus carboplatin compared with conventional solvent-based paclitaxel plus carboplatin in patients with advanced NSCLC (46). Patient populations, i.e., with squamous histology and those ≥70 years of age, had improved clinical outcomes with nab-paclitaxel plus carboplatin compared with solvent-based paclitaxel plus carboplatin (47, 48). No unexpected differences in toxicity were noted.

CLINICAL EFFICACY

In the multicenter, randomized, Phase III registrational trial, 1052 patients with advanced NSCLC were randomized to receive first-line weekly nab-paclitaxel (100 mg/m^2) plus carboplatin (AUC 6) every 3 weeks ($n = 521$) or solvent-based (sb) paclitaxel (200 mg/m^2) plus carboplatin (AUC 6) every 3 weeks ($n = 531$)

(46). Patients had to have stage IIIB/IV disease, ECOG PS of 0 or 1 and were previously untreated for metastatic NSCLC. Adjuvant chemotherapy was permitted if it was completed 12 months prior to study enrollment. The median age of the patients was 60 years, 75% were male, 81% were white, 73% were smokers, and 79% had stage IV disease. Patients in the nab-paclitaxel arm had a significantly greater ORR = the primary endpoint, compared with the sb-paclitaxel arm (33 vs. 25%, response rate ratio = 1.313, 95% CI = 1.082–1.593, $p = 0.005$). Patients on nab-paclitaxel had longer median PFS = 6.3 vs. 5.8 months, hazard ratio (HR) = 0.902, 95% CI = 0.767–1.060, $p = 0.214$). The median OS for nab-paclitaxel vs. sb-paclitaxel was 12.1 vs. 11.2 months (HR = 0.922, 95% CI = 0.797–1.066, $p = 0.271$), respectively.

An exploratory elderly subgroup analysis examined the efficacy and safety of these two regimens in patients ≥70 years of age enrolled in this phase III trial (46). In these patients ($n = 156$), the ORR in the nab-paclitaxel vs. sb-paclitaxel arms was 34 vs. 24% ($p = 0.196$). A trend toward improved PFS was also noted in elderly patients with nab-paclitaxel, 8.0 vs. 6.8 months, $p = 0.134$. Elderly patients in the nab-paclitaxel arm experienced an impressive 19.9 months median OS compared with 10.4 months in the sb-paclitaxel arm, $p = 0.009$.

Another analysis of this phase III trial examined efficacy of the regimens by histology (48). Patients with squamous NSCLC ($n = 450$), achieved a significantly higher ORR, $p < 0.001$, with nab-paclitaxel plus carboplatin (41%) vs. sb-paclitaxel plus carboplatin (24%) and a 1.2 month improvement in median OS (10.7 vs. 9.5 months, p = NS). A similar ORR was observed for nab-paclitaxel plus carboplatin vs. sb-paclitaxel in patients with non-squamous NSCLC ($n = 602$, 26 vs. 25%, p = NS), median OS was 13.1 vs. 13 months in each arm (p = NS). **Table 2** shows efficacy outcomes of the intent-to-treat (ITT) population as well as by age and histology from the phase III trial of nab-paclitaxel plus carboplatin in NSCLC.

SAFETY AND TOLERABILITY

The most common Grade 3–4 adverse events of the ITT population and select subgroups of the phase III trial are shown in Table S1 in Supplementary Material.

Pre-treatment with antihistamines and/or steroids is required for sb-paclitaxel and docetaxel to prevent hypersensitivity reactions but not for nab-paclitaxel (39, 42). Much of these reactions may be solvent-related because both Cremophor EL and polysorbate 80 have been associated with hypersensitivity reactions (40); nab-paclitaxel is not formulated with a chemical solvent (39). Taxanes are associated with the development of peripheral neuropathy (49). However, in the phase III trial of nab-paclitaxel plus carboplatin vs. sb-paclitaxel plus carboplatin, patients on nab-paclitaxel arm experienced significantly less grade 3–4 peripheral neuropathy compared with the sb-paclitaxel arm (46). Results based on the FACT-Taxane neuropathy, pain in hands/feet and hearing loss subscales demonstrated significantly less worsening of taxane-related symptoms in the nab-paclitaxel arm compared with the sb-paclitaxel arm, $p \leq 0.002$ for all (46, 50). The patient-reported symptom scores were consistent with physician assessments of peripheral neuropathy (50). In addition, patients in the nab-paclitaxel arm who experienced Grade 3–4 peripheral neuropathy experienced a faster median time-to-improvement to Grade 1 (38 vs. 104 days) compared to sb-paclitaxel, respectively.

Taxane use is frequently associated with increased muscle and joint pains (39, 42). In the phase III study, patients in the nab-paclitaxel arm experienced significantly less Grade 3–4 arthralgia and myalgia than patients in the sb-paclitaxel arm (46, 50). In the phase III trial, patients in the nab-paclitaxel arm experienced significantly less Grade 3–4 neutropenia, but *more* thrombocytopenia and anemia *than* patients in the sb-based arm (46, 50).

CONCLUSION

Nab-paclitaxel represents an important advancement especially as the treatment options for patients with squamous histology are

Table 2 | Select efficacy outcomes from the Phase III trial of nab-paclitaxel plus carboplatin in NSCLC.

Treatment	ITT (41)		≥70 years (42)		Histology (43)			
					SCC		NSCC	
	nab-P/C	sb-P/C	nab-P/C	sb-P/C	nab-P/C	sb-P/C	nab-P/C	sb-P/C
n	514	524	74	82	229	221	292	310
ORR (%)	33	25	34	24	41	24	26	25
Response rate ratio	1.313		1.385		1.680		1.034	
p-Value	0.005		0.196		<0.001		0.808	
Median PFS (months)	6.3	5.8	8.0	6.8	5.6	5.7	6.9	6.5
HR	0.902		0.687		0.865		0.933	
p-Value	0.214		0.134		0.245		0.532	
Median OS (months)	12.1	11.2	8.0	6.8	10.7	9.5	13.1	13.0
HR	0.922		0.583		0.890		0.950	
p-Value	0.271		0.009		0.284		0.611	

HR, hazard ratio; IIT, intent-to-treat; nab-P/C, nab-paclitaxel + carboplatin; NSCC, non-squamous cell carcinoma; NSCLC, non-small cell lung cancer; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; sb-P/C, solvent-based paclitaxel + carboplatin; SCC, squamous cell carcinoma.

limited and elderly patients are often undertreated due to toxicity concerns among other reasons. Based on these findings and its greater ease of administration, nab-paclitaxel plus carboplatin could be considered a first-line standard of care therapy in patients with advanced NSCLC. Targeted agents active for patients with squamous histology are in development and in the near future some of these agents could be assessed in combination with this regimen.

We must also select for each patient a treatment that is best suited to his individual comorbidities and treatment toxicities to ensure the best possible QOL during the last months of his life. Better safety and tolerability profile in addition to a greater RR makes nab-paclitaxel an excellent improvement to a paclitaxel combination for the first-line treatment of metastatic NSCLC especially for squamous cell carcinoma.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/Journal/10.3389/fonc.2014.00177/abstract

REFERENCES

1. Ettinger DS, Akerley W, Borghaei H, Chang AC, Cheney RT, Chirieac LR et al. Non–small cell lung cancer, version 2.2013. *J Natl Compr Canc Netw* (2013) **11**(6): 645–53.
2. Azzoli CG, Baker S Jr, Temin S, Pao W, Aliff T, Brahmer J, et al. American society of clinical oncology clinical practice guideline update on chemotherapy for stage IV non-small cell lung cancer. *J Clin Oncol* (2009) **27**(36):6251–66. doi:10.1200/JCO.2009.23.5622
3. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of four chemotherapy regimens for advanced non-small cell lung cancer. *N Engl J Med* (2002) **346**(2):92–8. doi:10.1056/NEJMoa011954
4. Kelly K, Crowley J, Bunn PA Jr, Presant CA, Grevstad PK, Moinpour CM, et al. Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non-small cell lung cancer: a Southwest Oncology Group trial. *J Clin Oncol* (2001) **19**(13):3210–8.
5. Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small cell lung cancer. *J Clin Oncol* (2008) **26**(21):3543–51. doi:10.1200/JCO.2007.15.0375
6. Scagliotti GV, De Marinis F, Rinaldi M, Crinò L, Gridelli C, Ricci S, et al. Phase III randomized trial comparing three platinum-based doublets in advanced non-small cell lung cancer. *J Clin Oncol* (2002) **20**(21):4285–91. doi:10.1200/JCO.2002.02.068
7. Fossella F, Pereira JR, von Pawel J, Pluzanska A, Gorbounova V, Kaukel E, et al. Randomized, multinational, phase III study of docetaxel plus platinum combinations versus vinorelbine plus cisplatin for advanced non-small cell lung cancer: the TAX 326 study group. *J Clin Oncol* (2003) **21**(16):3016–24. doi:10.1200/JCO.2003.12.046
8. Bonomi P, Kim K, Fairclough D, Cella D, Kugler J, Rowinsky E, et al. Comparison of survival and quality of life in advanced non-small cell lung cancer patients treated with two dose levels of paclitaxel combined with cisplatin versus etoposide with cisplatin: results of an Eastern Cooperative Oncology Group trial. *J Clin Oncol* (2000) **18**(3):623–31.
9. Gatzemeier U, von Pawel J, Gottfried M, ten Velde GP, Mattson K, de Marinis F, et al. Phase III comparative study of high-dose cisplatin versus a combination of paclitaxel and cisplatin in patients with advanced non-small cell lung cancer. *J Clin Oncol* (2000) **18**(19):3390–9.
10. Smit EF, van Meerbeeck JP, Lianes P, Debruyne C, Legrand C, Schramel F, et al. Three-arm randomized study of two cisplatin-based regimens and paclitaxel plus gemcitabine in advanced non-small cell lung cancer: a phase III trial of the European Organization for Research and Treatment of Cancer lung cancer group – EORTC 08975. *J Clin Oncol* (2003) **21**(21):3909–17. doi:10.1200/JCO.2003.03.195
11. Marchetti A, Martella C, Felicioni L, Barassi F, Salvatore S, Chella A, et al. EGFR mutations in non-small cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J Clin Oncol* (2005) **23**(4):857–65. doi:10.1200/JCO.2005.08.043
12. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small cell lung cancer to gefitinib. *N Engl J Med* (2004) **350**(21):2129–39. doi:10.1056/NEJMoa040938
13. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A* (2004) **101**(36):13306–11. doi:10.1073/pnas.0405220101
14. Paik PK, Hasanovic A, Wang L, Rekhtman N, Ladanyi M, Kris MG. Multiplex testing for driver mutations in squamous cell carcinomas of the lung. *J Clin Oncol* (2012) **30**(15 Suppl):Abstr 7505
15. Herbst RS, Heymach JV, Lippman SM. Lung cancer. *N Engl J Med* (2008) **359**(13):1367–80. doi:10.1056/NEJMra0802714
16. Kris MG, Johnson BE, Kwiatkowski DJ, Iafrate AJ, Wistuba II, Aronson SL, et al. Identification of driver mutations in tumor specimens from 1,000 patients with lung adenocarcinoma: the NCI's Lung Cancer Mutation Consortium (LCMC). *J Clin Oncol* (2011) **29**(18 Suppl):Abstr CRA-7506
17. Inamura K, Takeuchi K, Togashi Y, Nomura K, Ninomiya H, Okui M, et al. EML4-ALK fusion is linked to histological characteristics in a subset of lung cancers. *J Thorac Oncol* (2008) **3**(1):13–7. doi:10.1097/JTO.0b013e31815e8b60
18. Takeuchi K, Choi YL, Soda M, Inamura K, Togashi Y, Hatano S, et al. Multiplex reverse transcription-PCR screening for EML4-ALK fusion transcripts. *Clin Cancer Res* (2008) **14**(20):6618–24. doi:10.1158/1078-0432.CCR-08-1018
19. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, et al. Identification of the transforming EML4-ALK fusion gene in non-small cell lung cancer. *Nature* (2007) **448**(7153):561–6. doi:10.1038/nature05945
20. Saliba W, Corrales-Rodriquez L, Albadine R, Soulieres D, Weng X, Gorska I, et al. Retrospective analysis of the prevalence of NSCLC driver mutations in unselected samples. *J Thorac Oncol* (2013) **8**(Suppl 2):S672; Abstr P1.18-021, [poster].
21. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. *Nature* (2012) **489**(7417):519–25. doi:10.1038/nature11404
22. Rekhtman N, Paik PK, Arcila ME, Tafe LJ, Oxnard GR, Moreira AL, et al. Clarifying the spectrum of driver oncogene mutations in biomarker-verified squamous carcinoma of lung: lack of EGFR/KRAS and presence of PIK3CA/AKT1 mutations. *Clin Cancer Res* (2012) **18**(4):1167–76. doi:10.1158/1078-0432.CCR-11-2109
23. Pirker R, Pereira JR, Szczesna A, von Pawel J, Krzakowski M, Ramlau R, et al. Cetuximab plus chemotherapy in patients with advanced non-small cell lung cancer (FLEX): an open-label randomized phase III trial. *Lancet* (2009) **373**(9674):1525–31. doi:10.1016/S0140-6736(09)60569-9
24. Tan EH, Rolski J, Grodzki T, Schneider CP, Gatzemeier U, Zatloukal P, et al. Global lung oncology branch trial 3 (GLOB3): final results of a randomized multinational phase III study alternating oral and iv vinorelbine plus cisplatin versus docetaxel plus cisplatin as first-line treatment of advanced non-small cell lung cancer. *Ann Oncol* (2009) **20**(7):1249–56. doi:10.1093/annonc/mdn774
25. Scagliotti G, Novello S, von Pawel J, Reck M, Pereira JR, Thomas M, et al. Phase III study of carboplatin and paclitaxel alone or with sorafenib in advanced non-small cell lung cancer. *J Clin Oncol* (2010) **28**(11):1835–42. doi:10.1200/JCO.2009.26.1321
26. Lynch TJ, Patel T, Dreisbach L, McCleod M, Heim WJ, Hermann RC, et al. Cetuximab and first-line taxane/carboplatin chemotherapy in advanced non-small cell lung cancer: results of the randomized multicenter phase III trial BMS099. *J Clin Oncol* (2010) **28**(6):911–7. doi:10.1200/JCO.2009.21.9618
27. Rinaldi DA, Kuhn JG, Burris HA, Dorr FA, Rodriguez G, Eckhardt SG, et al. A phase I evaluation of multitargeted antifolate (MTA, LY231514) administered every 21 days utilizing the modified continual reassessment method for dose escalation. *Cancer Chemother Pharmacol* (1999) **44**(5):372–80. doi:10.1007/s002800050992
28. Hanna N, Shepherd FA, Fossella FV, Pereira JR, De Marinis F, von Pawel J, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *J Clin Oncol* (2004) **22**(9):1589–97. doi:10.1200/JCO.2004.08.163

29. Sigmond J, Backus HH, Wouters D, Temmink OH, Jansen G, Peters GJ. Induction of resistance to the multitargeted antifolate pemetrexed (ALIMTA) in WiDr human colon cancer cells is associated with thymidylate synthase overexpression. *Biochem Pharmacol* (2003) **66**(3):431–8. doi:10.1016/S0006-2952(03)00287-9
30. Ceppi P, Volante M, Saviozzi S, Rapa I, Novello S, Cambieri A, et al. Squamous cell carcinoma of the lung compared with other histotypes shows higher messenger RNA and protein levels for thymidylate synthase. *Cancer* (2006) **107**(7):1589–96. doi:10.1002/cncr.22208
31. Scagliotti GV, Shin DM, Kindler HL, Vasconcelles MJ, Keppler U, Manegold C, et al. Phase II study of pemetrexed with and without folic acid and vitamin B12 as front-line therapy in malignant pleural mesothelioma. *J Clin Oncol* (2003) **21**(8):1556–61. doi:10.1200/JCO.2003.06.122
32. Scagliotti G, Brodowicz T, Shepherd FA, Zielinski C, Vansteenkiste J, Manegold C, et al. Treatment-by-histology interaction analyses in three phase III trials show superiority of pemetrexed in nonsquamous non-small cell lung cancer. *J Thorac Oncol* (2011) **6**(1):64–70. doi:10.1097/JTO.0b013e3181f7c6d4
33. Paz-Ares LG, de Marinis F, Dediu M, Thomas M, Pujol JL, Bidoli P, et al. PARAMOUNT: final overall survival results of the phase III study of maintenance pemetrexed versus placebo immediately after induction treatment with pemetrexed plus cisplatin for advanced nonsquamous non-small-cell lung cancer. *J Clin Oncol* (2013) **31**(23):2895–902. doi:10.1200/JCO.2012.47.1102
34. Barlesi F, Scherpereel A, Rittmeyer A, Pazzola A, Ferrer Tur N, Kim JH, et al. Randomized phase III trial of maintenance bevacizumab with or without pemetrexed after first-line induction with bevacizumab, cisplatin, and pemetrexed in advanced nonsquamous non-small-cell lung cancer: AVAPERL (MO22089). *J Clin Oncol* (2013) **31**(24):3004–11. doi:10.1200/JCO.2012.42.3749
35. Ciuleanu T, Brodowicz T, Zielinski C, Kim JH, Krzakowski M, Laack E, et al. Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomized, double-blind, phase 3 study. *Lancet* (2009) **374**(9699):1432–40. doi:10.1016/S0140-6736(09)61497-5
36. Grønberg BH, Bremnes RM, Fløtten O, Amundsen T, Brunsvig PF, Hjelde HH, et al. Phase III study by the Norwegian lung cancer study group: pemetrexed plus carboplatin compared with gemcitabine plus carboplatin as first-line chemotherapy in advanced non-small-cell lung cancer. *J Clin Oncol* (2009) **27**(19):3217–24. doi:10.1200/JCO.2008.20.9114
37. Patel JD, Socinski MA, Garon EB, Reynolds CH, Spigel DR, Olsen MR, et al. Point break: a randomized phase III study of pemetrexed plus carboplatin and bevacizumab followed by maintenance pemetrexed and bevacizumab versus paclitaxel plus carboplatin and bevacizumab followed by maintenance bevacizumab in patients with stage IIIB or IV nonsquamous non-small-cell lung cancer. *J Clin Oncol* (2013) **31**(34):4349–57. doi:10.1200/JCO.2012.47.9626
38. Li N, Ou W, Yang H, Liu QW, Zhang SL, Wang BX, et al. A randomized phase 2 trial of erlotinib versus pemetrexed as second-line therapy in the treatment of patients with advanced EGFR wild-type and EGFR FISH-positive lung adenocarcinoma. *Cancer* (2014) **120**(9):1379–86. doi:10.1002/cncr.28591
39. *Abraxane for Injectable Suspension (Albumin-Bound), 100 mg/20 ml (Vial), Single-Use for Intravenous Infusion, Sterile, Lyophilized Solution.* Celgene Corporation (2005).
40. ten Tije AJ, Verweij J, Loos WJ, Sparreboom A. Pharmacological effects of formulation vehicles implications for cancer chemotherapy. *Clin Pharmacokinet* (2003) **42**(7):665–85. doi:10.2165/00003088-200342070-00005
41. *Taxol, a Paclitaxel Injection [Semi-Synthetic] Solution, for Intravenous Use, Multi-Dose Vial (MDV), 6 mg/ml.* Princeton, NJ: Bristol-Myers Squibb Co (2011).
42. *Taxotere, a Docetaxel for Injection, in a Concentrated Solution [20 mg/ml, 80 mg/4 ml, 160 mg/8 ml], One Vial, Single-Use, in Docetaxel Anhydrous, Sterile, Non-Pyrogenic, Non-Aqueous Solution, Intravenously Administered.* Bridgewater, NJ: Sanofi-Aventis (2010).
43. Weiss RB, Donehower RC, Wiernik PH, Ohnuma T, Gralla RJ, Trump DL, et al. Hypersensitivity reactions from taxol. *J Clin Oncol* (1990) **8**(7):1263–8.
44. Gelderblom H, Verweij J, Nooter K, Sparreboom A. Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation. *Eur J Cancer* (2001) **37**(13):1590–8. doi:10.1016/S0959-8049(01)00171-X
45. Desai N, Trieu V, Yao Z, Louie L, Ci S, Yang A, et al. Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel. *Clin Cancer Res* (2006) **12**(4):1317–24. doi:10.1158/1078-0432.CCR-05-1634
46. Socinski MA, Bondarenko I, Karaseva NA, Makhson AM, Vynnychenko I, Okamoto I, et al. Weekly nab-paclitaxel in combination with carboplatin versus solvent-based paclitaxel plus carboplatin as first-line therapy in patients with advanced non-small cell lung cancer: final results of a phase III trial. *J Clin Oncol* (2012) **30**(17):2055–62. doi:10.1200/JCO.2011.39.5848
47. Socinski MA, Langer CJ, Okamoto I, Hirsh V, Dakhil SR, Page RD, et al. Safety and efficacy of weekly nab-paclitaxel in combination with carboplatin as first-line therapy in elderly patients with advanced non-small cell lung cancer. *Ann Oncol* (2013) **24**(2):314–21. doi:10.1093/annonc/mdt235
48. Socinski MA, Okamoto I, Hon JK, Hirsh V, Dakhil SR, Page RD, et al. Safety and efficacy analysis by histology of weekly nab-paclitaxel in combination with carboplatin as first-line therapy in patients with advanced non-small cell lung cancer (NSCLC). *Ann Oncol* (2013) **24**(9):2390–6. doi:10.1093/annonc/mdt235
49. Lee JJ, Swain SM. Peripheral neuropathy induced by microtubule-stabilizing agents. *J Clin Oncol* (2006) **24**(10):1633–42. doi:10.1200/JCO.2005.04.0543
50. Hirsh V, Okamoto I, Hon JK, Page RD, Orsini J, Sakai H, et al. Patient-reported neuropathy and taxane-associated symptoms in a phase 3 trial of nab-paclitaxel plus carboplatin versus solvent-based paclitaxel plus carboplatin for advanced non-small cell lung cancer. *J Thorac Oncol* (2014) **9**(1):83–90. doi:10.1097/JTO.0000000000000011

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 14 May 2014; paper pending published: 11 June 2014; accepted: 23 June 2014; published online: 21 July 2014.

*Citation: Blais N and Hirsh V (2014) Chemotherapy in metastatic NSCLC – new regimens (pemetrexed, nab-paclitaxel). Front. Oncol. **4**:177. doi: 10.3389/fonc.2014.00177*

This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 19 August 2014
doi: 10.3389/fonc.2014.00213

Maintenance therapies for non-small cell lung cancer

Normand Blais* and Elie Kassouf

Medical Oncology, Centre Hospitalier de l'Université de Montréal, Montreal, QC, Canada

Edited by:
Vera Hirsh, McGill University Health Centre, Canada

Reviewed by:
Sacha I. Rothschild, University Hospital Basel, Switzerland
Janaki Deepak, University of Maryland School of Medicine, USA

****Correspondence:***
Normand Blais, Medical Oncology, Centre Hospitalier de l'Université de Montréal, 1560, Sherbrooke Est, Montreal, Quebec, Canada
e-mail: n.blais@umontreal.ca

Treatment of lung cancer had evolved during the last decade with the introduction of new chemotherapeutic regimens and targeted therapies. However, the maximum benefit reached after first-line therapy is limited by the cumulative toxicity of platinum drugs and the subsequent deterioration in performance status in a high percentage of patients who end up receiving not more than one line of treatment. Maintenance therapy had been introduced and evaluated in many large randomized trials showing a delay in tumor progression and an improvement in overall survival. This effective strategy should be taken into account when discussing the initial treatment plan and tailored according to the preferences of both patients and physicians.

Keywords: maintenance, pemetrexed, erlotinib, gefitinib, bevacizumab, lung cancer, docetaxel

INTRODUCTION

Standard first-line treatment for patients who are negative for the EGFR mutation and ALK rearrangement consists of platinum-based chemotherapy (1). The optimal number of cycles had been determined after Park et al. compared four to six cycles of treatment showing non-inferiority in terms of overall survival and thus, making four cycles a currently accepted standard (2).

During the past decade, research from different work groups has focused on finding alternative strategies to improve tumor response and extend survival.

The limited benefit from extending platinum-based chemotherapy beyond four cycles as well as the cumulative toxicities of these regimens leading to worsening of the quality of life (QoL) (2–4) had led to the re-emergence of a relatively new concept based on maintaining the response in patients who attain tumor control during first-line induction treatment.

Therefore, cytotoxic agents and molecularly targeted agents have been extensively evaluated in this setting and two practical applications of maintenance therapy have evolved: continuation maintenance and switch maintenance.

Maintenance therapy has potential advantages and inconveniences. Although many large randomized studies have shown that maintenance therapy is associated with a delay in tumor progression and an improvement in overall survival, prolonging therapy in a palliative intent also significantly increases the burden of medical interventions to a patient, prohibits the patient from having an often desired treatment holiday and may also increase treatment related toxicities, which may have a detrimental impact on QoL.

This mini review will expose the issues related to maintenance therapy and discuss a personalized approach to the implementation of such strategies in clinical practice.

CONTINUATION MAINTENANCE

Continuation maintenance refers to the continuation of one or more non-platinum agents, initially used during induction, until progression. This approach allows the discontinuation of platinum compounds known to cause cumulative toxicity that often becomes clinically meaningful after four to six doses of these drugs. It also has the advantage of continuing an agent for which tolerance has been defined in the induction phase of the treatment.

In 2006, Sandler et al. published the results of the ECOG 4599 trial, which showed that continuing bevacizumab (Bev) beyond six cycles of paclitaxel (Pac)/carboplatin (Carbo)/Bev added 2 months improvement in OS compared to six cycles of Carbo/Pac alone (HR, 0.79, $p = 0.003$) (5). Similarly, in 2009, Pirker et al. demonstrated in the FLEX study, that maintenance with cetuximab after chemotherapy with cisplatin/vinorelbine/cetuximab significantly improved OS (11.3 vs. 10.1 months; HR, 0.87, $p = 0.044$) compared to cisplatin/vinorelbine alone (6). It is not clear whether the benefit of bevacizumab or cetuximab in these studies is from their integration of the induction phase, the maintenance phase or both. Nonetheless, the design of these trials revived the concept of adapting maintenance as an appropriate clinical strategy.

Gemcitabine was evaluated in three randomized trials. CECOG (7) and IFCT-GFPC 0502 (8) met their primary endpoint showing longer PFS but with no significantly improved OS. These trials show interesting trends toward an increase in overall survival although this interpretation is limited by the small sample size of these trials. In a third trial, Belani et al. failed to demonstrate any advantage of maintenance gemcitabine on PFS and OS. These negative results have been attributed to the fact that most patients (64%) had a poor performance status (PS 2) at randomization in contrast to most other maintenance trials where most of the patients were PS 0 or 1 (9).

More recently, continuation maintenance with pemetrexed (pem) has been evaluated in two large randomized studies. In the PARAMOUNT trial, continuation pem after four cycles of induction with cisplatin/pem demonstrated significant improvement in PFS (4.1 vs. 2.8 months; HR, 0.62; $p < 0.0001$) and OS (16.9 vs. 14 months; HR, 0.78; $p = 0.019$) (10). In 2009, Patel et al. combined pem and bev as continuous maintenance in a phase II study. Their strategy was safe and resulted in a promising 14.1 months median OS (11). Maintenance with pem and bev was

thereafter tested in three major phase III studies. In AVAPERL, there was a 3.6 months improvement in PFS in the Pem/Bev arm compared to the Bev arm, and OS benefit was similar as that reported in PARAMOUNT although the sample size was too small to demonstrate statistical significance (19.8 vs. 15.9 months, HR, 0.88, $p = 0.32$) (12).

Although not a maintenance trial *per se*, PointBreak compared the ECOG 4599 regimen Pac/Carbo/Bev followed by Bev to Pem/Carbo/Bev followed by maintenance with Pem/Bev. Despite the fact that this study did not show significant improvement in OS (median OS, 12.6 vs. 13.4 months; $p = 0.949$) and a slight advantage in PFS favoring the pemetrexed containing regimen (median PFS, 6.0 vs. 5.6 months; HR, 0.83; $p = 0.012$), the study results highlighted different toxicity profiles between the paclitaxel and pemetrexed containing regimens that may lead to a better treatment selection (13). Fatigue and thrombocytopenia were more frequent in the pem arm and neutropenia, neuropathy, and alopecia more frequent in the paclitaxel arm.

The ongoing ECOG 5508 study may better define the optimal choice of maintenance agent after pac/carbo/bev as it randomizes patients for maintenance after induction into three arms: Bev alone, Pem alone, or combined Pem-Bev (**Table 1**).

SWITCH MAINTENANCE

Switch maintenance is the introduction of an additional, potentially non-cross-resistant agent, immediately after completion of first-line chemotherapy in patients who achieved an objective response or a stable disease. This strategy focuses on the early integration of drugs that have been shown to be useful in the second line setting and in this regard can be seen as "early second line" therapy. This exposes the patient to new toxicity that needs to be addressed before choosing such a strategy.

The first pivotal trial to report benefit with a cytotoxic agent using this strategy was presented by Fidias et al. who randomized 309 patients with advanced NSCLC who did not progress after front-line treatment with four cycles of carbo/gemcitabine to receive immediate docetaxel maintenance therapy vs. delayed docetaxel at disease progression. The study showed a significant 3 months improvement in PFS and a non-statistically significant 2.5 months increase in OS in favor of the "immediate" docetaxel arm, with no increase in toxicity or decrease in QOL. Even though the patients in the delayed arm were carefully assessed and followed and that docetaxel was available to all of these patients, 37.2% of the patients in this arm did not receive docetaxel due to a rapid disease progression or a rapid PS decline (14).

JMEN is a phase III trial that evaluated maintenance pem vs. BSC in 633 non-progressive stage IIIB/IV patients after non-pem containing platinum-doublet chemotherapy. The pemetrexed arm showed significantly improved PFS (4.3 vs. 2.6 months; HR, 0.5, $p < 0.0001$) and OS (13.4 vs. 10.6 months; HR, 0.79; $p = 0.012$). Subgroup analysis based on histology showed that the improvement in PFS (4.5 vs. 2.6 months; HR, 0.44; $p = 0.0001$) and OS (15.5 vs. 10.3 months; HR, 0.70; $p = 0.002$) was restricted to patients with tumors having a non-squamous histology (72.5% of the population) (15). In this trial, there was a limited access to pem in the BSC arm, as only 18% of patients were treated with this drug, thus creating a subsequent imbalance in interpreting the benefit of pem as a highly active drug in this particular population.

After showing an OS benefit in second and third line setting for advanced NSCLC (16), erlotinib was evaluated as a switch

Table 1 | Key studies addressing continuous maintenance.

Reference (study name)	*N* (pts)	Maintenance arms	PFS (mo)	HR	*p* Value	OS (mo)	HR	*p* Value
Sandler et al. (5) (ECOG 4599)	850	Bevacizumab Observation	6.2 4.5	0.66	<0.001	12.3 10.3	0.79	0.003
Pirker et al. (6) (FLEX)	850	Cetuximab Placebo	4.8 4.8	0.943	0.39	11.3 10.1	0.871	0.044
Brodowicz et al. (7) (CECOG)	206	Gemcitabine BSC	3.6 2.0		<0.001	13 11		0.195
Perol et al. (8) (IFCT-GFPC 0502)	464	Gemacitabine Observation	3.8 1.9	0.56	<0.001	12.1 10.8	0.89	0.3867
Belani et al. (9)	255	Gemacitabine Observation	3.9 3.8		0.58	8.0 9.3	0.97	0.84
Paz-Ares et al. (10) (PARAMOUNT)	539	Pemetrexed BSC	4.1 2.8	0.62	<0.001	13.9 11.0	0.78	0.0195
Barlesi et al. (12) (AVAPERL)	253	Pem/Bev Bev	10.2 6.6	0.5	<0.001	19.8 15.9	0.88	0.32
Patel et al. (13) (Point break)	590	Pem/Bev Bev	6.0 5.6	0.83	0.012	12.6 13.4	1	0.949

PFS = progression-free survival; OS = overall survival; BSC = best supportive care; HR = hazard ratio; Bev = bevacizumab; Pem = pemetrexed.

maintenance therapy in SATURN, a large phase III trial that randomized 889 patients who did not progress after four cycles of a platinum doublet, to erlotinib or placebo. There was a modest but statistically significant improvement in PFS (3 vs. 2.8 months; HR 0.71, $p < 0.0001$) and OS (12 vs. 11 months; HR, 0.81, $p = 0.0088$). Subgroup analyses showed larger treatment benefit in terms of OS in patients with stable disease (HR, 0.72) after induction than in responders (HR, 0.94). Progression-free survival was significantly higher in patients with EGFR activating mutations (HR, 0.23) than in patients EGFR WT (HR, 0.78) but a survival difference could not be demonstrated in these subgroups (17). In a similar fashion to the JMEN trial, erlotinib was not widely available to patients in the placebo arm and only 21% of these patients were actually treated with erlotinib. Similar results were seen in the Erlotinib maintenance arm of the smaller IFCT-GFPC 0502 study mentioned earlier, with a 1 month improvement in PFS but no statistically significant change in OS (8). Following these positive results, Johnson et al. studied the combination of bev and erlotinib in maintenance. In the ATLAS trial, 1160 patients received first-line platinum-based chemotherapy with bev, 768 patients had an objective response or SD, and were randomized to receive bev with erlotinib vs. bev alone. This trial showed 1 month improvement in PFS (4.8 vs. 3.7 months, HR 0.72, $p = 0.0012$) for patients in the combination maintenance arm but a non-statistically significant improvement in OS (14.4 vs. 13.3 months; HR, 0.92; $p = 0.5341$) (18).

The EORTC Lung Cancer Group and the Italian Lung Cancer Project evaluated maintenance with gefitinib vs. placebo in 173 patients who did not progress after four cycles of platinum-based chemotherapy. PFS was better in the treatment arm (4.1 vs. 2.9 months; HR, 0.61; $p = 0.0015$) but with no statistically significant improvement in OS (10.9 vs. 9.4 months; HR, 0.83; $p = 0.2$) (19). The INFORM trial, an Asian phase III trial, tested gefitinib in a similar setting in 296 patients (79 EGFR-mut). PFS was significantly higher in the gefitinib arm (4.8 vs. 2.6 months; HR, 0.42; $p < 0.0001$) with more benefit EGFR-mut subgroup (HR, 0.17) compared to EGFR WT (HR, 0.87). An OS benefit was not shown (18.7 vs. 16.9 months; HR, 0.84; $p = 0.2608$) (20). Finally, the West Japan Thoracic Oncology Group 0203 phase III trial compared prolonged chemotherapy with 6 cycles of a platinum doublet to a short course of 3 cycles followed by gefitinib maintenance in 604 patients. PFS was statistically better favoring gefitinib maintenance (4.6 vs. 4.3 months; HR, 0.68; $p < 0.001$) but no significant difference in OS was found (13.7 vs. 12.9 months; HR, 0.86; $p = 0.11$) (21) (**Table 2**).

DISCUSSION

Maintenance therapy, whether in switch or continuation approach, has proved to be beneficial in patients with advanced NSCLC who have received up to four cycles of a platinum-containing regimen. Despite much debate regarding the results of the different studies and the reserved improvement in survival, Pemetrexed and Erlotinib are already approved and used for maintenance in many countries.

ARGUMENTS AGAINST MAINTENANCE

In dealing with a population of patients with a non-curable disease, improvements in overall survival and QoL remain the primary

Table 2 | Key studies evaluating switch maintenance.

Reference (study name)	*N* (pts)	Maintenance arms	PFS (mo)	HR	*p* Value	OS (mo)	HR	*p* Value
Fidias et al. (14)	309	Immediate Docetaxel	5.7		0.0001	12.3		0.0853
		Delayed Docetaxel	2.7			9.7		
Ciuleanu et al. (15) (JMEN)	663	Pemetrexed	4.3	0.5	<0.0001	13.4	0.79	0.012
		Placebo	2.6			10.6		
Cappuzzo et al. (17) (SATURN)	889	Erlotinib	3.0	0.71	<0.0001	12.0	0.81	0.0088
		Placebo	2.8			11.0		
Perol et al. (8) (IFCT-GFPC 0502)	310	Erlotinib	2.9	0.69	0.003	11.4	0.87	0.3043
		Observation	1.9			10.8		
Kabbinavar et al. (18) (ATLAS)	768	Bev/Erlotinib	4.8	0.72	0.0012	14.4	0.92	0.5341
		Bevacizumab	3.7			13.3		
Gaafar et al. (19) (EORTC08021-LCP01/03)	173	Gefitinib	4.1	0.61	0.0015	10.9	0.83	0.2
		Placebo	2.9			9.4		
Zhang et al. (20) (INFORM)	296	Gefitinib	4.8	0.42	<0.0001	18.7	0.84	0.26
		Placebo	2.6			16.9		
Takeda et al. (21) (WJTOG 0203)	604	Gefitinib	4.6	0.68	<0.001	13.7	0.86	0.11
		Observation	4.3			12.9		

PFS = progression-free survival; OS = overall survival; BSC = best supportive care; HR = hazard ratio; Bev = bevacizumab; Pem = pemetrexed.

objective. Symptomatic patients who begin induction therapy with a platinum doublet for lung cancer are often looking forward to a symptom free and drug free holiday. In this regard, many patients who obtain a meaningful symptomatic response to induction are not enthusiastic about adding on more therapy, especially if maintenance therapy is discussed after induction. Compared to continuation maintenance, switch maintenance has the added inconvenience of exposing the patient to new toxicities not encountered during induction. The interpretation of many trials is also bound with controversy. In particular, the absence of broad availability of pemetrexed in the JMEN trial or erlotinib in SATURN limits the interpretation of any small OS gain observed in these studies as these drugs are now widely available in many countries, particularly in the second line setting. The question now becomes as to whether these agents are better given before radiological or clinical progression ("early second line") or at the time of clear progression.

ARGUMENTS FOR MAINTENANCE

The current data appear even more compelling for continuation maintenance, especially for non-squamous and EGFR-mut patients. The PARAMOUNT trial has shown a PFS and an OS benefit for continuation pem in responding and stable disease patients. As used in this trial, limiting therapy to four cycles of cisplatin therapy decreases platin-related toxicities and the absence of new drug exposure limits the risk of new unexpected toxicities. Considering a median time to progression of 6–12 weeks in many observation arms of maintenance strategy trials, delaying progression is a clinically meaningful endpoint to many patients.

The case for switch maintenance is more debatable. For reasons described above, the apparent OS benefit for switching to erlotinib or pemetrexed may be associated to study design not relevant to current practice. Nonetheless, progression in the placebo arms is often very rapid as reported in trials where radiological and clinical follow up is frequent. Leaving patients without treatment can thus expose them to rapid and early progression, often leading to a decline in performance status and inability to receive further therapy. As the biggest benefit in the JMEN and SATURN trial appears to be in patients obtaining no more than SD to induction therapy, it may be hypothesized that these patients may obtain more benefit from an earlier initiation of second line therapy. As such, it seems reasonable to consider switch therapy to patients that did not obtain palliative benefit from induction therapy in an attempt to better alleviate symptoms and prevent symptomatic progression.

Some patients are identified as having an actionable mutation during induction chemotherapy. The ideal timing of the beginning of targeted therapy in this particular situation is still a matter of debate. It seems reasonable to pursue induction chemotherapy in these patients, particularly if they are responding and tolerating treatment well. On the other hand, switching to a specific targeted agent is appropriate if induction is poorly tolerated or if symptoms are poorly controlled. Targeted agents, for instance EGFR and ALK inhibitors, are associated with rapid improvement in symptoms in patients harboring sensitive mutations to these agents.

CONCLUSION

Maintenance therapy has shown effectiveness in delaying progression in many studies as well as prolonging overall survival in some settings. Appropriate clinical decisions involve early discussions of these options with potentially eligible patients. Factors that may impact in the final decision to initiate maintenance include tumor histology, clinical and radiological response to induction, tumor mutations, and most importantly patient choice. Further improvements in treatment and patient selection will most likely arise with the improved refinement of the molecular diagnosis of lung cancers.

REFERENCES

1. National Comprehensive Cancer Network. *Non–small cell lung cancer (version 3.2014)*. (2014). Available: http://www.nccn.org/professionals/physician_gls/
2. Park JO, Kim SW, Ahn JS, Suh C, Lee JS, Jang JS, et al. Phase III trial of two versus four additional cycles in patients who are non progressive after two cycles of platinum-based chemotherapy in non small-cell lung cancer. *J Clin Oncol* (2007) **25**(33):5233–9. doi:10.1200/JCO.2007.10.8134
3. Soon YY, Stockler MR, Askie LM, Boyer MJ. Duration of chemotherapy for advanced non-small-cell lung cancer: a systematic review and meta-analysis of randomized trials. *J Clin Oncol* (2009) **27**:3277–83. doi:10.1200/JCO.2008.19.4522
4. Socinski MA, Schell MJ, Peterman A, Bakri K, Yates S, Gitten R, et al. Phase III trial comparing a defined duration of therapy versus continuous therapy followed by second-line therapy in advanced IIIB/IV non-small-cell lung cancer. *J Clin Oncol* (2002) **20**:1335–43. doi:10.1200/JCO.20.5.1335
5. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* (2006) **355**:2542–50. doi:10.1056/NEJMoa061884
6. Pirker R, Pereira JR, Szczesna A, von Pawel J, Krzakowski M, Ramlau R, et al. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. *Lancet* (2009) **373**:1525–31. doi:10.1016/S0140-6736(09)60569-9
7. Brodowicz T, Krzakowski M, Zwitter M, Tzekova V, Ramlau R, Ghilezan N, et al. Cisplatin and gemcitabine first-line chemotherapy followed by maintenance gemcitabine or best supportive care in advanced non-small cell lung cancer: a phase III trial. *Lung Cancer* (2006) **52**:155–63. doi:10.1016/j.lungcan.2006.01.006
8. Perol M, Chouaid C, Perol D, Barlesi F, Gervais R, Westeel V, et al. Randomized, phase III study of gemcitabine or erlotinib maintenance therapy versus observation, with predefined second-line treatment, after cisplatin gemcitabine induction chemotherapy in advanced non-small-cell lung cancer. *J Clin Oncol* (2012) **30**:3516–24. doi:10.1200/JCO.2011.39.9782
9. Belani CP, Waterhouse DM, Ghazal H, Ramalingam SS, Bordoni R, Greenberg R, et al. Phase III study of maintenance gemcitabine (G) and best supportive care (BSC) versus BSC, following standard combination therapy with gemcitabine-carboplatin (G-Cb) for patients with advanced non-small cell lung cancer (NSCLC). *J Clin Oncol* (2010) **28**(15 Suppl):abstr 7506.
10. Paz-Ares L, de Marinis F, Dediu M, Thomas M, Pujol JL, Bidoli P, et al. Maintenance therapy with pemetrexed plus best supportive care versus placebo plus best supportive care after induction therapy with pemetrexed plus cisplatin for advanced non-squamous non-small-cell lung cancer (PARAMOUNT): a double-blind, phase 3, randomized controlled trial. *Lancet Oncol* (2012) **13**:247–55. doi:10.1016/S1470-2045(12)70063-3
11. Patel JD, Hensing TA, Rademaker A, Hart EM, Blum MG, Milton DT, et al. Phase II study of pemetrexed and carboplatin plus bevacizumab with maintenance pemetrexed and bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer. *J Clin Oncol* (2009) **27**:3284–9. doi:10.1200/JCO.2008.20.8181
12. Barlesi F, De Castro J, Dvornichenko V, Kim J, Pazzola A, Rittmeyer A, et al. AVAPERL (MO22089): final efficacy outcomes for patients with advanced non-squamous non small cell lung cancer randomized to continuation maintenance with bevacizumab (Bev) or Bev + pemetrexed (Pem) after first-line Bev-cisplatin-Pem treatment. *Eur J Cancer (Oxford)* (2013) **47**:16. doi:10.1016/j.cllc.2011.05.002

13. Patel J, Socinski M, Garon E, Reynolds C, Spigel D, Olsen M, et al. PointBreak: a randomized phase III study of pemetrexed plus carboplatin and bevacizumab followed by maintenance pemetrexed and bevacizumab versus paclitaxel plus carboplatin and bevacizumab followed by maintenance bevacizumab in patients with stage IIIB or IV nonsquamous non–small-cell. *Lung Cancer JCO* (2013) **2012**:47.9626. doi:10.1200/JCO.2012.47.9626
14. Fidias PM, Dakhil SR, Lyss AP, Loesch DM, Waterhouse DM, Bromund JL, et al. Phase III study of immediate compared with delayed docetaxel after front-line therapy with gemcitabine plus carboplatin in advanced non-small-cell lung cancer. *J Clin Oncol* (2009) **27**:591–8. doi:10.1200/JCO.2008.17.1405
15. Ciuleanu T, Brodowicz T, Zielinski C, Kim JH, Krzakowski M, Laack E, et al. Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomized, double-blind, phase 3 study. *Lancet* (2009) **374**:1432–40. doi:10.1016/S0140-6736(09)61497-5
16. Sheperd F, Pereira JR, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non–small-cell lung cancer. *N Engl J Med* (2005) **353**:123–32. doi:10.1056/NEJMoa050753
17. Cappuzzo F, Ciuleanu T, Stelmakh L, Cicenas S, Szczesna A, Juhasz E, et al. Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomized, placebo-controlled phase 3 study. *Lancet Oncol* (2010) **11**:521–9. doi:10.1016/S1470-2045(10)70112-1
18. Johnson B, Kabbinavar F, Fehrenbacher L, Hainsworth J, Kasubhai S, Kressel B, et al. ATLAS: randomized, double-blind, placebo-controlled, phase IIIB trial comparing bevacizumab therapy with or without erlotinib, after completion of chemotherapy, with bevacizumab for first-line treatment of advanced non-small-cell lung cancer. *Lung Cancer JCO* (2013) **2012**:47.3983. doi:10.1200/JCO.2012.47.3983
19. Gaafar RM, Surmont VF, Scagliotti GV, Van Klaveren RJ, Papamichael D, Welch JJ, et al. A double-blind, randomized, placebo-controlled phase III intergroup study of gefitinib in patients with advanced NSCLC, non-progressing after first line platinum-based chemotherapy (EORTC 08021/ILCP 01/03). *Euro J Cancer* (2011) **47**(15):2331–40. doi:10.1016/j.ejca.2011.06.045
20. Zhang L, Shenglin M, Song X, Han B, Cheng Y, Huang C, et al. Efficacy, tolerability, and biomarker analyses from a phase III, randomized, placebo-controlled, parallel group study of gefitinib as maintenance therapy in patients with locally advanced or metastatic NSCLC. *J Clin Oncol* (2011) **29**:abstr LBA7511.
21. Takeda K, Hida T, Sato T, Ando M, Seto T, Satouchi M, et al. Randomized phase III trial of platinum-doublet chemotherapy followed by gefitinib compared with continued platinum-doublet chemotherapy in Japanese patients with advanced non-small-cell lung cancer: WJTOG0203. *J Clin Oncol* (2010) **28**:753–60. doi:10.1200/JCO.2009.23.3445

Conflict of Interest Statement: The Guest Associate Editor Vera Hirsh declares that, despite having collaborated with author Normand Blais, the review process was handled objectively and no conflict of interest exists. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 12 May 2014; accepted: 25 July 2014; published online: 19 August 2014.
*Citation: Blais N and Kassouf E (2014) Maintenance therapies for non-small cell lung cancer. Front. Oncol. **4**:213. doi: 10.3389/fonc.2014.00213*
This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 15 September 2014
doi: 10.3389/fonc.2014.00248

The management of brain metastases in non-small cell lung cancer

Scott Owen[1]* and Luis Souhami[2]

[1] Division of Medical Oncology, Department of Oncology, McGill University Health Centre, Montreal, QC, Canada
[2] Division of Radiation Oncology, Department of Oncology, McGill University Health Centre, Montreal, QC, Canada

Edited by:
Barbara Melosky, British Columbia Cancer Agency, Canada

Reviewed by:
Sacha I. Rothschild, University Hospital Basel, Switzerland
Weiqiang Zhao, The Ohio State University Wexner Medical Center, USA

***Correspondence:**
Scott Owen, Montreal General Hospital, 1650 Cedar Avenue, T7-402, Montreal, QC H3G 1A4, Canada
e-mail: scott.owen@mcgill.ca

Brain metastases (BM) are a common and lethal complication of non-small cell lung cancer (NSCLC), which portend a poor prognosis. In addition, their management implies several challenges including preservation of neurological and neurocognitive function during surgery or radiation-therapy, minimizing iatrogenic complications of supportive medications, and optimizing drug delivery across the blood–brain barrier. Despite these challenges, advancements in combined modality approaches can deliver hope of improved overall survival and quality of life for a subset of NSCLC patients with BM. Moreover, new drugs harnessing our greater understanding of tumor biology promise to build on this hope. In this mini-review, we revised the management of BM in NSCLC including advancements in neurosurgery, radiation therapy, as well as systemic and supportive therapy.

Keywords: brain metastases, lung cancer, targeted therapy, radiation therapy, chemotherapy, stereotactic radiosurgery, surgery

INTRODUCTION

Lung cancer is the leading cause of cancer mortality worldwide, accounting for 1.38 million annual deaths, representing 18.2% of total deaths from cancer (1). Among those, approximately 7.4% of non-small cell lung cancer (NSCLC) patients will have brain metastases (BM) at presentation (2), and 25–30% will develop BM during the course of their disease (3). Life-expectancy for these patients is poor, with a median survival of only 3.4 months (4). Moreover, many will suffer considerable loss of autonomy due to neurocognitive and functional deficits, as well as morbidity associated with medications such as steroids and anti-epileptic drugs.

Despite these grim realities, there is room for optimism among identifiable subsets of these patients. A recent published series of NSCLC patients with synchronous BM receiving surgery or radiosurgery to the brain and aggressive management of their extracranial disease reported a median overall survival (OS) of 12.1 months (5). Improved surgical techniques and radiation therapy (RT) technology, as well as more effective systemic treatments and multimodality approaches have led to these superior outcomes. Moreover, renewed hope has emerged from the use of small-molecule drugs targeting oncogenic mutations, which have shown promising activity both extra-cranially and intra-cranially (6).

PROGNOSTIC FACTORS

Several variables have been established of prognostic importance in determining potential outcomes for patients harboring BM. In 1997, the Radiation Therapy Oncology Group (RTOG) performed a recursive partitioning analysis (RPA) from a historical database of 1200 patients treated with whole-brain radiation therapy (WBRT) from three RTOG BM trials and published a prognostic scoring system (7). Three scoring classes were identified based on patients' Karnofsky performance score (KPS), age, status of primary tumor, and extent of extracranial disease (**Table 1**). Median survival ranged from 2.3 months for patients in class III to 7.1 months for those in class I.

Since then, several other scoring classifications have been described (4, 8–11) as shown in **Table 1**. All these classifications have limitations, but are able to consistently prognosticate outcomes based on the defined scoring. Irrespective of the scoring classification used, age, performance status, number of brain lesions, and the presence of extracranial metastases are the variables that better define prognosis. Given the high heterogeneity of the BM patient population, one should not rely exclusively on these indices when assessing the management for such patients. A comparative review of five of these prognostic indexes using an artificial neural network in patients with BM and receiving WBRT (12) suggests that the graded prognostic assessment index (10) was the most powerful in predicting survival.

Increasingly, molecular biomarkers are also being identified with prognostic significance in NSCLC, some with positive [e.g., EGFR (del-19 and L858R)] and others with negative (e.g., ERCC1, BRCA1, TP53, and KRAS) prognostic value (13). In addition, microarray-derived gene signatures provide the potential for even greater prognostic ability (14). However, many of these biomarkers require further validation, and are not yet ready for entry into routine clinical practice.

TREATMENT

SUPPORTIVE

Early integration of palliative care in the management of metastatic NSLCC has been demonstrated to improve both quality of life and mood, and is associated with improved survival despite less aggressive end of life treatment (15). In addition to general palliative measures, patients with BM often necessitate additional supportive medications such as steroids and anti-seizure medications.

Table 1 | Prognostic indexes for metastatic brain disease.

RECURSIVE PARTITIONING ANALYSIS	
Class 1	Age < 65; KPS ≥ 70, primary controlled; no extra-cranial disease
Class 2	Patients not in class 1 or 2
Class 3	KPS < 70
BASIC SCORE FOR BRAIN METASTASES	
Score 0	KPS 50–70; primary uncontrolled; extra-cranial disease present
Score 1	KPS 80–100; primary controlled; no extra-cranial disease
SCORE INDEX FOR RADIOSURGERY	
Score 0	KPS ≤ 50; age ≥ 60; extra-cranial disease progressive; lesions ≥ 3; volume > 13 ml (largest lesion)
Score 1	KPS 60–70; age 51–50; extra-cranial disease stable; lesion 2; volume 5–13 ml
Score 2	KPS > 80; age ≤ 50; systemic disease NED; lesion 1; volume < 5
GRADED PROGNOSTIC ASSESSMENT	
Score 0	KPS < 70; age > 60; lesions > 3; extra-cranial disease present
Score 0.5	KPS 70–80; age 50–59; lesions 2–3
Score 1	KPS 90–100; age < 50; lesion 1; no extra-cranial disease

KPS, Karnofsky performance status; NED, no evidence of disease.

Corticosteroids can be vital drugs in the control of intracranial edema from BM and the relief of related symptoms. However, in light of their considerable short- and long-term side effects, steroids should be used judiciously. Hence, a systematic review on the subject (16) has made the following recommendations:

- If corticosteroids are given, dexamethasone is the best choice (level 3).
- Starting doses of 4–8 mg of dexamethasone should be given for temporary relief of symptoms related to increased intracranial pressure. In more severe cases, where symptoms suggest impending herniation, doses of 16 mg/day or more may be considered (level 3).
- There is insufficient evidence to guide treatment recommendations for asymptomatic BM.

SURGERY

Up to few decades ago, surgical resection was mainly used to establish a diagnosis or to alleviate mass-effect symptoms. More recently, its definitive role in improving disease control for patients with single, resectable metastasis has been shown to be significant. Three randomized studies (17–19) have addressed the potential therapeutic value of surgical resection by comparing surgery followed by WBRT vs. WBRT alone in patients with a single brain metastasis (**Table 2**).

In two of these trials (17, 18), a survival benefit was reported for patients undergoing the combined approach. Patchell et al. (17) randomized 48 good-performing (KPS ≥70) patients with an MRI-diagnosed, tissue-proven single lesion to surgical resection plus WBRT (36 Gy in 12 fractions) vs. WBRT alone. Of interest, 11% of patients were excluded because no metastatic disease was seen on the biopsy specimens. The authors reported a statistically significant improvement in survival (median survival: 40 vs. 15 weeks, $p < 0.01$) favoring the combined therapy, as well as a reduction in brain recurrence rates and neurologic death. Vecht at al. (18) compared WBRT (40 Gy in 20 fractions) with the same WBRT preceded by surgery. Similarly, the combined approach showed a survival advantage (median survival: 10 vs. 6 months, $p = 0.04$). In this study, patients were stratified for progressive vs. stable extracranial disease, which proved to be the most important prognosticator for survival.

In contrast, the study by Mintz et al. (19) failed to show a survival benefit when WBRT (30 Gy in 10 fractions) followed surgical resection. The median survival for the WBRT group was 6.3 vs. 5.6 months for the combined modality group ($p = 0.24$). The median survival in the Mintz et al. (19) series was lower than the two other randomized studies and may be explained by the selection of patients with lower KPS or with more extensive extra cranial systemic disease (45% of patients). In addition, MRI was not routinely used to exclude multiple metastases.

It should be mentioned that all of these randomized studies had small patient numbers and did not include relatively radiosensitive tumors such as small cell lung cancer, lymphoma, myeloma, and germ cell tumors. Also, these trials were not specific for NSCLC patients, although this histology was the predominant one in all trials.

Despite these limitations, the current level 1 evidence supports the use of WBRT post-surgical resection in patients with a single, resectable lesion, good performance, and limited extracranial disease. For patients with multiple metastatic lesions, poor performance scores, and extensive systemic disease an evidence-based recommendation for the combined approach cannot be made.

A follow-up trial by Patchell and colleagues (24) addressed the real need of WBRT post-resection of a single brain metastasis. In a multi-center study, 95 patients (60% with NSCLC) with KPS ≥70 undergoing a complete resection of a single brain metastasis were randomized to WBRT (50.4 Gy in 28 fractions) or no further treatment for a primary end-point of tumor recurrence anywhere in the brain. A total of 95 patients were randomized and again NSCLC was the predominant tumor type. The group receiving post-operative WBRT experienced a significantly lower rate of brain recurrence (18 vs. 70%, $p < 0.001$). WBRT also decreased brain recurrence at the site of the original metastasis (10 vs. 41%, $p < 0.001$) and at other sites in the brain (14 vs. 37%, $p < 0.01$). Although OS was not different between groups, importantly, post-operative WBRT significantly prevented death from neurologic causes (14 vs. 44%, $p = 0.003$). This trial defined the need for adjuvant RT post-resection of a single brain metastasis.

RADIATION THERAPY

WHOLE-BRAIN RADIATION THERAPY

The use of WBRT for patients harboring BM is considered by many as the standard treatment. The rationale for treating the whole brain is based on the presumption that micro-metastatic deposits of tumor cells are present elsewhere in the brain. WBRT is the

Table 2 | Randomized trials of WBRT in brain metastases.

Author	No. patients	Randomization	Local control	Survival (months)	*p* Value
WBRT ± SURGERY					
Patchell (7)	48	WBRT	48%	3.6	$p < 0.001$
		S + WBRT	80%	9.5	
Vecht (8)	63	WBRT	NR	6.0	$p = 0.04$
		S + WBRT		10.0	
Mintz (9)	84	WBRT	NR	6.3	$p = 0.39$
		S + WBRT		5.6	
Author	**No. patients**	**Randomization**	**Local control (%)**	**Survival (months)**	**Neurologic death**
WBRT ± STEREOTACTIC RADIOSURGERY					
Chougule (23)	73	SRS	87	5	NR
		SRS + WBRT	91	9	
Aoyama (34)	132	SRS	72.5	7.5	19.3%
		SRS + WBRT	88.7	8	22.8%
Chang (35)	58	SRS	67	15.2	NR
		SRS + WBRT	100	5.7	
Kocher (36)	199	SRS	69	10.7	44%
		SRS + WBRT	81	10.9	28%

No, number; S, surgery; SRS, stereotactic radiosurgery; WBRT, whole brain radiation therapy; NR, not reported.

most frequently used treatment for the management of BM and its use is associated with improvement in neurologic symptoms and decreased neurologic death (25). The RTOG and other investigators (26–31) conducted several randomized trials evaluating different dose/fractionation regimens, but no particular regimen appears to be superior in terms of disease control or survival. Typically, a dose of 20 Gy in 5 fractions or 30 Gy in 10 fractions is recommended. Approximately 60% of patients will experience a complete or partial response with a similar rate for symptoms improvement, though usually transient.

One major concern with the use of WBRT is the risk of neurocognitive deficits, particularly short-term memory. Unfortunately, the real rate and magnitude of neurocognitive deficits post-WBRT has not been properly studied. It has been shown that over 90% of patients with BM had impairment in one or more neurocognitive tests at baseline and prior to WBRT (32). Proponents of WBRT argue that it is the disease progression in the brain *not* treated by WBRT that, in fact, compromises the patient's neurocognitive function. However, some patients develop cognitive problems that cannot be simply explained by disease progression elsewhere in the brain. Late effects from WBRT are usually seen after 6 months post-treatment and are secondary to white matter damage. Considering that many patients will not survive beyond 6 months, it is plausible to consider that cognitive deficits would be seen in larger proportion of patients should they survive longer. For a comprehensive review of the subject, we recommend the paper by McDuff et al. (33).

Recent approaches to reduce the potentially negative effects of WBRT on cognitive function include the concomitant use of memantine (20) and hippocampal sparing during WBRT (21). Memantine, a potential neuroprotector, was used during EBRT in a recent RTOG randomized trial (20). Patients receiving the drug had improved cognitive function in several domains. Gondi et al. (21) presented a phase II RTOG study of hippocampal sparing in patients undergoing WBRT for BM. Although this was a single arm trial, the declines in cognitive function are less than what was observed from historical controls.

STEREOTACTIC RADIOSURGERY

Stereotactic radiosurgery (SRS) delivers a single high dose of irradiation to the target volume while avoiding the surrounding normal tissues. A randomized trial conducted by the RTOG (22) showed that the addition of SRS to WBRT was superior to WBRT alone in patients with a newly diagnosed single brain lesion. A survival benefit was not seen for patients with two or three metastatic lesions, although local brain control was significantly improved with the addition of SRS. Given its focal delivery of irradiation, there have been concerns that its isolated use could lead to an increased rate of failure elsewhere in the brain. However, concerns with cognitive deficits from WBRT led investigators to use SRS alone in selected patients, reserving WBRT for a later date if necessary.

To address to this question, four randomized trials have, to date, compared SRS alone vs. SRS plus WBRT in patients with a

limited number of metastatic lesions (23, 34–36). One of them has only been reported in abstract form (23). **Table 2** summarizes the results of these trials.

Despite differences in patient selection and treatment design, all trials consistently show no significant difference in survival, but have shown a significant reduction in intracranial failures and death from brain causes. One study (35) had a neurocognitive endpoint – Hopkins Verbal Learning Test (HVLT) – at 4 months post-treatment. This small study was stopped prematurely because an interim analysis showed neurocognitive function at 4 months significantly worse after SRS + WBRT than after SRS alone, although brain control at 1 year was significantly better for the WBRT + SRS arm (73 vs. 27%, $p = 0.0003$). On the other hand, in the Japanese trial (34), there was a significant decline in mini-mental score when SRS was given alone making the authors conclude that BM control was the most important factor for preserving neurocognitive function.

Whether SRS can replace WBRT in newly diagnosed BM remains to be determined and treatment decisions should be individualized taking into consideration the patients' wishes, age, intra and extracranial disease extent, and prognosis.

CHEMOTHERAPY

Due to the failure of most drugs to cross the intact blood–brain barrier (BBB), the role of chemotherapy in the treatment of BM has been viewed critically (2). Chemotherapy drugs are generally large (>150 kDa), ionized, hydrophilic, and often protein-bound, and therefore, ill-suited to penetrate the tight-junctions, electrochemical barrier, astrocyte foot-processes, and highly regulated transmembrane transport proteins of the central nervous system's endothelial vasculature (37).

However, the effects of the BBB may be over-estimated. First, there is evidence that the BBB of BM is disrupted, as evidenced by the presence of peritumoral edema and the accumulation of contrast media during computed tomography or magnetic resonance assessments (38, 39). Second, there is evidence of intracranial tumor response, even to drugs that in healthy systems have little central nervous system penetration. In a recent review (37), the response rates (RRs) of BM to platinum-based regimens in seven clinical trials of treatment-naïve NSCLC patients were similar to those achieved extra-cranially, ranging from 30 to 50%. However, the median survival remained only 5–8 months in most cases. In the same review, three trials using temozolomide achieved a RR of only 0–10%, suggesting that the selection of chemotherapy drugs should be based mainly on their established anti-tumor activity to extracranial sites, and not on considerations of BBB penetrance.

More recently, two phase II trials have examined the use of cisplatin and pemetrexed for the treatment of NSCLC with BM. In one trial, 43 chemo-naive NSCLC patients (93% non-squamous histology) with BM received up to six cycles of cisplatin and pemetrexed at standard doses (40). WBRT was given in cases of disease progression or at chemotherapy completion. Cerebral, extracerebral, and objective RRs by intention to treat (ITT) were 41.9, 34.9, and 34.9%, respectively. Median OS and progression-free survival (PFS) were 7.4 and 4.0 months, respectively.

In another phase II trial (41), newly diagnosed NSCLC patients with BM received up to six cycles of cisplatin and pemetrexed concurrently with WBRT (30 Gy/10 fractions) during days 1–12 of the first cycle. Among the 41 patients evaluable for response (100% adenocarcinoma), the cerebral, extra-cerebral, and overall RRs were 68.3, 34.1, and 36.6%, respectively. The median PFS of BM and OS were 10.6 and 12.6 months, respectively. The hematologic toxicities were generally mild or moderate and there were no grade 4 or higher non-hematologic toxicities. The combined treatment was generally safe and well-tolerated.

TARGETED THERAPY

The use of drugs targeting the proteins of mutated EGFR and anaplastic lymphoma kinase (ALK) genes has become standard of care in the systemic treatment of metastatic NSCLC (42). In first-line clinical trials of the EGFR-targeted drugs gefitinib, erlotinib, and afatinib, objective response rates (ORRs) of 55–83% were observed, mostly clustering above 70% (43). In addition, large international phase III trials comparing EGFR tyrosine kinase inhibitors (TKIs) against platinum doublet chemotherapy have achieved significant PFS benefits of >4 months with hazard-ratios (HRs) ranging from 0.37 to 0.58, and improvements to symptoms and quality of life (44–47).

The ALK-inhibitor crizotinib has also demonstrated strong anti-tumor activity systemically. In a phase III second-line NSCLC trial of patients with ALK-rearranged tumors randomized to receive crizotinib vs. chemotherapy with docetaxel or pemetrexed, an ORR = 65% was demonstrated, as well as a PFS benefit of 4.7 months vs. chemotherapy (7.7 vs. 3.0 months, HR 0.49, $p < 0.001$) (48). Similarly, in a phase I study of the newer ALK-inhibitor ceritinib, an ORR = 58% was achieved, including an ORR = 56% in tumors that had progressed on crizotinib (49).

The mutation status of tumors is usually derived from biopsies obtained at extracranial sites, and thus, does not necessarily guarantee a mutation in the sub-clones within the brain. However, a Chinese study of 136 NSCLC patients with resected BM, in which an EGFR mutation was identified in 57% of the BM, found a concordance rate of 93.3% in the EGFR mutation status between the primary tumor and BM (50). This suggests that primary tumor EGFR status is a very good surrogate for EGFR mutation status of the BM. In this same cohort of patients, the median OS was 24.5 months in the EGFR mutation group, compared to 15 months in the wild-type group. This finding is consistent with other studies identifying EGFR mutation status as a positive prognostic factor among patients with BM (51).

Just as targeted therapy with EGFR and ALK inhibitors is highly active systemically among molecularly selected NSCLC patients, there is mounting evidence that this is also true for activity intracranially. A recent review has examined the use of the EGFR inhibitors gefitinib and erlotinib in BM among NSCLC patients (6). In the eight phase II clinical trials included in the review, the intracranial RRs with gefitinib were 27–32% in unselected patients, 43% in an Asian population without molecular selection, and 70–89% in molecularly selected patients. Similarly, intracranial RRs were 56 and 82% for erlotinib in clinically and molecularly selected patients, respectively. Taken together, these results highlight both robust intracranial activity and the importance of EGFR mutation status as a predictor of intracranial response. In addition, for the

three studies where OS data were presented, the median OS results were 12.9, 18.8, and 19.8 months, respectively.

CONCLUSION

The management of patients with BM has evolved over the years from an under-studied area to a field of exciting active research. Supportive therapy, surgery, and RT remain the mainstays of management for these patients. Additional areas of active research include techniques to preserve neurocognitive functions with radiotherapy (20, 52), improving the detection and clinical utility of circulating tumor cells (53), and novel systemic approaches including immunotherapy alone (54, 55) or in combination with radiotherapy (56), anti-metabolic agents (57), anti-angiogenesis drugs (58), and novel targeted therapies for a growing list of oncogenic mutations (59). Ultimately, the optimal management strategy will employ a multi-disciplinary approach accounting for individual characteristics of both patient and tumor.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* (2011) **61**(2):69–90. doi:10.3322/caac.20107
2. Schuette W. Treatment of brain metastases from lung cancer: chemotherapy. *Lung Cancer* (2004) **45**(Suppl 2):S253–7. doi:10.1016/j.lungcan.2004.07.967
3. Langer CJ, Mehta MP. Current management of brain metastases, with a focus on systemic options. *J Clin Oncol* (2005) **23**(25):6207–19. doi:10.1200/jco.2005.03.145
4. Lagerwaard FJ, Levendag PC, Nowak PJ, Eijkenboom WM, Hanssens PE, Schmitz PI. Identification of prognostic factors in patients with brain metastases: a review of 1292 patients. *Int J Radiat Oncol Biol Phys* (1999) **43**(4):795–803. doi:10.1016/S0360-3016(98)00442-8
5. Lind JS, Lagerwaard FJ, Smit EF, Postmus PE, Slotman BJ, Senan S. Time for reappraisal of extracranial treatment options? Synchronous brain metastases from nonsmall cell lung cancer. *Cancer* (2011) **117**(3):597–605. doi:10.1002/cncr.25416
6. Jamal-Hanjani M, Spicer J. Epidermal growth factor receptor tyrosine kinase inhibitors in the treatment of epidermal growth factor receptor-mutant non-small cell lung cancer metastatic to the brain. *Clin Cancer Res* (2012) **18**(4):938–44. doi:10.1158/1078-0432.ccr-11-2529
7. Gaspar L, Scott C, Rotman M, Asbell S, Phillips T, Wasserman T, et al. Recursive partitioning analysis (RPA) of prognostic factors in three Radiation Therapy Oncology Group (RTOG) brain metastases trials. *Int J Radiat Oncol Biol Phys* (1997) **37**(4):745–51. doi:10.1016/S0360-3016(96)00619-0
8. Weltman E, Salvajoli JV, Brandt RA, de Morais Hanriot R, Prisco FE, Cruz JC, et al. Radiosurgery for brain metastases: a score index for predicting prognosis. *Int J Radiat Oncol Biol Phys* (2000) **46**(5):1155–61. doi:10.1016/S0360-3016(99)00549-0
9. Lorenzoni J, Devriendt D, Massager N, David P, Ruiz S, Vanderlinden B, et al. Radiosurgery for treatment of brain metastases: estimation of patient eligibility using three stratification systems. *Int J Radiat Oncol Biol Phys* (2004) **60**(1):218–24. doi:10.1016/j.ijrobp.2004.02.017
10. Sperduto PW, Berkey B, Gaspar LE, Mehta M, Curran W. A new prognostic index and comparison to three other indices for patients with brain metastases: an analysis of 1,960 patients in the RTOG database. *Int J Radiat Oncol Biol Phys* (2008) **70**(2):510–4. doi:10.1016/j.ijrobp.2007.06.074
11. Rades D, Dunst J, Schild SE. A new scoring system to predicting the survival of patients treated with whole-brain radiotherapy for brain metastases. *Strahlenther Onkol* (2008) **184**(5):251–5. doi:10.1007/s00066-008-1831-5
12. Viani GA, da Silva LG, Stefano EJ. Prognostic indexes for brain metastases: which is the most powerful? *Int J Radiat Oncol Biol Phys* (2012) **83**(3):e325–30. doi:10.1016/j.ijrobp.2011.12.082
13. Coate LE, John T, Tsao M-S, Shepherd FA. Molecular predictive and prognostic markers in non-small-cell lung cancer. *Lancet Oncol* (2009) **10**(10):1001–10. doi:10.1016/S1470-2045(09)70155-X
14. Zhu CQ, Ding K, Strumpf D, Weir BA, Meyerson M, Pennell N, et al. Prognostic and predictive gene signature for adjuvant chemotherapy in resected non-small-cell lung cancer. *J Clin Oncol* (2010) **28**(29):4417–24. doi:10.1200/jco.2009.26.4325
15. Temel JS, Greer JA, Muzikansky A, Gallagher ER, Admane S, Jackson VA, et al. Early palliative care for patients with metastatic non-small-cell lung cancer. *N Engl J Med* (2010) **363**(8):733–42. doi:10.1056/NEJMoa1000678
16. Ryken TC, McDermott M, Robinson PD, Ammirati M, Andrews DW, Asher AL, et al. The role of steroids in the management of brain metastases: a systematic review and evidence-based clinical practice guideline. *J Neurooncol* (2010) **96**(1):103–14. doi:10.1007/s11060-009-0057-4
17. Patchell RA, Tibbs PA, Walsh JW, Dempsey RJ, Maruyama Y, Kryscio RJ, et al. A randomized trial of surgery in the treatment of single metastases to the brain. *N Engl J Med* (1990) **322**(8):494–500. doi:10.1056/nejm199002223220802
18. Vecht CJ, Haaxma-Reiche H, Noordijk EM, Padberg GW, Voormolen JH, Hoekstra FH, et al. Treatment of single brain metastasis: radiotherapy alone or combined with neurosurgery? *Ann Neurol* (1993) **33**(6):583–90. doi:10.1002/ana.410330605
19. Mintz AH, Kestle J, Rathbone MP, Gaspar L, Hugenholtz H, Fisher B, et al. A randomized trial to assess the efficacy of surgery in addition to radiotherapy in patients with a single cerebral metastasis. *Cancer* (1996) **78**(7):1470–6. doi:10.1002/(SICI)1097-0142(19961001)78:7<1470::AID-CNCR14>3.0.CO;2-X
20. Brown PD, Pugh S, Laack NN, Wefel JS, Khuntia D, Meyers C, et al. Memantine for the prevention of cognitive dysfunction in patients receiving whole-brain radiotherapy: a randomized, double-blind, placebo-controlled trial. *Neuro Oncol* (2013) **15**(10):1429–37. doi:10.1093/neuonc/not114
21. Gondi V, Tome WA, Marsh J, Struck A, Ghia A, Turian JV, et al. Estimated risk of perihippocampal disease progression after hippocampal avoidance during whole-brain radiotherapy: safety profile for RTOG 0933. *Radiother Oncol* (2010) **95**(3):327–31. doi:10.1016/j.radonc.2010.02.030
22. Andrews DW, Scott CB, Sperduto PW, Flanders AE, Gaspar LE, Schell MC, et al. Whole brain radiation therapy with or without stereotactic radiosurgery boost for patients with one to three brain metastases: phase III results of the RTOG 9508 randomised trial. *Lancet* (2004) **363**(9422):1665–72. doi:10.1016/s0140-6736(04)16250-8
23. Chougule P, Burton-Williams M, Saris M. Randomized treatment of brain metastases with gamma knife radiosurgery, whole brain radiotherapy or both. *Int J Radiat Oncol Biol Phys* (2000) **48**:114. doi:10.1016/S0360-3016(00)80024-3
24. Patchell RA, Tibbs PA, Regine WF, Dempsey RJ, Mohiuddin M, Kryscio RJ, et al. Postoperative radiotherapy in the treatment of single metastases to the brain: a randomized trial. *JAMA* (1998) **280**(17):1485–9.
25. Khuntia D, Brown P, Li J, Mehta MP. Whole-brain radiotherapy in the management of brain metastasis. *J Clin Oncol* (2006) **24**(8):1295–304. doi:10.1200/jco.2005.04.6185
26. Borgelt B, Gelber R, Kramer S, Brady LW, Chang CH, Davis LW, et al. The palliation of brain metastases: final results of the first two studies by the Radiation Therapy Oncology Group. *Int J Radiat Oncol Biol Phys* (1980) **6**(1):1–9. doi:10.1016/0360-3016(80)90195-9
27. Borgelt B, Gelber R, Larson M, Hendrickson F, Griffin T, Roth R. Ultra-rapid high dose irradiation schedules for the palliation of brain metastases: final results of the first two studies by the Radiation Therapy Oncology Group. *Int J Radiat Oncol Biol Phys* (1981) **7**(12):1633–8. doi:10.1016/0360-3016(81)90184-X
28. Kurtz JM, Gelber R, Brady LW, Carella RJ, Cooper JS. The palliation of brain metastases in a favorable patient population: a randomized clinical trial by the Radiation Therapy Oncology Group. *Int J Radiat Oncol Biol Phys* (1981) **7**(7):891–5. doi:10.1016/0360-3016(81)90005-5
29. Harwood AR, Simson WJ. Radiation therapy of cerebral metastases: a randomized prospective clinical trial. *Int J Radiat Oncol Biol Phys* (1977) **2**(11–12):1091–4. doi:10.1016/0360-3016(77)90114-6
30. Chatani M, Matayoshi Y, Masaki N, Inoue T. Radiation therapy for brain metastases from lung carcinoma. Prospective randomized trial according to the level of lactate dehydrogenase. *Strahlentherap Onkol* (1994) **170**(3):155–61.
31. Haie-Meder C, Pellae-Cosset B, Laplanche A, Lagrange JL, Tuchais C, Nogues C, et al. Results of a randomized clinical trial comparing two radiation schedules in the palliative treatment of brain metastases. *Radiother Oncol* (1993) **26**(2):111–6. doi:10.1016/0167-8140(93)90091-L
32. Meyers CA, Smith JA, Bezjak A, Mehta MP, Liebmann J, Illidge T, et al. Neurocognitive function and progression in patients with brain metastases treated

with whole-brain radiation and motexafin gadolinium: results of a randomized phase III trial. *J Clin Oncol* (2004) **22**(1):157–65. doi:10.1200/jco.2004.05.128
33. McDuff SG, Taich ZJ, Lawson JD, Sanghvi P, Wong ET, Barker FG II, et al. Neurocognitive assessment following whole brain radiation therapy and radiosurgery for patients with cerebral metastases. *J Neurol Neurosurg Psychiatry* (2013) **84**(12):1384–91. doi:10.1136/jnnp-2013-305166
34. Aoyama H, Shirato H, Tago M, Nakagawa K, Toyoda T, Hatano K, et al. Stereotactic radiosurgery plus whole-brain radiation therapy vs stereotactic radiosurgery alone for treatment of brain metastases: a randomized controlled trial. *JAMA* (2006) **295**(21):2483–91. doi:10.1001/jama.295.21.2483
35. Chang EL, Wefel JS, Hess KR, Allen PK, Lang FF, Kornguth DG, et al. Neurocognition in patients with brain metastases treated with radiosurgery or radiosurgery plus whole-brain irradiation: a randomised controlled trial. *Lancet Oncol* (2009) **10**(11):1037–44. doi:10.1016/s1470-2045(09)70263-3
36. Kocher M, Soffietti R, Abacioglu U, Villa S, Fauchon F, Baumert BG, et al. Adjuvant whole-brain radiotherapy versus observation after radiosurgery or surgical resection of one to three cerebral metastases: results of the EORTC 22952-26001 study. *J Clin Oncol* (2011) **29**(2):134–41. doi:10.1200/jco.2010.30.1655
37. Walbert T, Gilbert MR. The role of chemotherapy in the treatment of patients with brain metastases from solid tumors. *Int J Clin Oncol* (2009) **14**(4):299–306. doi:10.1007/s10147-009-0916-1
38. van den Bent MJ. The role of chemotherapy in brain metastases. *Eur J Cancer* (2003) **39**(15):2114–20. doi:10.1016/S0959-8049(03)00577-X
39. Gerstner ER, Fine RL. Increased permeability of the blood-brain barrier to chemotherapy in metastatic brain tumors: establishing a treatment paradigm. *J Clin Oncol* (2007) **25**(16):2306–12. doi:10.1200/jco.2006.10.0677
40. Barlesi F, Gervais R, Lena H, Hureaux J, Berard H, Paillotin D, et al. Pemetrexed and cisplatin as first-line chemotherapy for advanced non-small-cell lung cancer (NSCLC) with asymptomatic inoperable brain metastases: a multicenter phase II trial (GFPC 07-01). *Ann Oncol* (2011) **22**(11):2466–70. doi:10.1093/annonc/mdr003
41. Dinglin XX, Huang Y, Liu H, Zeng YD, Hou X, Chen LK. Pemetrexed and cisplatin combination with concurrent whole brain radiotherapy in patients with brain metastases of lung adenocarcinoma: a single-arm phase II clinical trial. *J Neurooncol* (2013) **112**(3):461–6. doi:10.1007/s11060-013-1079-5
42. Ettinger DS, Akerley W, Borghaei H, Chang AC, Cheney RT, Chirieac LR, et al. Non-small cell lung cancer, version 2.2013. *J Natl Compr Canc Netw* (2013) **11**(6):645–53.
43. Kohler J, Schuler M. Afatinib, erlotinib and gefitinib in the first-line therapy of EGFR mutation-positive lung adenocarcinoma: a review. *Onkologie* (2013) **36**(9):510–8. doi:10.1159/000354627
44. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* (2009) **361**(10):947–57. doi:10.1056/NEJMoa0810699
45. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* (2012) **13**(3):239–46. doi:10.1016/s1470-2045(11)70393-x
46. Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol* (2013) **31**(27):3327–34. doi:10.1200/jco.2012.44.2806
47. Yang JC, Hirsh V, Schuler M, Yamamoto N, O'Byrne KJ, Mok TS, et al. Symptom control and quality of life in LUX-lung 3: a phase III study of afatinib or cisplatin/pemetrexed in patients with advanced lung adenocarcinoma with EGFR mutations. *J Clin Oncol* (2013) **31**(27):3342–50. doi:10.1200/jco.2012.46.1764
48. Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, Ahn MJ, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med* (2013) **368**(25):2385–94. doi:10.1056/NEJMoa1214886
49. Shaw AT, Kim DW, Mehra R, Tan DS, Felip E, Chow LQ, et al. Ceritinib in ALK-rearranged non-small-cell lung cancer. *N Engl J Med* (2014) **370**(13):1189–97. doi:10.1056/NEJMoa1311107
50. Luo D, Ye X, Hu Z, Peng K, Song Y, Yin X, et al. EGFR mutation status and its impact on survival of Chinese non-small cell lung cancer patients with brain metastases. *Tumour Biol* (2014) **35**(3):2437–44. doi:10.1007/s13277-013-1323-9
51. Eichler AF, Kahle KT, Wang DL, Joshi VA, Willers H, Engelman JA, et al. EGFR mutation status and survival after diagnosis of brain metastasis in nonsmall cell lung cancer. *Neuro Oncol* (2010) **12**(11):1193–9. doi:10.1093/neuonc/noq076
52. Oskan F, Ganswindt U, Schwarz SB, Manapov F, Belka C, Niyazi M. Hippocampus sparing in whole-brain radiotherapy. *Strahlenther Onkol* (2014) **190**(4):337–41. doi:10.1007/s00066-013-0518-8
53. O'Flaherty JD, Gray S, Richard D, Fennell D, O'Leary JJ, Blackhall FH, et al. Circulating tumour cells, their role in metastasis and their clinical utility in lung cancer. *Lung Cancer* (2012) **76**(1):19–25. doi:10.1016/j.lungcan.2011.10.018
54. Sundar R, Soong R, Cho B-C, Brahmer JR, Soo RA. Immunotherapy in the treatment of non-small cell lung cancer. *Lung Cancer* (2014) **85**(2):101–9. doi:10.1016/j.lungcan.2014.05.005
55. Shepherd FA, Douillard JY, Blumenschein GR Jr. Immunotherapy for non-small cell lung cancer: novel approaches to improve patient outcome. *J Thorac Oncol* (2011) **6**(10):1763–73. doi:10.1097/JTO.0b013e31822e28fc
56. Eldredge HB, Denittis A, Duhadaway JB, Chernick M, Metz R, Prendergast GC. Concurrent whole brain radiotherapy and short-course chloroquine in patients with brain metastases: a pilot trial. *J Radiat Oncol* (2013) **2**:315–21. doi:10.1007/s13566-013-0111-x
57. Garon EB, Christofk HR, Hosmer W, Britten CD, Bahng A, Crabtree MJ, et al. Dichloroacetate should be considered with platinum-based chemotherapy in hypoxic tumors rather than as a single agent in advanced non-small cell lung cancer. *J Cancer Res Clin Oncol* (2014) **140**(3):443–52. doi:10.1007/s00432-014-1583-9
58. Blumenschein GR Jr. Developmental antiangiogenic agents for the treatment of non-small cell lung cancer (NSCLC). *Invest New Drugs* (2012) **30**(4):1802–11. doi:10.1007/s10637-011-9750-1
59. Berge EM, Doebele RC. Targeted therapies in non-small cell lung cancer: emerging oncogene targets following the success of epidermal growth factor receptor. *Semin Oncol* (2014) **41**(1):110–25. doi:10.1053/j.seminoncol.2013.12.006

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 April 2014; accepted: 01 September 2014; published online: 15 September 2014.
*Citation: Owen S and Souhami L (2014) The management of brain metastases in non-small cell lung cancer. Front. Oncol. **4**:248. doi: 10.3389/fonc.2014.00248*
This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

REVIEW ARTICLE
published: 16 June 2014
doi: 10.3389/fonc.2014.00146

Targeted treatments of bone metastases in patients with lung cancer

Vera Hirsh*

McGill University Health Centre, Royal Victoria Hospital, Montreal, QC, Canada

Edited by:
Miguel Angel Villalona, The Ohio State University, USA

Reviewed by:
Markus Joerger, Kantonsspital St. Gallen, Switzerland
Meng Xu Welliver, The Ohio State University James Cancer Center, USA

****Correspondence:***
Vera Hirsh, McGill University Health Centre, Royal Victoria Hospital, 687 Pine Avenue West, Montreal, QC H3A 1A1, Canada
e-mail: vera.hirsh@muhc.mcgill.ca

Until now ~30–40% of patients with advanced lung cancer develop bone metastases, but as the newer therapies are extending survival, the chance of developing bone metastases increases. Bone metastases cause skeletal-related events (SREs) such as pathologic fractures, spinal cord compression, radiation therapy or surgery to bone, or hypercalcemia, which can have debilitating consequences affecting patients' health-related quality of life (HR-QOL) and performance status (PS). Poor PS then prevents the patients to receive further lines of treatments, which are available today. SREs are associated with increased economic costs. In one clinical trial, the median time to first SRE was only 5 months. Early detection of bone metastases can prevent SREs and avoid inappropriate implementation of major surgery or chemoradiation therapy. With the new generation bisphosphonate zoledronic acid (ZA) or denosumab (anti-RANKL activity), one can reduce the number of patients who experience SREs, decrease the annual incidence of SREs and delay the median time to first SRE. These agents are effective even after the onset of SREs. They are well tolerated, with manageable side effects. The biochemical markers of bone metabolism especially N-telopeptide of type I collagen and bone specific alkaline phosphatase (BALP) can be both prognostic and predictive markers for the patients with bone metastases from non-small cell lung cancer (NSCLC). Anticancer activity of ZA and denosumab further supports their use as soon as bone metastases are diagnosed in patients with NSCLC. Further trials will inform us about the efficacy of these agents for prevention of bone metastases and even about possible effects on visceral metastases.

Keywords: bone metastases, denosumab, zoledronic acid, NSCLC, biomarkers, skeletal-related events, effect on pain and survival

INTRODUCTION

Approximately 30–40% of patients with lung cancer develop bone metastases (1), which can lead to skeletal-related event (SREs) such as pathologic fractures, spinal cord compression, radiation therapy or surgery to bone, or hypercalcemia. These SREs can affect the patient's health-related quality of life (HR-QOL). Bone metastases are the most common cause of cancer-associated pain in patients with advanced malignancies (2). The bone pain associated with bone metastases often requires palliative radiation therapy. Pathologic fracture, which may require surgery, spinal cord compression, and hypercalcemia of malignancy (HCM) can be life-threatening. In a large prospective trial, pathologic fractures were significantly and negatively correlated with survival among 460 patients with bone metastases from solid tumors, including breast, prostate, kidney, and lung cancers (3). SREs not only cause increased morbidity and deterioration of performance status (PS), but also increased economic costs (4), thus SRE prevention will not only decrease patient morbidity, improve HR-QOL, but will also be associated with decreased use of health care resources. The need to focus on bone metastases and their sequelae is heightened as the survival of patients with non-small cell lung cancer (NSCLC) increases with the newer therapies. In one clinical trial, median time to first SRE in patients with NSCLC was 5 months only (5). To prevent SREs, preserve patients' QOL, good PS, and functional independence are of great importance and will allow patients to receive all the lines of therapies now available.

PATHOPHYSIOLOGY OF BONE METASTASES

The release of growth factors from the bone matrix during osteoclast-mediated osteolysis is conducive to the development of metastatic lesions (6). In osteolytic lesions, factors secreted by tumor cells induce osteoclast recruitment and activation, leading to increased osteolysis (7). Elevated osteolysis decreases bone integrity, can cause bone pain and the release of minerals from the bone matrix, resulting in HCM (8). Bone resorption releases growth factors that stimulate tumor growth and increase of osteoclast-stimulating factors (9). In contrast, tumor cells in osteoblastic lesions secrete factors that stimulate osteoblasts, which are responsible for the formation of new bone tissue (osteogenesis). Levels of osteolysis are enhanced in response to increased osteogenesis, releasing growth factors from the bone matrix (7). Osteoblastic lesions may also contain a strong osteolytic component that can decrease bone integrity (9, 10). Aberrant new bone formation in osteoblastic lesions produces new bone tissue that is abnormal, malformed, and does not add to the overall bone strength (9, 11).

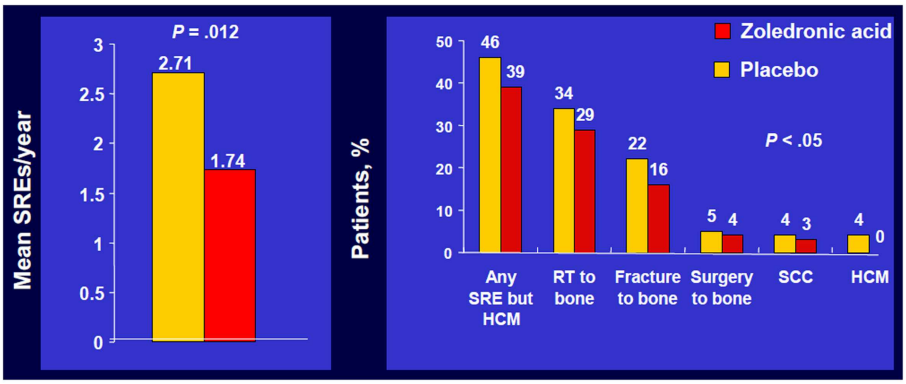


FIGURE 1 | Zoledronic Acid reduced percentage of patients with each SRE. Phase III trial of patients with bone metastases from NSCLC/OST who received ZOL or placebo every 3 weeks for up to 21 months. Approximately 50% of patients had NSCLC; ~7% of patients had SCLC. SRE, skeletal-related event; mets, metastases; NSCLC, non-small cell lung cancer; OST, other solid tumors; RT, radiotherapy; SCC, spinal cord compression; HCM, hypercalcemia of malignancy. Data from Rosen et al. (5).

EARLY DETECTION OF BONE METASTASES

The incorrect staging of patients with NSCLC can result in suboptimal treatment decisions such as major surgery or an aggressive chemoradiation without hope for a curative outcome.

Recently, PET scanning for accurate staging of NSCLC has been recognized as a valuable tool by the National Comprehensive Cancer Network (12). Fluorine-18 deoxyglucose (FDG)-PET scans for the detection of bone metastases in NSCLC have been shown to have a higher specificity compared with bone scans (~90 versus 70%, respectively) (13, 14) and a much lower rate of false negatives (6 versus 39%, respectively) (15). The sensitivity of FDG-PET and bone scans for the detection of bone metastases from NSCLC was comparable after appropriate follow-up imaging (13, 14).

CLINICAL IMPLICATIONS OF BONE METASTASES – BISPHOSPHONATES, ZOLEDRONIC ACID

Bisphosphonates are pyrophosphate analogs that are deposited at sites of bone remodeling. They bind to bone mineral surfaces and are ingested by osteoclasts wherein they inhibit osteolysis (16). Early bisphosphonates i.e., etidronate, clodronate, demonstrated efficacy for the treatment of HCM, but these agents are weak with limited utility in the oncology setting (16).

The introduction of a nitrogen group to the bisphosphonate backbone resulted (17) in increased potency and a different cellular target: farnesyl diphosphonate synthase, a key enzyme in the mevalonate pathway. These bisphosphonates inhibit protein prenylation and RAS signaling in osteoclasts, thereby inducing apoptosis (18). Zoledronic acid consistently achieved the greatest antiresorptive efficacy among the bisphosphonates tested in preclinical assays in human cancer cell lines and animal models (19, 20).

Regulatory approval for zoledronic acid (ZA) in patients with any solid tumors was based on results from a phase III randomized, placebo-controlled trial in which 773 patients with bone metastases from solid tumors other than breast or prostate cancer received ZA (4 or 8 mg) or placebo via 15 min intravenous infusion every 3 weeks for up to 21 months (5). Among the 507 patients randomized to the 4 mg ZA or placebo group of this trial, 249 had NSCLC and 36 had small cell lung cancer (SCLC).

In the overall trial population, ZA significantly reduced the number of patients who experienced at least one SRE, including HCM, 39 versus 48% with placebo, $p = 0.039$, and reduced the proportion of patients who experienced each type of SRE (**Figure 1**) (5).

Zoledronic acid also significantly decreased the annual incidence of SREs, 1.74 versus 1.71 per year for placebo, $p = 0.012$ and significantly delayed the median time to first SRE compared with placebo (236 versus 155 days, respectively, $p = 0.009$) (5). A multiple event analysis using a robust Andersen–Gill model was performed for the overall population. This analysis takes into account not only the number of SREs but also the timing between the SREs, thereby providing a sensitive comparison of the ongoing risk of SREs between two treatment groups.

Zoledronic acid reduced the risk of SREs by 31% versus placebo in the overall trial population (relative risk, RR = 0.693, $p = 0.003$). Many patients with lung cancer are diagnosed only after the first SRE. However, pre-existing skeletal morbidity does not preclude the benefits of subsequent therapy with ZA. Indeed, patients who have already experienced an SRE are at especially high risk for subsequent events. In an exploratory analysis of the ZA phase III trial in patients with NSCLC and other solid tumors, patients with a history of SRE before study entry had a 41% increased risk of experiencing an on-study SRE compared with patients with no history of prior SRE ($p = 0.036$) (21). In patients with a prior SRE, ZA produced a significant 31% reduction in the risk of developing an on-study SRE compared with placebo in a robust Andersen–Gill multiple event analysis, $p = 0.009$, and significantly reduced the skeletal morbidity rate, 1.96 versus 2.81 events per year for placebo, $p = 0.030$ (21).

Furthermore, ZA significantly prolonged the median time to first SRE on study by ~4 months compared with placebo in this prior-SRE cohort (215 versus 106 days, respectively, $p = 0.011$). Benefits were also seen in the subset of patients who had not experienced a prior SRE, but without a statistical significance

because of lack of the statistical power. This study suggests that ZA is effective and provides benefits even after the onset of SREs.

The most commonly reported adverse events (AEs) for ZA and placebo during the trial were bone pain including infusion of ZA-related pain (48 and 58%, respectively), nausea (47 and 32%, respectively), and dyspnea (45 and 30%, respectively) (22). There was no significantly lower incidence of palliative radiotherapy to bone in the 4 mg ZA group versus placebo (23). There were no grade 4 increases in serum creatinine in the NSCLC stratum. Monitoring of renal function and oral health during bisphosphonate therapy is recommended to avoid uncommon, but potentially serious AEs (24, 25). Because all intravenous bisphosphonates are cleared by the kidneys, renal function, and hydration status should be determined before each infusion to ensure renal safety. Reduced starting dose of ZA is recommended for patients with impaired renal function (26).

Osteonecrosis of the jaw (ONJ) has been reported as an uncommon event in patients receiving bisphosphonates and is characterized by exposed bone in the maxillofacial area with no evidence of healing after 6 weeks of appropriate dental care in the absence of metastatic disease or radiation to the jaw (25). The reports using the data obtained from retrospective analyses and reviews of medical records databases suggest that the frequency of ONJ in patients with malignant bone disease may be between 0.7 and 12.6% (27–29).

This wide range in ONJ frequency is likely due to variability in preventive dental measures before and during bisphosphonate therapy, variations in the duration of bisphosphonate treatment, and geographic differences. Preventive dental measures and appropriate oral hygiene have been identified that can significantly reduce the incidence of ONJ during bisphosphonate therapy (25, 30–32). A pilot study in patients with active ONJ lesions found that local application of a medical ozone oil suspension led to complete ONJ resolution (33).

ZOLEDRONIC ACID AND BIOCHEMICAL MARKERS

In a subset of patients with NSCLC or other solid tumors in the placebo group (238 patients), urinary levels of the bone resorption marker N-telopeptide of type I collagen (NTX) and the serum bone formation marker bone specific alkaline phosphatase (BALP) were assessed approximately every 3 months (34). High NTX levels (≥100 nmol/mmol creatinine) at baseline were associated with an increased risk of first SRE (RR = 1.85, $p = 0.076$) and bone disease progression (RR = 1.76, $p = 0.029$) compared with patients with low NTX levels (<100 nmol/mmol creatinine, **Figure 2**) (34). Moreover, compared with patients with low NTX levels, patients with high NTX levels had a more than threefold increased risk of death (RR = 3.03, $p < 0.001$) and a 5-month reduction in median survival (3.2 versus 8.2 months for patients with low baseline NTX levels) (34). Patients with high baseline BALP levels (≥146 IU/L) also had statistically significant increases in risk of disease progression (RR = 1.77, $p = 0.005$) and death (RR = 1.53, $p = 0.003$) compared with patients with low BALP levels (<146 IU/L) (34).

Exploratory analysis of the ZA phase III clinical trial database (36) showed that ZA reduced mean urinary NTX levels within 3 months in patients with bone metastases from NSCLC and other solid tumors who had bone marker assessment ($n = 204$) (35). ZA also significantly reduced the RR of death by 35% versus placebo (RR = 0.650, $p = 0.024$) among patients with NSCLC and high baseline NTX levels (NTX ≥ 64 nmol/mmol creatinine, $n = 144$) (37).

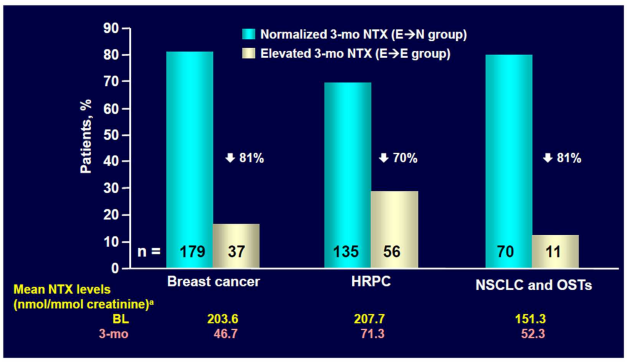


FIGURE 2 | ZOL normalized NTX levels within 3 months in most patients with elevated baseline NTX. NTX, N-telopeptide of type I collagen; HRPC, hormone-refractory prostate cancer; NSCLC, non-small cell lung cancer; OST, other solid tumors; BL, baseline. Data from Lipton et al. (35).

Differences in survival between the ZA and placebo groups did not reach statistical significance in the normal baseline NTX subset, consistent with the lower risks of SREs and death that have been reported for that subset (34, 37).

This benefit could result from reduced osteolysis, resulting in less release of growth factors from the bone matrix, reduced SRE rate or possibly also from direct and indirect antitumor effects of ZA i.e., increased apoptosis, synergism with chemotherapy, antiangiogenesis, and stimulation of immune system.

ANTICANCER ACTIVITY OF ZOLEDRONIC ACID

There is a preclinical evidence that ZA can inhibit proliferation and induce apoptosis in a broad range of human cancer cell lines (16, 38) ZA also exerts antitumor synergy with chemotherapy agents in the A549 lung cancer cell line (39, 40). In murine lung cancer cell line, ZA inhibited the growth of these tumors and mice treated with ZA survived significantly longer than the untreated mice ($p < 0.05$) (41).

Multiple effects may contribute to the antitumor activity of ZA that has been reported in preclinical models (42). In addition to direct antitumor effects, nitrogen-containing bisphosphonates appear to have immunomodulatory properties especially with regard to γδ T cells, a subset of T cells that plays a role in immunosurveillance for malignancies. In an *in vitro* model, ZA induced maturation and upregulated co-stimulating surface receptor expression (e.g., CD 40, CD 80, CD 83) on peripheral γδ T cells (43). In addition, bisphosphonates have been shown to activate the cytolytic activity of γδ T cells and therefore, may enhance the antitumor immune response (44).

There are ongoing clinical studies in patients with NSCLC evaluating the efficacy of ZA both for prevention of bone metastases and for antitumor activity.

DENOSUMAB AND ANTI-RANKL ACTIVITY

Denosumab is a fully human monoclonal antibody that binds to and neutralizes RANKL (receptor activator of nuclear factor kappa-B ligand) thereby inhibiting osteoclast function and preventing generalized bone resorption and local bone destruction.

It is hypothesized that tumor cells in the bone lead to increased expression of RANKL on osteoclasts and their precursors. RANKL is an essential mediator of osteoclast function, formation, and survival (45–47). Excessive RANKL-induced osteoclast activity results in resorption and local bone destruction with evidence of elevated levels of bone turnover markers, leading to SREs (34, 36).

Denosumab has been studied in two phase II trials of patients with bone metastases in advanced cancer and in one phase II trial with myeloma (48–50). These studies demonstrated that treatment with denosumab at doses ranging from 30 to 180 mg administered every 4 or 12 weeks was associated with a rapid and sustained suppression of bone turnover markers and delay of SREs similar to that seen with i.v. bisphosphonates.

In a randomized, double-blind phase III trial of denosumab versus ZA, in the treatment of bone metastases in patients with advanced cancer (excluding breast and prostate cancer) or multiple myeloma, 1779 patients were enrolled onto study, 890 patients analyzed on ZA, 886 on denosumab (51). Baseline characteristics were well balanced (**Table 1**). The primary endpoint was time to first on-study SRE comparing denosumab with ZA for non-inferiority. Secondary efficacy endpoints were to be evaluated only if non-inferiority was demonstrated, and were superiority tests comparing denosumab and ZA for time to first on-study SRE and time to first and subsequent SRE by multiple event analysis. A subsequent SRE was defined as an event occurring ≥21 days after the previous SRE.

The median number of doses was seven for ZA and seven for denosumab with cumulative drug exposure of 651.9 patient-years for ZA and 675.3 patient-years for denosumab. Median time on study was ~7 months.

Table 1 | Baseline characteristics.

Characteristic, *n* (%) or median	Zoledronic acid (*n* = 890)	Denosumab (*n* = 886)
Male	552 (62)	588 (66)
Age (years)	61	60
Primary tumor type		
Non-small cell lung cancer	345 (39)	343 (39)
Multiple myeloma	93 (10)	86 (10)
Other	452 (51)	457 (52)
ECOG performance status of 0 or 1	728 (82)	748 (84)
Time from first bone metastasis to randomization (months)	2	2
Previous SRE	446 (50)	440 (50)
Presence of visceral metastases	448 (50)	474 (53)

See Ref. (52).

Denosumab was non-inferior to ZA in delaying time to first on-study SRE (HR = 0.84, $p = 0.0007$) representing 16% reduction in hazard (**Figure 3**). The median time to first on-study SRE was 20.6 months for denosumab and 16.3 months for ZA. The test for superiority for time to first SRE showed $p = 0.06$ and therefore did not reach statistical significance. Time to first and subsequent SREs (multiple events) analysis demonstrated a rate ratio of 0.90 for denosumab compared with ZA, $p = 0.14$, which was not statistically significant. Overall survival (HR = 0.95, $p = 0.43$) and disease progression (HR = 1.00, $p = 1.0$) were similar between treatment groups (**Figures 4** and **5**).

The effect of denosumab on time to first on-study SRE relative to ZA by tumor stratification factors resulted in an HR = 0.84 for NSCLC, $p = 0.20$; 1.03 for myeloma, $p = 0.89$, and 0.79 for other solid tumors, $p = 0.04$. An *ad hoc* analysis examining overall survival demonstrated an HR = 0.79 for NSCLC, 2.26 for myeloma, and 1.08 for other solid tumors.

Patients in both arms experienced similar rates of AEs (**Table 2**). Rates of serious AEs are 13.4% for ZA versus 14.6% for denosumab. New primary malignancy occurred in three patients (0.3%) receiving ZA and in five patients (0.6%) receiving denosumab.

Adverse events of hypocalcemia occurred more frequently with denosumab (10.8% denosumab, 5.8% ZA). In general, the clinical consequences of hypocalcemia were not observed. Centrally determined grade 3 and 4 decreases in albumin-adjusted calcium values were reported in 9 patients (1%) receiving ZA and 20 patients (2.3%) receiving denosumab. IV calcium was administered on study to 2.7% of patients receiving ZA and 5.7% receiving denosumab.

Positive adjudicated ONJ occurred with cumulative incidence rates in the ZA and denosumab groups of 0.6 and 0.5% at 1 year, respectively, 0.9 and 1.1% at 2 years, and 1.3 and 1.1% at 3 years ($p = 1.0$).

Adverse events associated with acute phase reactions within the first 3 days after dose 1 occurred in 14.5% of patients receiving ZA versus 6.9% receiving denosumab. Most frequent reactions were pyrexia, arthralgia, and fatigue. One hundred fifty-two patients (17.3%) on ZA required dose adjustments to levels lower than 4 mg and doses were withheld because of elevated serum creatinine in 78 patients (8.9%). No dose adjustments or dose withholding for renal function were required for denosumab. Despite appropriate adjustments of the ZA dosing regimen for renal function, there was an evidence of an excess of renal AEs with ZA. Denosumab has no limitations with respect to renal impairment as it is a monoclonal antibody and is eliminated by intracellular catabolism in phagocytes, with no evidence of renal effects (53, 54).

BONE TURNOVER BIOMARKERS – DENOSUMAB VERSUS ZOLEDRONIC ACID

Patients treated with denosumab experienced a greater suppression of bone turnover markers than with ZA. Between baseline and study week 13 levels of urinary NTX/Cr decreased by a median of 76% for denosumab ($n = 546$) and 65% for ZA ($n = 543$), $p < 0.001$ and BALP decreased by 37% for denosumab ($n = 578$) and 29% for ZA ($n = 581$), $p < 0.001$.

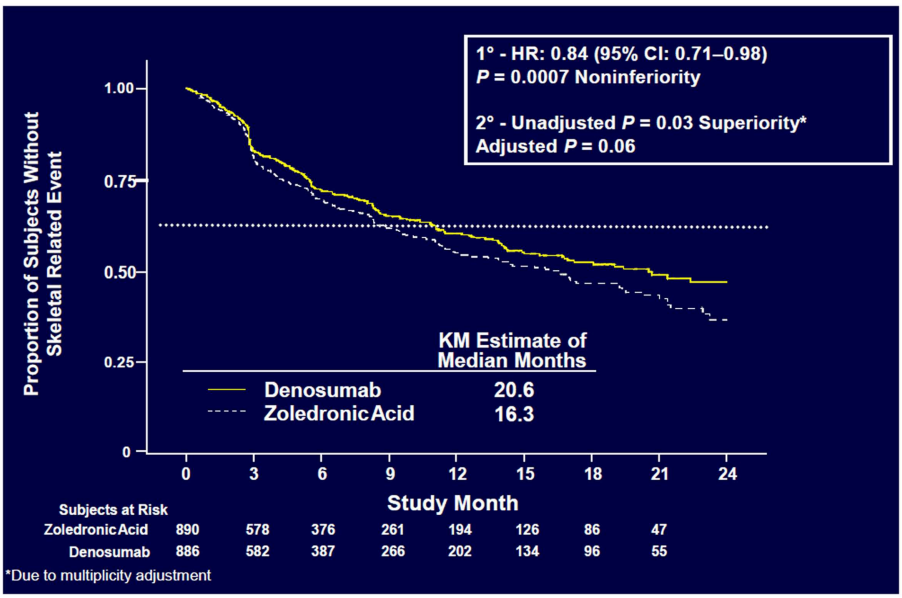


FIGURE 3 | Time to first on-study SRE (52).

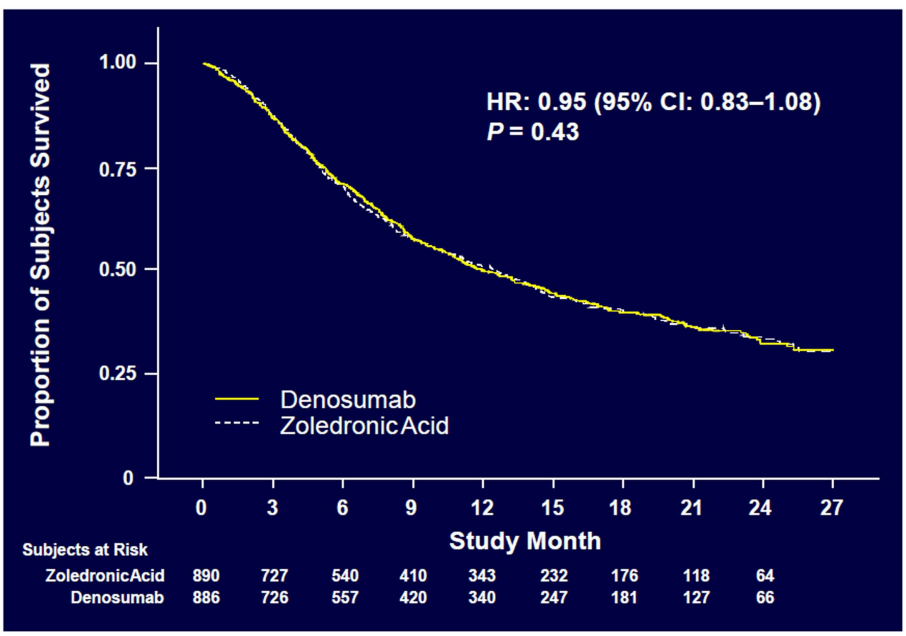


FIGURE 4 | Overall survival (52).

EXPLORATORY ANALYSIS OF OVERALL SURVIVAL IN LUNG CANCER

Sub-analysis of 811 patients with any lung cancer showed that denosumab was associated with significantly improved overall median survival compared with ZA, with a difference of 1.2 months (KM median = 8.9 versus 7.7 months, HR = 0.80, $p = 0.01$) (**Figure 6**) (55). Denosumab continued to show a significant survival advantage over ZA when overall survival was adjusted for relevant baseline covariates (age, sex, time from diagnosis of primary cancer to first evidence of metastasis or the first bone metastasis, visceral metastasis, and ECOG status) and stratified by the randomization stratification factors (previous SRE and systemic anticancer therapy), HR = 0.81, $p = 0.01$. In patients with visceral metastases (231 in denosumab group and 233 in

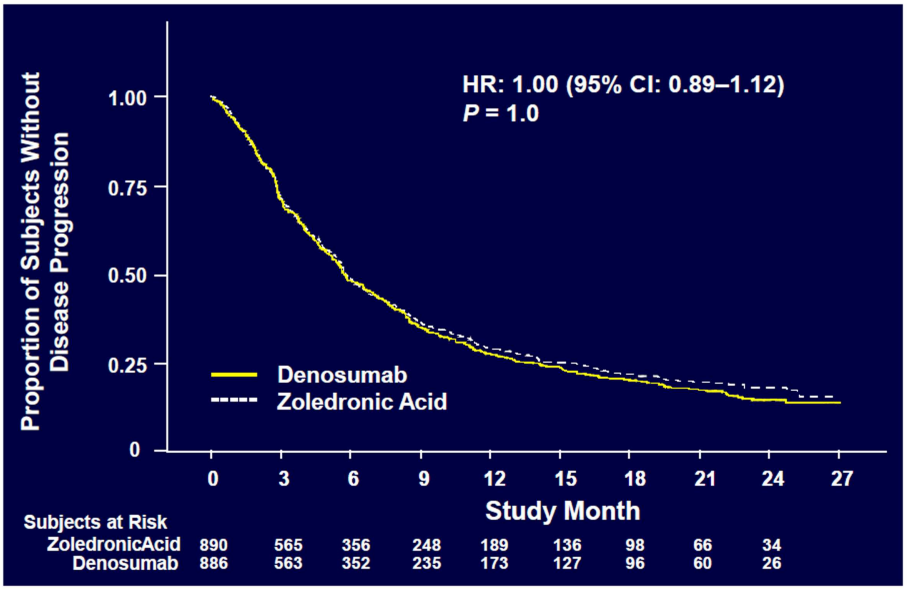


FIGURE 5 | Overall disease progression (52).

Table 2 | Adverse events of interest.

Event, *n* (%)	Zoledronic acid (*n* = 878)	Denosumab (*n* = 878)
Infectious AEs	349 (39.7)	358 (40.8)
Infectious serious AEs	118 (13.4)	128 (14.6)
Acute phase reaction (first 3 days)	127 (14.5)	61 (6.9)
Potential renal toxicity AEs[a]	96 (10.9)	73 (8.3)
Renal failure	25 (2.8)	20 (2.3)
Acute renal failure	16 (1.8)	11 (1.3)
Cumulative rates of ONJ*	11 (1.3)	10 (1.1)
Year 1	5 (0.6)	4 (0.5)
Year 2	8 (0.9)	10 (1.1)
New primary malignancy	3 (0.3)	5 (0.6)

[a] *Includes blood creatinine increased, renal failure, renal failure acute, proteinuria, blood urea increased, renal impairment, urine output decreased, anuria, oliguria, azotemia, hypercreatininemia, creatinine renal clearance decreased, renal failure chronic, blood creatinine abnormal.*

**p = 1.0.*

No neutralizing anti-denosumab antibodies were detected.

See Ref. (52).

ZA group), denosumab was also associated with improved overall median survival with a difference of 1.2 months (KM median = 7.7 versus 6.4 months, HR = 0.79, $p = 0.03$). Denosumab was associated with significantly improved survival in patients with NSCLC with a difference of 1.5 months (KM median = 9.5 versus 8.1 months, HR = 0.78, $p = 0.01$) (**Figure 7**).

Explanation for the longer survival with the denosumab treatment in these lung cancer patients includes both direct and indirect effects on tumor cells. An indirect effect may derive from the symbiotic relationship between tumor cells and the bone marrow microenvironment in which both bone destruction and tumor growth are promoted. Tumor cells secrete various factors that stimulate production of RANKL (45). The increased expression of RANKL in the tumor environment leads to increased formation, activation, and survival of osteoclasts and results in osteolytic lesions (56). Osteolysis then results in the release of growth factors derived from bone (45, 57).

These growth factors increase the production of parathyroid hormone-related protein or promote tumor growth directly (45). Bone destruction increases local extracellular calcium concentrations, which have also been shown to promote tumor growth and the production of parathyroid hormone-related protein (57). Denosumab may indirectly affect skeletal tumor progression by targeting osteoclasts and disrupting the interaction between tumor cells and the bone microenvironment. RANKL inhibition has been shown to reduce bone lesions/osteolysis, and skeletal tumor burden in a model of NSCLC (58) and to enhance antitumor efficacy of other therapies on skeletal tumors (59, 60).

Another hypothesis is that denosumab may improve survival by directly inhibiting RANKL on RANK-expressing tumor cells, which has been demonstrated for breast cancer cells in vivo (61) and for a number of tumor cell lines (including lung cancer cells) in vitro (62). RANKL inhibition may have a direct antineoplastic effect on lung cancer cells via apoptosis or anti-migration activity (63). The hypothesis of mechanism of anticancer activities, which inhibit RANKL or RANK-expressing tumor cells has been described in more detail in the review article of Peters and Meylan (64). These findings warrant further prospective clinical investigations, denosumab might have anticancer effects beyond the skeleton (65).

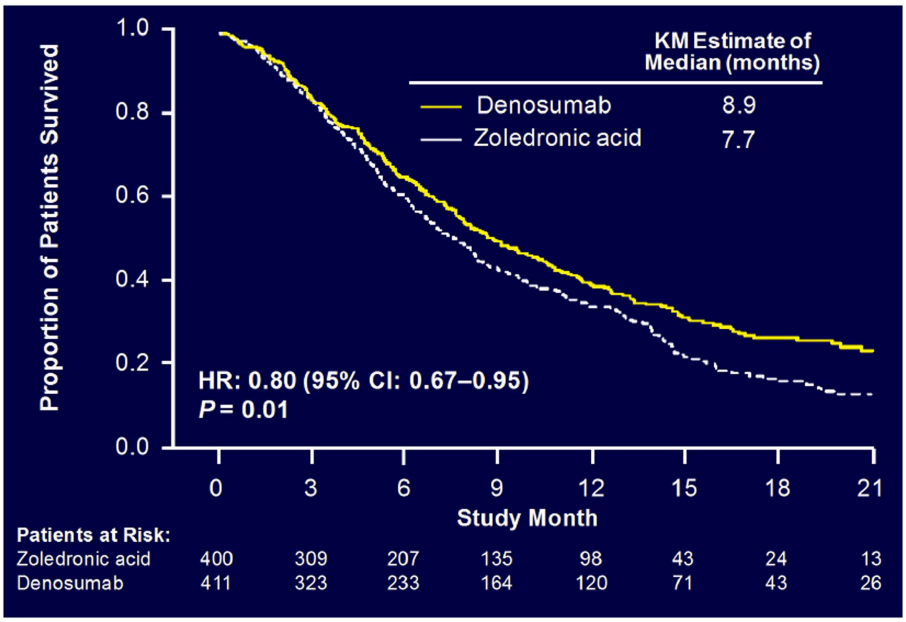


FIGURE 6 | Overall survival: patients with lung cancer.

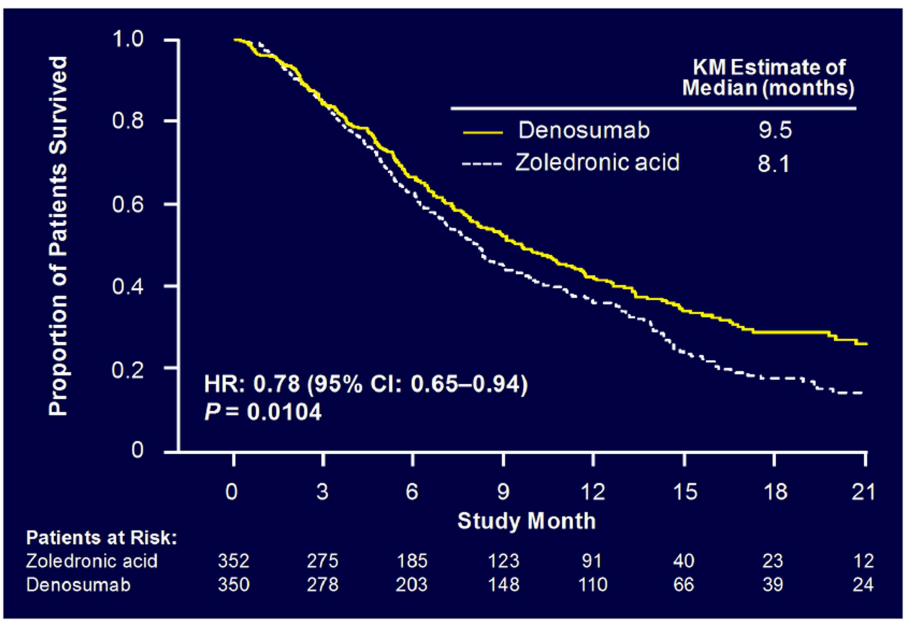


FIGURE 7 | Overall survival: patients with NSCLC.

PROMISING NEW BONE TARGETING AGENTS

Targeting bone agents in the early stage of investigation in NSCLC are Dasatinib (i.e., anti-src activity) (66), ACE-011 (Sotatercept – Activin TRAP) (67, 68), Cabozantinib (anti-RET agent) (69), and Radium 223 (targeted alpha emitter) (70).

CONCLUSION

In the palliative group of patients with metastatic lung cancer, the HR-QOL is extremely important. Preserving a good PS, which enables these patients to receive all the available lines of treatment for metastatic NSCLC is also desirable.

Early identification of bone metastases and management of SREs have become crucial for maintaining QOL and containing healthcare costs throughout the patient's care. The identification of risk factors for skeletal metastases in patients with NSCLC will help us to implement early treatment to prevent or delay the onset of debilitating SREs.

The safety profile for ZA and denosumab is similar but subcutaneous administration of denosumab offers advantages over intravenous administration with no need for renal monitoring. Denosumab is associated with fewer acute phase reactions, but has a higher incidence of hypocalcemia. ONJ is similar for both agents. Thus both agents are a reasonable option for targeted bone therapy.

Future trials are needed to inform us about efficacy of these agents for prevention of bone metastases and effects on visceral metastases, too, thus contributing to a longer survival in patients with metastatic NSCLC.

REFERENCES

1. Coleman RE. Skeletal complications of malignancy. *Cancer* (1997) **80**(Suppl):1588–94. doi:10.1002/(SICI)1097-0142(19971015)80:8+<1588::AID-CNCR9>3.3.CO;2-Z
2. Mercadante S. Malignant bone pain: pathophysiology and treatment. *Pain* (1997) **69**:1–18. doi:10.1016/S0304-3959(96)03267-8
3. Hansen BH, Keller J, Laitinen M, Berg P, Skjeldal S, Trovik C, et al. The Scandinavian Sarcoma Group Skeletal Metastasis Register. Survival after surgery for bone metastases in the pelvis and extremities. *Acta Orthop Scand Suppl* (2004) **75**:11–5. doi:10.1080/00016470410001708270
4. Delea TE, McKiernan JM, Liss M, Edelsberg JS, Brandman J, Sung JC, et al. Impact of skeletal complications on total medical care costs in lung cancer patients with bone metastases [abstract]. *J Clin Oncol* (2004) **22**(Suppl 14):6064.
5. Rosen LS, Gordon D, Tchekmedyian NS, Yanagihara R, Hirsh V, Krzakowski M, et al. Long-term efficacy and safety of zoledronic acid in the treatment of skeletal metastases in patients with non-small cell lung carcinoma and other solid tumors: a randomized, phase III, double-blind, placebo-controlled trial. *Cancer* (2004) **100**:2613–21. doi:10.1002/cncr.20308
6. Mundy GR. Mechanisms of bone metastasis. *Cancer* (1997) **80**(Suppl):1546–56. doi:10.1002/(SICI)1097-0142(19971015)80:8+<1546::AID-CNCR4>3.3.CO;2-R
7. Saad F, Schulman CC. Role of bisphosphonates in prostate cancer. *Eur Urol* (2004) **45**:26–34. doi:10.1016/j.eururo.2003.10.003
8. Coleman RE. Metastatic bone disease: clinical features, pathophysiology, and treatment strategies. *Cancer Treat Rev* (2001) **27**:165–76. doi:10.1053/ctrv.2000.0210
9. Kakonen SM, Mundy GR. Mechanisms of osteolytic bone metastases in breast carcinoma. *Cancer* (2003) **97**(Suppl):834–9. doi:10.1002/cncr.11132
10. Coleman RE. Bisphosphonates: clinical experience. *Oncologist* (2004) **9**(Suppl 4):14–27. doi:10.1634/theoncologist.9-90004-14
11. Lipton A. Pathophysiology of bone metastases: how this knowledge may lead to therapeutic intervention [Review]. *J Support Oncol* (2004) **2**(3):205–13.
12. National Comprehensive Cancer Network. *NCCN clinical practice guidelines in oncology: non-small cell lung cancer.* (2008). Available from: http://www.nccn.org/professionals/physician_gls/PDF/nsclc.pdf
13. Bury T, Barreto A, Daenen F, Barthelemy N, Ghaye B, Rigo P. Fluorine-18 deoxyglucose positron emission tomography for the detection of bone metastases in patients with non-small cell lung cancer. *Eur J Nucl Med* (1998) **25**:1244–7. doi:10.1007/s002590050291
14. Gayed I, Vu T, Johnson M, Macapinlac H, Podoloff D. Comparison of bone and 2-deoxy-2-[18F] fluoro-D-glucose positron emission tomography in the evaluation of bony metastases in lung cancer. *Mol Imaging Biol* (2003) **5**:26–31. doi:10.1016/S1536-1632(03)00036-2
15. Hetzel M, Arslandemir C, König HH, Buck AK, Nüssle K, Glatting G, et al. F-18 NaF PET for detection of bone metastases in lung cancer: accuracy, cost-effectiveness, and impact on patient management. *J Bone Miner Res* (2003) **18**:2206–14. doi:10.1359/jbmr.2003.18.12.2206
16. Green JR. Preclinical profile and anticancer potential of zoledronic acid. In: Birch EV editor. *Trends in Bone Cancer Research.* (Vol. 24), New York: Nova Science Publishers Inc (2006). p. 217–45.
17. Fleisch H. Development of bisphosphonates. *Breast Cancer Res* (2002) **4**:30–4. doi:10.1186/bcr487
18. Green JR. Bisphosphonates: preclinical review. *Oncologist* (2004) **9**(Suppl 4):3–13. doi:10.1634/theoncologist.9-90004-3
19. Green J. Zoledronate: the preclinical pharmacology. *Br J Clin Pract Suppl* (1996) **87**:16–8.
20. Green JR, Muller K, Jaeggi KA. Preclinical pharmacology of CGP 42'446, a new, potent, heterocyclic bisphosphonate compound. *J Bone Miner Res* (1994) **9**:745–51. doi:10.1002/jbmr.5650090521
21. Hirsh V, Tchekmedyian NS, Rosen LS, Zheng M, Hei YJ. Clinical benefit of zoledronic acid in patients with lung cancer and other solid tumors: analysis based on history of skeletal complications. *Clin Lung Cancer* (2004) **6**(3):170–4. doi:10.3816/CLC.2004.n.030
22. Bukowski R, Rosen L, Gordon D, et al. Long-term therapy with zoledronic acid is effective and safe in reducing the risk of skeletal complications in patients with bone metastases from non-small cell lung cancer (NSCLC) [poster]. *Proceedings of the 10th World Conference on Lung Cancer (WCLC)*; 2003 Aug 10–14. Vancouver (2003).
23. Rosen LS, Gordon D, Tchekmedyian S, Yanagihara R, Hirsh V, Krzakowski M, et al. Zoledronic acid versus placebo in the treatment of skeletal metastases in patients with lung cancer and other solid tumors: a phase III, double-blind, randomized trial – the Zoledronic Acid Lung Cancer and other Solid Tumors Study Group. *J Clin Oncol* (2003) **21**:3150–7. doi:10.1200/JCO.2003.04.105
24. Conte P, Guarneri V. Safety of intravenous and oral bisphosphonates and compliance with dosing regimens. *Oncologist* (2004) **9**(Suppl 4):28–37. doi:10.1634/theoncologist.9-90004-28
25. Weitzman R, Sauter N, Eriksen EF, Tarassoff PG, Lacerna LV, Dias R, et al. Critical review: updated recommendations for the prevention, diagnosis, and treatment of osteonecrosis of the jaw in cancer patients – May 2006. *Crit Rev Oncol Hematol* (2007) **62**:148–52. doi:10.1016/j.critrevonc.2006.12.005
26. Zometa (Zoledronic acid) Injection, sterile solution for IV use, 4 mg/100 ml, Monograph Citation NDA021223, *Chemical Ingredient: Zoledronic Acid Anhydrous.* East Hanover, NJ: Novartis Pharmaceuticals Corporation (2005).
27. Durie BG, Katz M, Crowley J. Osteonecrosis of the jaw and bisphosphonates. *N Engl J Med* (2005) **353**:99–102. doi:10.1056/NEJM200507073530120
28. Hoff AO, Toth BB, Altundag K, Johnson MM, Warneke CL, Hu M, et al. Frequency and risk factors associated with osteonecrosis of the jaw in cancer patients treated with intravenous bisphosphonates. *J Bone Miner Res* (2008) **23**:826–36. doi:10.1359/jbmr.080205
29. Pozzi S, Marcheselli R, Sacchi S, Baldini L, Angrilli F, Pennese E, et al. Bisphosphonate-associated osteonecrosis of the jaw: a review of 35 cases and an evaluation of its frequency in multiple myeloma patients. *Leuk Lymphoma* (2007) **48**:56–64. doi:10.1080/10428190600977690
30. Dimopoulos MA, Kastritis E, Bamia C, Melakopoulos I, Gika D, Roussou M, et al. Reduction of osteonecrosis of the jaw (ONJ) after implementation of preventive measures in patients with multiple myeloma treated with zoledronic acid. *Ann Oncol* (2009) **20**:117–20. doi:10.1093/annonc/mdn554
31. Montefusco V, Gay F, Spina F, Miceli R, Maniezzo M, Teresa Ambrosini M, et al. Antibiotic prophylaxis before dental procedures may reduce the incidence of osteonecrosis of the jaw in patients with multiple myeloma treated with bisphosphonates. *Leuk Lymphoma* (2008) **49**:2156–62. doi:10.1080/10428190802483778
32. Ripamonti CI, Maniezzo M, Campa T, Fagnoni E, Brunelli C, Saibene G, et al. Decreased occurrence of osteonecrosis of the jaw after implementation of dental preventive measures in solid tumour patients with bone metastases treated with bisphosphonates. The experience of the National Cancer Institute of Milan. *Ann Oncol* (2009) **20**:137–45. doi:10.1093/annonc/mdn526
33. Ripamonti C, Maniezzo M, Ghiringhelli R, et al. Medical oil suspension applications heal osteonecrosis of the jaw (ONJ) in patients treated with bisphosphonates (BPs): preliminary results of a single institution protocol [poster]. Primary therapy of early breast cancer. *Proceedings of the 11th International Conference [Poster 194]* (2009).
34. Brown JE, Cook RJ, Major P, Lipton A, Saad F, Smith M, et al. Bone turnover markers as predictors of skeletal complications in prostate cancer, lung cancer,

and other solid tumors. *J Natl Cancer Inst* (2005) **97**:59–69. doi:10.1093/jnci/dji002
35. Lipton A, Cook R, Saad F, Major P, Garnero P, Terpos E, et al. Normalization of bone markers is associated with improved survival in patients with bone metastases from solid tumors and elevated bone resorption receiving zoledronic acid. *Cancer* (2008) **113**:193–201. doi:10.1002/cncr.23529
36. Coleman RE, Major P, Lipton A, Brown JE, Lee KA, Smith M, et al. Predictive value of bone resorption and formation markers in cancer patients with bone metastases receiving the bisphosphonate zoledronic acid. *J Clin Oncol* (2005) **23**:4925–35. doi:10.1200/JCO.2005.06.091
37. Hirsh V, Major PP, Lipton A, Cook RJ, Langer CJ, Smith MR, et al. Zoledronic acid and survival in patients with metastatic bone disease from lung cancer and elevated markers of osteoclast activity. *J Thorac Oncol* (2008) **3**:228–36. doi:10.1097/JTO.0b013e3181651c0e
38. Green JR. Antitumor effects of bisphosphonates. *Cancer* (2003) **97**(Suppl):840–7. doi:10.1002/cncr.11128
39. Ozturk OH, Bozcuk H, Burgucu D, Ekinci D, Ozdogan M, Akca S, et al. Cisplatin cytotoxicity is enhanced with zoledronic acid in A549 lung cancer cell line: preliminary results of an in vitro study. *Cell Biol Int* (2007) **31**:1069–71. doi:10.1016/j.cellbi.2007.02.004
40. Gjyrezi A, O'Brate A, Chanel-Vos C, Giannakakou P. Zoledronic acid synergizes with Taxol in an HDAC6-dependant manner: novel mechanistic implications for combination anticancer therapy with taxanes [Poster – Anti-Tubulin Agents]. *The 98th AACR Annual Meeting*; Apr 14–18; Los Angeles, CA (2007). Abstract 1425.
41. Li YY, Chang JW, Chou WC, Liaw CC, Wang HM, Huang JS, et al. Zoledronic acid is unable to induce apoptosis but slows tumor growth and prolongs survival for non-small cell lung cancers. *Lung Cancer* (2008) **59**:180–91. doi:10.1016/j.lungcan.2007.08.026
42. Matsumoto S, Kimura S, Segawa H, Kuroda J, Yuasa T, Sato K, et al. Efficacy of the third-generation bisphosphonate, zoledronic acid alone and combined with anti-cancer agents against small cell lung cancer cell lines. *Lung Cancer* (2005) **47**:31–9. doi:10.1016/j.lungcan.2004.06.003
43. Landmeier S, Altvater B, Pscherer S, Varnholt L, Bollard CM, Juergens H, et al. Presentation of Epstein Barr virus (EBV) epitopes by activated human γδ T cells induces peptide-specific cytolytic CD8+ T cell expansion [Poster Session # 866-I]. *Blood* (2006) **108**:1738.
44. Fournier PG, Chirgwin JM, Guise TA. New insights into the role of T cells in the vicious cycle of bone metastases. *Curr Opin Rheumatol* (2006) **18**:396–404. doi:10.1097/01.bor.0000231909.35043.da
45. Roodman GD. Mechanisms of bone metastasis. *N Engl J Med* (2004) **350**:1655–64. doi:10.1056/NEJMra030831
46. Hofbauer LC, Neubauer A, Heufelder AE. Receptor activator of nuclear factor-kappa B ligand and osteoprotegerin: potential implications for the pathogenesis and treatment of malignant bone diseases. *Cancer* (2001) **92**:460–70. doi:10.1002/1097-0142(20010801)92:3<460::AID-CNCR1344>3.0.CO;2-D
47. Selvaggi G, Scagliotti GV. Management of bone metastases in cancer: a review. *Crit Rev Oncol Hematol* (2005) **56**:365–78. doi:10.1016/j.critrevonc.2005.03.011
48. Body JJ, Facon T, Coleman RE, Lipton A, Geurs F, Fan M, et al. A study of the biological receptor activator of nuclear factor-kappa B ligand inhibitor, denosumab, in patients with multiple myeloma or bone metastases from breast cancer. *Clin Cancer Res* (2006) **12**:1221–8. doi:10.1158/1078-0432.CCR-05-1933
49. Fizazi K, Lipton A, Mariette X, Body JJ, Rahim Y, Gralow JR, et al. Randomized phase II trial of denosumab in patients with bone metastases from prostate cancer, breast cancer, or other neoplasms after intravenous bisphosphonates. *J Clin Oncol* (2009) **27**:1564–71. doi:10.1200/JCO.2008.19.2146
50. Lipton A, Steger GG, Figueroa J, Alvarado C, Solal-Celigny P, Body JJ, et al. Randomized active-controlled phase II study of denosumab efficacy and safety in patients with breast cancer-related bone metastases. *J Clin Oncol* (2007) **25**:4431–7. doi:10.1200/JCO.2007.11.8604
51. Henry DH, Costa L, Goldwasser F, Hirsh V, Hungria V, Prausova J, et al. Randomized, double-blind study of denosumab versus zoledronic acid in the treatment of bone metastases in patients with advanced cancer (excluding breast and prostate cancer) or multiple myeloma. *J Clin Oncol* (2011) **29**(9):1125–32. doi:10.1200/JCO.2010.31.3304
52. Henry D, von Moos R, Vadhan-Raj S, Hungria V, Spencer A, Hirsh V, et al. A double-blind, randomized study of denosumab versus zoledronic acid for the treatment of bone metastases in patients with advanced cancer (excluding breast and prostate cancer) or multiple myeloma [Abstract 20LBA and Oral Presentation]. *Eur J Cancer Suppl* (2009) **7**(3):11
53. Tabrizi MA, Tseng CM, Roskos LK. Elimination mechanisms of therapeutic monoclonal antibodies. *Drug Discov Today* (2006) **11**:81–8. doi:10.1016/S1359-6446(05)03638-X
54. Wang W, Wang EQ, Balthasar JP. Monoclonal antibody pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther* (2008) **84**:548–58. doi:10.1038/clpt.2008.170
55. Scagliotti GV, Hirsh V, Siena S, Henry DH, Woll PJ, Manegold C, et al. Overall survival improvement in patients with lung cancer and bone metastases treated with denosumab versus zoledronic acid: subgroup analysis from a randomized, phase 3 study. *J Thorac Oncol* (2012) **7**(2):1823–9. doi:10.1097/JTO.0b013e31826aec2b
56. Kitazawa S, Kitazawa R. RANK ligand is a prerequisite for cancer-associated osteolytic lesions. *J Pathol* (2002) **198**:228–36. doi:10.1002/path.1199
57. Mundy GR. Metastasis to bone: causes, consequences, and therapeutic opportunities. *Nat Rev Cancer* (2002) **2**:584–93. doi:10.1038/nrc867
58. Feeley BT, Liu NQ, Conduah AH, Krenek L, Roth K, Dougall WC, et al. Mixed metastatic lung cancer lesions in bone are inhibited by noggin overexpression and Rank: Fc administration. *J Bone Miner Res* (2006) **21**:1571–80. doi:10.1359/jbmr.060706
59. Miller R, Jones J, Roudier M, et al. The RANKL inhibitor OPG-Fc either alone, or in combination with docetaxel, blocks lung cancer-induced osteolytic lesions or reduces skeletal tumor burden in a murine model of non-small cell lung cancer in bone. *Presented at the 9th International Conference on Cancer-Induced Bone Disease*, Arlington, VA (2009).
60. Miller RE, Roudier M, Jones J, Armstrong A, Canon J, Dougall WC. RANK ligand inhibition plus docetaxel improves survival and reduces tumor burden in a murine model of prostate cancer bone metastasis. *Mol Cancer Ther* (2008) **7**:2160–9. doi:10.1158/1535-7163.MCT-08-0046
61. Gonzalez-Suarez E, Jacob AP, Jones J, Miller R, Roudier-Meyer MP, Erwert R, et al. RANK ligand mediates progestin-induced mammary epithelial proliferation and carcinogenesis. *Nature* (2010) **468**:103–7. doi:10.1038/nature09495
62. Chen LM, Kuo CH, Lai TY, Lin YM, Su CC, Hsu HH, et al. RANKL increases migration of human lung cancer cells through intercellular adhesion molecule-1 up-regulation. *J Cell Biochem* (2011) **112**:933–41. doi:10.1002/jcb.23009
63. Jones DH, Nakashima T, Sanchez OH, Kozieradzki I, Komarova SV, Sarosi I, et al. Regulation of cancer cell migration and bone metastasis by RANKL. *Nature* (2006) **440**:692–6. doi:10.1038/nature04524
64. Peters S, Meylan E. Targeting receptor activator of nuclear factor-kappa B as a new therapy for bone metastases in non-small cell lung cancer. *Curr Opin Oncol* (2013) **25**(2):137–44. doi:10.1097/CCO.0b013e32835d720b
65. Hirsh V. Bisphosphonates in lung cancer: can they provide benefits beyond prevention of skeletal morbidity? *Anticancer Agents Med Chem* (2012) **12**(2):137–43. doi:10.2174/187152012799014922
66. Luo FR, Camuso A, McGlinchey K, Fager K, Flefleh C, Wen M-L, et al. Evaluation of anti-osteoclastic activity of the novel, oral multi-targeted kinase inhibitor Dasatinib (BMS-354825). *Proceedings of the American Association for Cancer Research (2005), Molecular Targets and Cancer Therapeutics – Discovery, Biology, and Clinical Applications, AACR-NCI-EORTC International Conference*; Nov 14–18; Philadelphia, PA. [Abstract B178] (2005). p. 173. [Poster Session B – Antiangiogenic/Antivascular Agents].
67. Fields SZ, Parshad S, Anne M, Raftopoulos H, Alexander MJ, Sherman ML, et al. Activin receptor antagonists for cancer- related anemia and bone disease. *Expert Opin Investig Drugs* (2013) **22**(1):87–101. doi:10.1517/13543784.2013.738666
68. Borgstein NG, Yang Y, Condon CH, Haltom E, Sherman ML. ACE-011, a soluble activin receptor type IIA IgG-Fc fusion protein decreases follicle stimulating hormone and increases bone- specific alkaline phosphatase, a marker of bone formation in postmenopausal healthy women [abstract]. *Cancer Res* (2009) **69**(2 Suppl 1):1160. doi:10.1158/0008-5472
69. Hellerstedt BA, Edelman G, Vogelzang NJ, Kluger HM, Yasenchak CA, Shen X, et al. Activitiy of cabozantinib (XL 184) in metastatic NSCLC: results from a phase II randomized discontinuation trial (RDT) [abstract]. *J Clin Oncol* (2012) **30**(Suppl 15):7514.
70. Parker C, Nilsson S, Heinrich D, Helle SI, O'Sullivan JM, Fosså SD, et al. Alpha emitter radium-223 and survival in metastatic prostate cancer. *N Engl J Med* (2013) **369**(3):213–23. doi:10.1056/NEJMoa1213755

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 April 2014; paper pending published: 18 May 2014; accepted: 28 May 2014; published online: 16 June 2014.
Citation: Hirsh V (2014) Targeted treatments of bone metastases in patients with lung cancer. Front. Oncol. ***4****:146. doi: 10.3389/fonc.2014.00146*

This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 10 July 2014
doi: 10.3389/fonc.2014.00178

The treatment of metastatic non-small cell lung cancer in the elderly: an evidence-based approach

David E. Dawe*[1] *** and Peter Michael Ellis*[2]***

[1] *Section of Hematology and Medical Oncology, Department of Internal Medicine, University of Manitoba, Winnipeg, MB, Canada*
[2] *Department of Oncology, McMaster University, Hamilton, ON, Canada*

Edited by:
Vera Hirsh, McGill University Health Centre, Canada

Reviewed by:
Sacha I. Rothschild, University Hospital Basel, Switzerland
Ajeet Gajra, SUNY Upstate Medical University, USA

***Correspondence:**
David E. Dawe, Section of Hematology and Medical Oncology, Department of Internal Medicine, University of Manitoba, ON 2052 – CancerCare Manitoba, 675 McDermot Avenue, Winnipeg, MB R3E 0V9, Canada
e-mail: david.dawe@cancercare.mb.ca

An increasing proportion of patients with advanced non-small cell lung cancer (NSCLC) are over 70 years old, raising unique challenges for treatment decision-making. While these patients are underrepresented in clinical trials, there is an emerging body of evidence associated with this group. The lesson of comprehensive geriatric assessment is that chronological age does not always correlate with physiological age and a variety of important co-morbidities and geriatric syndromes can go undetected in a typical history and physical. These co-morbidities and expected physiologic changes due to aging complicate decision-making around appropriate treatment. This review discusses geriatric assessment in elderly cancer patients and evaluates the current evidence for chemotherapy and targeted therapy for patients with advanced NSCLC aged ≥70 years.

Keywords: non-small cell lung cancer, chemotherapy, elderly, geriatric assessment, targeted therapy

INTRODUCTION

The number of seniors in Canada is projected to more than double between 2005 and 2036 (1) and global life expectancy has increased continuously over the last 40 years (2). Forty-three percent of cancers in 2010 were diagnosed in patients 70 years or older (3). Therefore, barring a significant change in cancer incidence, the absolute number of cancers diagnosed in elderly patients can be expected to increase substantially both in Canada and worldwide.

Worldwide, lung cancer is the leading cause of cancer-related mortality and by 2010 was the fifth overall leading cause of death (4, 5). Eighty-five percent of diagnosed lung cancer patients have non-small cell lung cancer (NSCLC) (6, 7). The median age of diagnosis is 70 years and has been increasing (7, 8). Lung cancer is therefore a disease of older adults and up to 70% of patients are diagnosed in advanced stage, where the standard treatment is systemic therapy (8).

This advanced age is an important treatment consideration due to the complex interplay of physiologic changes associated with aging, co-morbidities, competing mortality, and potential differences in priorities among younger vs. older individuals when prognosis is limited. These issues are compounded by difficulty in predicting both benefit from chemotherapy and risk of toxicity in older patients due to historical underrepresentation in clinical trials (9, 10).

This narrative review will discuss the complexity of treating geriatric patients and outline the current state of evidence for the use of chemotherapy in this population for the treatment of advanced lung cancer.

PHYSIOLOGIC CHANGES

Physiologic changes with aging occur in a number of organ systems that can affect the safety of chemotherapy (**Table 1**). Glomerular filtration rate is typically estimated to decrease by 1 mL/min/year beyond age 40 (11–14). In addition to this reduction in renal clearance, there is also impairment in the handling of water and electrolytes (13, 15). These changes can increase the risk to elderly lung cancer patients for toxicity from drugs primarily cleared by the kidneys, as well as dehydration and electrolyte imbalances.

The gastrointestinal system also changes with age, affecting drug absorption and the risk of mucositis (16, 17). Inconsistency in absorption results from reduced gastric blood flow, delayed gastric emptying, and a reduction in intestinal absorptive capacity (18–21). The vulnerability to mucosal injury arises from alteration of protective mechanisms, including a reduction in mucus and bicarbonate secretion (18). More importantly, elderly individuals generally show a decrease in hepatic mass and blood flow, which reduces drug metabolism (22). A reduction in activity of the cytochrome P450 system can also occur, resulting in a higher risk of drug interactions (23). The changes in metabolism can be further exacerbated by body composition changes that increase fat content and decrease water composition, thereby altering the volume of distribution for many drugs (24).

Finally, important changes occur in the bone marrow, with decreased cellularity, precursor proliferation, and cell mobilization (25, 26). These changes result in decreased bone marrow reserve. This altered bone marrow responsiveness increases the risk of marrow suppression and associated complications from chemotherapy and can delay further treatment administration (27, 28).

Table 1 | Physiologic changes with aging.

Organ system	Changes	Effect on chemotherapy
Renal	Decreased glomerular filtration	Decline in renal drug clearance that increases risk of drug toxicity
	Impaired water and electrolyte handling	Increased risk of dehydration
Gastrointestinal	Decreased gastric blood flow and delayed gastric emptying	Variable drug absorption
	Decreased absorptive capacity	Decreased absorption of oral drugs
	Decreased mucosal repair	Vulnerability to mucositis
Hepatobiliary	Decreased liver mass and blood flow	Reduced hepatic metabolism
	Reduction in cytochrome P450 activity	Greater vulnerability to P450 associated with drug interactions
Body composition	Increased fat and decreased water	Changes drug volume of distribution
Hematologic	Decreased marrow cellularity, proliferation, and mobilization	Impaired response to cytopenias, delayed blood count recovery, and higher risk of infection

PREDICTION OF TOXICITY

These physiologic shifts can increase the risk of chemotherapy toxicity in older individuals. However, clinical experience identifies many patients who seem much younger (or older) than their chronological age. This heterogeneity was strikingly demonstrated through comparison of life expectancies within geriatric age groups. Life expectancy for a 75-year-old woman ranged from 6.8 years (lowest 25th percentile) to 17 years (highest 25th percentile) and the same values for a man are 4.9 and 14.2 years (29). This variation in life expectancy reflects differences in baseline health, comorbidity, and genetics (30). It seems reasonable to hypothesize that the individual with better life expectancy has less risk of toxicity and more chance of benefit from chemotherapy, since they have less risk of competing causes of mortality. The challenge is identifying these patients and improving the up to 44% of lung cancer patients $\geq$70 years, who may require hospitalization during chemotherapy (31).

COMPREHENSIVE GERIATRIC ASSESSMENT

Historically, physicians used a combination of performance status (PS), measured using the Eastern Cooperative Oncology Group (ECOG) PS scale, and organ function as determined through blood work to determine, which patients qualified for chemotherapy treatment (32). This approach has been demonstrated to perform poorly when compared to more formal geriatric assessment (33–35). Comprehensive geriatric assessment (CGA) is usually composed of medical, functional, mental, social, and nutritional assessments, as well as explicit assessment of prescription drug use (36). A variety of studies have been completed to evaluate the usefulness of CGA in oncology patients. Systematic reviews of the available evidence show that CGA identifies problems that would otherwise be missed, leads to modifications in treatment plans, and helps predict toxicity from chemotherapy (37–42). Modification of treatment plans occurs in 21–53% of patients, suggesting that oncologists believe the additional information is valuable (42). CGA is also better than physician opinion for identifying frail elderly patients who experience greater toxicity (43). When tested in elderly NSCLC patients, CGA was feasible (44–46). Frail patients also exhibited poorer survival (46). However, when Corre et al. allocated patients to treatment based on CGA, survival was not different between groups, but toxicity was reduced in the arm allocating treatment using CGA (47).

OTHER PREDICTIVE TOOLS

While a CGA can be very useful, it has not become a routine part of oncologic care because it is time and labor intensive. The mean duration of CGA during one prospective study was 80 min/patient (48). Such a time commitment is difficult to undertake in lung cancer patients with metastatic disease, since it may delay patient throughput in clinic and/or delay commencement of treatment, a serious concern when patients have an average life expectancy of 10–12 months (8). In light of these concerns, a number of groups have attempted to shorten the CGA or provide a screening tool. Two groups have published new tools geared toward predicting chemotherapy toxicity and derived from multivariable analyses of CGAs conducted in cancer patients (**Table 2**) (33, 49). The chemotherapy risk assessment scale for high-age patients (CRASH) is actually composed of two scores, one for hematologic toxicity and another for non-hematologic toxicity (49). Diastolic blood pressure, instrumental activities of daily living (IADL), lactate dehydrogenase, and a proprietary Chemotox score help stratify the likelihood of experiencing Grade 3–4 hematologic toxicity into low (7%), medium-low (23%), medium-high (54%), and high (100%). For non-hematologic adverse events, the predictors are: ECOG PS, mini mental status, mini nutritional assessment, and the Chemotox score. The same predictive categories for non-hematologic toxicity predict risks of 33, 46, 67, and 93% (49). The Chemotox score is a quantitation of the toxicity of chemotherapy regimens that the group developed previously (50, 51). The cohort used for the CRASH score contained 21% lung cancer patients. The Cancer Aging Research Group (CARG) derived another predictive tool (33, 52). The CARG score uses 11 factors to stratify risk: age $\geq$72, cancer type, standard chemotherapy dosing, polychemotherapy, low hemoglobin, low creatinine clearance, fair or worse hearing, falls, needing help with medications, trouble walking 1 block, and decreased social activity.

Table 2 | Significant factors in scores predicting chemotherapy toxicity.

CRASH score (49)	**Hematologic**	**Non-hematologic**
	Diastolic blood pressure	ECOG performance status
	IADL	Mini mental status
	LDH	Mini nutritional assessment
	Chemotox score	Chemotox score
CARG score (33)	**Predictive factors**	
	Age ≥72 years	
	Standard chemotherapy dosing	
	Multi-drug chemotherapy	
	Low hemoglobin	
	Low creatinine clearance	
	Decreased hearing	
	Fall within 6 months	
	Needs help taking medications	
	Limited in walking 1 block	
	Decreased social activity	

IADL, instrumental activities of daily living; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group.

This score classified lung cancer patients into low (10%), intermediate (40%), or high (60%) risk of Grade 3–5 toxicity (52). This score was better able to stratify risk of toxicity than Karnofsky PS alone. Both of these predictive tools are exciting because they provide information that can be used to discuss chemotherapy treatment with elderly patients. Unfortunately, neither has been validated outside of the initial population and, therefore, widespread adoption is not yet justified.

EVIDENCE FOR CHEMOTHERAPY

Since 1995, standard first line chemotherapy for younger patients with stage IV NSCLC has been a platinum doublet. The meta-analysis supporting this recommendation demonstrated a 10% improvement in 1-year survival for patients treated with chemotherapy compared to supportive care (53). However, the first randomized trial focusing specifically on elderly patients was not published until 1999 (54, 55). Since then, only a few randomized trials have been conducted in patients 70 years and older with metastatic NSCLC. Most recommendations have been based on subgroup analyses or cohort studies. This lower level of evidence has likely contributed to uncertainty among health professionals regarding the standard of care in these patients.

The primary trial investigating the utility of single-agent chemotherapy compared to the best supportive care in elderly patients with metastatic NSCLC was the Elderly Lung Cancer Vinorelbine Italian Study (ELVIS) (55). The experimental arm of this RCT was single-agent vinorelbine (30 mg/m^2) administered on days 1 and 8 of a 21-day cycle. This treatment resulted in an improvement in median survival (28 vs. 21 weeks) and 1-year survival (32 vs. 14%), in addition to improvement in some lung cancer symptoms. Despite falling short of its 350 patient accrual target and closing prematurely, this established a new standard of care, which was incorporated as the control arm in further studies.

DOUBLET CHEMOTHERAPY

Subsequent trials have evaluated doublet chemotherapy regimens. Due to concerns about toxicity, these trials initially examined non-platinum chemotherapy combinations. The Southern Italy Cooperative Oncology Group (SICOG) conducted an RCT comparing gemcitabine and vinorelbine in combination to vinorelbine alone (56). The combination arm reported a median survival of 29 weeks compared to 18 weeks for vinorelbine alone. While this difference was statistically significant, there were concerns that the control group had worse survival than expected. These concerns prompted the multicenter Italian lung cancer in the elderly study (MILES), which compared three arms: vinorelbine plus gemcitabine, gemcitabine alone, and vinorelbine alone (57). Whereas, the SICOG trial enrolled 120 patients, MILES randomized 698 patients between the three arms. Median survivals were 30, 28, and 36 weeks for each of the arms, respectively. There was no statistically significant difference. There was, however, greater toxicity in the combination arm, specifically for neutropenia, thrombocytopenia, anemia, vomiting, constipation, and hepatic toxicity (57). These results do not support the use of the combination of vinorelbine and gemcitabine.

Further evaluation of doublet chemotherapy in patients ≥70 years was pursued. In one RCT of elderly patients or those with ECOG PS 2, the combination of gemcitabine and paclitaxel improved median survival to 9.2 months compared with 5.1 months for gemcitabine alone (58). However, a RCT in the same mixed population comparing gemcitabine/docetaxel to weekly docetaxel reported no difference in survival (59). A systematic review with meta-analysis of RCTs comparing non-platinum doublets with single-agent therapy for elderly patients showed no survival advantage to doublet therapy and higher risk of thrombocytopenia (60). Perhaps the most promising regimen was the combination of carboplatin with paclitaxel. One phase II trial demonstrated that weekly paclitaxel combined with carboplatin resulted in a 14-month median survival with quite manageable toxicity (61). Interestingly, when compared to standard paclitaxel, weekly paclitaxel appears to have equivalent benefit, but reduces the risk of neutropenia and peripheral neuropathy (62). The landmark trial investigating the use of platinum doublets in the elderly is Intergroupe Francophone Cancérologie Thoracique (IFCT)-0501 (63). This trial included 451 patients aged 70–89 years, with locally advanced or metastatic NSCLC and a PS of ECOG 0–2. Patients were randomized to carboplatin and weekly paclitaxel vs. monotherapy with either gemcitabine or vinorelbine. The trial was stopped early after interim analysis demonstrated superiority for the doublet regimen. Median overall survival was 10.3 months for carboplatin and paclitaxel compared to 6.2 months for monotherapy (hazard ratio 0.64, 95% CI 0.52–0.78, $p < 0.0001$). The largest

increases in toxicity for doublet chemotherapy were neutropenia (48.4 vs. 12.4%) and asthenia (10.3 vs. 5.8%) (63). Point estimates were quite consistent for all subgroups and multivariable analysis confirmed expected prognostic factors like sex, PS, adenocarcinoma histology, and smoking history.

One final trial, conducted in Japan by Takeda et al., has only been published in abstract form (64). The trial enrolled 276 patients, who were chemotherapy naïve, age >70 years, ECOG PS 0–1, and with stage III/IV NSCLC. Patients were randomized to receive either docetaxel every 3 weeks or weekly cisplatin–docetaxel. Enrollment was stopped early due to futility, with median survival times of 13.3 months for cisplatin–docetaxel and 17.3 months for docetaxel alone (hazard ratio 1.557, 95% CI 0.976–2.485). Interestingly, neutropenia was far more common with docetaxel alone than the doublet regimen 88 vs. 11%. The survival of the monotherapy group was remarkably high compared previous trials (65–67).

The majority of subgroup analyses from earlier trials suggest that survival is similar, though not always equal, between younger and older patients with advanced NSCLC who receive the same chemotherapy (68–71). The evidence suggests that the results of IFCT-0501 should form the standard of care for first line chemotherapy treatment in fit elderly patients with advanced NSCLC, especially when no molecular abnormalities are detected.

In the second line setting, there are no elder-specific trials. A retrospective analysis of the JMEI trial comparing docetaxel to pemetrexed was completed for patients ≥70 years old vs. younger patients. Median survival of 9.5 and 7.7 months was reported for elderly patients receiving pemetrexed ($n = 47$) and docetaxel ($n = 39$). In younger patients, these values were 7.8 and 8.0 months. Febrile neutropenia occurred in only 2.5% of elderly patients receiving pemetrexed, but 19% of those being treated with docetaxel ($p = 0.025$) (72).

TARGETED THERAPIES

BEVACIZUMAB

Bevacizumab is a monoclonal antibody that targets vascular endothelial growth factor (VEGF) and can be used in combination with first line platinum-based chemotherapy. Two trials, ECOG 4599 and AVAiL, originally tested the addition of bevacizumab to standard chemotherapy (73, 74). Analyses of the elderly patients in both of these trials were conducted. The AVAiL trial, comparing cisplatin and gemcitabine with or without bevacizumab, showed no improvement in overall survival in the elderly population ($n = 304$), with the addition of bevacizumab (73). An analysis of patients ≥70 years in the ECOG 4599 trial of carboplatin and paclitaxel with or without bevacizumab reported overall survival was 11.3 months with bevacizumab and 12.1 months without. There was a higher incidence of bleeding, neutropenia, and proteinuria in older compared to younger patients (74). There does not appear to be compelling evidence to include bevacizumab for those older than 65–70 years of age.

EGFR TYROSINE KINASE INHIBITORS

Molecularly defined subtypes of NSCLC have become incredibly important to management over the last 5 years. Epidermal growth factor receptor (EGFR) mutations are detected in approximately 15% of Caucasian patients with advanced NSCLC and these mutations are found more often, but not exclusively in younger, never smoking women, or those of Asian ethnicity (75). The presence of an *EGFR* mutation is highly predictive of benefit from EGFR tyrosine kinase inhibitors (TKIs) (76). More widespread screening of all NSCLC tumor samples for molecular abnormalities will increase the number of *EGFR* mutations identified in the elderly. Available data demonstrate EGFR TKIs (erlotinib, gefitinib, or afatinib) result in better progression free survival (PFS) and favorable toxicity compared to chemotherapy in NSCLC patients with an *EGFR* mutation (76–79). While few studies have examined the effect of this strategy exclusively in elderly patients, available data would suggest that elderly patients have similar response rate and PFS (80–84). Toxicities reported were the expected diarrhea, rash, and risk of transaminitis.

The NCIC BR.21 trial evaluated erlotinib in NSCLC patients who progressed after one or two prior chemotherapy treatments regardless of *EGFR* mutation status. The improvement in overall survival was seen in both *EGFR* mutated and wild type patients. A retrospective analysis of treatment effect and age in BR.21 found no statistically significant difference in treatment effect between younger and older patients for overall survival. Elderly patients did experience more Grade 3–4 toxicity (35 vs. 18%, $p < 0.001$). Based on this subgroup analysis, erlotinib seems to be a reasonable option for elderly patients in the second or third line setting (85). A trial in vulnerable elderly patients by CGA adds further support to this opinion, since both gemcitabine followed by erlotinib or the reverse on progression showed similar survival and tolerability (45).

ALK TYROSINE KINASE INHIBITORS

The other actionable mutation found in NSCLC is a translocation in echinoderm microtubule associated protein-like 4 – anaplastic lymphoma kinase (*EML4–ALK*) gene, which is found in approximately 4% of patients with adenocarcinoma (75). Data in younger patients have been extremely promising with the use of crizotinib for *EML4–ALK* translocated NSCLC (86–88). Few patients were older than 70 years. A phase I study including 149 patients did report a response rate of 65% (40.8–84.6%) in patients ≥65 years (87). More recently, two other early-phase clinical trials with different ALK TKIs demonstrated response rates >50%, with ceritinib showing impressive responses even in crizotinib resistant disease (89, 90). While there are little data in elderly patients, there is no reason to believe this group would derive less benefit from ALK TKI therapy.

CONCLUSION

An increasing proportion of patients with advanced NSCLC are over 70 years old, raising unique challenges for treatment decision-making. While these patients are underrepresented in clinical trials, there is an emerging body of evidence associated with this group. The lesson of CGA is that chronological age does not always correlate with physiological age and a variety of important co-morbidities and geriatric syndromes can go undetected in a typical history and physical. Geriatric assessment provides medical oncologists with information that can affect treatment decision and help predict chemotherapy toxicity. Abbreviated

CGAs or newly derived tools offer the promise of more widespread implementation of appropriate assessment of elderly patients.

For patients fit enough to consider first line chemotherapy, a platinum doublet appears to be a reasonable standard of care. Adding bevacizumab does not appear to improve overall survival. In the second line, pemetrexed, docetaxel, or erlotinib are all options for consideration. Pemetrexed would be the preferred option for patients with non-squamous histology. For patients with *EGFR* mutated disease, using an EGFR TKI as a first line treatment is a reasonable approach, though there is little evidence specific to elderly populations.

Further research is needed on the validation of tools that predict chemotherapy toxicity and prognosis to facilitate informed consent and treatment decisions. More studies focusing on elderly patients are also essential to help account for the physiologic changes inherent in this population. As we move forward, medical oncology is becoming geriatric oncology in many ways.

REFERENCES

1. Turcotte M, Schellenber G. Demographic trends and the geography of aging. In: Canada S, editor. *A Portrait of Seniors in Canada*. Ottawa, ON: Minister of Industry (2007) p. 11–42.
2. Wang H, Dwyer-Lindgren L, Lofgren KT, Rajaratnam JK, Marcus JR, Levin-Rector A, et al. Age-specific and sex-specific mortality in 187 countries, 1970-2010: a systematic analysis for the global burden of disease study 2010. *Lancet* (2012) **380**(9859):2071–94. doi:10.1016/S0140-6736(12)61719-X
3. Canadian Cancer Society's Steering Committee. *Canadian Cancer Statistics 2010*. Toronto, ON: Canadian Cancer Society, Statistics Canada, Public Health Agency of Canada (2010).
4. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* (2011) **61**(2):69–90. doi:10.3322/caac.20107
5. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the global burden of disease study 2010. *Lancet* (2012) **380**(9859):2095–128. doi:10.1016/S0140-6736(12)61728-0
6. Govindan R, Page N, Morgensztern D, Read W, Tierney R, Vlahiotis A, et al. Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: analysis of the surveillance, epidemiologic, and end results database. *J Clin Oncol* (2006) **24**(28):4539–44. doi:10.1200/JCO.2005.04.4859
7. Owonikoko TK, Ragin CC, Belani CP, Oton AB, Gooding WE, Taioli E, et al. Lung cancer in elderly patients: an analysis of the surveillance, epidemiology, and end results database. *J Clin Oncol* (2007) **25**(35):5570–7. doi:10.1200/JCO.2007.12.5435
8. Howlader N, Noone AM, Krapcho M, Garshell J, Miller D, Altekruse SF, et al. *SEER Cancer Statistics Review, 1975-2011*. Bethesda, MD: National Cancer Institute (2014). Available from: http://seer.cancer.gov/csr/1975_2011/
9. Sacher AG, Le LW, Leighl NB, Coate LE. Elderly patients with advanced NSCLC in phase III clinical trials: are the elderly excluded from practice-changing trials in advanced NSCLC? *J Thorac Oncol* (2013) **8**(3):366–8. doi:10.1097/JTO.0b013e31827e2145
10. Hutchins LF, Unger JM, Crowley JJ, Coltman CA Jr, Albain KS. Underrepresentation of patients 65 years of age or older in cancer-treatment trials. *N Engl J Med* (1999) **341**(27):2061–7. doi:10.1056/NEJM199912303412706
11. Sokoll LJ, Russell RM, Sadowski JA, Morrow FD. Establishment of creatinine clearance reference values for older women. *Clin Chem* (1994) **40**(12):2276–81.
12. Rowe JW, Andres R, Tobin JD, Norris AH, Shock NW. The effect of age on creatinine clearance in men: a cross sectional and longitudinal study. *J Gerontol* (1976) **31**(2):155–63. doi:10.1093/geronj/31.2.155
13. Lubran MM. Renal function in the elderly. *Ann Clin Lab Sci* (1995) **25**(2):122–33.
14. Fehrman-Ekholm I, Skeppholm L. Renal function in the elderly (>70 years old) measured by means of lohexol clearance, serum creatinine, serum urea and estimated clearance. *Scand J Urol Nephrol* (2004) **38**(1):73–7. doi:10.1080/00365590310015750
15. DeSanto NG, Anastasio P, Coppola S, Barba G, Jandanza A, Capasso G. Age-related changes in renal reserve and renal tubular function in healthy humans. *Child Nephrol Urol* (1991) **11**(1):33–40.
16. Sawhney R, Sehl M, Naeim A. Physiologic aspects of aging: impact on cancer management and decision making, part I. *Cancer J* (2005) **11**(6):449–60. doi:10.1097/00130404-200511000-00004
17. Balducci L. Geriatric oncology. *Crit Rev Oncol Hematol* (2003) **46**(3):211–20. doi:10.1016/S1040-8428(03)00020-9
18. Newton JL. Changes in upper gastrointestinal physiology with age. *Mech Ageing Dev* (2004) **125**(12):867–70. doi:10.1016/j.mad.2004.05.007
19. Mayersohn M. The 'xylose test' to assess gastrointestinal absorption in the elderly: a pharmacokinetic evaluation of the literature. *J Gerontol* (1982) **37**(3):300–5. doi:10.1093/geronj/37.3.300
20. Fabbri A, Marchesini G, Bianchi G, Bugianesi E, Zoli M, Pisi E. Kinetics of hepatic amino-nitrogen conversion in ageing man. *Liver* (1994) **14**(6):288–94. doi:10.1111/j.1600-0676.1994.tb00091.x
21. Wegener M, Borsch G, Schaffstein J, Luth I, Rickels R, Ricken D. Effect of ageing on the gastro-intestinal transit of a lactulose-supplemented mixed solid-liquid meal in humans. *Digestion* (1988) **39**(1):40–6. doi:10.1159/000199606
22. Wynne HA, Cope LH, Mutch E, Rawlins MD, Woodhouse KW, James OFW. The effect of age upon liver volume and apparent liver blood flow in healthy men. *Hepatology* (1989) **9**(2):297–301. doi:10.1002/hep.1840090222
23. Wilcock A, Thomas J, Frisby J, Webster M, Keeley V, Finn G, et al. Potential for drug interactions involving cytochrome P450 in patients attending palliative day care centres: a multicentre audit. *Br J Clin Pharmacol* (2005) **60**(3):326–9. doi:10.1111/j.1365-2125.2005.02428.x
24. Beaufrère B, Morio B. Fat and protein redistribution with aging: metabolic considerations. *Eur J Clin Nutr* (2000) **54**(Suppl 3):S48–53. doi:10.1038/sj.ejcn.1601025
25. Lipschitz DA, Udupa KB, Milton KY, Thompson CO. Effect of age on hematopoiesis in man. *Blood* (1984) **63**(3):502–9.
26. Ogawa T, Kitagawa M, Hirokawa K. Age-related changes of human bone marrow: a histometric estimation of proliferative cells, apoptotic cells, T cells, B cells and macrophages. *Mech Ageing Dev* (2000) **117**(1–3):57–68. doi:10.1016/S0047-6374(00)00137-8
27. Zinzani PL, Pavone E, Storti S, Moretti L, Fattori PP, Guardigni L, et al. Randomized trial with or without granulocyte colony-stimulating factor as adjunct to induction VNCOP-B treatment of elderly high-grade non-Hodgkin's lymphoma. *Blood* (1997) **89**(11):3974–9.
28. Gómez H, Mas L, Casanova L, Pen DLK, Santillana S, Valdivia S, et al. Elderly patients with aggressive non-Hodgkin's lymphoma treated with CHOP chemotherapy plus granulocyte-macrophage colony-stimulating factor: identification of two age subgroups with differing hematologic toxicity. *J Clin Oncol* (1998) **16**(7):2352–8.
29. Walter LC, Covinsky KE. Cancer screening in elderly patients: a framework for individualized decision making. *JAMA* (2001) **285**(21):2750–6. doi:10.1001/jama.285.21.2750
30. Pallis AG, Hatse S, Brouwers B, Pawelec G, Falandry C, Wedding U, et al. Evaluating the physiological reserves of older patients with cancer: the value of potential biomarkers of aging? *J Geriatr Oncol* (2014) **5**(2):204–18. doi:10.1016/j.jgo.2013.09.001
31. Zauderer MG, Sima CS, Korc-Grodzicki B, Kris MG, Krug LM. Toxicity of initial chemotherapy in older patients with lung cancers. *J Geriatr Oncol* (2013) **4**(1):64–70. doi:10.1016/j.jgo.2012.09.003
32. Orr ST, Aisner J. Performance status assessment among oncology patients: a review. *Cancer Treat Rep* (1986) **70**(12):1423–9.
33. Hurria A, Togawa K, Mohile SG, Owusu C, Klepin HD, Gross CP, et al. Predicting chemotherapy toxicity in older adults with cancer: a prospective multicenter study. *J Clin Oncol* (2011) **29**(25):3457–65. doi:10.1200/JCO.2011.34.7625
34. Repetto L, Fratino L, Audisio RA, Venturino A, Gianni W, Vercelli M, et al. Comprehensive geriatric assessment adds information to Eastern Cooperative Oncology Group performance status in elderly cancer patients: an Italian Group for Geriatric Oncology Study. *J Clin Oncol* (2002) **20**(2):494–502. doi:10.1200/JCO.20.2.494
35. Wedding U, Kodding D, Pientka L, Steinmetz HT, Schmitz S. Physicians' judgement and comprehensive geriatric assessment (CGA) select different patients as fit for chemotherapy. *Crit Rev Oncol Hematol* (2007) **64**(1):1–9. doi:10.1016/j.critrevonc.2007.05.001

36. Maas HA, Janssen-Heijnen ML, Olde Rikkert MG, Machteld Wymenga AN. Comprehensive geriatric assessment and its clinical impact in oncology. *Eur J Cancer* (2007) **43**(15):2161–9. doi:10.1016/j.ejca.2007.08.002
37. Puts MT, Hardt J, Monette J, Girre V, Springall E, Alibhai SM. Use of geriatric assessment for older adults in the oncology setting: a systematic review. *J Natl Cancer Inst* (2012) **104**(15):1133–63. doi:10.1093/jnci/djs285
38. Ramjaun A, Nassif MO, Krotneva S, Huang AR, Meguerditchian AN. Improved targeting of cancer care for older patients: a systematic review of the utility of comprehensive geriatric assessment. *J Geriatr Oncol* (2013) **4**(3):271–81. doi:10.1016/j.jgo.2013.04.002
39. Hamaker ME, Schiphorst AH, ten Bokkel Huinink D, Schaar C, van Munster BC. The effect of a geriatric evaluation on treatment decisions for older cancer patients – a systematic review. *Acta Oncol* (2014) **53**(3):289–96. doi:10.3109/0284186X.2013.840741
40. Hamaker ME, Seynaeve C, Wymenga AN, van Tinteren H, Nortier JW, Maartense E, et al. Baseline comprehensive geriatric assessment is associated with toxicity and survival in elderly metastatic breast cancer patients receiving single-agent chemotherapy: results from the OMEGA study of the Dutch Breast Cancer Trialists' Group. *Breast* (2014) **23**(1):81–7. doi:10.1016/j.breast.2013.11.004
41. Puts MT, Santos B, Hardt J, Monette J, Girre V, Atenafu EG, et al. An update on a systematic review of the use of geriatric assessment for older adults in oncology. *Ann Oncol* (2014) **25**(2):307–15. doi:10.1093/annonc/mdt386
42. Versteeg KS, Konings IR, Lagaay AM, van de Loosdrecht AA, Verheul HM. Prediction of treatment-related toxicity and outcome with geriatric assessment in elderly patients with solid malignancies treated with chemotherapy: a systematic review. *Ann Oncol* (2014). doi:10.1093/annonc/mdu052
43. Tucci A, Ferrari S, Bottelli C, Borlenghi E, Drera M, Rossi G. A comprehensive geriatric assessment is more effective than clinical judgment to identify elderly diffuse large cell lymphoma patients who benefit from aggressive therapy. *Cancer* (2009) **115**(19):4547–53. doi:10.1002/cncr.24490
44. LeCaer H, Barlesi F, Corre R, Jullian H, Bota S, Falchero L, et al. A multicentre phase II randomised trial of weekly docetaxel/gemcitabine followed by erlotinib on progression, vs the reverse sequence, in elderly patients with advanced non small-cell lung cancer selected with a comprehensive geriatric assessment (the GFPC 0504 study). *Br J Cancer* (2011) **105**(8):1123–30. doi:10.1038/bjc.2011.331
45. LeCaer H, Greillier L, Corre R, Jullian H, Crequit J, Falchero L, et al. A multicenter phase II randomized trial of gemcitabine followed by erlotinib at progression, versus the reverse sequence, in vulnerable elderly patients with advanced non small-cell lung cancer selected with a comprehensive geriatric assessment (the GFPC 0505 study). *Lung Cancer* (2012) **77**(1):97–103. doi:10.1016/j.lungcan.2012.02.004
46. Gironés R, Torregrosa D, Maestu I, Gómez-Codina J, Tenias JM, Costa RR. Comprehensive geriatric assessment (CGA) of elderly lung cancer patients: a single-center experience. *J Geriatr Oncol* (2012) **3**(2):98–103. doi:10.1016/j.jgo.2011.12.005
47. Corre R, Chouaid C, Greillier L, Le Caer H, Audigier-Valette C, Baize N, et al. Phase III, randomized, multicenter study comparing in elderly patients (>70 years) with stage iv non small-cell lung cancer (NSCLC) a standard strategy of treatment allocation (carboplatin based bi-therapy or monotherapy with docetaxel) based on performance status (PS) and age with an experimental strategy allocating the same chemotherapies or best supportive care (BSC) according to a comprehensive geriatric assessment (CGA)-study ESOGIA-GFPC-GECP 08-02. *Proceedings of the 15th World Conference on Lung Cancer*; 2013 Oct 27–30; Sydney, NSW Australia: International Association for the Study of Lung Cancer (2013).
48. Horgan AM, Leighl NB, Coate L, Liu G, Palepu P, Knox JJ, et al. Impact and feasibility of a comprehensive geriatric assessment in the oncology setting: a pilot study. *Am J Clin Oncol* (2012) **35**(4):322–8. doi:10.1097/COC.0b013e318210f9ce
49. Extermann M, Boler I, Reich RR, Lyman GH, Brown RH, DeFelice J, et al. Predicting the risk of chemotherapy toxicity in older patients: the chemotherapy risk assessment scale for high-age patients (CRASH) score. *Cancer* (2012) **118**(13):3377–86. doi:10.1002/cncr.26646
50. Extermann M, Bonetti M, Sledge GW, O'Dwyer PJ, Bonomi P, Benson AB III. MAX2 – a convenient index to estimate the average per patient risk for chemotherapy toxicity; validation in ECOG trials. *Eur J Cancer* (2004) **40**(8):1193–8. doi:10.1016/j.ejca.2004.01.028
51. Extermann M, Chen H, Cantor AB, Corcoran MB, Meyer J, Grendys E, et al. Predictors of tolerance to chemotherapy in older cancer patients: a prospective pilot study. *Eur J Cancer* (2002) **38**(11):1466–73. doi:10.1016/S0959-8049(02)00090-4
52. Nie X, Liu D, Li Q, Bai C. Predicting chemotherapy toxicity in older adults with lung cancer. *J Geriatr Oncol* (2013) **4**(4):334–9. doi:10.1016/j.jgo.2013.05.002
53. Non-Small Cell Lung Cancer Collaborative Group. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. Non-small cell lung cancer collaborative group. *BMJ* (1995) **311**(7010):899–909. doi:10.1136/bmj.311.7010.899
54. Rapp E, Pater JL, Willan A, Cormier Y, Murray N, Evans WK, et al. Chemotherapy can prolong survival in patients with advanced non-small-cell lung cancer – report of a Canadian multicenter randomized trial. *J Clin Oncol* (1988) **6**(4):633–41.
55. Gridelli C. The ELVIS trial: a phase III study of single-agent vinorelbine as first-line treatment in elderly patients with advanced non-small cell lung cancer. Elderly Lung Cancer Vinorelbine Italian Study. *Oncologist* (2001) **6**(Suppl 1):4–7. doi:10.1634/theoncologist.6-suppl_1-4
56. Frasci G, Lorusso V, Panza N, Comella P, Nicolella G, Bianco A, et al. Gemcitabine plus vinorelbine versus vinorelbine alone in elderly patients with advanced non-small-cell lung cancer. *J Clin Oncol* (2000) **18**(13):2529–36.
57. Gridelli C, Perrone F, Gallo C, Cigolari S, Rossi A, Piantedosi F, et al. Chemotherapy for elderly patients with advanced non-small-cell lung cancer: the multicenter Italian lung cancer in the elderly study (MILES) phase III randomized trial. *J Natl Cancer Inst* (2003) **95**(5):362–72. doi:10.1093/jnci/95.5.362
58. Comella P, Frasci G, Carnicelli P, Massidda B, Buzzi F, Filippelli G, et al. Gemcitabine with either paclitaxel or vinorelbine vs paclitaxel or gemcitabine alone for elderly or unfit advanced non-small-cell lung cancer patients. *Br J Cancer* (2004) **91**(3):489–97. doi:10.1038/sj.bjc.6602011
59. Hainsworth JD, Spigel DR, Farley C, Shipley DL, Bearden JD, Gandhi J, et al. Weekly docetaxel versus docetaxel/gemcitabine in the treatment of elderly or poor performance status patients with advanced nonsmall cell lung cancer: a randomized phase 3 trial of the Minnie Pearl Cancer Research Network. *Cancer* (2007) **110**(9):2027–34. doi:10.1002/cncr.23019
60. Russo A, Rizzo S, Fulfaro F, Adamo V, Santini D, Vincenzi B, et al. Gemcitabine-based doublets versus single-agent therapy for elderly patients with advanced nonsmall cell lung cancer: a literature-based meta-analysis. *Cancer* (2009) **115**(9):1924–31. doi:10.1002/cncr.24207
61. Inoue A, Usui K, Ishimoto O, Matsubara N, Tanaka M, Kanbe M, et al. A phase II study of weekly paclitaxel combined with carboplatin for elderly patients with advanced non-small cell lung cancer. *Lung Cancer* (2006) **52**(1):83–7. doi:10.1016/j.lungcan.2005.11.014
62. Sakakibara T, Inoue A, Sugawara S, Maemondo M, Ishida T, Usui K, et al. Randomized phase II trial of weekly paclitaxel combined with carboplatin versus standard paclitaxel combined with carboplatin for elderly patients with advanced non-small-cell lung cancer. *Ann Oncol* (2010) **21**(4):795–9. doi:10.1093/annonc/mdp401
63. Quoix E, Zalcman G, Oster J-P, Westeel V, Pichon E, Lavole A, et al. Carboplatin and weekly paclitaxel doublet chemotherapy compared with monotherapy in elderly patients with advanced non-small-cell lung cancer: IFCT-0501 randomised, phase 3 trial. *Lancet* (2011) **378**(9796):1079–88. doi:10.1016/S0140-6736(11)60780-0
64. Takeda K, Abe T, Yokoyama A, Ohe Y, Kudoh S, Ichinose Y, et al. Randomized phase III trial comparing weekly docetaxel (DTX) and cisplatin (CDDP) combination (DP) with triweekly DTX alone in elderly patients with advanced nonsmall cell lung cancer (NSCLC): results of an intergroup trial of JCOG0803/WJOG4307L: International Association for the Study of Lung Cancer (2011). Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed11&NEWS=N&AN=71235515
65. Roszkowski K, Pluzanska A, Krzakowski M, Smith AP, Saigi E, Aasebo U, et al. A multicenter, randomized, phase III study of docetaxel plus best supportive care versus best supportive care in chemotherapy-naive patients with metastatic or non-resectable localized non-small cell lung cancer (NSCLC). *Lung Cancer* (2000) **27**(3):145–57. doi:10.1016/S0169-5002(00)00094-5
66. Georgoulias V, Ardavanis A, Agelidou A, Agelidou M, Chandrinos V, Tsaroucha E, et al. Docetaxel versus docetaxel plus cisplatin as front-line treatment of patients

with advanced non-small-cell lung cancer: a randomized, multicenter phase III trial. *J Clin Oncol* (2004) **22**(13):2602–9. doi:10.1200/JCO.2004.11.004
67. Georgoulias V, Androulakis N, Kotsakis A, Hatzidaki D, Syrigos K, Polyzos A, et al. Docetaxel versus docetaxel plus gemcitabine as front-line treatment of patients with advanced non-small cell lung cancer: a randomized, multicenter phase III trial. *Lung Cancer* (2008) **59**(1):57–63. doi:10.1016/j.lungcan.2007.07.021
68. Ansari RH, Socinski MA, Edelman MJ, Belani CP, Gonin R, Catalano RB, et al. A retrospective analysis of outcomes by age in a three-arm phase III trial of gemcitabine in combination with carboplatin or paclitaxel vs. paclitaxel plus carboplatin for advanced non-small cell lung cancer. *Crit Rev Oncol Hematol* (2011) **78**(2):162–71. doi:10.1016/j.critrevonc.2010.03.003
69. Belani CP, Fossella F. Elderly subgroup analysis of a randomized phase III study of docetaxel plus platinum combinations versus vinorelbine plus cisplatin for first-line treatment of advanced nonsmall cell lung carcinoma (TAX 326). *Cancer* (2005) **104**(12):2766–74. doi:10.1002/cncr.21495
70. Gridelli C, Brodowicz T, Langer CJ, Peterson P, Islam M, Guba SC, et al. Pemetrexed therapy in elderly patients with good performance status: analysis of two phase III trials of patients with nonsquamous non-small-cell lung cancer. *Clin Lung Cancer* (2012) **13**(5):340–6. doi:10.1016/j.cllc.2011.12.002
71. Pallis AG, Karampeazis A, Vamvakas L, Vardakis N, Kotsakis A, Bozionelou V, et al. Efficacy and treatment tolerance in older patients with NSCLC: a meta-analysis of five phase III randomized trials conducted by the Hellenic Oncology Research Group. *Ann Oncol* (2011) **22**(11):2448–55. doi:10.1093/annonc/mdq772
72. Weiss GJ, Langer C, Rosell R, Hanna N, Shepherd F, Einhorn LH, et al. Elderly patients benefit from second-line cytotoxic chemotherapy: a subset analysis of a randomized phase III trial of pemetrexed compared with docetaxel in patients with previously treated advanced non-small-cell lung cancer. *J Clin Oncol* (2006) **24**(27):4405–11. doi:10.1200/JCO.2006.06.7835
73. Leighl NB, Zatloukal P, Mezger J, Ramlau R, Moore N, Reck M, et al. Efficacy and safety of bevacizumab-based therapy in elderly patients with advanced or recurrent nonsquamous non-small cell lung cancer in the phase III BO17704 study (AVAiL). *J Thorac Oncol* (2010) **5**(12):1970–6. doi:10.1097/JTO.0b013e3181f49c22
74. Ramalingam SS, Dahlberg SE, Langer CJ, Gray R, Belani CP, Brahmer JR, et al. Outcomes for elderly, advanced-stage non small-cell lung cancer patients treated with bevacizumab in combination with carboplatin and paclitaxel: analysis of Eastern Cooperative Oncology Group Trial 4599. *J Clin Oncol* (2008) **26**(1):60–5. doi:10.1200/JCO.2007.13.1144
75. Cooper WA, Lam DC, O'Toole SA, Minna JD. Molecular biology of lung cancer. *J Thorac Dis* (2013) **5**(Suppl 5):S479–90. doi:10.3978/j.issn.2072-1439.2013.08.03
76. Lee CK, Brown C, Gralla RJ, Hirsh V, Thongprasert S, Tsai C-M, et al. Impact of EGFR inhibitor in non-small cell lung cancer on progression-free and overall survival: a meta-analysis. *J Natl Cancer Inst* (2013) **105**(9):595–605. doi:10.1093/jnci/djt072
77. Ku GY, Haaland BA, de Lima Lopes G Jr. Gefitinib vs. chemotherapy as first-line therapy in advanced non-small cell lung cancer: meta-analysis of phase III trials. *Lung Cancer* (2011) **74**(3):469–73. doi:10.1016/j.lungcan.2011.04.008
78. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* (2012) **13**(3):239–46. doi:10.1016/S1470-2045(11)70393-X
79. Yang JC-H, Schuler MH, Yamamoto N, O'Byrne KJ, Hirsh V, Mok T, et al. LUX-Lung 3: a randomized, open-label, phase III study of afatinib versus pemetrexed and cisplatin as first-line treatment for patients with advanced adenocarcinoma of the lung harboring EGFR-activating mutations. *ASCO Meeting Abstracts*, Alexandria, VA (Vol. 30) (2012). p. LBA7500.
80. Tateishi K, Ichiyama T, Hirai K, Agatsuma T, Koyama S, Hachiya T, et al. Clinical outcomes in elderly patients administered gefitinib as first-line treatment in epidermal growth factor receptor-mutated non-small-cell lung cancer: retrospective analysis in a Nagano Lung Cancer Research Group study. *Med Oncol* (2013) **30**(1):450. doi:10.1007/s12032-012-0450-2
81. Maemondo M, Minegishi Y, Inoue A, Kobayashi K, Harada M, Okinaga S, et al. First-line gefitinib in patients aged 75 or older with advanced non-small cell lung cancer harboring epidermal growth factor receptor mutations: NEJ 003 study. *J Thorac Oncol* (2012) **7**(9):1417–22. doi:10.1097/JTO.0b013e318260de8b
82. Ebi N, Semba H, Tokunaga SJ, Takayama K, Wataya H, Kuraki T, et al. A phase II trial of gefitinib monotherapy in chemotherapy-naive patients of 75 years or older with advanced non-small cell lung cancer. *J Thorac Oncol* (2008) **3**(10):1166–71. doi:10.1097/JTO.0b013e318186a88d
83. Jackman DM, Yeap BY, Lindeman NI, Fidias P, Rabin MS, Temel J, et al. Phase II clinical trial of chemotherapy-naive patients > or = 70 years of age treated with erlotinib for advanced non-small-cell lung cancer. *J Clin Oncol* (2007) **25**(7):760–6. doi:10.1200/JCO.2006.07.5754
84. Asami K, Koizumi T, Hirai K, Ameshima S, Tsukadaira A, Morozumi N, et al. Gefitinib as first-line treatment in elderly epidermal growth factor receptor-mutated patients with advanced lung adenocarcinoma: results of a Nagano Lung Cancer Research Group study. *Clin Lung Cancer* (2011) **12**(6):387–92. doi:10.1016/j.cllc.2011.02.004
85. Wheatley-Price P, Ding K, Seymour L, Clark GM, Shepherd FA. Erlotinib for advanced non-small-cell lung cancer in the elderly: an analysis of the National Cancer Institute of Canada Clinical Trials Group Study BR.21. *J Clin Oncol* (2008) **26**(14):2350–7. doi:10.1200/JCO.2007.15.2280
86. Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, Ahn MJ, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med* (2013) **368**(25):2385–94. doi:10.1056/NEJMoa1214886
87. Camidge DR, Bang YJ, Kwak EL, Iafrate AJ, Varella-Garcia M, Fox SB, et al. Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a phase 1 study. *Lancet Oncol* (2012) **13**(10):1011–9. doi:10.1016/S1470-2045(12)70344-3
88. Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. *J Clin Oncol* (2009) **27**(26):4247–53. doi:10.1200/JCO.2009.22.6993
89. Seto T, Kiura K, Nishio M, Nakagawa K, Maemondo M, Inoue A, et al. CH5424802 (RO5424802) for patients with ALK-rearranged advanced non-small-cell lung cancer (AF-001JP study): a single-arm, open-label, phase 1-2 study. *Lancet Oncol* (2013) **14**(7):590–8. doi:10.1016/S1470-2045(13)70142-6
90. Shaw AT, Kim DW, Mehra R, Tan DS, Felip E, Chow LQ, et al. Ceritinib in ALK-rearranged non-small-cell lung cancer. *N Engl J Med* (2014) **370**(13):1189–97. doi:10.1056/NEJMoa1311107

Conflict of Interest Statement: Dr. David E. Dawe has no conflicts of interest to report. Dr. Peter Michael Ellis reports having received honoraria from Lilly, Roche, and Boehringer Ingelheim for advisory boards.

Received: 01 May 2014; accepted: 25 June 2014; published online: 10 July 2014.
*Citation: Dawe DE and Ellis PM (2014) The treatment of metastatic non-small cell lung cancer in the elderly: an evidence-based approach. Front. Oncol. **4**:178. doi: 10.3389/fonc.2014.00178*
This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 11 August 2014
doi: 10.3389/fonc.2014.00204

Biomarkers that currently affect clinical practice in lung cancer: EGFR, ALK, MET, ROS-1, and KRAS

Grzegorz J. Korpanty[1], Donna M. Graham[1], Mark D. Vincent[2] and Natasha B. Leighl[1]*

[1] *Division of Medical Oncology and Hematology, Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada*
[2] *London Regional Cancer Program, Department of Medical Oncology, London Health Sciences Centre, London, ON, Canada*

Edited by:
Barbara Melosky, British Columbia Cancer Agency, Canada

Reviewed by:
Sacha I. Rothschild, University Hospital Basel, Switzerland
Ricardo Martinez, Eli Lilly, USA

Correspondence:
Natasha B. Leighl, Division of Medical Oncology, Princess Margaret Cancer Centre, 5-105 610 University Avenue, Toronto, ON M5G 2M9, Canada
e-mail: natasha.leighl@uhn.ca

Lung cancer remains the most lethal malignancy in the world. Despite improvements in surgical treatment, systemic therapy, and radiotherapy, the 5-year survival rate for all patients diagnosed with lung cancer remains between 15 and 20%. Newer therapeutic strategies rely on specific molecular alterations, or biomarkers, that provide opportunities for a personalized approach to specific patient populations. Classification of lung cancer is becoming increasingly focused on these biomarkers, which renders the term "non-small cell lung" cancer less clinically useful. Non-small cell lung cancer is now recognized as a complex malignancy and its molecular and genomic diversity allows for patient-centered treatment options. Here, we review advances in targeted treatment of lung adenocarcinoma with respect to five clinically relevant biomarkers – *EGFR*, *ALK*, *MET*, *ROS-1*, and *KRAS*.

Keywords: EGFR, ALK, Met, ROS-1, KRAS, lung adenocarcinoma, biomarkers

INTRODUCTION

Lung cancer remains one of the most commonly diagnosed malignancy worldwide and the leading cause of cancer-related death (1). Until the last decade, non-small cell lung cancer (NSCLC) was considered a single disease, and systemic treatment of metastatic NSCLC was limited to platinum-based chemotherapy doublets resulting in approximately 20% response rates and median survival of 8 months (2). Only recently, we have realized that recognition of histological subtypes of NSCLC is clinically relevant when choosing systemic, platinum-based chemotherapy (3). In recent years, the oncology community has seen a paradigm shift in the molecular diagnosis and treatment of lung cancer thanks to identification of sensitizing mutations within the epidermal growth factor receptor gene *(EGFR)* to EGFR tyrosine kinase inhibitors (EGFR-TKIs) (erlotinib, gefitinib, and afatinib), and anaplastic lymphoma kinase gene *(ALK)* rearrangements (i.e., *EML4-ALK*) to ALK inhibitors (crizotinib and ceritinib) (4).

These breakthrough discoveries provide the unique opportunity for molecularly selected lung cancer patients to receive targeted, personalized treatment options that translate into clinically meaningful benefit (4). Molecular testing of NSCLC is now widely recommended by oncology societies because it provides personalized treatment options and better outcomes for patients with metastatic disease (5, 6).

To improve outcome, molecular profiling of lung cancer tumors should be available to all NSCLC patients in order to make targeted therapy available to patients with actionable/"druggable" driver mutations (7–9). Currently, we can offer these treatments routinely to patients with *EGFR*-mutated and *ALK*-rearranged NSCLC, the vast majority of whom have adenocarcinoma histology.

This review summarizes the most recent data on efficacy, risks, and benefits of novel biologic therapies in NSCLC focusing on *EGFR, ALK, MET, ROS-1*, and *KRAS* (**Table 1**).

EGFR

The epidermal growth factor receptor family (ERBB family) comprises four tyrosine kinase receptors: HER-1 (EGFR), HER-2/neu (ERBB2), HER-3 (ERBB3), and HER-4 (ERBB4) (38, 39). Following ligand-binding, EGFR receptors homo- and hetero-dimerize and promote autophosphorylation of the intracellular tyrosine kinase domain and initiate molecular cascade of events involved in growth, cell proliferation, differentiation, and survival (10, 11, 40). Small-molecule receptor tyrosine kinase inhibitors (TKIs) bind to the intracellular catalytic domain of the tyrosine kinase and inhibit receptor autophosphorylation and activation of downstream signaling pathways by competing with adenosine triphosphate (ATP) (41). Gefitinib and erlotinib are the most extensively studied reversible EGFR-TKIs in patients with metastatic NSCLC (42, 43). The majority of unselected NSCLC patients will not respond to treatment with EGFR-TKIs. Patients of Asian ethnicity, females, never-smokers, or those with adenocarcinoma histology, were initially identified as a population with the most substantial clinical benefit from EGFR-TKIs (12, 44–53). The marker of sensitivity to EGFR-TKIs was unknown until 2004 when activating mutations in exon 18, 19, and 21 of the *EGFR* gene were discovered (54–56). The majority of mutations are either point mutations leading to amino acid substitutions (exon 18 and 21) or in-frame deletions (exon 19) clustered around the ATP-binding pocket of the intracellular tyrosine kinase domain (13). A kinetic analysis of the intracellular domains of mutant *EGFR* has shown that the mutant receptor compared with a wild-type shows reduced affinity for ATP in the presence of EGFR-TKI (57).

The Iressa Pan-Asia Study (IPASS) was the first phase III randomized trial that demonstrated superior outcome with first-line EGFR-TKI treatment in patients with *EGFR*-mutant NSCLC when compared with platinum-based chemotherapy in a retrospective subgroup analysis (58). Other trials have employed a similar approach to the IPASS study and reported similar results (59, 60).

Table 1 | Clinically relevant biomarkers in NSCLC.

Biomarker	Treatment	Genomic aberration	Prevalence in NSCLC patients	Reference
EGFR	1. Tyrosine kinase inhibitors (e.g., gefitinib, erlotinib, and afatinib) 2. Monoclonal antibodies (e.g., cetuximab and necitumumab)	1. Activating mutation within intracellular catalytic domain of *EGFR* 2. Over-expression of extracellular part of EGFR	*EGFR m*utations (non-squamous histology) 1. ~15% in Caucasians 2. ~40% in Asians 3. ~75–80% in never-smoker Asians *EGFR* mutations (squamous histology) 1. ~5% EGFR over-expression 1. 39% in adenocarcinoma 2. 58% in squamous cell carcinoma 3. 38% in large-cell carcinoma	(10–14)
ALK	Tyrosine kinase inhibitors (e.g., crizotinib and ceritinib)	Chromosomal translocation and fusion of *ALK* gene	1. 3–5% in unselected NSCLC 2. ~10% in non-never-smokers 3. <1% in squamous carcinoma	(15–19)
MET	1. Tyrosine kinase inhibitors (e.g., tivantinib, cabozantinib, and crizotinib) 2. Monoclonal antibodies (onartuzumab, AMG 102, ficlatuzumab)	1. Increased *MET* copy number 2. Over-expression of extracellular part of MET receptor	1. 2–4% *MET* amplification (untreated) 2. 5–20% *MET* amplification in EGFR-TKI-resistant tumors 3. 25–75% over-expression of extracellular part of MET receptor	(20–23)
ROS-1	Tyrosine kinase inhibitor (crizotinib)	Chromosomal translocation and fusion of *ROS-1* gene	1–2% in unselected population	(24–27)
KRAS	Downstream pathway inhibitors (e.g., MEK inhibitors selumetinib and trametinib)	Activating mutation within catalytic *RAS* domain	1. *KRAS* are rare in never-smokers 2. ~25–30% in adenocarcinoma 3. ~5% in squamous cell carcinoma	(28–37)

Four randomized phase III trials prospectively compared the efficacy of first generation EGFR-TKIs against standard platinum-based chemotherapy in patients with *EGFR* mutation-positive NSCLC (61–67). In all four trials, *EGFR*-mutated NSCLC patients treated with TKIs (erlotinib or gefitinib) had significantly better ORR, PFS, and quality of life (QOL) when compared with patients treated with platinum-based chemotherapy (58, 61, 63, 65, 67–70). Despite significant PFS benefit of EGFR-TKIs in *EGFR*-mutant NSCLC patients, none of the trials showed statistically significant survival improvement, which is likely related to a high rate of patient crossover to EGFR-TKI from first-line chemotherapy upon progression or development of acquired resistance.

Afatinib is a second-generation EGFR-TKI that irreversibly blocks EGFR and Her-2 (71, 72). LUX-Lung 3 was a phase III clinical trial of afatinib compared to cisplatin-pemetrexed chemotherapy in treatment-naïve patients with *EGFR*-mutant advanced lung adenocarcinoma (73). Both median PFS and ORR were significantly better in patients treated with afatinib compared with chemotherapy. A pooled, retrospective subgroup analysis of LUX-Lung 3 and LUX-Lung 6 trial at 2014 ASCO annual meeting demonstrated better OS for patients with *EGFR* exon 19 deletion vs. *EGFR L858R* exon 21 insertion mutations (HR = 0.59; CI 0.45–0.77; $p < 0.001$ vs. HR = 1.25; CI 0.92–1.71; $p = 0.16$) (74). First-line treatment of *EGFR* mutation-positive NSCLC with EGFR-TKIs (gefitinib, erlotinib, and afatinib) is now recommended worldwide (5, 9). AZD9291 and CO-1686 are irreversible selective EGFR inhibitors, which demonstrate significant activity in patients with acquired resistance to first-generation EGFR-TKI, and are currently under development. One of the most common mechanisms of resistance to EGFR-TKIs is the development of T790M mutation (~50% of patients), which prevents binding of reversible EGFR-TKI to the EGFR kinase domain while preserving its catalytic activity (75). In patients with tumors harboring T790M mutation, AZD9291 and CO-1686 show promising 64 and 58% ORR, respectively (76, 77).

ALK

The *EML4-ALK* fusion gene is a product of inversion within the short arm of chromosome 2, where *ALK (anaplastic large-cell lymphoma kinase)* joins *EML4* (*echinoderm microtubule-associated protein-like 4*) to form a fusion gene (15). The product of *EML4-ALK* fusion is a chimeric protein with constitutive ALK activity and is detected in 3–6% of unselected NSCLC and especially among never-smokers or light ex-smokers who have adenocarcinoma histology (16–19). *ALK* rearrangements are nearly almost mutually exclusive with *EGFR* or *KRAS* mutations, although some rare exceptions exist (78). *ALK*-positive NSCLC represents a distinct molecular subtype that can be targeted with ALK-specific treatments (15, 24). Crizotinib is an oral small-molecule TKI that targets ALK, MET, and ROS1 tyrosine kinases (79–82). Crizotinib received accelerated US Food and Drug Administration (FDA) approval for treatment of

ALK-positive NSCLC based on an objective response rate of 60% and median PFS of 8–10 months in single-arm studies (16, 79, 83, 84).

A first-line phase III study (PROFILE 1014) assessed efficacy of crizotinib vs. cisplatin/carboplatin-pemetrexed chemotherapy in patients with *ALK*-positive NSCLC. Recently presented data at the 2014 ASCO Annual Meeting demonstrated significantly better median PFS and ORR when compared with patients who received chemotherapy – 10.9 vs. 7.0 months and 74 vs. 45%, respectively (85). No survival benefit was demonstrated at the time of data cut-off and may never be, since patients who progressed on chemotherapy were allowed to crossover to crizotinib. The PROFILE 1007 phase III study investigated the efficacy of crizotinib vs. standard of care second-line chemotherapy (pemetrexed or docetaxel) in previously treated *ALK*-positive NSCLC (86). Patients treated with crizotinib demonstrated significantly improved median PFS when compared with chemotherapy – 7.0 vs. 3.0 months. No overall survival benefit was noted likely due to a high rate of patient crossover to the crizotinib arm from chemotherapy. Patients treated with single-agent pemetrexed had higher ORR when compared with docetaxel (29 vs. 13%).

After clinical recognition of acquired resistance to crizotinib, multiple second-generation ALK inhibitors (LKD378, AP26113, and TSR-011) entered early phase clinical trials for patients with ALK-positive solid tumors, including NSCLC (87, 88). Recently published results of a phase I clinical trial of ceritinib (LDK378) in patients with *ALK*-rearranged NSCLC demonstrated a ORR of 58% in all patients and 56% in crizotinib-resistant patients (88). Median PFS in crizotinib-naïve patients was 10.4 and 6.9 months in the crizotinib-pretreated population. Ceritinib received accelerated FDA approval in April 2014 and confirmatory trials with ceritinib in this group of patients are ongoing (http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm395299).

MET

MET is a proto-oncogene that encodes for the heterodimeric transmembrane MET tyrosine receptor kinase. Its only known ligand – hepatocyte growth factor (HGF) (89). Binding of HGF to the MET receptor activates the tyrosine kinase and downstream signaling pathways including PI3K/AKT, Ras-Rac/Rho, mitogen-activated protein kinase (MAPK), and phospholipase C (PLC) involved in cell motility and invasion (20, 21, 89). The MET receptor is expressed in approximately 40–50% of NSCLC tumors; high levels of receptor expression, as well as high *MET* gene copy number are independent prognostic factors of poor outcome in patients with resected NSCLC (22, 23). *MET* amplification is recognized as one of the potential molecular mechanisms of acquired resistance in *EGFR*-mutated NSCLC to EGFR-TKIs (90, 91).

Pre-clinical studies showed promising results of combined blockade of EGFR and MET signaling pathways in NSCLC (92). MET inhibitors can be divided into mAbs targeting HGF or the MET receptor (AMG 102, ficlatuzumab, and onartuzumab) or MET TKIs (tivantinib, cabozantinib, foretinib, and crizotinib) (93).

A phase II randomized study compared onartuzumab plus erlotinib vs. erlotinib alone in second- and third-line treatment. Onartuzumab, in combination with erlotinib, significantly improved PFS and OS in patients with increased *MET* gene copy ($\geq$5) assessed by FISH (*MET*-FISH positive) as well as in patients with over-expression of MET receptor as assessed by immunohistochemistry (MET-IHC positive) regardless of gene amplification status (94). Unfortunately, a confirmatory phase III MET-Lung trial that randomized MET-IHC-positive NSCLC patients to combination onartuzumab/erlotinib vs. erlotinib alone was stopped prematurely due to lack of clinically meaningful efficacy in the combination arm (95).

Tivantinib was investigated in combination with erlotinib (EGFR-TKI) in patients with previously treated NSCLC in both phase II and phase III trials (96, 97). In the phase II trial, an exploratory subgroup analysis showed that MET-IHC-positive patients with non-squamous histology harboring *KRAS* mutations had better PFS and OS with tivantinib and erlotinib treatment when compared with erlotinib and placebo. MARQUEE, a phase III, double-blind trial randomized 1048 patients with metastatic pre-treated non-squamous NSCLC to tivantinib plus erlotinib vs. tivantinib plus placebo (98). While median PFS and ORR significantly favored tivantinib plus erlotinib (3.6 vs. 1.9 months; 10.3 vs. 6.5%, respectively), MARQUEE did not reach its primary endpoint of improved overall survival (http://eccamsterdam2013.ecco-org.eu/Scientific-Programme/Abstract-search.aspx?abstractid=6904). A subgroup analysis of patients with 2+-positive MET immunostaining demonstrated better OS, PFS, and ORR when compared to patients who had lower levels of tumoral MET expression. A further retrospective molecular subset analysis is underway to identify other potential biomarkers (*MET* copy number, *KRAS*, and *EGFR* mutations) that may help to select a target population for MET-directed treatments.

Crizotinib, which inhibits both ALK and MET, demonstrated promising results in a small pilot study ($N = 13$) of patients with *MET*-amplified NSCLC (99).

ROS-1

ROS-1 is an orphan receptor tyrosine kinase that is phylogenetically related to ALK (100–103). *ROS-1* chromosomal rearrangements with *CD74*, *EZR*, *SLC24A2*, and *FIG* genes define a new genomic driver in 1–2.5% of NSCLC patients (25, 26). Clinical characteristics of NSCLC patients with *ROS-1* rearrangements are similar to patients with *ALK*-rearranged NSCLC – more commonly seen in patients of Asian ethnicity, young age (median age 49.8 years), female sex, never-smokers, and adenocarcinoma histology (25). *ROS-1* rearrangements appear mutually exclusive of other known oncogenic drivers like *EGFR*, *KRAS*, *HER-2*, *ALK*, *RET*, and *MET* aberrations (27, 104). Pre-clinical data showed activity of ALK inhibitors (i.e., crizotinib and TAE684) in *ROS-1*-rearranged NSCLC cell lines given the high degree of homology between *ALK* and *ROS-1* tyrosine kinase domains (25). This led investigators to assess the benefit of crizotinib in this unique patient subset. Efficacy has been demonstrated with an overall response rate of 56% and 6-month PFS of 71% in 25 evaluable patients (105). There are a number of currently ongoing phase I and II studies investigating activity of crizotinib, dual ALK/ROS1 inhibitor PF-06463922, and ceritinib in *ROS-1*-rearranged NSCLC.

Since *ROS-1*-rearranged NSCLC is rare and detection of *ROS1* fusions by a break-apart FISH assay is expensive and labor intensive, diagnostic algorithms and simpler screening methods (e.g., by immunohistochemistry) are needed to identify patients with *ROS-1*-rearranged NSCLC (104, 106). At this moment, patients without driver mutations like *EGFR*, *KRAS*, *HER-2*, *ALK*, and *RET* rearrangements and *MET* amplifications should be screened for *ROS-1* fusions (preferentially never-smokers) since they can be offered targeted treatment with crizotinib.

KRAS

The *RAS* oncogene family, *HRAS*, *KRAS*, and *NRAS*, encodes intracellular transducer proteins (small GTPases) that are involved in transmitting signals from extracellular growth factor receptors like EGFR to the cell (107, 108). As G proteins, they are located on the intracellular side of the plasma membrane, bind guanine nucleotides, and have GTP-ase activity (109). In the resting state, RAS proteins are bound to GDP and are inactive. Upon exchange of GDP to GTP, the RAS-GTP complex activates multiple downstream pathways (MAPK, STAT, and PI3K) that regulate cell proliferation, motility, and apoptosis (110). After a short period, the signaling configuration of RAS is halted by intrinsic GTP-ase activity. Activating *RAS* mutations prevent GTP hydrolysis to GDP, thus the RAS protein is rendered constitutively active with uncontrolled activation of downstream signaling pathways (111).

KRAS mutations are present in approximately 30% of lung adenocarcinomas and less commonly in squamous NSCLC (~5%) (28). They are found more frequently in Caucasians with lung cancer than in the Asian population and in current- or ex-smokers when compared with never-smokers (29, 110). Most *KRAS* mutations in NSCLC are single amino acid substitutions in codon 12 (80%) and to a lesser extent in codons 13 and 61 (30). In current- or ex-smokers, *KRAS* mutations are usually transversions (pyrimidine nucleotide is exchanged for purine or *vice versa*; e.g., G → T or G → C) and transitions in never-smokers (purine nucleotide is exchanged for another purine or pyrimidine for another pyrimidine; e.g., G → A or C → T) (29). *KRAS* mutations are nearly always mutually exclusive with *EGFR* and *BRAF* mutations although rare co-existence of *EGFR* and *KRAS* mutations has been observed (12, 31–33). *KRAS* mutations co-exist with *PIK3CA* mutations in approximately 19% of *PIK3CA*-mutant NSCLC (32).

It has been postulated for over 20 years that *KRAS*-mutant NSCLC may be associated with poor outcome. However, multiple studies have shown conflicting results due to heterogeneity among the studies, including tumor type, stage, treatment, and study end points (28, 34). A meta-analysis of 28 studies published in 2005 demonstrated that *KRAS* mutation was a significant prognostic marker when polymerase chain reaction sequencing was used as a detection method (35). Recently published results of a LACE-Bio pooled retrospective analysis reported no prognostic or predictive (in regard to benefit from adjuvant chemotherapy) effect of *KRAS* mutations in patients with resected NSCLC (36). A subset analysis of patients with NSCLC with *KRAS* codon 13 mutations suggests that adjuvant chemotherapy may have a deleterious effect in this subgroup, but needs to be further validated (HR – 5.78; 95% CI, 2.06–16.2) (36). In the absence of prospective, large, randomized clinical trials, *KRAS* mutation status in NSCLC cannot be used as a prognostic nor predictive biomarker for treatment with exception of negative predictive value of *KRAS* mutations and response to EGFR-TKI (37, 112).

Direct inhibition of KRAS has been unsuccessful so far due to its molecular and functional complexity (113). The activation of the RAS-RAF-MEK-ERK signaling pathway as a consequence of *KRAS* mutations renders it an attractive target for small-molecule inhibition in *KRAS*-mutated NSCLC. Given the critical location in this signaling pathway, MEK has been recognized as an important target, downstream from KRAS, for anti-cancer therapy (114).

The efficacy of treatment with a combination of the orally available potent MEK inhibitor selumetinib plus docetaxel chemotherapy has been demonstrated in the treatment of patients with advanced *KRAS*-mutant NSCLC (115). Median PFS was 5.3 months in the selumetinib group and 2.1 months in the placebo group ($p = 0.014$), with a 37% ORR in the selumetinib/docetaxel arm and no response in the docetaxel alone arm ($p < 0.0001$).

Trametinib is another orally available MEK inhibitor that has been combined with docetaxel or pemetrexed in phase I/Ib trial in patients stratified by *KRAS* mutation status (116). While no difference in response rate was seen between the pemetrexed-treated groups, these response rates compare favorably with historical data for second-line chemotherapy treatment and support the absence of any negative interaction between these agents (117). Given these promising findings, ongoing studies are investigating the optimal combination of MEK inhibition and chemotherapeutic agents (www.clinicaltrials.gov).

Early studies have also suggested that the subgroup of *KRAS*-mutant NSCLC patients may benefit from targeting the PI3K-AKT-mTOR signaling pathway, downstream from KRAS. The mTOR inhibitor, ridaforolimus, has been investigated in patients with disease progression following chemotherapy with randomization to continued therapy or placebo after 8 weeks of treatment. Improved PFS was seen in the ongoing therapy group (4 vs. 2 months) with a trend toward survival benefit (18 vs. 5 months; $p = 0.09$) (118).

KRAS mutations in NSCLC, despite being the most common, remain the most intriguing and elusive of therapeutic targets. At present, targeted treatment is not available for KRAS-mutated NSCLC outside clinical trials. However, novel agents targeting downstream effector signaling pathways are under clinical development (119).

CONCLUSION

In the addition to the emergence of histological subtypes as key factors in the treatment decision-making process for patients with advanced NSCLC, identification of certain genomic abnormalities and protein expression signatures that drive progression and metastasis of lung cancer have led to a completely new approach to treatment of NSCLC patients (120). For the first time, we recognize NSCLC as a heterogenous entity and are able to use the differences within tumors to tailor treatment with clear improvements in outcome for patients.

Biomarker-driven treatment has proven to be a major breakthrough in the modern management of lung cancer. New therapeutic modalities target specific genomic aberrations resulting

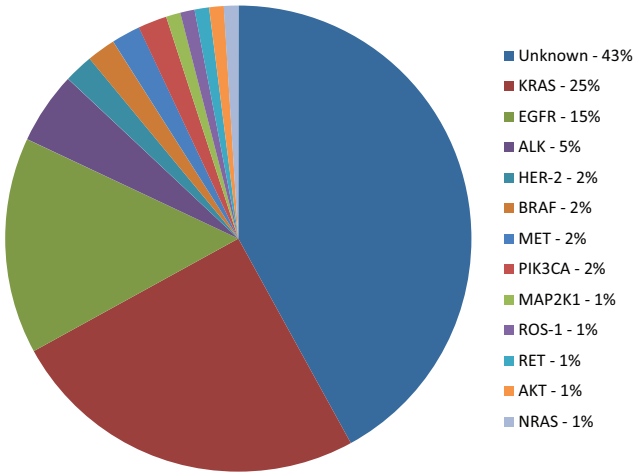


FIGURE 1 | Molecular subsets of lung adenocarcinoma. KRAS: v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; EGFR: epidermal growth factor receptor; ALK: anaplastic lymphoma kinase fusion; HER-2: human epidermal growth factor receptor 2; BRAF: v-raf murine sarcoma viral oncogene homolog B1; PIK3CA: phosphoinositide-3-kinase, catalytic, α polypeptide; MAP2K1: mitogen-activated protein kinase kinase 1; RET: rearranged during transfection; AKT1: v-akt murine thymoma viral oncogene homolog 1; NRAS: neuroblastoma RAS viral (v-ras) oncogene homolog (4, 9, 14, 18, 24, 31, 104).

in deregulation of select signaling pathways that are crucial for proliferation and metastasis of lung cancer.

There are a number of clinically and therapeutically relevant molecular changes within the lung cancer genome that can be now effectively targeted with systemic therapy in specific subgroups of patients (14). Ongoing research involving genomic efforts to elucidate further molecular subsets of NSCLC with ongoing development of biomarker-guided targeted therapies hopefully will continue to expand the therapeutic options for NSCLC patients.

Unfortunately, the number of patients for whom targeted therapy is suitable is still very small (**Figure 1**). The access to tumor tissue for biomarker assessment and *de novo* molecular and genomic tumor heterogeneity (that may be further increased during the biomarker-driven therapy) remain a serious challenge. Ongoing research in detection of cell-free circulating tumor DNA (cfDNA) and circulating tumor cells (CTCs) may become clinically relevant alternatives for tumor biopsy that will provide measurements of the total tumor burden as well as identify mutations arising during therapy that may be responsible for development of acquired resistance (121). Genomic screening of NSCLC tumors will continue to facilitate identification of molecular mechanisms of acquired resistance to targeted therapies. Ongoing translational and clinical research will facilitate a greater understanding of genomic alterations within lung cancer, with the aim of increasing benefit to wider population of lung cancer patients.

REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* (2013) **63**(1):11–30. doi:10.3322/caac.21166
2. Azzoli CG, Temin S, Aliff T, Baker S Jr, Brahmer J, Johnson DH, et al. 2011 focused update of 2009 American society of clinical oncology clinical practice guideline update on chemotherapy for stage IV non-small-cell lung cancer. *J Clin Oncol* (2010) **29**(28):3825–31. doi:10.1200/JCO.2010.34.2774
3. Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* (2008) **26**(21):3543–51. doi:10.1200/JCO.2007.15.0375
4. Mok TS. Personalized medicine in lung cancer: what we need to know. *Nat Rev Clin Oncol* (2011) **8**(11):661–8. doi:10.1038/nrclinonc.2011.126
5. Keedy VL, Temin S, Somerfield MR, Beasley MB, Johnson DH, McShane LM, et al. American society of clinical oncology provisional clinical opinion: epidermal growth factor receptor (EGFR) mutation testing for patients with advanced non-small-cell lung cancer considering first-line EGFR tyrosine kinase inhibitor therapy. *J Clin Oncol* (2011) **29**(15):2121–7. doi:10.1200/JCO.2010.31.8923
6. Pirker R, Herth FJ, Kerr KM, Filipits M, Taron M, Gandara D, et al. Consensus for EGFR mutation testing in non-small cell lung cancer: results from a European workshop. *J Thorac Oncol* (2010) **5**(10):1706–13. doi:10.1097/JTO.0b013e3181f1c8de
7. Gandara DR, Li T, Lara PN Jr, Mack PC, Kelly K, Miyamoto S, et al. Algorithm for codevelopment of new drug-predictive biomarker combinations: accounting for inter- and intrapatient tumor heterogeneity. *Clin Lung Cancer* (2012) **13**(5):321–5. doi:10.1016/j.cllc.2012.05.004
8. Haber DA, Gray NS, Baselga J. The evolving war on cancer. *Cell* (2011) **145**(1):19–24. doi:10.1016/j.cell.2011.03.026
9. Peters S, Adjei AA, Gridelli C, Reck M, Kerr K, Felip E. Metastatic non-small-cell lung cancer (NSCLC): ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* (2012) **23**(Suppl 7):vii56–64. doi:10.1093/annonc/mds226
10. Scagliotti GV, Selvaggi G, Novello S, Hirsch FR. The biology of epidermal growth factor receptor in lung cancer. *Clin Cancer Res* (2004) **10**(12 Pt 2):4227s–32s. doi:10.1158/1078-0432.CCR-040007
11. Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* (2007) **7**(3):169–81. doi:10.1038/nrc2088
12. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* (2005) **97**(5):339–46. doi:10.1093/jnci/dji055
13. Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. *Cancer Res* (2004) **64**(24):8919–23. doi:10.1158/0008-5472.CAN-04-2818
14. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. *Lancet Oncol* (2011) **12**(2):175–80. doi:10.1016/S1470-2045(10)70087-5
15. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* (2007) **448**(7153):561–6. doi:10.1038/nature05945
16. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* (2010) **363**(18):1693–703. doi:10.1056/NEJMoa1006448
17. Horn L, Pao W. EML4-ALK: honing in on a new target in non-small-cell lung cancer. *J Clin Oncol* (2009) **27**(26):4232–5. doi:10.1200/JCO.2009.23.6661
18. Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. *J Clin Oncol* (2009) **27**(26):4247–53. doi:10.1200/JCO.2009.22.6993
19. Inamura K, Takeuchi K, Togashi Y, Hatano S, Ninomiya H, Motoi N, et al. EML4-ALK lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. *Mod Pathol* (2009) **22**(4):508–15. doi:10.1038/modpathol.2009.2
20. Cipriani NA, Abidoye OO, Vokes E, Salgia R. MET as a target for treatment of chest tumors. *Lung Cancer* (2009) **63**(2):169–79. doi:10.1016/j.lungcan.2008.06.011
21. Sadiq AA, Geynisman DM, Salgia R. Inhibition of MET receptor tyrosine kinase and its ligand hepatocyte growth factor. *J Thorac Oncol* (2011) **6**(11 Suppl 4):S1810–1. doi:10.1097/01.JTO.0000407568.45147.43

22. Park S, Choi YL, Sung CO, An J, Seo J, Ahn MJ, et al. High MET copy number and MET overexpression: poor outcome in non-small cell lung cancer patients. *Histol Histopathol* (2012) **27**(2):197–207.
23. Cappuzzo F, Marchetti A, Skokan M, Rossi E, Gajapathy S, Felicioni L, et al. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. *J Clin Oncol* (2009) **27**(10):1667–74. doi:10.1200/JCO.2008.19.1635
24. Rikova K, Guo A, Zeng Q, Possemato A, Yu J, Haack H, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell* (2007) **131**(6):1190–203. doi:10.1016/j.cell.2007.11.025
25. Bergethon K, Shaw AT, Ou SH, Katayama R, Lovly CM, McDonald NT, et al. ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol* (2012) **30**(8):863–70. doi:10.1200/JCO.2011.35.6345
26. Kim HR, Lim SM, Kim HJ, Hwang SK, Park JK, Shin E, et al. The frequency and impact of ROS1 rearrangement on clinical outcomes in never smokers with lung adenocarcinoma. *Ann Oncol* (2012) **24**(9):2364–70. doi:10.1093/annonc/mdt220
27. Yoshida A, Kohno T, Tsuta K, Wakai S, Arai Y, Shimada Y, et al. ROS1-rearranged lung cancer: a clinicopathologic and molecular study of 15 surgical cases. *Am J Surg Pathol* (2013) **37**(4):554–62. doi:10.1097/PAS.0b013e3182758fe6
28. Slebos RJ, Kibbelaar RE, Dalesio O, Kooistra A, Stam J, Meijer CJ, et al. K-ras oncogene activation as a prognostic marker in adenocarcinoma of the lung. *N Engl J Med* (1990) **323**(9):561–5.
29. Riely GJ, Kris MG, Rosenbaum D, Marks J, Li A, Chitale DA, et al. Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. *Clin Cancer Res* (2008) **14**(18):5731–4. doi:10.1158/1078-0432.CCR-08-0646
30. Prior IA, Lewis PD, Mattos C. A comprehensive survey of RAS mutations in cancer. *Cancer Res* (2012) **72**(10):2457–67. doi:10.1158/0008-5472.CAN-11-2612
31. Schmid K, Oehl N, Wrba F, Pirker R, Pirker C, Filipits M. EGFR/KRAS/BRAF mutations in primary lung adenocarcinomas and corresponding locoregional lymph node metastases. *Clin Cancer Res* (2009) **15**(14):4554–60. doi:10.1158/1078-0432.CCR-09-0089
32. Wang L, Hu H, Pan Y, Wang R, Li Y, Shen L, et al. PIK3CA mutations frequently coexist with EGFR/KRAS mutations in non-small cell lung cancer and suggest poor prognosis in EGFR/KRAS wildtype subgroup. *PLoS One* (2014) **9**(2):e88291. doi:10.1371/journal.pone.0088291
33. Eberhard DA, Johnson BE, Amler LC, Goddard AD, Heldens SL, Herbst RS, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol* (2005) **23**(25):5900–9. doi:10.1200/JCO.2005.02.857
34. Rodenhuis S, van de Wetering ML, Mooi WJ, Evers SG, van Zandwijk N, Bos JL. Mutational activation of the K-ras oncogene. A possible pathogenetic factor in adenocarcinoma of the lung. *N Engl J Med* (1987) **317**(15):929–35. doi:10.1056/NEJM198710083171504
35. Mascaux C, Iannino N, Martin B, Paesmans M, Berghmans T, Dusart M, et al. The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis. *Br J Cancer* (2005) **92**(1):131–9. doi:10.1038/sj.bjc.6602258
36. Shepherd FA, Domerg C, Hainaut P, Janne PA, Pignon JP, Graziano S, et al. Pooled analysis of the prognostic and predictive effects of KRAS mutation status and KRAS mutation subtype in early-stage resected non-small-cell lung cancer in four trials of adjuvant chemotherapy. *J Clin Oncol* (2013) **31**(17):2173–81. doi:10.1200/JCO.2012.48.1390
37. Martin P, Leighl NB, Tsao MS, Shepherd FA. KRAS mutations as prognostic and predictive markers in non-small cell lung cancer. *J Thorac Oncol* (2013) **8**(5):530–42. doi:10.1097/JTO.0b013e318283d958
38. Bazley LA, Gullick WJ. The epidermal growth factor receptor family. *Endocr Relat Cancer* (2005) **12**(Suppl 1):S17–27. doi:10.1677/erc.1.01032
39. Hsieh AC, Moasser MM. Targeting HER proteins in cancer therapy and the role of the non-target HER3. *Br J Cancer* (2007) **97**(4):453–7. doi:10.1038/sj.bjc.6603910
40. Mendelsohn J, Baselga J. Epidermal growth factor receptor targeting in cancer. *Semin Oncol* (2006) **33**(4):369–85. doi:10.1053/j.seminoncol.2006.04.003
41. Gschwind A, Fischer OM, Ullrich A. The discovery of receptor tyrosine kinases: targets for cancer therapy. *Nat Rev Cancer* (2004) **4**(5):361–70. doi:10.1038/nrc1360
42. Giaccone G. The role of gefitinib in lung cancer treatment. *Clin Cancer Res* (2004) **10**(12 t 2):4233s–7s. doi:10.1158/1078-0432.CCR-040005
43. Perez-Soler R. The role of erlotinib (Tarceva, OSI 774) in the treatment of non-small cell lung cancer. *Clin Cancer Res* (2004) **10**(12 Pt 2):4238s–40s. doi:10.1158/1078-0432.CCR-040017
44. Miller VA, Kris MG, Shah N, Patel J, Azzoli C, Gomez J, et al. Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. *J Clin Oncol* (2004) **22**(6):1103–9. doi:10.1200/JCO.2004.08.158
45. Pao W, Zakowski M, Cordon-Cardo C, Ben-Porat L, Kris MG, Miller VA. Molecular characteristics of non-small cell lung cancer (NSCLC) patients sensitive to gefitinib. *ASCO Meet Abstr* (2004) **22**(14 Suppl):7025.
46. Kris MG, Sandler A, Miller V, Cespon M, Zakowski M, Pizzo B, et al. Cigarette smoking history predicts sensitivity to erlotinib: results of a phase II trial in patients with bronchioloalveolar carcinoma (BAC). *ASCO Meet Abstr* (2004) **22**(14 Suppl):7062.
47. Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (the IDEAL 1 trial) [corrected]. *J Clin Oncol* (2003) **21**(12):2237–46. doi:10.1200/JCO.2003.10.038
48. Kris MG, Natale RB, Herbst RS, Lynch TJ Jr, Prager D, Belani CP, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* (2003) **290**(16):2149–58. doi:10.1001/jama.290.16.2149
49. Perez-Soler R. Phase II clinical trial data with the epidermal growth factor receptor tyrosine kinase inhibitor erlotinib (OSI-774) in non-small-cell lung cancer. *Clin Lung Cancer* (2004) **6**(Suppl 1):S20–3. doi:10.3816/CLC.2004.s.010
50. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* (2005) **353**(2):123–32. doi:10.1056/NEJMoa050753
51. Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (iressa survival evaluation in lung cancer). *Lancet* (2005) **366**(9496):1527–37. doi:10.1016/S0140-6736(05)67625-8
52. Herbst RS, Prager D, Hermann R, Fehrenbacher L, Johnson BE, Sandler A, et al. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. *J Clin Oncol* (2005) **23**(25):5892–9. doi:10.1200/JCO.2005.02.840
53. Lee DH, Han JY, Lee HG, Lee JJ, Lee EK, Kim HY, et al. Gefitinib as a first-line therapy of advanced or metastatic adenocarcinoma of the lung in never-smokers. *Clin Cancer Res* (2005) **11**(8):3032–7. doi:10.1158/1078-0432.CCR-04-2149
54. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* (2004) **350**(21):2129–39. doi:10.1056/NEJMoa040938
55. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* (2004) **304**(5676):1497–500. doi:10.1126/science.1099314
56. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A* (2004) **101**(36):13306–11. doi:10.1073/pnas.0405220101
57. Carey KD, Garton AJ, Romero MS, Kahler J, Thomson S, Ross S, et al. Kinetic analysis of epidermal growth factor receptor somatic mutant proteins shows increased sensitivity to the epidermal growth factor receptor tyrosine kinase inhibitor, erlotinib. *Cancer Res* (2006) **66**(16):8163–71. doi:10.1158/0008-5472.CAN-06-0453
58. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* (2009) **361**(10):947–57. doi:10.1056/NEJMoa0810699
59. Janne PA, Wang X, Socinski MA, Crawford J, Stinchcombe TE, Gu L, et al. Randomized phase II trial of erlotinib alone or with carboplatin and paclitaxel in patients who were never or light former smokers with advanced lung adenocarcinoma: CALGB 30406 trial. *J Clin Oncol* (2012) **30**(17):2063–9. doi:10.1200/JCO.2011.40.1315

60. Han JY, Park K, Kim SW, Lee DH, Kim HY, Kim HT, et al. First-SIGNAL: first-line single-agent iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. *J Clin Oncol* (2012) **30**(10):1122–8. doi:10.1200/JCO.2011.36.8456
61. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol* (2010) **11**(2):121–8. doi:10.1016/S1470-2045(09)70364-X
62. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Seto T, et al. Updated overall survival results of WJTOG 3405, a randomized phase III trial comparing gefitinib (G) with cisplatin plus docetaxel (CD) as the first-line treatment for patients with non-small cell lung cancer harboring mutations of the epidermal growth factor receptor (EGFR). *ASCO Meet Abstr* (2012) **30**(15 Suppl):7521.
63. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* (2010) **362**(25):2380–8. doi:10.1056/NEJMoa0909530
64. Inoue A, Kobayashi K, Maemondo M, Sugawara S, Oizumi S, Isobe H, et al. Final overall survival results of NEJ002, a phase III trial comparing gefitinib to carboplatin (CBDCA) plus paclitaxel (TXL) as the first-line treatment for advanced non-small cell lung cancer (NSCLC) with EGFR mutations. *ASCO Meet Abstr* (2011) **29**(15 Suppl):7519. doi:10.1093/annonc/mds214
65. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* (2011) **12**(8):735–42. doi:10.1016/S1470-2045(11)70184-X
66. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* (2012) **13**(3):239–46. doi:10.1016/S1470-2045(11)70393-X
67. Rosell R, Gervais R, Vergnenegre A, Massuti B, Felip E, Cardenal F, et al. Erlotinib versus chemotherapy (CT) in advanced non-small cell lung cancer (NSCLC) patients (p) with epidermal growth factor receptor (EGFR) mutations: interim results of the European erlotinib versus chemotherapy (EURTAC) phase III randomized trial. *ASCO Meet Abstr* (2011) **29**(15 Suppl):7503.
68. Sequist LV, Martins RG, Spigel D, Grunberg SM, Spira A, Janne PA, et al. First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. *J Clin Oncol* (2008) **26**(15):2442–9. doi:10.1200/JCO.2007.14.8494
69. Inoue A, Suzuki T, Fukuhara T, Maemondo M, Kimura Y, Morikawa N, et al. Prospective phase II study of gefitinib for chemotherapy-naive patients with advanced non-small-cell lung cancer with epidermal growth factor receptor gene mutations. *J Clin Oncol* (2006) **24**(21):3340–6. doi:10.1200/JCO.2005.05.4692
70. Asahina H, Yamazaki K, Kinoshita I, Sukoh N, Harada M, Yokouchi H, et al. A phase II trial of gefitinib as first-line therapy for advanced non-small cell lung cancer with epidermal growth factor receptor mutations. *Br J Cancer* (2006) **95**(8):998–1004. doi:10.1038/sj.bjc.6603393
71. Minkovsky N, Berezov A. BIBW-2992, a dual receptor tyrosine kinase inhibitor for the treatment of solid tumors. *Curr Opin Investig Drugs* (2008) **9**(12):1336–46.
72. Li D, Ambrogio L, Shimamura T, Kubo S, Takahashi M, Chirieac LR, et al. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. *Oncogene* (2008) **27**(34):4702–11. doi:10.1038/onc.2008.109
73. Yang JC-H, Schuler MH, Yamamoto N, O'Byrne KJ, Hirsh V, Mok T, et al. LUX-Lung 3: a randomized, open-label, phase III study of afatinib versus pemetrexed and cisplatin as first-line treatment for patients with advanced adenocarcinoma of the lung harboring EGFR-activating mutations. *ASCO Meet Abstr* (2012) **30**(18 Suppl):LBA7500.
74. Yang JC-H, Sequist LV, Schuler MH, Mok T, Yamamoto N, O'Byrne KJ, et al. Overall survival (OS) in patients (pts) with advanced non-small cell lung cancer (NSCLC) harboring common (Del19/L858R) epidermal growth factor receptor mutations (EGFR mut): pooled analysis of two large open-label phase III studies (LUX-Lung 3 [LL3] and LUX-Lung 6 [LL6]) comparing afatinib with chemotherapy (CT). *ASCO Meet Abstr* (2014) **32**(15 Suppl):8004.
75. Godin-Heymann N, Ulkus L, Brannigan BW, McDermott U, Lamb J, Maheswaran S, et al. The T790M "gatekeeper" mutation in EGFR mediates resistance to low concentrations of an irreversible EGFR inhibitor. *Mol Cancer Ther* (2008) **7**(4):874–9. doi:10.1158/1535-7163.MCT-07-2387
76. Janne PA, Ramalingam SS, Yang JC-H, Ahn M-J, Kim D-W, Kim S-W, et al. Clinical activity of the mutant-selective EGFR inhibitor AZD9291 in patients (pts) with EGFR inhibitor-resistant non-small cell lung cancer (NSCLC). *ASCO Meet Abstr* (2014) **32**(15 Suppl):8009.
77. Sequist LV, Soria J-C, Gadgeel SM, Wakelee HA, Camidge DR, Varga A, et al. First-in-human evaluation of CO-1686, an irreversible, highly selective tyrosine kinase inhibitor of mutations of EGFR (activating and T790M). *ASCO Meet Abstr* (2014) **32**(15 Suppl):8010.
78. Camidge DR, Kono SA, Flacco A, Tan AC, Doebele RC, Zhou Q, et al. Optimizing the detection of lung cancer patients harboring anaplastic lymphoma kinase (ALK) gene rearrangements potentially suitable for ALK inhibitor treatment. *Clin Cancer Res* (2010) **16**(22):5581–90. doi:10.1158/1078-0432.CCR-10-0851
79. Camidge DR, Bang YJ, Kwak EL, Iafrate AJ, Varella-Garcia M, Fox SB, et al. Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a phase 1 study. *Lancet Oncol* (2012) **13**(10):1011–9. doi:10.1016/S1470-2045(12)70344-3
80. Ou SH, Bazhenova L, Camidge DR, Solomon BJ, Herman J, Kain T, et al. Rapid and dramatic radiographic and clinical response to an ALK inhibitor (crizotinib, PF02341066) in an ALK translocation-positive patient with non-small cell lung cancer. *J Thorac Oncol* (2010) **5**(12):2044–6. doi:10.1097/JTO.0b013e318200f9ff
81. Ou SH, Kwak EL, Siwak-Tapp C, Dy J, Bergethon K, Clark JW, et al. Activity of crizotinib (PF02341066), a dual mesenchymal-epithelial transition (MET) and anaplastic lymphoma kinase (ALK) inhibitor, in a non-small cell lung cancer patient with de novo MET amplification. *J Thorac Oncol* (2011) **6**(5):942–6. doi:10.1097/JTO.0b013e31821528d3
82. Shaw AT, Yeap BY, Solomon BJ, Riely GJ, Gainor J, Engelman JA, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. *Lancet Oncol* (2011) **12**(11):1004–12. doi:10.1016/S1470-2045(11)70232-7
83. Kim D-W, Ahn M-J, Shi Y, De Pas TM, Yang P-C, Riely GJ, et al. Results of a global phase II study with crizotinib in advanced ALK-positive non-small cell lung cancer (NSCLC). *ASCO Meet Abstr* (2011) **30**(15 Suppl):7533. doi:10.1007/s40265-013-0142-z
84. Crino L, Kim D, Riely GJ, Janne PA, Blackhall FH, Camidge DR, et al. Initial phase II results with crizotinib in advanced ALK-positive non-small cell lung cancer (NSCLC): profile 1005. *ASCO Meet Abstr* (2011) **29**(15 Suppl):7514.
85. Mok T, Kim D-W, Wu Y-L, Solomon BJ, Nakagawa K, Mekhail T, et al. First-line crizotinib versus pemetrexed-cisplatin or pemetrexed-carboplatin in patients (pts) with advanced ALK-positive non-squamous non-small cell lung cancer (NSCLC): results of a phase III study (PROFILE 1014). *ASCO Meet Abstr* (2014) **32**(15 Suppl):8002.
86. Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, Ahn MJ, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med* (2013) **368**(25):2385–94. doi:10.1056/NEJMoa1214886
87. Camidge DR, Bazhenova L, Salgia R, Weiss GJ, Langer CJ, Shaw AT, et al. First-in-human dose-finding study of the ALK/EGFR inhibitor AP26113 in patients with advanced malignancies: updated results. *ASCO Meet Abstr* (2013) **31**(15 Suppl):8031.
88. Shaw AT, Kim DW, Mehra R, Tan DS, Felip E, Chow LQ, et al. Ceritinib in ALK-rearranged non-small-cell lung cancer. *N Engl J Med* (2014) **370**(13):1189–97. doi:10.1056/NEJMoa1311107
89. Ma PC, Maulik G, Christensen J, Salgia R. c-Met: structure, functions and potential for therapeutic inhibition. *Cancer Metastasis Rev* (2003) **22**(4):309–25. doi:10.1023/A:1023768811842
90. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* (2007) **316**(5827):1039–43. doi:10.1126/science.1141478
91. Xu L, Kikuchi E, Xu C, Ebi H, Ercan D, Cheng KA, et al. Combined EGFR/MET or EGFR/HSP90 inhibition is effective in the treatment of lung cancers codriven by mutant EGFR containing T790M and MET. *Cancer Res* (2012) **72**(13):3302–11. doi:10.1158/0008-5472.CAN-11-3720

92. Robinson KW, Sandler AB. The role of MET receptor tyrosine kinase in non-small cell lung cancer and clinical development of targeted anti-MET agents. *Oncologist* (2013) **18**(2):115–22. doi:10.1634/theoncologist.2012-0262
93. Sadiq AA, Salgia R. MET as a possible target for non-small-cell lung cancer. *J Clin Oncol* (2013) **31**(8):1089–96. doi:10.1200/JCO.2012.43.9422
94. Spigel DR, Ervin TJ, Ramlau R, Daniel DB, Goldschmidt JH, Blumenschein GR, et al. Final efficacy results from OAM4558g, a randomized phase II study evaluating MetMAb or placebo in combination with erlotinib in advanced NSCLC. *ASCO Meet Abstr* (2011) **29**(15 Suppl):7505.
95. Spigel DR, Edelman MJ, O'Byrne K, Paz-Ares L, Shames DS, Yu W, et al. Onartuzumab plus erlotinib versus erlotinib in previously treated stage IIIb or IV NSCLC: results from the pivotal phase III randomized, multicenter, placebo-controlled METLung (OAM4971g) global trial. *ASCO Meet Abstr* (2014) **32**(15 Suppl):8000.
96. Sequist LV, von Pawel J, Garmey EG, Akerley WL, Brugger W, Ferrari D, et al. Randomized phase II study of erlotinib plus tivantinib versus erlotinib plus placebo in previously treated non-small-cell lung cancer. *J Clin Oncol* (2011) **29**(24):3307–15. doi:10.1200/JCO.2010.34.0570
97. Munshi N, Jeay S, Li Y, Chen CR, France DS, Ashwell MA, et al. ARQ 197, a novel and selective inhibitor of the human c-Met receptor tyrosine kinase with antitumor activity. *Mol Cancer Ther* (2010) **9**(6):1544–53. doi:10.1158/1535-7163.MCT-09-1173
98. Scagliotti GV, Novello S, Schiller JH, Hirsh V, Sequist LV, Soria JC, et al. Rationale and design of MARQUEE: a phase III, randomized, double-blind study of tivantinib plus erlotinib versus placebo plus erlotinib in previously treated patients with locally advanced or metastatic, nonsquamous, non-small-cell lung cancer. *Clin Lung Cancer* (2012) **13**(5):391–5. doi:10.1016/j.cllc.2012.01.003
99. Camidge DR, Ou S-HI, Shapiro G, Otterson GA, Villaruz LC, Villalona-Calero MA, et al. Efficacy and safety of crizotinib in patients with advanced c-MET-amplified non-small cell lung cancer (NSCLC). *ASCO Meet Abstr* (2014) **32**(15 Suppl):8001.
100. Acquaviva J, Wong R, Charest A. The multifaceted roles of the receptor tyrosine kinase ROS in development and cancer. *Biochim Biophys Acta* (2009) **1795**(1):37–52. doi:10.1016/j.bbcan.2008.07.006
101. Birchmeier C, Sharma S, Wigler M. Expression and rearrangement of the ROS1 gene in human glioblastoma cells. *Proc Natl Acad Sci U S A* (1987) **84**(24):9270–4. doi:10.1073/pnas.84.24.9270
102. Gu TL, Deng X, Huang F, Tucker M, Crosby K, Rimkunas V, et al. Survey of tyrosine kinase signaling reveals ROS kinase fusions in human cholangiocarcinoma. *PLoS One* (2011) **6**(1):e15640. doi:10.1371/journal.pone.0015640
103. Birch AH, Arcand SL, Oros KK, Rahimi K, Watters AK, Provencher D, et al. Chromosome 3 anomalies investigated by genome wide SNP analysis of benign, low malignant potential and low grade ovarian serous tumours. *PLoS One* (2011) **6**(12):e28250. doi:10.1371/journal.pone.0028250
104. Go H, Kim DW, Kim D, Keam B, Kim TM, Lee SH, et al. Clinicopathologic analysis of ROS1-rearranged non-small-cell lung cancer and proposal of a diagnostic algorithm. *J Thorac Oncol* (2013) **8**(11):1445–50. doi:10.1097/JTO.0b013e3182a4dd6e
105. Shaw AT, Camidge DR, Engelman JA, Solomon BJ, Kwak EL, Clark JW, et al. Clinical activity of crizotinib in advanced non-small cell lung cancer (NSCLC) harboring ROS1 gene rearrangement. *ASCO Meet Abstr* (2012) **30**(15 Suppl):7508. doi:10.1097/CCO.0b013e32835d8175
106. Mescam-Mancini L, Lantuejoul S, Moro-Sibilot D, Rouquette I, Souquet PJ, Audigier-Valette C, et al. On the relevance of a testing algorithm for the detection of ROS1-rearranged lung adenocarcinomas. *Lung Cancer* (2014) **83**(2):168–73. doi:10.1016/j.lungcan.2013.11.019
107. Rodenhuis S, Slebos RJ. The ras oncogenes in human lung cancer. *Am Rev Respir Dis* (1990) **142**(6 Pt 2):S27–30. doi:10.1164/ajrccm/142.6_Pt_2.S27
108. Rodenhuis S. Ras and human tumors. *Semin Cancer Biol* (1992) **3**(4):241–7.
109. Harvey JJ. An unidentified virus which causes the rapid production of tumours in mice. *Nature* (1964) **204**:1104–5. doi:10.1038/2041104b0
110. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene* (2007) **26**(22):3291–310. doi:10.1038/sj.onc.1210422
111. Karnoub AE, Weinberg RA. Ras oncogenes: split personalities. *Nat Rev Mol Cell Biol* (2008) **9**(7):517–31. doi:10.1038/nrm2438
112. Raponi M, Winkler H, Dracopoli NC. KRAS mutations predict response to EGFR inhibitors. *Curr Opin Pharmacol* (2008) **8**(4):413–8. doi:10.1016/j.coph.2008.06.006
113. Ahearn IM, Haigis K, Bar-Sagi D, Philips MR. Regulating the regulator: post-translational modification of RAS. *Nat Rev Mol Cell Biol* (2011) **13**(1):39–51. doi:10.1038/nrm3255
114. Hoshino R, Chatani Y, Yamori T, Tsuruo T, Oka H, Yoshida O, et al. Constitutive activation of the 41-/43-kDa mitogen-activated protein kinase signaling pathway in human tumors. *Oncogene* (1999) **18**(3):813–22. doi:10.1038/sj.onc.1202367
115. Janne PA, Shaw AT, Pereira JR, Jeannin G, Vansteenkiste J, Barrios C, et al. Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. *Lancet Oncol* (2013) **14**(1):38–47. doi:10.1016/S1470-2045(12)70489-8
116. Kelly K, Mazieres J, Leighl NB, Barlesi F, Zalcman G, Gordon MS, et al. Oral MEK1/MEK2 inhibitor trametinib (GSK1120212) in combination with pemetrexed for KRAS-mutant and wild-type (WT) advanced non-small cell lung cancer (NSCLC): a phase I/Ib trial. *ASCO Meet Abstr* (2013) **31**(15 Suppl):8027.
117. Hanna N, Shepherd FA, Fossella FV, Pereira JR, De Marinis F, von Pawel J, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *J Clin Oncol* (2004) **22**(9):1589–97. doi:10.1200/JCO.2004.08.163
118. Riely GJ, Brahmer JR, Planchard D, Crino L, Doebele RC, Mas Lopez LA, et al. A randomized discontinuation phase II trial of ridaforolimus in non-small cell lung cancer (NSCLC) patients with KRAS mutations. *ASCO Meet Abstr* (2012) **30**(15 Suppl):7531.
119. Roberts PJ, Stinchcombe TE. KRAS mutation: should we test for it, and does it matter? *J Clin Oncol* (2013) **31**(8):1112–21. doi:10.1200/JCO.2012.43.0454
120. Langer CJ, Besse B, Gualberto A, Brambilla E, Soria JC. The evolving role of histology in the management of advanced non-small-cell lung cancer. *J Clin Oncol* (2010) **28**(36):5311–20. doi:10.1200/JCO.2010.28.8126
121. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* (2014) **6**(224):224ra24. doi:10.1126/scitranslmed.3007094

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 26 May 2014; accepted: 16 July 2014; published online: 11 August 2014.
*Citation: Korpanty GJ, Graham DM, Vincent MD and Leighl NB (2014) Biomarkers that currently affect clinical practice in lung cancer: EGFR, ALK, MET, ROS-1, and KRAS. Front. Oncol. **4**:204. doi: 10.3389/fonc.2014.00204*
This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 15 September 2014
doi: 10.3389/fonc.2014.00244

Review of EGFR TKIs in metastatic NSCLC, including ongoing trials

Barbara Melosky*

Medical Oncology, British Columbia Cancer Agency – Vancouver Centre, Vancouver, BC, Canada

Edited by:
Vera Hirsh, McGill University Health Centre, Canada

Reviewed by:
Rabab Mohamed Gaafar, Cairo University, Egypt
Shahab Babakoohi, Medstar Good Samaritan Hospital, USA

****Correspondence:***
Barbara Melosky, Medical Oncology, British Columbia Cancer Agency – Vancouver Centre, 600-10th Avenue West, Vancouver, BC V5Z 4E6, Canada
e-mail: bmelosky@bccancer.bc.ca

Recent clinical trials have demonstrated the efficacy of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) in the treatment of patients with advanced metastatic non-small cell lung cancer. Most of these recent trials were conducted in patients with EGFR mutation-positive tumors. As our knowledge of the EGFR mutation and its resistant pathways develops, the complexity of the situation expands. This article briefly reviews the pivotal trials leading to approval of EGFR TKIs in the first-line setting for patients with EGFR mutation-positive non-small cell lung carcinomas. It discusses the historical use of EGFR TKIs after the first-line setting in unselected patients and briefly describes ongoing trials.

Keywords: EGFR TKI, first-line therapy, erlotinib, gefitinib, afatinib, dacomitinib

BACKGROUND

For many years, standard first-line systemic treatment for metastatic NSCLC has consisted of chemotherapy with a two drug combination including a platinum compound and a non-platinum drug such as pemetrexed, gemcitabine, vinorelbine, or a taxane. The typical median time to progression for chemotherapy-treated patients is 4–6 months and median survival is 10–12 months. The advent of epidermal growth factor receptor (EGFR) molecular testing changed the treatment paradigm.

The EGFR or human epidermal growth factor receptor (HER) family contains four members: EGFR (otherwise known as HER1), HER2, HER 3, and HER4. In a normal cell, binding of the epidermal growth factor ligand causes dimerization, phosphorylation, activation of the receptor, and triggering of signaling cascades through pathways such as PI3-Kinase-AKT and RAS/RAF. The presence of an EGFR gene mutation is activating, causing a constant signal to be generated, which leads to cell proliferation and other cancer processes.

Approximately 10–30% of NSCLC patients have an EGFR gene mutation. This mutation is observed at a higher frequency in some subpopulations. In Asian NSCLC cancer patients who never smoked or were only light smokers, this percentage may be as high as 60% (1). For NSCLC patients whose tumors test positive for any EGFR mutations, an oral tyrosine kinase inhibitor (TKI) is now the preferred first-line therapy.

FIRST-GENERATION EGFR TKIs

First-generation EGFR TKIs such as erlotinib and gefitinib reversibly compete with adenosine triphosphate (ATP) binding at the tyrosine kinase domain of EGFR. This inhibits ligand-induced EGFR tyrosine phosphorylation, EGFR/HER1 activation, and subsequent activation of the downstream signaling networks (2). Pivotal randomized trials with these first-generation TKIs are chronologically described in the sections below. Although it is tempting to directly compare the results of these studies, a recent publication (3) argues that this type of comparison is invalid due to differences in trial design, comparator choice, and inclusion criteria; readers are urged to refer to Sebastian et al.'s elegant description and critical analysis of these trials (3).

IDEAL 1 AND IDEAL 2 – GEFITINIB PROVIDES A SURVIVAL ADVANTAGE IN EGFR MUTATION-UNSELECTED PATIENTS

The IDEAL 1 (4) and IDEAL 2 (5) phase II trials were two of the first studies to test gefitinib in patients with stage IV NSCLC. These trials demonstrated that both 250 and 500 mg doses of gefitinib were equally active in an EGFR mutation-unselected patient population, resulting in response rates of approximately 20% and median progression-free survival of 2.7 and 2.8 months for the 250 and 500 mg doses of gefitinib, respectively (4). Because both doses showed equivalent results, the lower 250 mg dose was put forward for the registration phase III trials. A subset of patients treated with gefitinib demonstrated a very positive response, but it was unclear why that was the case. At the time, the implications of EGFR mutations were not understood, but we now know that most of these patients likely harbored an EGFR gene mutation.

NCIC BR.21: ERLOTINIB FOR AN EGFR MUTATION-UNSELECTED PATIENT POPULATION IMPROVES SURVIVAL

The NCIC BR.21 phase III trial demonstrated that erlotinib prolonged survival in NCSLC following the failure of first-line or second-line chemotherapy (6). This multicenter, randomized control trial compared erlotinib to placebo in 731 patients with stage IIIB/IV recurrent NSCLC. Study participants who had failed first- or second-line chemotherapy were randomized 2:1 to receive

Abbreviations: ATP, adenosine triphosphate; CI, confidence interval; EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; HRQoL, health-related quality of life; NCI, National Cancer Institute; NSCLC, non-small cell lung cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RCT, randomized clinical trial; TKI, tyrosine kinase inhibitor.

either erlotinib or placebo. One half of the patients had received one prior regimen, and half had received two prior regimens. Patient selection was not based on EGFR mutation status, gender, smoking history, or type of NSCLC.

This study met its primary endpoint of improving overall survival, 6.7 months for erlotinib compared to 4.7 months for placebo (HR 0.70, CI 0.58–0.85, $P < 0.001$). The study demonstrated statistically significant effects in secondary endpoints including progression-free survival of 2.23 months for patients treated with erlotinib compared to 1.84 months for those treated with placebo (HR 0.61, CI 0.51–0.73, $P < 0.001$), time to symptom deterioration, and response rate. Overall, 8.9% of patients achieved an objective response to erlotinib ($P < 0.001$), although mutational analysis was retrospective and only positive in approximately 40 patients. This trial demonstrated a survival benefit in all patients regardless of whether their tumors had an EGFR gene mutation.

Why an EGFR inhibitor was efficacious in the absence of an EGFR mutation is unclear. This reflects the complexity of the EGFR mutation and other downstream signaling pathways, many of which are still to be delineated. As a result of the NCIC BR.21 trial (6), erlotinib was approved and became standard of care in the second or third line setting for patients with NSCLC.

ISEL: GEFITINIB PROVIDES NO SURVIVAL ADVANTAGE IN AN EGFR MUTATION-UNSELECTED POPULATION

The Iressa Survival Evaluation in Lung Cancer (ISEL) phase III study was similar to the NCIC BR.21 trial design as it compared an EGFR TKI to placebo in EGFR mutation-unselected NSCLC patients in the second and third line setting (7). Unlike NCIC BR.21, this study failed to meet its endpoint of improved overall survival, with median survival of 5.6 months for patients treated with gefitinib as compared to 5.1 months for patients treated with placebo (HR 0.89, CI 0.77–1.02, $P = 0.087$). There was a pronounced heterogeneity in survival outcomes between groups of patients, most notably those who were never smokers (HR 0.67, CI 0.49–0.92, $P = 0.012$) and those of Asian ancestry (HR 0.66, CI 0.48–0.91, $P = 0.01$). Due to the negative primary results of this trial, gefitinib fell out of use for EGFR mutation-unselected patients in North America.

DISCOVERY OF EGFR MUTATIONS

In 2004, two articles were published in prestigious journals by Paez et al. (8) and Lynch et al. (9). Both publications demonstrated that patients who responded well to gefitinib had EGFR gene mutations, and the mutations were located in the region of the gene that encoded the tyrosine kinase domain. Although much discussion centered on whether the presence of the mutation should influence treatment decisions, clarity about the importance of EGFR mutations did not occur until the Iressa Pan Asian Study (IPASS) trial was completed, the mutation status of patients was analyzed, and the biomarker story became clear.

IPASS TRIAL: GEFITINIB IMPROVES SURVIVAL IN THE FIRST LINE, IN AN EGFR MUTATION-ENHANCED POPULATION

The IPASS trial was the study attributed to changing practice. The goal of the IPASS trial was to evaluate the benefit of gefitinib as compared to carboplatin/paclitaxel as first-line treatment for patients with advanced NSCLC (10). Patients selected with this trial had favorable clinical characteristics and included Asian patients with adenocarcinoma, who were non-smokers or former light smokers. Patients treated with gefitinib demonstrated superior progression-free survival as compared to those treated with chemotherapy (HR 0.74, CI 0.65–0.85, $P < 0.001$).

An EGFR biomarker analysis was specified in this protocol, but was retrospective and exploratory. Of 1200 patients, 437 had a tumor specimen that was evaluable for EGFR mutation analysis and of these, 261 patients (59.7%) had tumors that contained EGFR gene mutations. In the subset of EGFR mutation-positive patients, the response rate to gefitinib was 71.2% as compared to 47.3% for carboplatin/paclitaxel. PFS was significantly superior for the EGFR mutation-positive patients treated with gefitinib, 9.5 months as compared to 6.3 months for those treated with chemotherapy (HR 0.48, CI 0.36–0.64, $P < 0.001$). Overall survival was not different, most likely due to crossover; 21.6 months for gefitinib as compared to 21.9 months for carboplatin/paclitaxel.

Iressa Pan Asian Study demonstrated that an EGFR was the most appropriate biomarker for the use of EGFR TKI inhibitors in stage IV non-small cell lung carcinomas and with a significant improvement in PFS and quality of life, gefitinib became standard of care first-line option for NSCLC patients with EGFR-mutated tumor. From this point onward, all TKI trials were conducted in EGFR mutation selected populations and European authorities restricted the use of gefitinib to patients with an EGFR mutation only, regardless of therapeutic line.

WJOG AND NEJSG: JAPANESE TRIALS TESTING GEFITINIB IN EGFR MUTATION SELECTED POPULATIONS

Two randomized phase III studies compared gefitinib to chemotherapy in the first-line setting (11, 12). Both of these trials, involving NSCLC patients selected on the basis of EGFR mutations, demonstrated a statistically significant increase in progression-free survival for patients treated with gefitinib over chemotherapy. In the West Japan Oncology Group (WJOG) trial, patients treated with gefitinib experienced a median PFS of 9.2 as compared to 6.3 months for those treated with chemotherapy (HR = 0.489, CI 0.336–0.710, $P < 0.0001$) (11). Results were similar in the North-East Japan Study Group (NEJSG), where patients treated with gefitinib experienced a median PFS of 10.8 months compared to 5.4 months for those treated with chemotherapy (HR = 0.30, CI 0.22–0.41, $P < 0.001$) (12). This study was stopped following the results of a planned interim analysis as the gefitinib arm had significantly superior PFS compared to the chemotherapy arm. A high number of patients crossed over to gefitinib (98%); this is the most likely explanation for no difference is overall survival.

EURTAC TRIAL: ERLOTINIB IN THE FIRST-LINE IMPROVES PROGRESSION-FREE SURVIVAL

The European Tarceva vs. Chemotherapy (EURTAC) trial was conducted in patients with EGFR mutation positive tumors, and was the first to demonstrate the benefits of an EGFR TKI in a Caucasian population (13). Patients were randomized to receive erlotinib or chemotherapy (cisplatin/gemcitabine or cisplatin/docetaxel) in the first-line setting. Response rate was 58% in the erlotinib

arm compared to 15% in the chemotherapy arm ($P < 0.0001$). Progression-free survival was 9.7 months for patients treated with erlotinib and 5.2 months for patients treated with chemotherapy (HR = 0.37, CI 0.25–0.54, $P < 0.0001$) (13). Overall survival was 22.9 months in the erlotinib arm as compared to 18.8 months in the chemotherapy arm (HR = 0.80; $P = 0.42$), most likely confounded by second-line therapy and crossover to erlotinib.

SECOND-GENERATION TKIs

Afatinib and dacomitinib are second-generation EGFR TKIs, and block all HER-family ligands, including HER1 (EGFR), as well as HER2 and HER4. These agents form permanent covalent bonds with the target, irreversibly inhibiting ATP binding at the tyrosine kinase domain. As a result, second-generation TKIs are theoretically more effective in inhibiting EGFR signaling than first-generation erlotinib or gefitinib because the inhibition of EGFR signaling is prolonged for the entire lifespan of the drug-bound receptor molecule (14).

Two phase III trials were conducted to test dacomitinib in EGFR mutation-unselected populations. The Archer 1009 phase III trial compared dacomitinib with erlotinib in EGFR mutation-unselected patients who were previously treated with chemotherapy. The trial did not demonstrate statistically significant improvement in progression-free survival and was discontinued. The NCIC BR.26 trial phase III trial compared dacomitinib with placebo in 736 EGFR mutation-unselected patients with advanced NSCLC previously treated with both chemotherapy and an EGFR TKI. This study also did not meet its objective of prolonging overall survival. Subgroup analysis is currently being conducted in order to understand if there was a difference in response between patients whose tumors harbored an EGFR mutation and those whose tumors did not.

A number of other trials testing the second-generation EGFR TKI dacomitinib are underway and have yet to be published. Archer 1050 is a phase III randomized, open-label trial comparing dacomitinib to gefitinib in a first-line treatment setting in EGFR mutation-positive NSCLC patients. In this trial, approximately 440 patients were randomized 1:1 to dacomitinib or gefitinib. The primary endpoint is PFS by independent review, while the secondary endpoints include PFS by investigator assessment, overall survival, best overall response, duration of response, safety and tolerability, and patient-reported outcomes. As phase II studies of dacomitinib in the first-line treatment setting were promising, we look forward to the results of this phase III study, which will be revealed in mid-2015.

AFATINIB FOR PATIENTS WITH EGFR MUTATION POSITIVE TUMORS

LUX-Lung 1 was a phase 2b/3 randomized trial comparing afatinib to best supportive care in unselected patients who had received both a platinum doublet and 3 months of an EGFR TKI, gefitinib, or erlotinib (15). Although progression-free survival was increased, the primary endpoint of overall survival was not. Because of this negative trial, the use of afatinib in patients with an acquired resistance to EGFR TKIs was not approved in any country except Japan.

The pivotal afatinib trial is LUX-Lung 3 (16). This phase III trial randomized 345 patients with NSCLC in the first-line setting who had EGFR mutation-positive tumors to receive either afatinib or cisplatin/pemetrexed. For this study, all EGFR mutations from codons 18–21 were analyzed. While the majority of patient tumors harbored common EGFR mutations (Del-19 and Point 21 L858R), approximately 10% of patients had uncommon EGFR mutations. The primary endpoint of this trial was progression-free survival and secondary endpoints included overall survival, objective response rate, and quality of life.

Afatinib treatment led to an increase in the objective response rate compared with chemotherapy treatment (56.1 vs. 22.6%). Patients randomized to afatinib experienced a significant improvement in median progression-free survival compared with those randomized to chemotherapy, 11.1 vs. 6.9 months, respectively (HR 0.58, CI 0.43–0.78, $P = 0.0004$). The treatment effect of afatinib was more pronounced when comparing progression-free survival in the pre-defined subgroup of patients with the common Del-19 or Point 21 L858R EGFR mutations. In this subgroup, patients treated with afatinib experienced progression-free survival of 13.6 months as compared to 6.9 months for those treated with chemotherapy (HR 0.47, CI 0.34–0.65, $P < 0.0001$) (16).

The LUX-Lung 6 trial, conducted in Asia, confirmed the value of afatinib in the population of patients with EGFR mutation-positive tumors (17). This phase III, open-label trial randomized 364 NSCLC patients in a 2:1 fashion to receive afatinib or gemcitabine/cisplatin. The primary endpoint in this study was progression-free survival and secondary endpoints included objective response rate, disease control rate, patient-reported outcomes, and safety.

A statistically significant improvement in progression-free survival was demonstrated between patients treated with afatinib as compared to those treated with chemotherapy, 11.0 vs. 5.6 months, respectively (HR 0.28, CI 0.20–0.39, $P < 0.0001$) (17). The progression-free survival benefit was consistent across all subgroups, including all mutation categories. The percentage of LUX-Lung 6 patients with a confirmed objective response was 67% in the afatinib group as compared to 23% in the chemotherapy group. Overall, the results of the LUX-Lung 6 trial support the efficacy observations (progression-free survival and objective response rate) demonstrated in the LUX-Lung 3 trial.

To date, none of the published randomized EGFR TKI trials have demonstrated a statistically significant improvement in overall survival. In the American Society of Clinical Oncology meeting in Chicago 2014, a pooled analysis of LUX-Lung 3 and LUX-Lung 6 was presented (18). Although the pooling of clinical trial results in this way is controversial, the results are interesting. According to this analysis, the overall survival of LUX-Lung 3 was 31.6 months for patients treated with afatinib as compared to 28.2 months for those treated with chemotherapy (pemetrexed/cisplatin) (HR: 0.78). The pooled overall survival analysis of LUX-Lung 6 showed that patients treated with afatinib had a median survival of 23.6 months as compared to 23.5 months when treated with gemcitabine/cisplatin (HR 0.8). Although both hazard ratios are approximately 0.8, neither of the P values were significant.

The pooled analysis showed an important improvement in overall survival in patients whose tumors had the most

common EGFR mutations, Del-19 and Point 21 L858R. In the sub-population of patients with these mutations, the median overall survival in the afatinib arm was 27.3 months, which was significantly improved over median overall survival of 24.3 months in the chemotherapy arm (HR 0.81, $p = 0.037$).

The most interesting analysis concerned the subpopulation of patients whose tumors had harbored the Del-19 deletion, where a significant improvement in overall survival was seen in both LUX-Lung 3 and LUX-Lung 6 trials. In the LUX-Lung 3 trail, the median survival was 33.2 months for Del-19 patients treated with afatinib as compared to 21.1 months with chemotherapy (pemetrexed/cisplatin) (HR 0.54). In LUX-Lung 6, the median survival was 31.4 months for Del-19 patients treated with afatinib as compared to 18.4 months for patients treated with chemotherapy (gemcitabine/cisplatin) (HR 0.64). The author concluded that the patients with Del-19 and Point 21 L858R mutations may constitute very different populations, and may require different treatment strategies (18).

A highly anticipated trial is LUX-Lung 7. This phase III, open-label trial randomized 316 patients with EGFR mutation-positive advanced adenocarcinoma to receive either afatinib or gefitinib. The primary endpoint for the trial, which completed in July 2013, was overall survival. We await the results eagerly.

Clinical trials with the third-generation EGFR TKIs are underway. These inhibitors work to selectively inhibit tumors that harbor the acquired T790 mutation.

Currently, there are more than 350 open trials for EGFRs in NSCLC, and at least 20 of these are phase III. Indeed, this is a very exciting time in the evolution of our knowledge of the EGFR TKI inhibitors, and we expect outstanding advances in the care of our patients with non-small cell lung carcinoma.

REFERENCES

1. Janku F, Garrido-Laguna I, Petruzelka LB, Stewart DJ, Kurzrock R. Novel therapeutic targets in non-small cell lung cancer. *J Thorac Oncol* (2011) **6**(9):1601–12. doi:10.1097/JTO.0b013e31822944b3
2. Zhang X, Chang A. Molecular predictors of EGFR-TKI sensitivity in advanced non-small cell lung cancer. *Int J Med Sci* (2008) **5**(4):209–17. doi:10.7150/ijms.5.209
3. Sebastian M, Schmittel A, Reck M. First-line treatment of EGFR-mutated non-small cell lung cancer: critical review on study methodology. *Eur Respir Rev* (2014) **23**(131):92–105. doi:10.1183/09059180.00008413
4. Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard J-Y, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non–small-cell lung cancer. *J Clin Oncol* (2003) **21**(12):2237–46. doi:10.1200/JCO.2003.10.038
5. Kris MG, Natale RB, Herbst RS, Lynch TJ Jr, Prager D, Belani CP, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* (2003) **290**(16):2149–58. doi:10.1001/jama.290.16.2149
6. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* (2005) **353**(2):123–32. doi:10.1056/NEJMoa050753
7. Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* (2005) **366**(9496):1527–37. doi:10.1016/S0140-6736(05)67625-8
8. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* (2004) **304**(5676):1497–500. doi:10.1126/science.1099314
9. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non–small-cell lung cancer to gefitinib. *N Engl J Med* (2004) **350**(21):2129–39. doi:10.1056/NEJMoa040938
10. Mok TSWY, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, et al. Gefitinib or carboplatin–paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* (2009) **361**(10):947–57. doi:10.1056/NEJMoa0810699
11. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol* (2010) **11**(2):121–8. doi:10.1016/S1470-2045(09)70364-X
12. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non–small-cell lung cancer with mutated EGFR. *N Engl J Med* (2010) **362**(25):2380–8. doi:10.1056/NEJMoa0909530
13. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* (2012) **13**(3):239–46. doi:10.1016/S1470-2045(11)70393-X
14. Kosaka T, Yamaki E, Mogi A, Kuwano H. Mechanisms of resistance to EGFR TKIs and development of a new generation of drugs in non-small-cell lung cancer. *J Biomed Biotechnol* (2011) **165214**(10):2. doi:10.1155/2011/165214
15. Miller VA, Hirsh V, Cadranel J, Chen YM, Park K, Kim SW, et al. Afatinib versus placebo for patients with advanced, metastatic non-small-cell lung cancer after failure of erlotinib, gefitinib, or both, and one or two lines of chemotherapy (LUX-Lung 1): a phase 2b/3 randomised trial. *Lancet Oncol* (2012) **13**(5):528–38. doi:10.1016/S1470-2045(12)70087-6
16. Yang JC, Hirsh V, Schuler M, Yamamoto N, O'Byrne KJ, Mok TS, et al. Symptom control and quality of life in LUX-Lung 3: a phase III study of afatinib or cisplatin/pemetrexed in patients with advanced lung adenocarcinoma with EGFR mutations. *J Clin Oncol* (2013) **31**(27):3342–50. doi:10.1200/JCO.2012.46.1764
17. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. *Lancet Oncol* (2014) **15**(2):213–22. doi:10.1016/S1470-2045(13)70604-1
18. Yang JC-H, Sequist LV, Schuler MH, Mok T, Yamamoto N, O'Byrne KJ, et al. Overall survival (OS) in patients (pts) with advanced non-small cell lung cancer (NSCLC) harboring common (Del19/L858R) epidermal growth factor receptor mutations (EGFR mut): pooled analysis of two large open-label phase III studies (LUX-Lung 3 [LL3] and LUX-Lung 6 [LL6]) comparing afatinib with chemotherapy (CT). *J Clin Oncol* (2014).

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The Guest Associate Editor Vera Hirsh declares that, despite having collaborated with author Barbara Melosky, the review process was handled objectively and no conflict of interest exists.

Received: 28 April 2014; accepted: 26 August 2014; published online: 15 September 2014.

*Citation: Melosky B (2014) Review of EGFR TKIs in metastatic NSCLC, including ongoing trials. Front. Oncol. **4**:244. doi: 10.3389/fonc.2014.00244*

This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 23 July 2014
doi: 10.3389/fonc.2014.00190

Drug resistance to molecular targeted therapy and its consequences for treatment decisions in non-small-cell lung cancer

Johanna N. Spaans*[1] *and Glenwood D. Goss*[1,2,3]

[1] *Ottawa Hospital Research Institute, Ottawa, ON, Canada*
[2] *Ottawa Hospital Cancer Centre, Ottawa, ON, Canada*
[3] *Department of Medicine, University of Ottawa, Ottawa, ON, Canada*

Edited by:
Vera Hirsh, McGill University Health Centre, Canada

Reviewed by:
Gregory Masters, Medical Oncology Hematology Consultants, PA, USA
Janaki Deepak, University of Maryland School of Medicine, USA

***Correspondence:**
Glenwood D. Goss, 501 Smyth Road, Ottawa, ON K1H 8L6, Canada
e-mail: ggoss@toh.on.ca

Our ability to detect and directly target the oncogenic alterations responsible for tumor proliferation has contributed significantly to the management of lung cancer in the last decade. The therapeutic efficacy of molecularly targeted therapy is, however, mainly limited to patients harboring certain genetic mutations and is generally short-lived. Herein, we review primary and secondary drug resistance using the most well-studied of the molecularly targeted agents, the tyrosine kinase inhibitors targeting the epidermal growth factor (EGF) receptor, and the anaplastic lymphoma kinase (ALK) rearrangement, the current limitations of targeted therapies and their consequences on the management of patients with lung cancer.

Keywords: EGFR, inhibition, primary resistance, acquired resistance, molecular biology

The treatment of advanced non-small-cell lung cancer (NSCLC) had reached a therapeutic plateau prior to the introduction of molecularly targeted agents (MTAs), with a median survival of 8–12 months (1, 2). With an improved understanding of the molecular biology of lung cancer, enabled by advances in high-throughput technology, have come molecular therapies that target specific receptors and oncogenic pathways responsible for tumor growth and proliferation. Despite the demonstrated superiority of these MTAs over standard chemotherapy in subgroups of patients (3, 4), their therapeutic efficacy is limited to patients harboring the targeted genetic aberration and is generally short-lived. Any future advances in the survival of patients with advanced NSCLC will hinge on our ability to expand on the percentage of patients eligible and responsive to targeted therapy and our capacity to mitigate the mechanisms of acquired resistance that prevent long-term disease control.

As the most well-studied of the MTAs, tyrosine kinase inhibitors in NSCLC targeting the epidermal growth factor (EGF) receptor (erlotonib, gefitinib, and afatinib) and the anaplastic lymphoma kinase (ALK) rearrangement (crizotinib) provide a useful framework in which to understand the current limitations of molecularly targeted therapy and their consequences in the management of patients with NSCLC.

PRIMARY RESISTANCE TO EGFR INHIBITORS

The EGFR pathway is known to be active in NSCLC (5) and protein overexpression is known to be associated with poorer prognosis (6). Early on in the clinical development of EGFR-tyrosine kinase inhibitors (EGFR-TKIs), which targeted this pathway, it was realized that patients whose tumor harbored an activating mutation in the EGFR gene at exons 19 and 21 had more dramatic responses and better clinical outcomes than their EGFR wild-type (W/T) counterparts (7, 8). This has resulted in some countries limiting regulatory approval in the first-line setting to patients whose tumors harbor these sensitizing mutations (9). Although common among lung cancer patients of Asian descent (10), sensitizing EGFR mutations are relatively uncommon in North American and European NSCLC populations with a prevalence of ~15% in patients with advanced non-squamous histology (11). Further, despite their heightened sensitivity to EGFR-TKIs, as many as one third of NSCLC patients with tumors with sensitizing EGFR mutations do not respond to targeted therapy (12, 13).

The mechanisms of primary resistance to EGFR-TKIs are best considered in terms of patients with tumors with (EGFR mutant) and without (EGFR W/T) sensitizing mutations. In the latter case, patients may not respond to EGFR-TKIs because their tumors are being driven by other oncogenic pathways that are not sensitive to EGFR inhibition. Indeed, different oncogenic alterations have been identified in up to half of patients with EGFR W/T disease (14). Importantly, the successful targeting of one such alteration, namely the ALK gene rearrangement with crizotinib (15) demonstrates the feasibility of addressing this form of primary resistance in the EGFR W/T population. Therapies targeting other mutations that commonly occur among EGFR W/T patients such as c-ros oncogene 1 (ROS1), ret proto-oncogene (RET), v-raf murine sarcoma viral oncogene homolog B (BRAF), and the human EGF 2 (HER2) are currently under development.

In addition, primary resistance in the EGFR W/T population may be the result of activation of alternate parallel signaling pathways, which can overcome EGFR blockade, that are independent of a pathway-specific activating mutation. Activation of the insulin-like growth factor receptor (IGFR) pathway (16) is one such potential mechanisms of primary resistance. Blockade of these alternate pathways to enhance EGFR TKI efficacy is a strategy that is being investigated, but to date has yielded mixed results (17–19). Lack of response to EGFR-TKIs among patients with

W/T tumors may also be due to incomplete binding of the drug to the EGF receptor or because of insufficient drug concentration necessary for effective pathway blockade (20). Further, as only one of four receptor tyrosine kinases in the ERbB family (21, 22), the isolated targeting of the EGF/Erb1 receptor may not prevent autophosphorylation and downstream pathway activation by the other receptors (i.e., Erb2, Erb3, Erb4). Newer second-generation irreversible pan-HER tyrosine kinase inhibitors such as dacomitinib and afatinib that target multiple receptors are being evaluated in both EGFR W/T and EGFR-mutant populations as a strategy to enhance and prolong treatment response (23, 24).

In patients with tumors with EGFR sensitizing mutations a number of factors have been identified in the primary resistance setting, which may modulate or blunt the therapeutic efficacy of EGFR-TKIs. While most oncogenic driver mutations are mutually exclusive in context of lung cancer, the co-existence of EGFR mutations with other oncogenic alterations, including class A phosphoinositide 3-kinase (PI3KCA), have been reported (25). Activation of compensatory signaling pathways by these other mutations may afford continued disease progression and negate or circumvent clinical benefit derived from EGFR TKIs in patients whose tumors harbor EGFR mutations. The dual targeting of co-existing mutations with combination therapy in EGFR-mutant disease is currently under investigation (clinical trials.gov: NCT01570296).

In a similar fashion, exogenous factors, including MED12-mediated transforming growth factor beta (TGF-β) activation (26) and hepatocyte growth factor (HGF) ligand overexpression (27), may enable the activation of alternate signaling pathways in tumors that may override the pathways inhibited by the EGFR-TKIs. The inhibition of these compensatory pathways in tumors with EGFR sensitizing mutations is a hot topic in clinical research with the testing of a number of combination therapies that include agents to overcome TGF-β and HGF-mediated resistance [e.g., heat shock protein (Hsp) 90 inhibitors] in both the primary (clinicaltrials.gov: NCT01714037) and resistance setting (clinicaltrials.gov: NCT01259089, NCT01288430, NCT01851096).

Finally, exogenous apoptotic factors have been identified that may modulate the impact of EGFR TKIs in patients with tumors with sensitizing EGFR mutations and may explain their variable treatment response. Increasing evidence suggests that expression levels of proapototic BH-3 only molecule (BIM) can influence treatment-induced apoptosis (28, 29) and further that pre-treatment BIM expression may play a role in treatment response to many kinase inhibitors across many disease sites (30, 31). While in lung cancer, it has been shown that low pre-treatment BIM levels are associated with shorter time to progression (29), available pro-apoptotic assays are not currently being used in clinical practice to predict treatment response. Targeted therapies with B-cell lymphoma 2 (blc2) inhibitors that enhance apoptosis, however, are currently being evaluated in combination with EGFR-TKIs as a strategy to enhance treatment response (clinicaltrials.gov: NCT00988169).

Despite early and dramatic treatment responses in up to two thirds of patients with EGFR sensitizing mutations, most patients will eventually progress while on therapy within a year of treatment initiation (12). Beyond the level of pre-treatment apoptotic factors, such as BIM discussed previously, a number of other factors have been suggested to influence the development of clonal and sub-clonal EGFR-resistant cell populations. Specifically, both factors affecting the drug metabolism and characteristics of the treatment schedules may impact the development of acquired resistance in previously responsive patients (32). While the higher metabolic clearance of EGFR-TKIs among smokers and fast metabolizers has long been recognized as a negative predictor for time to progression (33), only recently have the pharmacokinetics of different dosing schedules been considered for their potential influence on the evolution of drug resistance to EGFR TKIs. Specifically, based on evolutionary modeling and clinical data, it has been proposed that pulsed high dose with continuous low dose EGFR-TKI treatment helps to maintain sensitive cell populations and may extend the therapeutic benefit of EGFR-TKI therapy beyond progression (34). While standard once daily dosing continues to be used in the clinic for approved MTAs, research is on-going to define characteristics of the treatment regimen that may delay disease progression and optimize therapeutic outcomes with EGFR-TKI therapy (clinicaltrials.gov: NCT01967095).

Although criteria for acquired resistance have now been developed (35), resistant disease is best considered along an evolutionary continuum, where resistant clones eventually overrun EGFR-sensitive cells, leading to the clinical characteristics of disease progression. The existence of EGFR-sensitive cells in tumors that progress is supported by reports of clinical response in patients re-challenged with EGFR-TKIs (36) and also by reports of disease flare in up to 15% of patients who are taken off EGFR-TKI therapy at disease progression (37).

SECONDARY EGFR-TKI RESISTANCE

Genetic adaptations and altered network signaling pathways invariably lead to drug resistance in patients whose tumors harbor EGFR sensitizing mutations who initially respond to EGFR-targeted therapy (acquired resistance). Molecular profiling of tumors with acquired resistance to EGFR-TKIs has identified a number of resistance mechanisms and dominant acquired resistance phenotypes, which may be useful in guiding future treatment. The most common mechanism of acquired resistance to EGFR-TKI is the development of a second mutation of the EGFR that is resistant to therapy. While a number of secondary mutations have been identified (38), the most common "gatekeeper" mutation is that of the T790M, which occurs in 50–60% of patients with acquired resistance to EGFR-TKIs (39). This secondary mutation is believed to exert its effect by enhancing ATP kinase affinity, thereby decreasing sensitivity to the ATP-competitive EGFR TKIs (40). Importantly, the development of secondary resistance mutations in the EGFR kinase domain has implications in the re-challenging of patients with previously sensitive disease and has fueled research in the development of second and third generation inhibitors (41–45). Despite encouraging phase II data of one such second-generation inhibitor (dacomitinib) in previously treated patients (41, 42), emerging phase III data suggests that there is no overall survival benefit associated with its use in previously treated EGFR W/T patients or those with acquired EGFR-TKI resistance (23). Similarly, while interim analysis of

another second-generation irreversible ErbB family blocker (Afatinib) in patients with acquired resistance to EGFR-TKIs suggested improved progression-free survival (PFS) (43), the lack to an overall survival benefit observed in this phase 2b/3 randomized trial (24) does not support the strategy of extended EGFR blockade in the EGFR-resistant population.

Most recently, the finding of a T790M mutation in tumors at the time of initial diagnosis (44, 45) has implicated the mutation in primary EGFR-TKI resistance, suggesting that up-front treatment with second/third generation EGFR-TKIs may confer added benefit over their use in the second-line setting in patients with T790M-mediated acquired resistance. Indeed, preliminary clinical reports of second-generation EGFR inhibitors in the first-line treatment setting support their up-front use in patients whose tumors harbor EGFR mutations (46, 47).

An alternate mechanism of secondary resistance is the activation of other signaling pathways by adaptive *de novo* alterations that develop outside the EGFR kinase domain in response to treatment. A number of these alterations have been identified; the most well-studied being MET amplification, which occurs in 10–20% of patients with EGFR-TKI resistant disease (48). Other less common mutations include HER2 amplification (49, 50), activation of PIK3Ca (51) and BRAF (52), and loss of phosphatase and tensin homolog (PTEN) function (53).

Crosstalk between key signaling pathways may also play a role in the development of acquired resistance to EGFR inhibitors. Specifically, the activity of the angiogenic VEGF pathway has been suggested to play a role in resistance to EGFR-TKIs (54), which is not surprisingly given the common downstream effectors shared by these parallel pathways (55). Although preclinical data across different tumor types (56, 57) and early phase lung cancer clinical trials (58, 59) pointed to the potential utility of dual inhibition of VEGF and EGFR, phase III data of the dual inhibitor vandetanib suggest that this is not a promising approach to overcome acquired resistance to EGFR-TKIs in advanced NSCLC (60).

Finally, a less common but well documented mechanism of acquired resistance to EGFR-TKIs is histological transformation from NSCLC to SCLC or epithelial–mesenchymal transition (EMT), which has been reported in up to 3% of EGFR-TKI resistant patients (61). Increasing evidence suggest that these transformations are linked to the activation of the AXL kinase, the inhibition of which may restore EGFR-TKI sensitivity in previously resistant cells (62). Collectively, these mechanisms clearly demonstrate the multitude of adaptive strategies developed by the tumor to ensure its continued growth and underscores the complexity of treating EGFR-TKI-resistance disease.

While the above resistant disease phenotypes are useful in the classification of acquired resistance, these adaptive mechanisms may not be mutually exclusive. Indeed, it has recently been proposed that T790M mutations and MET amplifications are complementary and may co-exist in the development of drug resistance (63). In addition, oncogenic driver mutations may be tumor specific, as different driver mutations from different tumor sites within the same individual have been identified in patients with EGFR-TKI resistant disease (39), further illustrating the challenges in managing patients with acquired resistance.

PRIMARY RESISTANCE TO ALK INHIBITORS

Between 1 and 3% of patients with advanced NSCLC have tumors that harbor sensitizing chromosomal rearrangements of the ALK gene (64–66). The ALK inhibitor crizotinib has recently been approved for the treatment of patients with ALK-positive tumors, however, as with EGFR-TKIs not all patients respond to therapy. Specifically, while phase III studies have shown that crizotinib improves PFS compared to chemotherapy in previously treated NSCLC patients with ALK-positive disease (HR = 0.49 95% CI: 0.37–0.64, $p < 0.001$), only 65% of patients were shown to respond to therapy (67). While primary resistance among patients with ALK-positive tumors is less well-understood, the occurrence of drug-resistant ALK mutations and compensatory mechanisms have been advanced as potential mechanisms of primary resistance (68).

In summary, less than 20% of patients have tumors with an EGFR or ALK mutation at the time of diagnosis, and of these, only 60–70% of patients respond to currently available MTAs. Therefore, we are mandated to address the approximately 80% of patients whose tumors are *de novo* resistant to EGFR and ALK inhibition, a percentage of whom are resistant due to other oncogenic driver mutations such as Kirsten rat sarcoma viral oncogene homolog (KRAS), BRAF, and RET, among others. Many investigations targeting these mutations are on-going.

SECONDARY RESISTANCE TO ALK INHIBITORS

While secondary mutations in the ALK domain have been identified in approximately one-third of the patients with acquired resistance to ALK inhibitors (69), unlike acquired resistance to EGFR-TKIs, there does not appear to be a dominant secondary mutation. Further complicating the management of such patients, multiple mutations within the same individual have also been reported in patients with acquired resistance (70). Of note, second-generation ALK inhibitors have, however, recently shown high response rates (48% confirmed responses) in patients previously treated with crizotinib, in tumors with and without secondary mutations in ALK (71). These results support the importance of ALK in crizotinib-resistant disease and the continued effort in targeting the ALK domain.

TREATMENT IMPLICATIONS

Since the publication of the initial reports over a decade ago, we now have a much better understanding of which patients stand to benefit most from targeted therapies with EGFR and ALK tyrosine kinase inhibitors and the intrinsic and adaptive mechanisms that limit treatment response and inevitably lead to acquired drug resistance. Despite these treatment advances, there are currently a limited number of therapeutic options available to patients not harboring sensitizing EGFR mutations or rearrangement of the ALK gene. Further, the mechanisms of acquired resistance among those who initially respond to treatment remain uncharacterized in almost 40% of patients with acquired resistance (72). That said, many agents targeting other oncogenic mutations are in phase III development, and in the near future will expand the armamentarium of targeted therapies available in the treatment of advanced NSCLC. The following strategies are proposed in the current and future management of patients with NSCLC.

All patients presenting with advanced NSCLC should be screened for all known oncogenic driver mutations with treatment assigned accordingly based on available molecularly targeted therapies. With the approval of second line and third line (T790M specific) EGFR-TKIs, it may also be useful to screen up-front for T790M mutations and preferentially treat patients harboring these mutations with these second and third generation therapies, given the shorter PFS that patients harboring this mutation experience with first-line reversible EGFR-TKIs (44). In addition, for patients harboring sensitizing EGFR mutations, the assessment of pre-treatment BIM expression may be a useful approach to help to optimize EGFR-TKI treatment outcomes, with the addition of anti-apoptotic inhibitors such as Blc2 to the treatment regimen.

While alternate dosing schedules, such as pulsed high dose with continuous low dose may be shown to delay time to disease progression, current treatment regimens of approved targeted agents are limited to once daily dosing. EGFR-TKI treatment should ideally be continued in the case of disease progression until the initiation of second-line therapy, given the potential for disease flare (37) and data that suggest that patients may benefit from continued treatment beyond progression (73, 74). As it has been shown that isolated sites of disease progression may be successfully treated while continuing on EGFR-TKIs (75), the decision to discontinue EGFR-TKI therapy at disease progression should be considered in the context of available therapeutic alternatives and the potential benefit of continued EGFR-TKI therapy. For example, treatment with afatinib in addition to chemotherapy has recently been shown to delay progression over chemotherapy alone (5.6 vs. 2.8 months) in patients who had progressed on afatinib (76).

The optimal treatment of patients with tumors that harbor EGFR mutations and ALK gene rearrangement who develop acquired resistance to EGFR and ALK tyrosine kinase inhibitors has yet to be defined. While most patients are managed with chemotherapy, the evidence to support this therapeutic approach is limited and the documented response rate with chemotherapy in patients with EGFR-resistance disease is l0–20% (77). As more targeted therapies become available, a more informed approach to the treatment of acquired disease to targeted therapies may emerge through rebiopsy at the time of disease progression and tailoring of subsequent mechanism-based therapies.

Lung cancer is a heterogenous disease and resistance mechanisms to targeted molecular therapy are many. Given the multitude of signaling pathways and the evolving characteristics of resistant disease, an up-front combination therapy that simultaneously inhibits multiple resistance pathways is likely to yield better clinical outcomes. Personalized targeted therapy at the time of disease recurrence may further improve survival. Importantly, an aggressive front-line strategy and a tailored management approach in the case of resistant disease has been successfully employed in the management of other diseases, including HIV (78), which has now come to be considered a chronic disease. Whether advanced lung cancer may someday have a similar clinical outcome remains to be seen. To achieve this, attention must be directed at reducing the toxicity of combination therapies and greater efforts made to define the molecular basis of acquired resistance.

REFERENCES

1. Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* (2008) **26**:3543–51. doi:10.1200/JCO.2007.15.0375
2. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* (2002) **346**:92–8. doi:10.1056/NEJMoa011954
3. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non small-cell lung cancer (OPTIMAL, CTONG-0802): a multicenter, open-label, randomized, phase 3 study. *Lancet Oncol* (2011) **12**:735–42. doi:10.1016/S1470-2045(11)70184-X
4. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non small-cell lung cancer (EURTAC): a multicentre, open-label, randomized phase 3 trial. *Lancet Oncol* (2012) **13**:239–46. doi:10.1016/S1470-2045(11)70393-X
5. Sechler M, Cizmic AD, Avasarala S, Van Scoyk M, Brzezinski C, Kelley N, et al. Non-small-cell lung cancer: molecular targeted therapy and personalized medicine – drug resistance, mechanisms, and strategies. *Pharmacogenom Personal Med* (2013) **6**:25–36. doi:10.2147/PGPM.S26058
6. Meert AP, Martin B, Verdebout JM, Noël S, Ninane V, Sculier JP. Is there a relationship between c-erbB-1 and c-erbB-2 amplification and protein expression in NSCLC? *Lung Cancer* (2005) **47**:325–36. doi:10.1016/j.lungcan.2004.07.047
7. Zhu CQ, da CunhaSantos G, Ding K, Sakurada A, Cutz JC, Liu N, et al. Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR 21. *J Clin Oncol* (2008) **26**(26):4268–75. doi:10.1200/JCO.2007.14.8924
8. Hirsch FR, Varella-Garcia M, Bunn PAJr, Franklin WA, Dziadziuszko R, Thatcher N, et al. Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non small cell lung cancer. *J Clin Oncol* (2006) **24**(31):5034–42. doi:10.1200/JCO.2006.06.3958
9. Ellis PM, Verma S, Sehdev S, Younas J, Leighl N. Implementation of a national EGFR testing strategy in a publicly funded health system. *J Thorac Oncol* (2011) **6**:4.199. doi:10.1097/JTO.0b013e31829f6a43
10. Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, Mitsudomi T. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. *Cancer Res* (2004) **64**:8919–23. doi:10.1158/0008-5472.CAN-04-2818
11. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* (2005) **98**:339–46. doi:10.1093/jnci/dji055
12. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Eng J Med* (2009) **361**:947–57. doi:10.1056/NEJMoa0810699
13. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harboring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised Phase 3 trial. *Lancet Oncol* (2009) **11**:121–8. doi:10.1016/S1470-2045(09)70364-X
14. Pao G, Girard N. New driver mutations in non-small-cell lung cancer. *Lancet Oncol* (2011) **12**(2):175–80. doi:10.1016/S1470-2045(10)70087-5
15. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small cell lung cancer. *NEJM* (2010) **363**:1693–703. doi:10.1056/NEJMoa1006448
16. Gong Y, Yao E, Shen R, Goel A, Arcila M, Teruya-Feldstein J, et al. High expression levels of total IGF-1R and sensitivity of NSCLC cells in vitro to anti-IGF-IR antibody (R1507). *PLoS One* (2009) **4**:e7273. doi:10.1371/journal.pone.0007273
17. Li H, Schmid-Bindert G, Wang D, Zhao Y, Yang X, Su B, et al. Blocking the PI3K/AKT and MEK/ERK signaling pathways can overcome gefitinib resistance in non-small cell lung cancer cell lines. *Adv Med Sci* (2011) **56**:275–84. doi:10.2478/v10039-011-0043-x
18. Weickhardt A, Doebele R, Oton A, Lettieri J, Maxson D, Reynolds M, et al. A phase I/II study of erlotinib in combination with the anti-insulin-like growth factor-1 receptor monoclonal antibody IMC-A12 (cixutumumab) in patients with advanced non-small cell lung cancer. *J Thorac Oncol* (2012) **7**:419–26. doi:10.1097/JTO.0b013e31823c5b11

19. Ramalingam SS, Spigel DR, Chen D, Steins MB, Engelman JA, Schneider CP, et al. Randomized phase II study of erlotinib in combination with placebo or R1507, a monoclonal antibody to insulin-like growth factor-1 receptor, for advanced-stage non-small-cell lung cancer. *J Clin Oncol* (2011) **29**:4574–80. doi:10.1200/JCO.2011.36.6799
20. Mir O, Blanchet B, Goldwasser F. Drug-induced effects on erlotinib metabolism. *N Eng J Med* (2011) **365**:379–80. doi:10.1056/NEJMc1105083
21. Tebbutt N, Pedersen MW, Johns TG. Targeting the ERBB family in cancer: couples therapy. *Nat Rev Cancer* (2013) **13**:663–73. doi:10.1038/nrc3559
22. Carpenter G. Receptors for epidermal growth factor and other polypeptide mitogens. *Ann Rev Biochem* (1987) **56**:881–914. doi:10.1146/annurev.bi.56.070187.004313
23. Ellis PM, Liu G, Millward M, Perrone F, Shepherd FA, Sun S, et al. NCIC-CTG BR 26: a phase III randomized, double-blind, placebo controlled trial of dacomitinib versus placebo in patients with advanced/metastatic non-small cell lung cancer (NSCLC) who received prior chemotherapy and a EGFR TKI (Abst 8036). *50th ASCO Annual Meeting*. Chicago, IL (2014).
24. Miller VA, Hirsh V, Cadranel J, Chen YM, Park K, Kim SW, et al. Afatinib versus placebo for patients with advanced, metastatic non-small cell lung cancer after failure of erlotinib, gefitinib, or both, and one or two lines of chemotherapy (LUX-LUNG 1): a phase 2b/3 randomised trial. *Lancet Oncol* (2012) **13**(5):528–38. doi:10.1016/S1470-2045(12)70087-6
25. Kawano O, Sasaki H, Endo K, Suzuki E, Haneda H, Yukiue H, et al. PIK3CA mutation status is Japanese lung cancer patients. *Lung Cancer* (2006) **54**:209–15. doi:10.1016/j.lungcan.2006.07.006
26. Huang S, Hölzel M, Knijnenburg T, Schlicker A, Roepman P, McDermott U, et al. MED12 controls the response to multiple cancer drugs through regulation of TGF-β receptor signaling. *Cell* (2012) **151**(5):937–50. doi:10.1016/j.cell.2012.10.035
27. Yano S, Yamada T, Takeuchi S, Tachibana K, Minami Y, Yatabe Y, et al. Hepatocyte growth factor expression in EGFR mutant lung cancer with intrinsic and acquired resistance to tyrosine kinase inhibitors in a Japanese cohort. *J Thorac Oncol* (2011) **6**:2011–7. doi:10.1097/JTO.0b013e31823ab0dd
28. Li Z, Zhou S, Zhang L, Su C, Hang J, Zhao Y, et al. BIM induction of apoptosis triggered by EGFR-sensitive and resistance cell lines of non-small cell lung cancer. *Med Oncol* (2011) **28**(2):572–7. doi:10.1007/s12032-010-9470-y
29. Costa DB, Halmos B, Kumar A, Schumer ST, Huberman MS, Boggon TJ, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. *PLoS Med* (2007) **4**:e315. doi:10.1371/journal.pmed.0040315
30. Faber AC, Corcoran RB, Ebi H, Sequist LV, Waltman BA, Chung E, et al. BIM expression in treatment-naïve cancers predicts responsiveness to kinase inhibitors. *Cancer Discovery* (2011) **1**:352–65. doi:10.1158/2159-8290.CD-11-0106
31. Costa C, Molina MA, Drozdowskyj A, Giménez-Capitán A, Bertran-Alamillo J, Karachaliou N, et al. The impact of EGFRT790 mutations and BIM mRNA expression on outcome in patients with EGFR-mutant NSCLC treated with erlotinib or chemotherapy in the randomized phase III EURTAC trial. *Clin Cancer Res* (2014) **20**(7):2001–10. doi:10.1158/1078-0432.CCR-13-2233
32. Foo J, Chmielecki J, Pao W, Michor F. Effects of pharmacokinetic processes and varied dosing schedules on the dynamics of acquired resistance to erlotinib in EGFR-mutant lung cancer. *J Thorac Oncol* (2012) **7**:1583–93. doi:10.1097/JTO.0b013e31826146ee
33. Hamilton M, Wolf JL, Rusk J, Beard SE, Clark GM, Witt K, et al. Effects of smoking on the pharmacokinetics of erlotinib. *Clin Cancer Res* (2006) **12**:2166–71. doi:10.1158/1078-0432.CCR-05-2235
34. Chmielecki J, Foo J, Oxnard GR, Hutchinson K, Ohashi K, Somwar R, et al. Optimization of dosing for EGFR-mutant non-small cell lung cancer with evolutionary cancer modeling. *Sci Transl Med* (2011) **3**:90ra59. doi:10.1126/scitranslmed.3002356
35. Jackman D, Pao W, Riely GJ, Engelman JA, Kris MG, Jänne PA, et al. Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. *J Clin Oncol* (2010) **28**:357–60. doi:10.1200/JCO.2009.24.7049
36. Watanabe S, Tanaka J, Ota T, Kondo R, Tanaka H, Kagamu H, et al. Clinical responses to EGFR-tyrosine kinase inhibitor retreatment in non small-cell lung cancer patients who benefited from prior effective gefitinib therapy: a retrospective analysis. *BMC Cancer* (2011) **11**:1. doi:10.1186/1471-2407-11-1
37. Chaft JE, Oxnard GR, Sima CS, Kris MG, Miller VA, Riely GJ. Disease flare after tyrosine kinase inhibitor (TKI) discontinuation in patients with EGFR mutant lung cancer and acquired resistance. *J Clin Oncol* (2011) **29**:A#e18001. doi:10.1158/1078-0432.CCR-11-1468
38. Kosaka T, Yatabe Y, Endoh H, Yoshida K, Hida T, Tsuboi M, et al. Analysis of epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer and acquired resistance to gefitinib. *Clin Cancer Res* (2006) **12**:5764–9. doi:10.1158/1078-0432.CCR-06-0714
39. Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res* (2013) **19**:2240–7. doi:10.1158/1078-0432.CCR-12-2246
40. Bell DW, Gore I, Okimoto RA, Godin-Heymann N, Sordella R, Mulloy R, et al. Inherited susceptibility to lung cancer may be associated with the T790M drug resistance mutation in EGFR. *Nat Genet* (2005) **37**:1315–6. doi:10.1038/ng1671
41. Ramalingam SS, Blackhall F, Krzakowski M, Barrios CH, Park K, Bover I, et al. Randomized Phase II study of dacomitinib (PF-00299804), and Irreversible Pan-Human Epidermal Growth factor receptor inhibitor, versus erlotinib in patients with advanced non-small cell lung cancer. *J Clin Oncol* (2012) **30**(27):3337–44. doi:10.1200/JCO.2011.40.9433
42. Reckamp KL, Giaconne G, Camidge DR. A phase 2 trial of dacomitinib (PF-00299804) an oral, irreversible pan-HER (human epidermal growth factor receptor) inhibitor, in patients with advanced non-small cell lung cancer after failure of prior chemotherapy and erlotinib. *Cancer* (2014) **120**(8):1145–54. doi:10.1002/cncr.28561
43. Schuler MH, Planchard D, Chih-Hsin J, Kim J-H, De Marinis F, Chen Y-M, et al. Interim analysis of afatinib monotherapy in patients with metastatic NSCLC progressing after chemotherapy and erlotinib/gefitinib (E/G) in a trial of afatinib plus paclitaxel versus investigator's choice chemotherapy following progression on afatinib monotherapy. *J Clin Oncol* (2012) **30**(Suppl 15):Abst 7557.
44. Su KY, Chen HY, Li KC, Kuo ML, Yang JC, Chan WK, et al. Pretreatment epidermal growth factor receptor (EGFR) T790M mutation predicts shorter EGFR tyrosine kinase inhibitor response duration in patients with non-small cell lung cancer. *J Clin Oncol* (2012) **30**:433–40. doi:10.1200/JCO.2011.38.3224
45. Rosell R, Molina MA, Costa C, Simonetti S, Gimenez-Capitan A, Bertran-Alamillo J, et al. Pretreatment EGFR T790M mutation and BRCA1 mRNA expression in erlotinib-treated advanced non-small-cell lung cancer patients with EGFR mutations. *Clin Cancer Res* (2011) **17**:1160–8. doi:10.1158/1078-0432.CCR-10-2158
46. Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol* (2013) **31**(27):3327–34. doi:10.1200/JCO.2012.44.2806
47. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non small cell lung cancer harbouring EGFR mutations (LUX-lung 6): an open-label, randomized phase 3 trial. *Lancet Oncol* (2014) **15**(2):213–22. doi:10.1016/S1470-2045(13)70604-1
48. Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci U S A* (2007) **104**:20932–7. doi:10.1073/pnas.0710370104
49. Takezawa K, Pirazzoli V, Arcila ME, Nebhan CA, Song X, de Stanchina E, et al. HER2 amplification: a potential mechanism of acquired resistance to EGFR inhibition in EGFR-mutant lung cancers that lack the second site EGFR 790M mutation. *Cancer Discovery* (2012) **2**:922–33. doi:10.1158/2159-8290.CD-12-0108
50. Takezawa K, Pirazzoli V, Arcila ME, Nebhan CA, Song X, de Stanchina E, et al. HER2 amplification: a potential mechanism of acquired resistance to EGFR inhibition in EGFR-mutant lung cancers that lack second-site EGFRT790M mutation. *Cancer Discovery* (2012) **2**(10):922–33. doi:10.1158/2159-8290.CD-12-0108
51. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and Histological Evolution of Lung Cancers Acquiring Resistance to EGFR Inhibitors. *Sci Transl Med* (2011) **3**:75ra26. doi:10.1126/scitranslmed.3002003
52. Ohashi K, Sequist LV, Arcila ME, Moran T, Chmielecki J, Lin YL, et al. Lung cancers with acquired resistance to EGFR inhibitors occasionally harbor BRAF

mutations but lack mutations in KRAS, NRAS or MEK1. *Proc Natl Acad Sci U S A* (2012) **109**:E2127–33. doi:10.1073/pnas.1203530109
53. Sos ML, Koker M, Weir BA, Heynck S, Rabinovsky R, Zander T, et al. PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of AKT and EGFR. *Cancer Res* (2009) **69**:3256–61. doi:10.1158/0008-5472.CAN-08-4055
54. Viloria-Petit AM, Kerbel RS. Acquired resistance to EGFR inhibitors: mechanisms and prevention strategies. *Int J Radiat Oncol Phys* (2004) **58**:914–26. doi:10.1016/j.ijrobp.2003.09.091
55. Tabernero J. The role of VEGF and EGFR inhibition: implications for combining anti-VEGF and anti-EGFR agents. *Mol Cancer Res* (2007) **5**:203–20. doi:10.1158/1541-7786.MCR-06-0404
56. Ciardiello F, Bianco R, Damiano V, Fontanini G, Caputo R, Pomatico G, et al. Antiangiogenic and antitumor activity of anti-epidermal growth factor receptor C225 monoclonal antibody in combination with vascular endothelial growth factor anti-sense oligonucleotide in human GEO colon cancer cells. *Clin Cancer Res* (2000) **6**:3739–47.
57. Jung YD, Mansfield PF, Akagi M, Takeda A, Liu W, Bucana CD, et al. Effects of combination anti-vascular endothelial growth factor receptor and anti-epidermal growth factor receptor therapies on the growth of gastric cancer in a nude mouse model. *Eur J Cancer* (2002) **38**:1133–40. doi:10.1016/S0959-8049(02)00013-8
58. Herbst RS, Johnson DH, Mininberg E, Carbone DP, Henderson T, Kim ES, et al. Phase I/II trial evaluating the anti-vascular endothelial growth factor monoclonal antibody bevacizumab in combination with the HER-1/epidermal growth factor receptor tyrosine kinase inhibitor erlotinib for patients with recurrent non-small cell lung cancer. *J Clin Oncol* (2005) **23**:2544–55.
59. Herbst RS, O'Neill VJ, Fehrenbacher L, Belani CP, Bonomi PD, Hart L, et al. Phase II study of efficacy and safety of bevacizumab in combination with chemotherapy or erlotinib compared with chemotherapy alone for treatment of recurrent or refractory non small cell lung cancer. *J Clin Oncol* (2007) **25**:4743–50. doi:10.1200/JCO.2007.12.3026
60. Lee JS, Hirsh V, Park K, Qin S, Blajman CR, Perng RP, et al. Vandetanib versus placebo in patients with advanced non-small cell lung cancer after prior therapy with an epidermal growth factor tyrosine kinase inhibitor: a randomized, double-blind phase III trial (ZEPHYR). *J Clin Oncol* (2012) **30**(1):1114–21. doi:10.1200/JCO.2011.36.1709
61. Arcila ME, Oxnard GR, Nafa K, Riely GJ, Solomon SB, Zakowski MF, et al. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. *Clin Cancer Res* (2011) **17**:1169–80. doi:10.1158/1078-0432.CCR-10-2277
62. Uramoto H, Iwata T, Onitsuka T, Shimokawa H, Hanagiri T, Oyama T. Epithelial-mesenchymal transition in EGFR-TKI acquired resistant lung adenocarcinoma. *Anticancer Res* (2010) **30**:2513–7.
63. Suda K, Murakami I, Katayama T, Tomizawa K, Osada H, Sekido Y, et al. Reciprocal and complementary role of MET amplification and EGFR T790M mutation in acquired resistance to kinase inhibitors in lung cancer. *Clin Cancer Res* (2010) **16**:5489–98. doi:10.1158/1078-0432.CCR-10-1371
64. Perner S, Wagner PL, Demichelis F, Mehra R, Lafargue CJ, Moss BJ, et al. EML4-ALK fusion lung cancer: a rare acquired event. *Neoplasia* (2008) **10**:298–302.
65. Pan Y, Zhang Y, Li Y, Hu H, Wang L, Li H, et al. ALK, ROS1 and RET fusions in 1139 lung adenocarcinomas: a comprehensive study of common and fusion pattern-specific clinicopathologic, histologic and cytologic features. *Lung Cancer* (2014) **84**(2):121–6. doi:10.1016/j.lungcan.2014.02.007
66. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* (2007) **448**:561–6. doi:10.1038/nature05945
67. Shaw AT, Kim DW, Nakagawa K, Seto T, Crinó L, Ahn MJ, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Eng J Med* (2013) **368**(25):2385–94. doi:10.1056/NEJMoa1214886
68. Zhang S, Wang F, Keats J, Zhu X, Ning Y, Wardwell SD, et al. Crizotinib-resistant mutants of EML4-ALK identified through an accelerated mutagenesis screen. *Chem Biol Drug Des* (2011) **78**(6):999–1005. doi:10.1111/j.1747-0285.2011.01239.x
69. Gainor JF, Varghese AM, Ou SH, Kabraji S, Awad MM, Katayama R, et al. ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer. *Clin Cancer Res* (2013) **19**:4273–81. doi:10.1158/1078-0432.CCR-13-0318
70. Choi YL, Soda M, Yamashita Y, Ueno T, Takashima J, Nakajima T, et al. ELM4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *N Eng J Med* (2010) **363**:1734–9. doi:10.1056/NEJMoa1007478
71. Shaw AT, Mehra R, Kim DW, Felip E, Chow LQM, Camidge DR, et al. Clinical activity of the ALK inhibitor LDK378 in advanced ALK-positive NSCLC. *J Clin Oncol* (2013) **31**(Suppl):Abst8010.
72. Ohashi K, Maruvka YE, Michor R, Pao W. Epidermal growth factor receptor tyrosine kinase inhibitor-resistant disease. *J Clin Oncol* (2013) **31**:1070–80. doi:10.1200/JCO.2012.43.3912
73. Nishie K, Kawaguchi T, Tamiya A, Mimori T, Takeuchi N, Matsuda Y, et al. Epidermal growth factor receptor tyrosine kinase inhibitors beyond progressive disease: a retrospective analysis for Japanese patients with activating EGFR mutations. *J Thoracic Oncol* (2012) **7**(11):1722–7. doi:10.1097/JTO.0b013e31826913f7
74. Asami K, Okuma T, Hirashima T, Kawahara M, Atagi S, Kawaguchi T, et al. Continued treatment with gefitinib beyond progressive disease benefits patients with activating EGFR mutations. *Lung Cancer* (2013) **79**(3):276–82. doi:10.1016/j.lungcancer.2012.11.022
75. Yu HA, Sima CS, Huang J, Solomon SB, Rimner A, Paik P, et al. Local therapy with continued EGFR tyrosine kinase inhibitor therapy as a treatment strategy in EGFR-mutant advanced lung cancers that have developed acquired resistance to EGFR tyrosine kinase inhibitors. *J Thorac Oncol* (2013) **8**(3):346–51. doi:10.1097/JTO.0b013e31827e1f83
76. Schuler MH, Yang CH, Park K, et al. Continuation of afatinib beyond progression: results of a randomized, open label, phase III trial of afatinib plus paclitaxel (P) versus investigator's choice chemotherapy (CT) in patients (pts) with metastatic non-small cell lung cancer (NSCLC) progressed on erlotinib/gefitinib (E/G) and afatinib – LUXLUNG-5 (LL5) (Abst 8019). *50th ASCO Annual Meeting.* Chicago, IL (2014).
77. Gridelli C, Cardiello F, Gallo C, Feld R, Butts C, Gebbia V, et al. First-line erlotinib followed by second-line cisplatin-gemcitabine chemotherapy in advanced non-small cell lung cancer: the TORCH randomized trial. *J Clin Oncol* (2012) **30**:3002–11. doi:10.1200/JCO.2011.41.2056
78. Bock C, Lengauer T. Managing drug resistance in cancer: lessons from HIV therapy. *Nat Rev Cancer* (2012) **12**:494–501. doi:10.1038/nrc3297

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 26 May 2014; accepted: 07 July 2014; published online: 23 July 2014.
*Citation: Spaans JN and Goss GD (2014) Drug resistance to molecular targeted therapy and its consequences for treatment decisions in non-small-cell lung cancer. Front. Oncol. **4**:190. doi: 10.3389/fonc.2014.00190*
This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 27 August 2014
doi: 10.3389/fonc.2014.00233

Trials to overcome drug resistance to EGFR and ALK targeted therapies – past, present, and future

Johanna N. Spaans[1] and Glenwood D. Goss[1,2,3]*

[1] *Ottawa Hospital Research Institute, Ottawa, ON, Canada*
[2] *Ottawa Hospital Cancer Centre, Ottawa, ON, Canada*
[3] *Department of Medicine, University of Ottawa, Ottawa, ON, Canada*

Edited by:
Vera Hirsh, McGill University Health Centre, Canada

Reviewed by:
Meng Xu Welliver, The Ohio State University James Cancer Center, USA
Nathalie Heuzé-Vourc'h, CEPR INSERM U1100/EA6305, France

***Correspondence:**
Glenwood D. Goss, 501 Smyth Road, Ottawa, ON K1H 8L6, Canada
e-mail: ggoss@toh.on.ca

Molecularly targeted agents are changing the therapeutic landscape in advanced non-small cell lung cancer. Since the discovery of sensitizing mutations in the epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) domain, clinical investigations have focused on optimizing the efficacy of EGFR and ALK tyrosine kinase inhibitors by addressing therapeutic resistance that commonly develops within a year of treatment initiation. Here, we review the clinical trials of novel therapies and combination regimens that have been undertaken in response to our evolving understanding of the mechanisms of resistance to targeted therapy. The aim of these trials was to enhance the therapeutic efficacy of targeted therapies by improving blockade and/or inhibiting parallel or compensatory signaling pathways. We have documented the sequential conduct of EGFR and ALK biomarker-driven trials in order to highlight particular pitfalls and successes, which should be considered in the design of future trials. Although there remain significant challenges, substantial gains have been made in our understanding of cellular resistance. This knowledge will drive the design of future trials to the benefit of lung cancer patients.

Keywords: EGFR, ALK, resistance, molecular therapy, clinical trial

Lung cancer is the leading cause of cancer related deaths worldwide (1). Non-small cell lung cancer (NSCLC) represents approximately 85% of all lung cancers (2). In the advanced disease setting, systemic platinum-based chemotherapy yields survival rates of approximately 1 year (3). In the last decade, the targeted inhibition of oncogenic driver mutations with molecular therapies of which the epidermal growth factor receptor (EGFR) and the anaplastic lymphoma kinase (ALK) are the most studied targets, has seen dramatic improvements in overall survival in defined subsets of patients (4, 5). However, despite impressive early response rates, most patients progress within a year (4). Herein, we review clinical trials undertaken in response to our evolving understanding of the oncogenic drivers and molecular mechanisms of drug resistance.

BACKGROUND

Given its important role in tumor growth and proliferation, the EGFR pathway has been the focus of intense clinical investigation across many tumor sites (6, 7). The high protein expression levels observed in NSCLC across all histologies (8), particularly among those with advanced disease (9), provided the initial impetus for the early lung cancer trials targeting the EGFR pathway by small molecule tyrosine kinase inhibitors (EGFR-TKIs) and anti-EGFR monoclonal antibodies.

Early trials in advanced NSCLC evaluated EGFR-TKIs as both monotherapy after chemotherapy failure and in combination with chemotherapy in the first-line setting. As a monotherapy, the EGFR-TKI erlotinib, was shown to improve progression free survival (PFS) (2.2 vs. 1.8 months, $p < 0.001$) and overall survival (OS) over best supportive care (6.7 vs. 4.7, $p < 0.001$) in unselected NSCLC patients with advanced disease who had failed one or two prior lines of chemotherapy (BR 21) (10). Although gefitinib, another EGFR-TKI, was similarly able to delay disease progression over placebo in the second/third-line setting (3.0 vs. 2.6 months, $p = 0.0006$), the lack of overall survival benefit (5.6 vs. 5.1 months, $p = 0.087$) in the definitive phase III trial (ISEL) (11)resulted in the withdrawal of gefitinib's accelerated FDA approval, which was based on encouraging phase II data (IDEAL-1, IDEAL-2) (12, 13). Disappointingly, when used in combination with upfront chemotherapy in unselected patients with advanced NSCLC, neither erlotinib nor gefitinib was shown to improve overall survival (14–17). The strategy of combining EGFR-TKIs and chemotherapy, therefore, has since largely been abandoned.

Objective responses in most of the early EGFR-TKI trials in unselected patients was quite variable, with many trials independently identifying a small subgroup of extreme responders (10, 11), against a large background of patients with primary resistance to EGFR inhibition. In these trials, the extreme responders were most commonly defined by their clinical and ethnic characteristics (Asian, non-smokers) and not by their pre-treatment EGFR protein expression levels. With the discovery of the activating EGFR mutations in exon 19 and 21 of the kinase domain (18, 19), came a number of retrospective mutational studies of the earlier anti-EGFR trials, which confirmed the importance of these mutations (20, 21). These studies lead to the molecular characterization of EGFR-TKI responders and the subsequent EGFR mutation-positive biomarker-driven trials, detailed below.

PAST CLINICAL TRIALS ADDRESSING RESISTANCE

The restriction of EGFR-TKI trials to patients whose tumors harbored activating EGFR mutations represented the first attempt to address primary drug resistance, by limiting exposure in patients unlikely to benefit from EGFR-TKIs. Six large randomized phase III trials that enriched or selectively enrolled patients with activating EGFR mutations definitively confirmed the benefit of first-generation reversible EGFR-TKIs over standard chemotherapy in the first-line setting (**Table 1**) (22–27). These trials, which collectively enrolled over 2200 patients, showed a doubling of response rate to 60–80% over chemotherapy alone in patients whose tumors harbored the EGFR mutation (22–27). In the four trials that only enrolled EGFR-mutant positive NSCLC patients (two gefitinib, two erlonitib), EGFR-TKIs were shown to extend PFS by 3–8 months (24–27). Consequently, targeted monotherapy with erlotinib or gefitinib has now become standard of care in the first-line setting in patients with EGFR mutation-positive tumors.

Due to their different mechanism of action, anti-EGFR monoclonal antibodies have also been evaluated in the management of advanced NSCLC. The most well studied of these agents is cetuximab, a monoclonal chimeric IgG1 antibody, that inhibits EGFR pathway activation by binding to the EGF receptor (28). Unlike its small molecule counterpart, monotherapy trials of cetuximab were disappointing in advanced NSCLC (29). However, cetuximab has been successfully combined with chemotherapy in the first-line setting. In a large phase III trial that enrolled 1125 patients with advanced NSCLC (FLEX) (30), cetuximab was shown to improve overall survival (11.3 vs. 10.1 months, $p = 0.004$) when combined with cisplatin and vinorelbine, with greater efficacy noted in patients with higher EGFR protein expression (31). While a similar smaller study ($n = 676$) of cetuximab with a carboplatin/paclitaxel regimen (BMS099) (32) did not demonstrate improved PFS or OS with the addition of cetuximab, a meta-analysis of four randomized phase II&III trials (which included the BMS099 study results) did show that cetuximab with first-line platinum-based chemotherapy improved both PFS and OS (33). Cetuximab's inconsistent and limited clinical efficacy, however, has restricted the uptake and regulatory approval of this anti-EGFR monoclonal antibody in advanced NSCLC. While other anti-EGFR monoclonal IgG1 antibodies, such as matuzumab, have demonstrated some efficacy in combination with chemotherapy in the second-line setting in phase II trials (34), other anti-EGFR monoclonal IgG2 antibodies (e.g., panitumumab) in combination with chemotherapy have shown little activity, even in patients with EGFR-mutant disease (35). Newer fully human recombinant anti-EGFR IgG1 monoclonal antibodies (e.g., necitumumab), which lack the immunoreactivity of earlier chimeric (human/mouse) monoclonal antibodies, are currently under investigation in certain histological subgroups in combination with chemotherapy, as detailed below.

Table 1 | Randomized phase III trials of first-generation EGFR-TKIs in EGFR mutation (+)/enriched populations.

Study	Agent	EGFR+/N	PFS (EGFR+) (months)	OS (EGFR+) (months)
NEJ002 (24)	Gefitinib	224/224	10.8 vs. 5.4 (HR 0.3)	27.7 vs. 26.6 (HR 0.89)
WJTOG-3405 (25)	Gefitinib	192/192	9.2 vs. 6.3 (HR 0.5)	36 vs. 39 (HR 1.19)
OPTIMAL (26)	Erlotinib	154/154	13.1 vs. 4.6 HR 0.16	HR 1.065
EURTAC (27)	Erlotinib	153/153	9.7 vs. 5.2 HR 0.37	19.3 vs. 19.5 HR 1.04
IPASS (22)	Gefitinib	261/1217	9.5 vs. 6.3 HR 0.48	21.6 vs. 21.9 HR 1.0
SIGNAL (23)	Gefitinib	42/309	8.0 vs. 6.3 HR 0.54	27.2 vs. 25.6 HR 1.04

As noted previously, even in the presence of sensitizing EGFR mutations, only 60–80% of patients with advanced NSCLC respond to EGFR-TKIs. Despite earlier failed attempts to combine EGFR-TKIs with chemotherapy, other combination regimens were evaluated early in the clinical development of EGFR-TKIs, in an attempt to expand and enhance their therapeutic efficacy. To understand the rationale for these, and other combination regimens evaluated in molecular oncology clinical trials, it is useful to consider the therapeutic strategies advanced by Dancey and colleagues (36) to address targeted therapy drug resistance, namely to (1) augment the first agent's activity; (2) enhance single target blockade; (3) inhibit multiple targets or multiple pathways; and (4) inhibit compensatory pathways. While combination therapies with chemotherapy used the first of these strategies, strategies two to four have guided most clinical research in the acquired resistance setting, as detailed below.

PAST TRIALS OF COMBINATION THERAPIES

Dual targeting of a single receptor was the rationale behind the early trials of combined EGFR-TKI and anti-EGFR monoclonal antibody therapies, which, due to the off-target effects of the first-generation EGFR-TKIs, proved very toxic (37). Multiple pathway inhibition was the guiding strategy behind combining EGFR-TKIs with the anti-VEGF monoclonal antibody, bevacizumab, given its earlier success in improving overall survival in advanced NSCLC when given with chemotherapy (38). Although early trials of dual VEGF and EGFR inhibition were encouraging (39, 40), more recent trials in unselected populations of VEGF/EGFR inhibitors, such as vandetanib, do not support this approach in the management of advanced NSCLC (41).

CURRENT CLINICAL TRIALS ADDRESSING RESISTANCE

Despite dramatic responses in patients whose tumors harbor EGFR activating mutations, most patients become resistant to EGFR-TKIs within the first year (10, 11). The majority of current trials are, therefore, focused on addressing the mechanisms of acquired resistance (**Table 2**). The most common of these mechanisms is the development of a second mutation of the EGFR, namely the T790M mutation (T790M), which occurs in up to 60% of those with EGFR-TKI resistant disease (42). The newer second-generation pan-HER irreversible inhibitors provide compensatory pathway inhibition by direct targeting of the resistant T790M

Table 2 | Select trials addressing acquired resistance to targeted therapy.

Line of therapy	Agents	Trial	PFS (months)
MONOTHERAPY TRIALS TARGETING THE EGFR DOMAIN			
First line	Afatinib vs. pem/cispl	LuxLung 3 (45)	11.1 vs. 6.9 ($p = 0.001$)
First line	Afatinib vs. gem/cispl	LuxLung 6 (46)	11.0 vs. 5.6 ($p < 0.0001$)
First line	Dacomitinib vs. gefitinib	ARCHER 1050	Results pending
Second line	Afatinib vs. placebo	LuxLung 1 (43)	3.3 vs. 1.1 ($p < 0.0001$)
Second line	Dacomitinib vs. placebo	BR 26 (44)	2.7 vs. 1.4 ($p < 0.0001$)
COMBINATION THERAPIES TARGETING THE MET COMPENSATORY PATHWAY			
Second/third line	Tivantinib + erlotinib vs. erlotinib + placebo	Marquee[a] (47)	3.6 vs. 1.9 ($p < 0.0001$)
Second/third line	Onartuzumab + erlotinib vs. erlotinib + placebo	METLung[a] (48)	2.7 vs. 2.6 ($p = 0.92$)

[a]*Trial stopped early for futility to meet primary endpoint.*

mutation and bind irreversibly to 2 or more receptors of the EGFR domain, thus providing enhanced EGFR pathway blockade. Of the second-generation inhibitors, afatinib and dacomitinib have been the most extensively studied, in both heavily pre-treated patients and in the first-line setting. Afatinib has been evaluated in an EGFR-mutant enriched population who had failed one to two lines of chemotherapy and an EGFR-TKI (LUX-Lung 1) (43). In this placebo-controlled phase III trial, afatinib was shown to improve PFS (3.3 vs. 1.1 months, $p < 0.0001$) but not overall survival (10.8 vs. 12.0 months, $p = 0.87$) (43). A similar study was also undertaken with dacomitinib (BR. 26) (44) in patients who had failed one to three lines of chemotherapy and an EGFR-TKI. As with afatinib, dacomitinib showed improved PFS (2.7 vs. 1.4 months, $p < 0.0001$) but did not improve OS (6.8 vs. 6.3 months, $p = 0.099$) (44).

Unlike the disappointing results observed in the second/third-line setting, second-generation EGFR inhibitors have proven effective in the first-line setting in patients whose tumors harbor EGFR activating mutations. Specifically, afatinib has been shown to improve PFS compared to pemetrexed/cisplatin (LUX-Lung 3) (11.1 vs. 6.9 months, $p = 0.001$) (45) and, more recently, compared to gemcitabine/cisplatin (LUX-Lung 6) (11.0 vs. 5.6 months, $p < 0.0001$) (46). Dacomitinib is also being evaluated in a head-to-head phase III trial against gefitinib (ARCHER 1050), with the results expected next year (www.clinicaltrials.gov: NCT01774721).

In the context of acquired resistance to anti-EGFR therapies, the activation of alternate signaling pathways by both adaptive mutations that develop outside the EGFR kinase domain and mutation-independent factors have also been described (49), and are informing novel combination therapies to address these acquired mechanisms of resistance. The most common of these alterations is MET activation, which occurs in up to 20% of patients with acquired resistance (50). Both small molecule inhibitors targeting MET and anti-MET monoclonal antibodies have been combined with first-generation EGFR-TKIs. The small molecule, tivantinib, and the monoclonal antibody onartuzumab have both been evaluated in the second-line setting in EGFR-TKI naïve patients after chemotherapy failure. In phase II trials, patients were screened and stratified by EGFR mutation status with planned subgroup analysis by both EGFR mutation and pre-treatment tumor MET expression levels. Despite promising phase II data in patients whose tumors were strongly positive for MET by immunohistochemistry (IHC) (51), the combination of onartuzumab and erlotinib in the phase III trial was not shown to improve PFS (2.7 vs. 2.6 months, $p = 0.92$) or objective response rate (ORR) (8.4 vs. 9.6%, $p = 0.63$) and was stopped early for futility to meet its primary endpoint of improved OS (48). The small molecule tivantinib had a similar outcome, with the phase III trial discontinued prematurely for futility for the primary outcome of improved overall survival, which at the planned interim analysis was comparable to EGFR monotherapy (8.5 vs. 7.8, HR = 0.98, $p = 0.81$) (47). Given the failure of these phase III trials to improve survival, direct targeting of MET as a strategy to enhance EGFR-TKI efficacy has an uncertain future.

The failure of dual MET/EGFR inhibition to improve OS in the second-line setting can, in part, be attributed to fact that none of these trials, which were designed to address acquired resistance to EGFR-TKIs, restricted enrollment to patients with tumors harboring EGFR activating mutations and due to the lack of a robust biomarker predictive of efficacy to MET inhibition. The more focused strategy applied in the first-line setting to evaluate of the first and second-generation EGFR-TKIs, and more recently to ALK inhibitors, which limited enrollment to EGFR mutation-positive and ALK mutation-positive NSCLC, respectively, have been far more successful. In the case of the ALK inhibitor, crizotinib, accelerated approval was granted based on clinical activity observed in a phase I dose-escalation and expansion study, which screened over 1500 pre-treated patients and selectively enrolled 82 patients whose tumors screened positive for the ALK rearrangement (5% prevalence) (52). Results of the definitive phase III trial of first-line crizotinib compared to chemotherapy have recently confirmed these initial findings (53). Using a similar approach of selective enrollment, the second-generation ALK inhibitor ceretinib (LDK378) has also recently achieved regulatory FDA drug approval in patients who have failed the crizotinib, by demonstrating antitumor activity and ORR of ~60% in a heavily pre-treated populations in a phase I trial (ASCEND-1) (54).

THE FUTURE OF CLINICAL TRIALS OF ACQUIRED RESISTANCE

Moving forward, clinical trials of acquired resistance will continue to focus on the testing of novel monotherapies and combination therapies targeting the EGFR kinase domain. In the former

case, phase III clinical trials are currently on-going comparing the efficacy of the second-generation irreversible EGFR-TKIs against the first-generation inhibitors, in the second-line setting in advanced squamous NSCLC (LuxLung 8, afatinib vs. erlotinib, www.clinicaltrials.gov: NCT01523587) and in the first-line treatment of patients with EGFR-mutant disease (ARCHER 1050, dacomitinib vs. gefitinib, www.clinicaltrials.gov: NCT01774721). Disappointingly, the results of another phase III trial comparing first and second-generation EGFR-TKIs in the second/third-line setting (ARCHER 1009: dacomitinib vs. erlotinib) do not support the greater clinical efficacy of the newer EGFR-TKIs in unselected patients with advanced NSCLC (55). However, the results from the two on-going phase III trials, mentioned above, are eagerly awaited.

THIRD-GENERATION EGFR-TKIs AND NEWER RECOMBINANT ANTI-EGFR MONOCLONAL ANTIBODIES

A number of third-generation EGFR-TKIs are also in development, with AZ9291 and CO-1686 being the most advanced of these T790M-specific small molecule inhibitors. Results of early phase I trials of both AZ9291 and CO-1686 in EGFR mutation-positive NSCLC patients previously treated with EGFR-TKIs are encouraging. In both trials, which required screening biopsies for centralized T790M mutation testing, objectives responses of ~60% in patients testing T790M positive at screening have been observed (56, 57), with responses also noted in patients lacking the T790M mutation receiving AZ9291, albeit less frequently (23%) (56). Definitive phase III trials of these molecularly targeted agents are planned.

The recent success of the newer recombinant anti-EGFR monoclonal antibody, necitumumab, in combination with gemcitabine–cisplatin chemotherapy over chemotherapy alone in the first-line treatment of patients with advanced squamous NSCLC (58) (OS: 11.5 vs. 9.9, $p = 0.012$) is also of interest in advanced NSCLC. Although potentially effective in the primary resistance setting, what role necitumumab will play in the acquired resistance setting in patients with squamous and non-squamous NSCLC histology remains to be determined.

DUAL TARGETING OF THE EGFR KINASE DOMAIN

Dual targeting of the EGFR kinase domain, although historically quite toxic, will also likely continue to be explored as a strategy to optimize EGFR-TKI therapy given the phase I dose-escalation and expansion trial demonstrating ORR of ~30% with dual therapy with the anti-EGFR monoclonal antibody cetuximab and afatinib in patients with acquired resistance to first-generation EGFR-TKIs (59). These encouraging results, however, are tempered by the continued toxicity of this combination regimen with 77 and 69% of patients experiencing rash and diarrhea (any grade), respectively (59).

SECOND-GENERATION ALK INHIBITORS

As secondary mutations in the ALK domain have been identified in approximately one third of patients with acquired resistance to the ALK inhibitor crizotinib (60), clinical trials in the ALK resistance setting are also focused on the evaluation of more potent second-generation molecular therapies. Of note, high response rates with the newer second-generation ALK inhibitor, ceretinib, have been observed in patients with and without secondary ALK mutations (54), suggesting that their benefit in ALK-resistant disease may not be limited to patients with secondary ALK mutations. A number of other second-generation ALK inhibitors are also in early clinical development (e.g., AP26113), with promising phase I/II results emerging (61).

DUAL EGFR AND MET INHIBITION

While the dual inhibition of EGFR and MET-mediated pathway inhibition by direct MET targeting has not been successful to date, other c-MET inhibitors are being investigated (e.g., INC 280, XL184). A related mechanism of EGFT-TKI resistance is MET activation by its ligand hepatocyte growth factor (HGF) (62) that when overexpressed enables this compensatory mechanism of pathway activation. Preliminary results of a phase I clinical trial evaluating dual inhibition of EGFR and HGF with erlotinib and rilotumumab (AMG 102), a HGF-binding monoclonal antibody, have recently been reported (63) and a phase II study is on-going.

EGFR AND HSP 90 INHIBITORS

As a molecular chaperone for proteins involved in MET, HGF, and EML4/ALK fusion, the inhibition of heat shock protein 90 (HSP90) is also being considered as a target for compensatory pathway inhibition and has fueled combination therapies in both EGFR-TKI and ALK inhibitor-resistant disease. In the advanced NSCLC EGFR-TKI resistant setting, the HSP90 small molecule inhibitor AUY922 is being evaluated as monotherapy in a phase II clinical trial vs. pemetrexed or docetaxel in patients with tumors with activating EGFR mutations (www.clinicaltrials.gov: NCT016461250). This agent is also being assessed in a phase II trial in patients with *de novo* resistant T790M mutations not previously treated with EGFR-TKIs (www.clinicaltrials.gov: NCT01854034) and in patients with EGFR mutations and/or EGFR-TKI resistant disease, as part of a phase II cluster study in Chinese patients evaluating five novel inhibitors of HSP90, PI3K, ALK, MET, and MEK (64). Further, AUY922 is also being assessed in combination with erlotinib in patients who have previously responded to EGFR-TKIs and/or whose tumors harbor activating EGFR mutations (www.clinicaltrials.gov: NCT01259089), with results expected in the near future. The safety and activity of another HSP90 inhibitor, ganetespib (STA-9090), has also been assessed in a heavily pre-treated population with NSCLC in a phase II single arm trial with three cohorts ($EGFR^+$, $KRAS^+$, EGFR/KRAS wild-type) (65). In this study, partial responses were noted in 4/66 patients in the EGFR/KRAS wild-type cohort, all of whom were retrospectively confirmed to have disease that harbored the ALK gene rearrangement (65). Despite interest in this HSP90 inhibitor in combination with chemotherapy (GALAXY-1, GALAXY-2) (66, 67), ganetespib's role in inhibiting EGFR is unclear. Given encouraging preclinical data in ALK-driven tumors resistant to crizotinib (68), ganetespib is being investigated in clinical trials in NSCLC patients with ALK-driven tumors, as a monotherapy in heavily treated (crizotinib naïve) patients (www.clinicaltrials.gov: NCT01562015) and in combination with crizotinib in patients with prior exposure to crizotinib (www.clinicaltrials.gov: NCT01579994).

CONCLUSION

Over the last decade, our understanding of the EGF receptor and our ability to target it has evolved significantly, from single receptor first-generation inhibitors in unselected populations to biomarker-driven clinical trials of more potent second and third-generation irreversible multi-targeted EGFR-TKIs and humanized monoclonal antibodies. The failure of earlier trials targeting the EGF receptor was in part due to the lack of good predictive biomarkers of efficacy. The future success of targeted strategies addressing resistance will hinge on our ability to identify these biomarkers and selectively enroll patients to clinical trials, a strategy that has been more successfully applied in the approval of ALK inhibitors. Furthermore, in order to be successful in the acquired resistance setting, rebiopsy, and tailored mechanism-driven strategies will be required at the time of progression, with a concurrent reduction in the toxicity of multi-targeted and combination therapies. Importantly, the knowledge gained from investigations of EGFR and ALK inhibition over the last decade can be applied to the testing of novel therapies targeting newly discovered oncogenic drivers in NSCLC (69) in order to optimize study designs and streamline regulatory approval, to the benefit of all patients with NSCLC.

REFERENCES

1. World Health Organization. *Factsheet No 310: The Top Ten Causes of Death* (2008). Available from: http://www.who.int/mediacentre/factsheets/fs310_2008.pdf
2. American Cancer Society. *Cancer Facts & Figures 2009.* Atlanta, GA: American Cancer Society, Inc (2009).
3. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of four chemotherapy regimens for advanced non-small-cell-lung-cancer. *N Engl J Med* (2002) **346**:92–8. doi:10.1056/NEJMoa011954
4. Lee CK, Brown C, Gralla RJ, Hirsh V, Thongprasert S, Tsai CM, et al. Impact of EGFR inhibitor in non-small cell lung cancer on prognosis-free and overall survival: a meta-analysis. *J Natl Cancer Inst* (2013) **105**(9):595–605. doi:10.1093/jnci/djt072
5. Kris MG, Johnson BE, Berry LD, Kwiatkowski DJ, Iafrate AJ, Wistuba II, et al. Treatment with therapies matched to oncogenic drivers improves survival in patients with lung cancers: results from the lung cancer mutation consortium (LCMC). *J Thorac Oncol* (2013) **8**(2):S3–4. doi:10.1001/jama.2014.3741
6. Krasinskas AM. EGFR signalling in colorectal carcinoma. *Patholog Res Int* (2011) **2011**:932932. doi:10.4061/2011/932932
7. Bethune G, Bethune D, Ridgway N, Xu Z. Epidermal growth factor receptor (EGFR) in lung cancer: an overview and update. *J Thorac Oncol* (2010) **2**:48–51.
8. Hirsch FR, Varella-Garcia M, Bunn PA Jr, Di Maria MV, Veve R, Bremmes RM, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. *J Clin Oncol* (2003) **21**(20):3798–807. doi:10.1200/JCO.2003.11.069
9. Fujino S, Enokibori T, Tezuka N, Asada Y, Inoue S, Kato H, et al. A comparison of epidermal growth factor receptor levels and other prognostic parameters in non-small cell lung cancer. *Eur J Cancer* (1996) **32A**:2070–4. doi:10.1016/S0959-8049(96)00243-2
10. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small cell lung cancer. *N Engl J Med* (2005) **353**:123–32. doi:10.1056/NEJMoa050753
11. Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small cell lung cancer: results from a randomized placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* (2005) **366**:1527–37. doi:10.1016/S0140-6736(05)67625-8
12. Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. *J Clin Oncol* (2003) **21**:2237–46. doi:10.1200/JCO.2003.10.038
13. Kris MG, Natale RB, Herbst RS, Lynch TJ Jr, Prager D, Belani CP, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* (2003) **290**:2149–58. doi:10.1001/jama.290.16.2149
14. Giaccone G, Herbst RS, Manegold C, Scagliotti G, Rosell R, Miller V, et al. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small cell lung cancer: a phase III trial – INTACT 1. *J Clin Oncol* (2004) **22**:777–84. doi:10.1200/JCO.2004.08.001
15. Herbst RS, Giaccone G, Schiller JH, Natale RB, Miller V, Manegold C, et al. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small cell lung cancer: phase III trial: INTACT 2. *J Clin Oncol* (2004) **22**:785–94. doi:10.1200/JCO.2004.07.215
16. Herbst RS, Prager D, Hermann F, Fehrenbacher L, Johnson BE, Sandler A, et al. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small cell lung cancer. *J Clin Oncol* (2005) **23**:5892–9. doi:10.1200/JCO.2005.02.840
17. Gatzemeier U, Pluzanska A, Szczesna A, Kaukel E, Roubec J, De Rosa F, et al. Phase III study of erlotinib in combination with cisplatin and gemcitabine in advanced non small cell lung cancer: the tarceva lung cancer investigation trial. *J Clin Oncol* (2007) **25**:1545–52. doi:10.1200/JCO.2005.05.1474
18. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small cell lung cancer to gefitinib. *N Engl J Med* (2004) **350**:2129–39. doi:10.1056/NEJMoa040938
19. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* (2004) **304**:1497–500. doi:10.1126/science.1099314
20. Hirsch F, Varella-Garcia M, Bunn P, Franklin WA, Dziadziuszko R, Thatcher N, et al. Molecular analysis of EGFR gene copy number, EGFR expression and AKT activation in advanced non-small cell lung cancer treated with gefitinib or placebo [ISEL trial]: AACR-NCI-EORTC. *International Conference: Molecular Targets and Therapeutics*, Philidelphia, PA (2005).
21. Tsao MS, Sakurada A, Cutz JC, Zhu CQ, Kamel-Reid S, Squire J, et al. Erlotinib in lung cancer-molecular and clinical predictors of outcome. *N Engl J Med* (2005) **353**:133–44. doi:10.1056/NEJMoa050736
22. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* (2009) **361**:947–57. doi:10.1056/NEJMoa0810699
23. Han JY, Park K, Kim SW, Lee DH, Kim HY, Kim HT, et al. First-SIGNAL: first-line single-agent Iressa versus gemcitabine and cisplatin trial in never smokers with adenocarcinoma of the lung. *J Clin Oncol* (2012) **30**(10):1122–8. doi:10.1200/JCO.2011.36.8456
24. Inoue A, Kobayashi K, Maemondo M, Sugawara S, Oizumi S, Isobe H, et al. Updated overall survival results from a randomized phase III trial comparing gefitinib with carboplatin-paclitaxel for chemo-naïve non-small cell lung cancer with sensitive EGFR gene mutations (NEJ002). *Ann Oncol* (2013) **24**(1):54–9. doi:10.1093/annonc/mds214
25. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomized phase 3 trial. *Lancet Oncol* (2010) **11**:121–8. doi:10.1016/S1470-2045(09)70364-X
26. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR-mutation positive non-small cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* (2011) **12**(8):735–42. doi:10.1016/S1470-2045(11)70184-X
27. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* (2012) **13**:239–46. doi:10.1016/S1470-2045(11)70393-X
28. Pirker R. EGFR-directed monoclonal antibodies in non-small cell lung cancer. *Target Oncol* (2013) **8**:47–53. doi:10.1007/s11523-012-0244-7
29. Hanna N, Lilenbaum R, Ansari R, Lynch T, Govindan R, Jänne PA, et al. Phase II trial of cetuximab in patients with previously treated non-small cell lung cancer. *J Clin Oncol* (2006) **24**(33):5253–8. doi:10.1200/JCO.2006.08.2263
30. Pirker R, Pereira JR, Szczesna A, von Pawel J, Krzakowski M, Ramlau R, et al. Cetuximab plus chemotherapy in patients advanced non-small-cell lung cancer

(FLEX): an open-label randomised phase III trial. *Lancet* (2009) **373**:1525–31. doi:10.1016/S0140-6736(09)60569-9

31. Pirker R, Pereira JR, von Pawel J, Krzakowski M, Ramlau R, Park K, et al. EGFR expression as a predictor of survival for first-line chemotherapy plus cetuximab in patients with advanced non-small-cell lung cancer: analysis of data from the phase 3 FLEX study. *Lancet Oncol* (2011) **13**(1):33–42. doi:10.1016/S1470-2045(11)70318-7
32. Lynch TJ, Patel T, Dreisbach L, McCleod M, Heim WJ, Hermann RC, et al. Cetuximab and first-line taxane/carboplatin chemotherapy in advanced non-small-cell lung cancer: results of the randomized multicenter phase III trials BMS099. *J Clin Oncol* (2010) **28**:911–7. doi:10.1200/JCO.2009.21.9618
33. Thatcher N Linchy TJ. Cetuximab plus platinum-based chemotherapy as 1st line treatment in patients with non-small cell lung cancer (NSCLC): a meta-analysis of randomized phase II/III trials. *13th World Conference on Lung Cancer*; San Francisco (2009). abstr. A3.7.
34. Schiller JH, von Pawel J, Schütt P, Ansari RH, Thomas M, Saleh M, et al. Pemetrexed with or without matuzumab as second-line treatment for patients with a stage IIIB/IV non small cell lung cancer. *J Thorac Oncol* (2010) **5**:1977–85. doi:10.1097/JTO.0b013e3181f4a5c9
35. Weiner LH, Belldegrun A, Rowinsky E, Crawford J, Lockbaum P, Huang S, et al. Updated results from a dose and schedule study of panitumumab (ABX-EGF) monotherapy in patients with advanced solid malignancies. *J Clin Oncol* (2005) **23**:abstr.3059.
36. Dancey JE, Chen XE. Strategies for optimizing combinations of molecularly targeted anticancer agents. *Nat Rev Drug Discov* (2006) **5**:649–59. doi:10.1038/nrd2089
37. Ramalingam S, Forster J, Naret C, Evans T, Sulecki M, Lu H, et al. Dual inhibition of the epidermal growth factor receptor with cetuximab, an IgG1 monoclonal antibody, and gefitinib, a tyrosine kinase inhibitor, in patients with refractor non-small cell lung cancer (NSCLC): a phase I study. *J Thorac Oncol* (2008) **3**(3):258–64. doi:10.1097/JTO.0b013e3181653d1b
38. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel-carboplatin alone or with bevacizumab for non small cell lung cancer. *N Engl J Med* (2006) **355**:2542–50. doi:10.1056/NEJMoa061884
39. Herbst RS, Johnson DH, Mininberg E, Carbone DP, Henderson T, Kim ES, et al. Phase I/II trial evaluating the anti-vascular endothelial growth factor monoclonal antibody bevacizumab in combination with the HER-1/epidermal growth factor receptor tyrosine kinase inhibitor erlotinib for patients with recurrent non-small cell lung cancer. *J Clin Oncol* (2005) **23**:2544–55. doi:10.1200/JCO.2005.02.477
40. Herbst RS, O'Neill VJ, Fehrenbacher L, Belani CP, Bonomi PD, Hart L, et al. Phase II study of efficacy and safety of bevacizumab in combination with chemotherapy or erlotinib compared with chemotherapy alone for treatment of recurrent or refractory non small cell lung cancer. *J Clin Oncol* (2007) **25**:4743–50. doi:10.1200/JCO.2007.12.3026
41. Lee JS, Hirsh V, Park K, Qin S, Blajman CR, Perng RP, et al. Vandetanib versus placebo in patients with advanced non-small cell lung cancer after prior therapy with an epidermal growth factor tyrosine kinase inhibitor: a randomized, double-blind phase III trial (ZEPHYR). *J Clin Oncol* (2012) **30**(1):1114–21. doi:10.1200/JCO.2011.36.1709
42. Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res* (2013) **19**:2240–7. doi:10.1158/1078-0432.CCR-12-2246
43. Miller VA, Hirsh V, Cadranel J, Chen YM, Park K, Kim SW, et al. Afatinib versus placebo for patients with advanced, metastatic non small cell lung cancer after failure of erlotinib, gefitinib or both and one or two lines of chemotherapy (LUXLUNG-1): a phase 2b/3 randomized trial. *Lancet Oncol* (2012) **13**(5):528–38. doi:10.1016/S1470-2045(12)70087-6
44. Ellis PM, Liu G, Millward M, Perrone F, Shepherd FA, Sun S, et al. NCIC-CTG BR 26: a phase III randomized, double-blind, placebo controlled trial of dacomitinib versus placebo in patients with advanced/metastatic non-small cell lung cancer (NSCLC) who received prior chemotherapy and a EGFR TKI. *50th ASCO Annual Meeting*; Chicago, IL (2014). abstr. 8036.
45. Sequist LV, Yan JCH, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol* (2013) **31**(37):3327–34. doi:10.1200/JCO.2012.44.2806
46. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small cell lung cancer harbouring EGFR mutations (LUX-lung 6): an open-label, randomized phase 3 trial. *Lancet Oncol* (2014) **15**(2):213–22. doi:10.1016/S1470-2045(13)70604-1
47. Scagliotti G, Novello S, Ramlau R, Favaretto A, Barlesi F, Akerley W, et al. MARQUEE: a randomized, double-blind, placebo-controlled, phase 3 trial of tivantinib (ARQ 197) plus erlotinib versus placebo plus erlonitib in previously treated patients with locally advanced or metastatic, non-squamous, non-small cell lung cancer (NSCLC). *The European Cancer Congress* 2013; Amsterdam (2013). abstr. 3410.
48. Spigel DR, Edelman MJ, O'Byrne K, Paz-Ares L, Shames DS, Yu W, et al. Onartuzumab plus erlotinib versus erlotinib in previously treated stage IIIb or IV NSCLC: results from the pivotal phase III randomized, multicenter, placebo-controlled METlung (OAM4971g) global trial. *ASCO Annual Meeting*; Chicago, IL (2014). abstr. 8000.
49. Chong CR, Janne PA. The quest to overcome resistance to EGFR-targeted therapies in cancer. *Nat Med* (2013) **19**(11):1389–99. doi:10.1038/nm.3388
50. Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci U S A* (2007) **104**:20932–7. doi:10.1073/pnas.0710370104
51. Spigel DR, Ervin TJ, Ramlau RA, Daniel DB, Goldschmidt JHJr, Blumenschein GRJr, et al. Randomized phase II trial of onartuzumab combination with erlotinib in patients with advanced non-small cell lung cancer. *J Clin Oncol* (2013) **31**(32):4105–14. doi:10.1200/JCO.2012.47.4189
52. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* (2010) **363**(18):1693–703. doi:10.1056/NEJMoa1006448
53. Mok T, Kim DW, Wu YL, Solomon BJ, Nakagawa K, Mekhail T, et al. First-line crizotinib versus pemetrexed-cisplatin or pemetrexed-carboplatin in patients with advanced ALK-positive non-squamous non-small cell lung cancer (NSCLC): results of a phase III study (PROFILE 1014). *ASCO Annual Meeting*; Chicago, IL (2014). abstr. 8002.
54. Kim DW, Mehra R, Tan DSW, Felip E, Chow LQM, Camidge DR, et al. Ceritinib in advanced anaplastic lymphoma kinase (ALK)-rearranged (ALK+) non-small cell lung cancer (NSCLC): results of the ASCEND-1 trial. *ASCO Annual Meeting*; Chicago, IL (2014). abstr 8003.
55. Ramalingam SS, Janne PA, Mok T, O'Byrne K, Boyer MJ, Zhang H, Liang JQ, et al. Randomized, double-blinded study of dacomitinib, an irreversible pan-human epidermal growth factor receptor (HER) inhibitor, versus erlotinib for second-line/third-line therapy of locally advanced/metastatic non-small cell lung cancer (ARCHER 1009). *ASCO Annual Meeting*; Chicago, IL (2014). abstr. 8018.
56. Janne PA, Ramalingam SS, Yang JCH, Ahn MJ, Kim DW, Kim SW, et al. Clinical activity of the mutant-selective EGFR inhibitor AZD 9291 in patients with EGFR inhibitor-resistant non-small cell lung cancer (NSCLC). *ASCO Annual Meeting*; Chicago, IL (2014). abstr. 8009.
57. Sequist LV, Soria JC, Gadgeel SM, Wakelee HA, Camidge DR, Varga A, et al. First-in-human evaluation of CO-1686, an irreversible, highly selective tyrosine kinase inhibitor of mutations of EGFR (activating and T790M). *ASCO Annual Meeting*; Chicago, IL (2014). Abstr. 8010.
58. Thatcher N, Hirsch FR, Szczesna A, Ciuleanu TE, Szafranski W, Dediu M, et al. A randomized, multicenter, open-label, phase III study of gemcitabine-cisplatin (GC) chemotherapy plus necitumumab (IMC-11F8/LY3012211) versus GC alone in the first-line treatment of patients (pts) with stage IV squamous non-small cell lung cancer (sq-NSCLC). *ASCO Annual Meeting 2014*; Chicago, IL (2014). abstr. 8008.
59. Janjigian YY, Smit EF, Horn L, Groen HJM, Camidge R, Gettinger S, et al. Activity of afatinib/cetuximab in patients (pts) with EGFR mutant non-small cell lung cancer (NSCLC) and acquired resistance (AR) to EGFR inhibitors. *Ann Oncol* (2012) **23**(Suppl 9):abstr. 12270. doi:10.1093/annonc/mds466
60. Gainor JF, Varghese AM, Ou SH, Kabraji S, Awad MM, Katayama R, et al. ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer. *Clin Cancer Res* (2013) **19**:4273–81. doi:10.1158/1078-0432.CCR-13-0318
61. Gettinger SN, Bazhenova L, Salgia R, Langer CJ, Gold KA, Rosell R, et al. Updated efficacy and safety of the ALK inhibitor AP26113 in patients (pts) with advanced malignancies, including ALK+ non-small cell lung cancer (NSCLC). *ASCO Annual Meeting*; Chicago, IL (2014). abstr. 8047.

62. Yano S, Yamada T, Takeuchi S, Tachibana K, Minami Y, Yatabe Y, et al. Hepatocyte growth factor expression in EGFR mutant lung cancer with intrinsic and acquired resistance to tyrosine kinase inhibitors in a Japanese cohort. *J Thorac Oncol* (2011) **6**:2011–7. doi:10.1097/JTO.0b013e31823ab0dd
63. Tran P, Gooding WE, Villaruz LC, Burns TF, Socinski MA, Tarhini AA et al. Phase I study of rilotumumab (AMG 102), an HGF inhibitor, and erlotinib in patients with advanced non-small cell lung cancer (NSCLC). *ASCO Annual Meeting*; Chicago, IL (2014). abstr. e19065.
64. Zhou Q, Zhang XC, Peng B, Yu X, Akimov M, Weber BL, et al. A phase II cluster study of single agent AUY922, BYL719, INC 280, LDK378 and MEK162 in Chinese patients with advanced non-small cell lung cancer (NSCLC). *ASCO Annual Meeting*; Chicago, IL (2014). abstr. TPS8122.
65. Socinski MA, Goldman J, El-Hariry I, Koczywas M, Vukovic V, Horn L, et al. A multicenter phase II study of ganestespib monotherapy in patients with genotypically defined advanced non-small cell lung cancer. *Clin Cancer Res* (2013) **19**(11). doi:10.1158/1078-0432.CCR-12-3381
66. Ramalingam SS, Goss GD, Andric ZG, Bondarenko I, Zaric B, Ceric T, et al. A randomized study of ganetespib, a heat shock protein 90 inhibitor, in combination with docetaxel versus docetaxel alone for second-line therapy of lung adenocarcinoma (GALAXY-1). *J Clin Oncol* (2013) **31**(Suppl):abstr:CRA8007.
67. Ramalingam SS, Zaric B, Ceric T, Ciuleanu TE, Spicer JF, Bondarenko I, et al. Galaxy-2: a randomized phase 3 study of ganetespib in combination with docetaxel versus docetaxel along in patients with advanced lung adenocarcinoma. *J Clin Oncol* (2014) **32**(Suppl):TPS8118.
68. Sang J, Acquaviva J, Friedland JC, Smith DL, Sequeira M, Zhang C, et al. Targeted inhibition of the molecular chaperone Hsp90 overcomes ALK inhibitor resistance in non-small cell lung cancer. *Cancer Discov* (2013) **3**(4):1–14. doi:10.1158/2159-8290.CD-12-0440
69. Pao W, Hutchinson K. Chipping away at the lung cancer genome. *Nat Med* (2012) **18**(2):349–51. doi:10.1038/nm.2697

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 06 June 2014; accepted: 11 August 2014; published online: 27 August 2014.
*Citation: Spaans JN and Goss GD (2014) Trials to overcome drug resistance to EGFR and ALK targeted therapies – past, present, and future. Front. Oncol. **4**:233. doi: 10.3389/fonc.2014.00233*
This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 21 July 2014
doi: 10.3389/fonc.2014.00174

A systemic review of resistance mechanisms and ongoing clinical trials in ALK-rearranged non-small cell lung cancer

***Khashayar Esfahani*[1], *Jason Scott Agulnik*[2] *and Victor Cohen*[1]*

[1] Department of Oncology, Segal Cancer Center, Sir Mortimer B. Davis Jewish General Hospital, Montreal, QC, Canada
[2] Division of Pulmonary Diseases, Department of Oncology, Peter Brojde Cancer Center, Montreal, QC, Canada

Edited by:
Barbara Melosky, British Columbia Cancer Agency, Canada

Reviewed by:
Sacha I. Rothschild, University Hospital Basel, Switzerland
Ajeet Gajra, SUNY Upstate Medical University, USA

***Correspondence:**
Victor Cohen, 3755 Cote-Ste-Catherine Road, Pav E, Suite E-714, Montreal, QC, Canada
e-mail: vcohen@jgh.mcgill.ca

The identification of oncogenic driver mutations in non-small cell lung cancer (NSCLC) has led to a paradigm shift and the development of specific molecular treatments. Tumors harboring a rearranged EML4–ALK fusion oncogene are highly sensitive to therapy with ALK-targeted inhibitors. Crizotinib is the first approved treatment for advanced lung tumors containing this genetic abnormality. In this mini review, we discuss the existing data on crizotinib as well as ongoing trials involving this medication. A brief overview of the known resistance mechanisms to crizotinib will also be presented followed by a summary of the ongoing trials involving next-generation ALK-inhibitors or other targeted therapies in patients with ALK+ NSCLC.

Keywords: non-small cell lung cancer, driver mutations, anaplastic lymphoma kinase, crizotinib, ALK-inhibitors, clinical trials as topic

INTRODUCTION

Treatment for non-small cell lung cancer (NSCLC) has historically consisted of cytotoxic chemotherapy. Recent advances in molecular biology had led to the discovery of oncogenic driver mutations with subsequent development of oral agents that target these molecular pathways. In NSCLC, the main two driver mutations, with FDA approved targeted therapies, consist of echinoderm microtubule protein like-4/anaplastic lymphoma kinase (EML-4/ALK) translocations and epidermal growth factor receptor (EGFR) mutations. Crizotinib is the first FDA approved treatment for patients with ALK+ NSCLC. To this date, only the final data from one phase III randomized trial has been published, evaluating the use of crizotinib as a second-line therapy (1). Multiple phase III randomized trials are in progress to assess the efficacy of crizotinib as first-line chemotherapy. Eventually, most patients on treatment with crizotinib develop resistance to this drug within 1 year of treatment. Most clinical trials in progress in the ALK+ patient population involve "new-generation ALK-inhibitors," or crizotinib in combination with novel drugs to bypass known resistance mechanisms.

The scope of this review article is twofold. First, the existing data on crizotinib will be presented as well ongoing trials involving this medication. Second, a brief overview of the known resistance mechanisms to crizotinib will be presented followed by a summary of the ongoing trials involving newer generation ALK-inhibitors or other targeted therapies in patients with ALK+ NSCLC.

ALK FUSION GENE AND ITS TARGET CRIZOTINIB

Crizotinib is an orally active inhibitor of multiple tyrosine kinase inhibitors (TKIs), including ALK, c-Met, hepatocyte growth factor receptor (HGFR), and c-ros oncogene 1 (ROS1) (**Figure 1**) (2). In 2007, the ALK gene rearrangement in which the 5′ end of EML4 gene is fused to the 3′ end of ALK was first identified by Soda et al. in NSCLC cell lines (3). The fusion protein resulting from this translocation has constitutive kinase activity, leading to downstream activation of multiple diverse signaling cascades involved in cell proliferation and carcinogenesis. Currently, multiple EML4–ALK fusion combinations have been identified (4). All these fusion proteins have a similar ALK kinase domain, but differ in the EML4 breakpoint. Pre-clinical data from *in vitro* studies suggested different crizotinib sensitivity for each variant of the EML4–ALK fusion protein (5). However, a subgroup analysis from the phase I trial of crizotinib failed to demonstrate such correlation between variant fusion proteins and clinical response to therapy (6). In addition, fusions of ALK with other partners including TRK-fused gene TFG and KIF5B have also been described in lung cancer patients, but appear to be much less common than EML4-ALK (7).

CLINICAL TRIALS INVOLVING CRIZOTINIB

In 2013, Shaw et al. published the first phase III randomized trial involving crizotinib in the second-line setting (1). Patients with locally advanced or metastatic ALK+ NSCLC were randomly assigned to receive oral treatment with crizotinib (250 mg) twice daily or intravenous chemotherapy with either pemetrexed or docetaxel. The median progression-free survival was 7.7 months in the crizotinib group and 3.0 months in the chemotherapy group. An interim analysis of overall survival showed no significant improvement with crizotinib as compared with chemotherapy. This analysis was nevertheless immature with a total of 96 deaths (40% of the required events) and censoring of over 70% of patients in either treatment arm. In addition, the analysis was likely confounded by the high crossover rate of patients in the chemotherapy group, with nearly 90% of patients on the chemotherapy arm crossing over to the other arm upon disease progression. The response rates were 65% with crizotinib, as compared with 20% with chemotherapy. Common adverse events associated with crizotinib were visual disorder, gastrointestinal side effects, and elevated liver

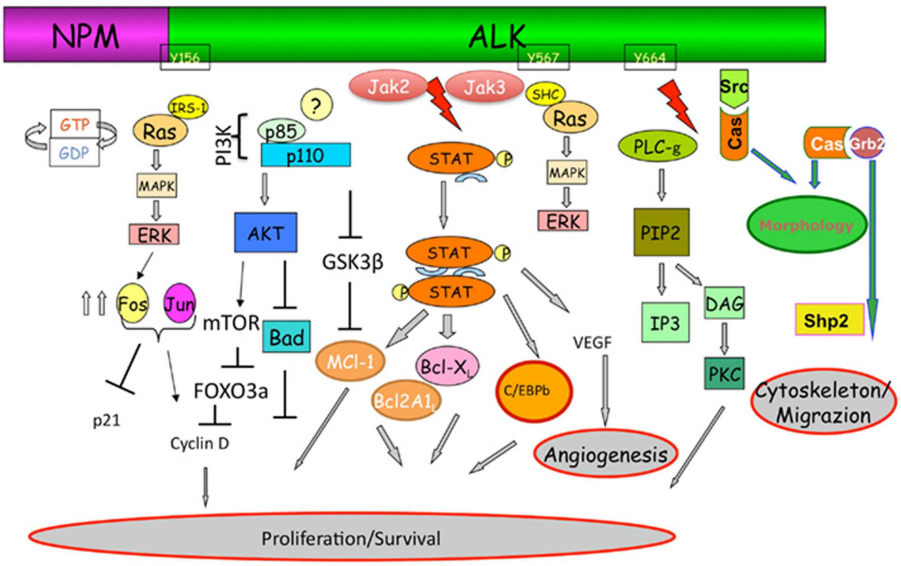


FIGURE 1 | The ALK signaling pathway with its cross-talk with other pathways involved in the resistance to ALK-inhibitors. Adapted from Tabbo et al. (47).

aminotransferase levels. Given the positive response rates with crizotinib, multiple phase III trials are currently in progress to address the efficacy of crizotinib as first-line therapy. Details of these trials, including patient population and their respective primary endpoints, are summarized in Table S1 in Supplementary Material.

One challenging clinical problem remains the treatment of ALK+ NSCLC patients with brain metastasis. These patients suffer from an adverse impact on quality of life and survival. Although it has been shown that crizotinib is effective for brain metastasis, it is penetration into cerebrospinal fluid (CSF) has been demonstrated to be very poor (8). Costa et al. measured the CSF-to-plasma ratio of crizotinib being only at 0.0026 (9). Past experience with erlotinib and gefitinib in patients with EGFR-mutated lung cancer has uncovered similar challenges: despite good systemic control of disease, a subset of patients would progress in the CNS, without any new acquired resistance mechanism, owing to the poor penetration of these TKIs in the CSF. Although pulse EGFR–TKIs doses have been used in this setting, there is limited data to support its use with crizotinib (10). Newer generation of ALK-inhibitors with better CSF penetration are currently under study.

RESISTANCE MECHANISMS OF CRIZOTINIB

In order to understand the rationale behind the majority of ongoing clinical trials involving ALK+ NSCLC patients, it is important to survey the currently known mechanism of resistance to crizotinib. ALK-dependant resistance mechanism occurs upon mutations in the tyrosine kinase (TK) domain, and activation of alternative signaling pathways. Alternatively, true ALK-independent resistance may arise through the outgrowth of clones that do not harbor an ALK gene fusion and contain a separate activated oncogene (11). Given that multiple resistance mechanisms are occasionally found within the same biopsy specimen, as well as different resistance mechanisms may be found in separate tumor deposits within the same patient, it is important to consider re-biopsy of the tumor upon progression on treatment, whenever technically feasible, to correctly identify the resistance mechanism accounting for progression of disease (12).

MUTATIONS IN TARGET TYROSINE KINASES

Past experience with the use of TKIs in chronic myelogenous leukemia as well as EGFR-mutated lung cancer teaches us that most common mechanisms of resistance to this class of medications are secondary mutations in the TK domains (13). This also holds true for crizotinib, given that mutations in the TK domains of the different targets of crizotinib is currently the best studied and most prevalent form of resistance to this drug, accounting for up to 25% of all cases resistant to ALK therapy (14).

The first major "gatekeeper" mutation identified in the TK domain of EML4–ALK involves the substitution of leucine for a methionine at position 1196 (L1196M) of the kinase domain of ALK, thus creating a mutant bulky amino-acid side chain in the ATP-binding pocket of the receptor, ultimately interfering with the binding of crizotinib to its receptor (15). This is analogous to the EGFR T790M mutation in the ATP biding pocket of the EGFR, which is the most common mechanism of resistance in EGFR-mutated lung cancer (12).

Tissue analysis of harvested tumor cells from patients resistant to crizotinib has demonstrated other non-gatekeeper secondary mutations in the ALK TK receptor. ALK secondary mutations in NSCLC are distributed throughout the kinase domain, including the solvent front (G1202R, S1206Y), ATP-binding pocket (G1269A), and N-terminal to the C-helix (1151Tins, F1174L, L1152R, and C1156Y) (11, 15–20). The prevalence and clinical significance of these secondary mutations remains to

be elucidated. Of interest, a separate secondary ALK mutation, F1174L, has also been identified in inflammatory myofibroblastic tumors (21).

Crizotinib is an inhibitor of multiple TKIs beside ALK, including ROS1 oncogene. A recent report of a G2032R mutation in the ROS1 TK domain leading to crizotinib resistance has also been identified (22). Although this mutation does not lie at the gatekeeper position, it confers resistance to ROS1 kinase inhibition through steric interference with drug binding.

ACTIVATION OF ALTERNATIVE PATHWAYS

Activation of alternative downstream signaling pathways, even in the setting of complete ALK receptor inhibition, is increasingly recognized as mechanisms of resistance to crizotinib. These include activation of the EGFR, heat shock protein 90 (HSP90), and the PI3K/AKT/mTOR pathways. The activation of these alternative pathways is present in up to 20% of patients (23).

Recent data from cell line experiments showed the activation of EGFR to be associated with ALK resistance (18). This was further corroborated with clinical analysis of tumor cells biopsied from patients resistant to ALK therapy (24). In most studies, the activation of EGFR occurred through increased phosphorylation and upregulation of EGFR ligands, such as amphiregulin, rather than being caused by mutations in the EGFR gene itself (18, 25). Although ALK mutations are usually mutually exclusive to other driver mutations such as EGFR, there have been reports of *de novo* mutation of the EGFR gene in patients and cell lines treated with crizotinib, accounting for resistance to this drug (11, 24, 26, 27).

The PI3K/AKT/mTOR pathway is an intracellular signaling pathway important in cell cycle regulation and apoptosis also implicated in resistance to ALK-targeted therapy. Recent ALK-resistant cell line analysis revealed that the activation of the mTOR pathways was associated with increased autophagy of the ALK receptor thus leading to decreased response to crizotinib treatment (28). The exact mechanism by which the ALK receptor induces activation of the mTOR pathway remains to be elucidated, but inhibition of AKT by phosphorylation seems to play a key factor. One study showed synergistic *in vitro* growth inhibitory activity of ALK inhibitor when combined with an mTOR inhibitor (29). The clinical significance of mTOR activation in NSCLC patients remains to be elucidated.

HSP90 is a highly abundant and ubiquitous molecular chaperone, which plays an essential role in many cellular processes including cell cycle control, hormone signaling, as well as protein folding, and degradation. The ALK receptor is one of the many client proteins of HSP90. HSP90 inhibition induced loss of EML4-ALK expression and depletion of multiple oncogenic signaling proteins in ALK-driven NSCLC cell lines (30). These results were further corroborated in murine models of NSCLC as well as anecdotal case reports of tissues derived from ALK therapy resistant patients (31, 32).

ONGOING CLINICAL TRIALS INVOLVING NEW-GENERATION ALK-INHIBITORS AND COMBINATION THERAPY

Most ongoing trials in ALK+ NSCLC patients involve newer generation ALK-inhibitors or combination therapy targeting currently known resistance mechanism to crizotinib. These include agents with activity against NSCLC with the L1196M gatekeeper mutation or the ROS1 mutation, as well as combination therapy targeting the EGFR and HSP90 proteins/pathways. A summary of these trials are presented in **Table 1**. Current evidence for some of these new-generation ALK-inhibitors are presented here.

CERITINIB (LDK378)

Ceritinib (LDK378, Novartis) is a novel and potent selective TKI targeting ALK. Results from a recent phase I/II study of this drug in both crizotinib-naïve and crizotinib-resistant patients have been published (33). The maximum tolerated dose of ceritinib was 750 mg once daily. Dose-limiting toxic events included diarrhea, vomiting, dehydration, elevated aminotransferase levels, and hypophosphatemia. Among 114 patients with NSCLC who received at least 400 mg of Ceritinib per day, the overall response rate was 58%. For 80 patients previously treated with crizotinib, the response rate was 56%. Responses were observed in patients with various resistance mutations in ALK, including L1196M, and in patients without detectable mutations. The median progression-free survival of patients receiving at least 400 mg of ceritinib was 7 months. Based on these findings, Ceritinib has recently been granted FDA approval for treatment of patients with ALK+ NSCLC in the second-line setting following failure or intolerance to crizotinib.

Multiple trials are nevertheless ongoing for this drug. These include three phase II trials of ceritinib for crizotinib-resistant patients (NCT01685060; NCT02040870) and crizotinib-naïve patients (NCT01685138). One phase II study is looking specifically at patients with ROS1 mutation (NCT01964157). Two phase III trials are comparing ceritinib with standard chemotherapy in patients previously treated with platinum-based chemotherapy (NCT01828112), and chemotherapy-naïve patients (NCT01828099). One phase I study is assessing the combination of ceritinib with a HSP90 inhibitor (NCT01772797).

ALECTINIB (CH5424802/RO5424802)

Alectinib (Chugai and Roche Pharmaceuticals) is a highly potent selective ALK inhibitor with activity against L1196M gatekeeper mutation as well as other secondary mutations such as F1174L and R1275Q (34, 35). Results from a recent phase I/II study with alectinib in a Japanese population have been published (36). In the phase I study, alectinib was given up to a maximum dose of 300 mg twice daily without dose-limiting toxicity. In the phase II part of the study, 40/46 (87%) of patients achieved a partial response within 6 weeks of treatment. Two patients had a complete response. Median PFS had not been reached at the time of the report. Responses were seen in brain metastases in three patients. Grade 3 adverse events including neutropenia and increase creatine phosphokinase occurred in 12 (26%) of patients.

Three phase II/III trials with alectinib are in progress in crizotinib-naïve (ALEXA trial-NCT02075840) as well as in crizotinib-resistant (NCT01871805; NCT01801111) patients.

AP26113

AP26113 (Ariad Pharmaceuticals) is a novel inhibitor of ALK with activity against L1196M gatekeeper mutation as well as

Table 1 | Ongoing clinical trials involving novel ALK- and HSP90-inhibitors in NSCLC.

ONGOING CLINICAL TRIALS INVOLVING NOVEL ALK-INHIBITORS						
Drug	**Company**	**Activity against other kinases**	**Activity against L1196M mutation**	**Ongoing trials**	**Study phase**	**Previous treatment**
LDK378 (ceritinib)	Novartis	IFG-1R c-MET	Yes	NCT01772797 NCT02040870 NCT01685138 NCT01685060 NCT01947608 NCT01964157 NCT01828099 NCT01828112	Phase I Phase I/II Phase II Phase II Phase II Phase II Phase III Phase III	None Crizotinib/chemotherapy 0-3 lines of chemotherapy Crizotinib or 1-3 lines of chemotherapy Crizotinib 1 line of chemotherapy None Crizotinib
CH5424802/ RO5424802 (alectinib)	Roche/Chugai	ROS1	Yes	NCT01588028 NCT01871805 NCT01801111 NCT02075840	Phase I Phase II Phase II Phase III	 Crizotinib Crizotinib
AP26113	Ariad	EGFR ROS1	Unknown	NCT01449461 NCT02094573	Phase I/II Phase II	Refractory to standard therapy Crizotinib
ASP3026	Astellas	ROS1	Yes	NCT01401504 NCT01284192	Phase I Phase I	Refractory to standard therapy Refractory to standard therapy
TSR-001	Tesaro	Unknown	Yes	NCT02048488	Phase I	None
PF-06463922	Pfizer	EGFR ROS1	Unknown	NCT01970865	Phase I/II	None
X-396	Xcovery	Unknown	Yes	NCT01625234	Phase I	None

ONGOING CLINICAL TRIALS INVOLVING HSP90 INHIBITORS					
Drug	**Company**	**Ongoing trials**	**Study phase**	**Combination therapy**	**Previous treatment**
AUY922	Novartis	NCT01772797 NCT01752400 NCT01124864 NCT01922583	Phase I Phase II Phase II Phase II	LDK378	None Crizotinib Two lines of chemotherapy One line of chemotherapy
STA-9090	Synta	NCT01579994 NCT01562015	Phase I/II Phase II	Crizotinib	Standard chemotherapy Three lines of therapy
IPI-504	Infinity	NCT01228435	Phase II		Refractory to standard therapy
AT13387	Astex	NCT01712217	Phase I/II	Crizotinib	Crizotinib
DS-2248	Daiichi Sankyo	NCT01288430	Phase I		Crizotinib

against ROS1 and EGFR (including mutant form with the T790M gatekeeper mutation) (37). In an ongoing phase I/II study (NCT01449461), the established dose was at 180 mg once daily with good antitumor activity in ALK+ NSLC patients (38). Objective response was observed in 15/24 (63%) patients (1 complete response and 14 partial responses), including 12/16 (75%) in patients resistant to crizotinib. Of interest, 4/5 patients with brain metastasis had objective responses as well. The most common treatment-related adverse events were nausea (33%), fatigue (22%), and diarrhea (20%). A confirmatory phase II studies in crizotinib-resistant (NCT02094573) is currently in progress.

OTHER NEW-GENERATION ALK AGENTS

Multiple other new-generation ALK agents are currently under phase I study. These include TSR-001, ASP3026, PF-06463922, as well as X-396 (39–42). Details about these studies can be found in **Table 1**.

HSP90 INHIBITORS

Pre-clinical studies involving HSP90 inhibitors in ALK+ NSCLC led to decreased ALK fusion protein levels *in vitro*, and led to tumor regression in *in vivo* models (43). Currently, multiple HSP90 inhibitors are in phase I/II clinical trials involving ALK+ NSCLC patients, either as stand-alone drugs or in combination with other ALK-inhibitors such as crizotinib. Details about ongoing studies can be found in **Table 1**.

Early results from phase II studies involving IPI-504 (Infinity Pharma) in three NSCLC patients resulted in two partial responses and one case of prolonged stable disease (44). In another phase II study of ganetespib (STA-9090), 4/8 patients had partial responses and three patients had stable disease (45). The most common adverse effects were diarrhea, fatigue, nausea, and anorexia. Two patients in this study died of cardiac arrest and renal failure associated with ganetespib. There has also been one case report of a crizotinib-resistant ALK+ NSCLC with an objective response to this drug (31). AUY922 (Novartis) has been studied as a single agent in both crizotinib-resistant and – naïve patients. Objective response was achieved in 6/21 (29%) of patients, 4 of which were crizotinib-naïve and remainder 2 had been previously treated with crizotinib (46).

SUMMARY AND CONCLUSION

The identification of the ALK fusion protein in 2007 and the fast development and approval of a FDA targeted treatment in <5 years constitutes a remarkable feat in the field of targeted therapies. Crizotinib holds many promises from its early debut in treatment-refractory NSCLC patients. Final results from phase III randomized trials using Crizotinib as a first-line therapy are eagerly awaited for. Despite excellent response in the initial stages, most patients develop resistance to crizotinib. Elucidating resistance mechanism and subsequently developing therapeutic strategies to overcome resistance to ALK-inhibitors constitutes a priority, with the vast majority of ongoing clinical trials in this field involving new-generation ALK-inhibitors or combination therapy with other targeted agents. Key questions remain on how to correctly identify the resistance mechanism of a tumor progressing on ALK-targeted therapy given re-biopsy is often technically challenging and resource intensive, as well as on how to correctly identify and stream the correct combination of therapies to the appropriate patient populations as first-line therapy. The ongoing Q-CROC-05 multicenter phase IV clinical trial (NCT02041468) will aim to shed some light on these important clinical issues.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/Journal/10.3389/fonc.2014.00174/abstract

REFERENCES

1. Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, Ahn MJ, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med* (2013) **368**(25):2385–94. doi:10.1056/NEJMoa1214886
2. Frampton JE. Crizotinib: a review of its use in the treatment of anaplastic lymphoma kinase-positive, advanced non-small cell lung cancer. *Drugs* (2013) **73**(18):2031–51. doi:10.1007/s40265-013-0142-z
3. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* (2007) **448**(7153):561–6. doi:10.1038/nature05945
4. Heuckmann JM, Holzel M, Sos ML, Heynck S, Balke-Want H, Koker M, et al. ALK mutations conferring differential resistance to structurally diverse ALK inhibitors. *Clin Cancer Res* (2011) **17**(23):7394–401. doi:10.1158/1078-0432.CCR-11-1648
5. Heuckmann JM, Balke-Want H, Malchers F, Peifer M, Sos ML, Koker M, et al. Differential protein stability and ALK inhibitor sensitivity of EML4-ALK fusion variants. *Clin Cancer Res* (2012) **18**(17):4682–90. doi:10.1158/1078-0432.CCR-11-3260
6. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* (2010) **363**(18):1693–703. doi:10.1056/NEJMoa1006448
7. Takeuchi K, Choi YL, Togashi Y, Soda M, Hatano S, Inamura K, et al. KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. *Clin Cancer Res* (2009) **15**(9):3143–9. doi:10.1158/1078-0432.CCR-08-3248
8. Kaneda H, Okamoto I, Nakagawa K. Rapid response of brain metastasis to crizotinib in a patient with ALK rearrangement-positive non-small-cell lung cancer. *J Thorac Oncol* (2013) **8**(4):e32–3. doi:10.1097/JTO.0b013e3182843771
9. Costa DB, Kobayashi S, Pandya SS, Yeo WL, Shen Z, Tan W, et al. CSF concentration of the anaplastic lymphoma kinase inhibitor crizotinib. *J Clin Oncol* (2011) **29**(15):e443–5. doi:10.1200/JCO.2010.34.1313
10. Kim YH, Ozasa H, Nagai H, Sakamori Y, Yoshida H, Yagi Y, et al. High-dose crizotinib for brain metastases refractory to standard-dose crizotinib. *J Thorac Oncol* (2013) **8**(9):e85–6. doi:10.1097/JTO.0b013e31829cebbb
11. Doebele RC, Pilling AB, Aisner DL, Kutateladze TG, Le AT, Weickhardt AJ, et al. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. *Clin Cancer Res* (2012) **18**(5):1472–82. doi:10.1158/1078-0432.CCR-11-2906
12. Gainor JF, Shaw AT. Emerging paradigms in the development of resistance to tyrosine kinase inhibitors in lung cancer. *J Clin Oncol* (2013) **31**(31):3987–96. doi:10.1200/JCO.2012.45.2029
13. Chong CR, Janne PA. The quest to overcome resistance to EGFR-targeted therapies in cancer. *Nat Med* (2013) **19**(11):1389–400. doi:10.1038/nm.3388
14. Perez CA, Velez M, Raez LE, Santos ES. Overcoming the resistance to crizotinib in patients with non-small cell lung cancer harboring EML4/ALK translocation. *Lung Cancer* (2014) **84**(2):110–5. doi:10.1016/j.lungcan.2014.02.001
15. Choi YL, Soda M, Yamashita Y, Ueno T, Takashima J, Nakajima T, et al. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *N Engl J Med* (2010) **363**(18):1734–9. doi:10.1056/NEJMoa1007478
16. Huang D, Kim DW, Kotsakis A, Deng S, Lira P, Ho SN, et al. Multiplexed deep sequencing analysis of ALK kinase domain identifies resistance mutations in relapsed patients following crizotinib treatment. *Genomics* (2013) **102**(3):157–62. doi:10.1016/j.ygeno.2013.02.006
17. Kim S, Kim TM, Kim DW, Go H, Keam B, Lee SH, et al. Heterogeneity of genetic changes associated with acquired crizotinib resistance in ALK-rearranged lung cancer. *J Thorac Oncol* (2013) **8**(4):415–22. doi:10.1097/JTO.0b013e318283dcc0
18. Sasaki T, Koivunen J, Ogino A, Yanagita M, Nikiforow S, Zheng W, et al. A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. *Cancer Res* (2011) **71**(18):6051–60. doi:10.1158/0008-5472.CAN-11-1340
19. Zhang S, Wang F, Keats J, Zhu X, Ning Y, Wardwell SD, et al. Crizotinib-resistant mutants of EML4-ALK identified through an accelerated mutagenesis screen. *Chem Biol Drug Des* (2011) **78**(6):999–1005. doi:10.1111/j.1747-0285.2011.01239.x
20. Lovly CM, Pao W. Escaping ALK inhibition: mechanisms of and strategies to overcome resistance. *Sci Transl Med* (2012) **4**(120):120–2. doi:10.1126/scitranslmed.3003728
21. Sasaki T, Okuda K, Zheng W, Butrynski J, Capelletti M, Wang L, et al. The neuroblastoma-associated F1174L ALK mutation causes resistance to an ALK kinase inhibitor in ALK-translocated cancers. *Cancer Res* (2010) **70**(24):10038–43. doi:10.1158/0008-5472.CAN-10-2956
22. Awad MM, Katayama R, McTigue M, Liu W, Deng YL, Brooun A, et al. Acquired resistance to crizotinib from a mutation in CD74-ROS1. *N Engl J Med* (2013) **368**(25):2395–401. doi:10.1056/NEJMoa1215530

23. Boland JM, Jang JS, Li J, Lee AM, Wampfler JA, Erickson-Johnson MR, et al. MET and EGFR mutations identified in ALK-rearranged pulmonary adenocarcinoma: molecular analysis of 25 ALK-positive cases. *J Thorac Oncol* (2013) **8**(5):574–81. doi:10.1097/JTO.0b013e318287c395
24. Katayama R, Shaw AT, Khan TM, Mino-Kenudson M, Solomon BJ, Halmos B, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung cancers. *Sci Transl Med* (2012) **4**(120):120ra17. doi:10.1126/scitranslmed.3003316
25. Tanizaki J, Okamoto I, Okabe T, Sakai K, Tanaka K, Hayashi H, et al. Activation of HER family signaling as a mechanism of acquired resistance to ALK inhibitors in EML4-ALK-positive non-small cell lung cancer. *Clin Cancer Res* (2012) **18**(22):6219–26. doi:10.1158/1078-0432.CCR-12-0392
26. Rossing HH, Grauslund M, Urbanska EM, Melchior LC, Rask CK, Costa JC, et al. Concomitant occurrence of EGFR (epidermal growth factor receptor) and KRAS (V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) mutations in an ALK (anaplastic lymphoma kinase)-positive lung adenocarcinoma patient with acquired resistance to crizotinib: a case report. *BMC Res Notes* (2013) **6**:489. doi:10.1186/1756-0500-6-489
27. Gainor JF, Varghese AM, Ou SH, Kabraji S, Awad MM, Katayama R, et al. ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer. *Clin Cancer Res* (2013) **19**(15):4273–81. doi:10.1158/1078-0432.CCR-13-0318
28. Yamaguchi N, Lucena-Araujo AR, Nakayama S, de Figueiredo-Pontes LL, Gonzalez DA, Yasuda H, et al. Dual ALK and EGFR inhibition targets a mechanism of acquired resistance to the tyrosine kinase inhibitor crizotinib in ALK rearranged lung cancer. *Lung Cancer* (2014) **83**(1):37–43. doi:10.1016/j.lungcan.2013.09.019
29. Ji C, Zhang L, Cheng Y, Patel R, Wu H, Zhang Y, et al. Induction of autophagy contributes to crizotinib resistance in ALK-positive lung cancer. *Cancer Biol Ther* (2014) **15**:5. doi:10.4161/cbt.28162
30. Gao J, Yin M, Zhu Y, Gu L, Zhang Y, Li Q, et al. Prognostic significance and therapeutic potential of the activation of anaplastic lymphoma kinase/protein kinase B/mammalian target of rapamycin signaling pathway in anaplastic large cell lymphoma. *BMC Cancer* (2013) **13**:471. doi:10.1186/1471-2407-13-471
31. Sang J, Acquaviva J, Friedland JC, Smith DL, Sequeira M, Zhang C, et al. Targeted inhibition of the molecular chaperone Hsp90 overcomes ALK inhibitor resistance in non-small cell lung cancer. *Cancer Discov* (2013) **3**(4):430–43. doi:10.1158/2159-8290.CD-12-0440
32. Chen Z, Sasaki T, Tan X, Carretero J, Shimamura T, Li D, et al. Inhibition of ALK, PI3K/MEK, and HSP90 in murine lung adenocarcinoma induced by EML4-ALK fusion oncogene. *Cancer Res* (2010) **70**(23):9827–36. doi:10.1158/0008-5472.CAN-10-1671
33. Shaw AT, Kim DW, Mehra R, Tan DS, Felip E, Chow LQ, et al. Ceritinib in ALK-rearranged non-small-cell lung cancer. *N Engl J Med* (2014) **370**(13):1189–97. doi:10.1056/NEJMoa1311107
34. Sakamoto H, Tsukaguchi T, Hiroshima S, Kodama T, Kobayashi T, Fukami TA, et al. CH5424802, a selective ALK inhibitor capable of blocking the resistant gatekeeper mutant. *Cancer Cell* (2011) **19**(5):679–90. doi:10.1016/j.ccr.2011.04.004
35. Kinoshita K, Asoh K, Furuichi N, Ito T, Kawada H, Hara S, et al. Design and synthesis of a highly selective, orally active and potent anaplastic lymphoma kinase inhibitor (CH5424802). *Bioorg Med Chem* (2012) **20**(3):1271–80. doi:10.1016/j.bmc.2011.12.021
36. Seto T, Kiura K, Nishio M, Nakagawa K, Maemondo M, Inoue A, et al. CH5424802 (RO5424802) for patients with ALK-rearranged advanced non-small-cell lung cancer (AF-001JP study): a single-arm, open-label, phase 1-2 study. *Lancet Oncol* (2013) **14**(7):590–8. doi:10.1016/S1470-2045(13)70142-6
37. Rivera VM, Wang F, Anjum R, Zhang S, Squillace R, Keats J, et al. AP26113 is a dual ALK/EGFR inhibitor: characterization against EGFR T790M in cell and mouse models of NSCLC. *Proceedings: AACR 103rd Annual Meeting.* Chicago: AACR (2012).
38. Camidge DR, Bazhenova L, Salgia R, Weiss GJ, Langer CJ, Shaw AT, et al. First-in-human dose-finding study of the ALK/EGFR inhibitor AP26113 in patients with advanced malignancies: updated results. *J Clin Oncol* (2013) **31**(Suppl):Abstr.8031.
39. Wilcoxen KM, Brake RL, Saffran D, Teffera Y, Choquette D, Whittington D, et al. Proceedings of AACR, 15 April 2012. *Cancer Res* (2012) **71**(Suppl 1):Abstr.1795. doi:10.1158/1538-7445.AM2012-1795
40. Lovly CM, Heuckmann JM, de Stanchina E, Chen H, Thomas RK, Liang C, et al. Insights into ALK-driven cancers revealed through development of novel ALK tyrosine kinase inhibitors. *Cancer Res* (2011) **71**(14):4920–31. doi:10.1158/0008-5472.CAN-10-3879
41. Patnaik APA. Pharmacokinetics and safety of an oral ALK inhibitor, ASP3026, observed in a phase I dose escalation trial. *J Clin Oncol* (2013) **31**(Suppl):Abstr.2602.
42. Kuromitsu S, Mori M, Shimada I, Kondoh Y, Shindoh N, Soga T, et al. Antitumor activities of ASP3026 against EML4-ALK-dependent tumor models. *Mol Cancer Ther* (2011) **10**(Suppl 11):Abstr.A227.
43. Pillai RN, Ramalingam SS. Heat shock protein 90 inhibitors in non-small-cell lung cancer. *Curr Opin Oncol* (2014) **26**(2):159–64. doi:10.1097/CCO.0000000000000047
44. Sequist LV, Gettinger S, Senzer NN, Martins RG, Janne PA, Lilenbaum R, et al. Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non-small-cell lung cancer. *J Clin Oncol* (2010) **28**(33):4953–60. doi:10.1200/JCO.2010.30.8338
45. Socinski MA, Goldman J, El-Hariry I, Koczywas M, Vukovic V, Horn L, et al. A multicenter phase II study of ganetespib monotherapy in patients with genotypically defined advanced non-small cell lung cancer. *Clin Cancer Res* (2013) **19**(11):3068–77. doi:10.1158/1078-0432.CCR-12-3381
46. Felip E, Carcereny E, Barlesi F, Gandhi L, Sequist LV, Kim SW, et al. Phase II activity of the HSP90 inhibitor AUY922 in patients with ALK-rearranged (ALK+) or EGFR-mutated advanced non-small cell lung cancer. *Ann Oncol* (2012) **23**(ix152):Abstr.4380. doi:10.1093/annonc/mds395
47. Tabbo F, Barreca A, Piva R, Inghirami G. ALK signaling and target therapy in anaplastic large cell lymphoma. *Front Oncol* (2012) **2**:41. doi:10.3389/fonc.2012.00041

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 01 May 2014; paper pending published: 06 June 2014; accepted: 19 June 2014; published online: 21 July 2014.

*Citation: Esfahani K, Agulnik JS and Cohen V (2014) A systemic review of resistance mechanisms and ongoing clinical trials in ALK-rearranged non-small cell lung cancer. Front. Oncol. **4**:174. doi: 10.3389/fonc.2014.00174*

This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

REVIEW ARTICLE
published: 22 October 2014
doi: 10.3389/fonc.2014.00288

Immunotherapy for lung cancer: has it finally arrived?

Ahmed A. Mostafa and Don G. Morris*

Department of Oncology, University of Calgary, Calgary, AB, Canada

Edited by:
Vera Hirsh, McGill University Health Centre, Canada

Reviewed by:
Sacha I. Rothschild, University Hospital Basel, Switzerland
Rabab Mohamed Gaafar, Cairo University, Egypt

***Correspondence:**
Don G. Morris, Department of Oncology, Tom Baker Cancer Center, University of Calgary, 1331 29th Street NW, T2N 4N2, Calgary, AB, Canada
e-mail: don.morris@albertahealthservices.ca

The possible link between infection/inflammation/immune activation and a cancer patient's outcome from both a causative and outcome point of view has long been postulated. Substantial progress in the understanding of tumor-associated antigens/epitopes, immune cellular subpopulations, cytokine pathways/expression, the tumor microenvironment, and the balance between tumor-immune suppression and stimulation have been made over the past decade. This knowledge has heralded a new era of tumor immunotherapy utilizing vaccines, immune checkpoint inhibition, and oncolytic viruses. Despite significant progress in the molecular era now with targeted therapeutics such as EGFR tyrosine kinase inhibitors and ALK fusion protein inhibitors that have significantly improved the outcome of these specific lung cancer subpopulations, the overall 5 year survival for all non-small cell lung cancer (NSCLC) is still <20%. Unlike malignancies such as malignant melanoma, renal cell carcinoma, and neuroblastoma given their documented spontaneous remission rates lung cancer historically has been felt to be resistant to immune approaches likely related to an immunosuppressive tumor microenvironment and/or lack of immune recognition. Defining responding populations, understanding the mechanism(s) underlying durable immune responses, and the role of chemotherapy, radiation, oncolytic viruses, and other tumor disrupting agents in augmenting immune responses have led to improved optimization of immune therapeutic strategies. The purpose of this review is to focus on the recent advances in lung immunotherapy with an emphasis on recent clinical trials in the last 5 years in NSCLC.

Keywords: lung cancer, vaccines, immune checkpoint inhibitors, clinical trials

INTRODUCTION

Lung cancer is the number one cause of cancer mortality globally and has an estimated incidence of 1.3 million new cases every year (1). Approximately 80–85% of the newly diagnosed cases of lung cancer are non-small cell lung cancer (NSCLC) (adenocarcinoma, squamous carcinoma, and large cell carcinoma) and 15–20% small cell lung carcinoma. In the majority of cases, patients present with unresectable and/or non-curable disease (2). Locally advanced, good performance status NSCLC patients may be offered concurrent chemotherapy, radical radiotherapy, and/or surgery, with a resultant 8-month progression-free survival rate and <15% 5-year survival (3). Patients diagnosed with metastatic disease newer cytotoxic chemotherapies such as pemetrexed [17-month median overall survival (OS)] and treatment with molecularly targeted therapeutics for adenocarcinomas, such as next generation small molecules targeting the EGFR (24 months median OS) and ALK inhibitors (20 months median OS), the survival rate for advanced disease has improved only marginally (4–6). In the last decade, there has been a better understanding on how cancer interacts with the immune cells and the ways that the cancer have developed to evade the immune system, resulting in a new era of cancer immunotherapy protocols, which may aid in overcoming the limitations of conventional therapeutic strategies (7).

Two such immunotherapeutic strategies in NSCLC are currently in clinical trials that involve increasing tumor immunogenicity by using cancer vaccines to augment tumor-immune recognition and overcoming tumor immunosuppression by using immune checkpoint inhibitors (**Figure 1**).

CANCER VACCINES

Cancer vaccines are biologically active antigenic preparations that ideally educate the immune system about an existing cancer (8, 9). For a cancer vaccine to be effective, it should target an antigen specific to the cancer cell, i.e., tumor-associated antigens (TAA), which are frequently elevated in the circulation of cancer patients (10). Vaccines have historically been (glyco) peptides, recombinant proteins, or whole cancer cell preparations (that have been rendered replication incompetent); however, since antigenic peptides suboptimally activate antigen presenting cells (APCs), vaccines are usually augmented by an immunoadjuvant or immunostimulant in the form of inactive pathogen or other non-specific immune stimulant. Cancer vaccines are taken up APCs, which later migrate to the nearest draining lymph node and consequently activate T- and B-lymphocytes. Specific T-cells will differentiate and expand to become tumor specific effector cells that will home to the tumor microenvironment which hosts the original antigens (11). It is of interest to speculate whether immune targeted therapeutics will be more effective if the tumor is initially disrupted by cytotoxic chemotherapy and/or radiation or some other cellular disrupting strategy, i.e., radiofrequency ablation/cryotherapy/oncolytic virus in order to augment antigen/epitope exposure to the immune system. There are numerous types of cancer vaccines that have

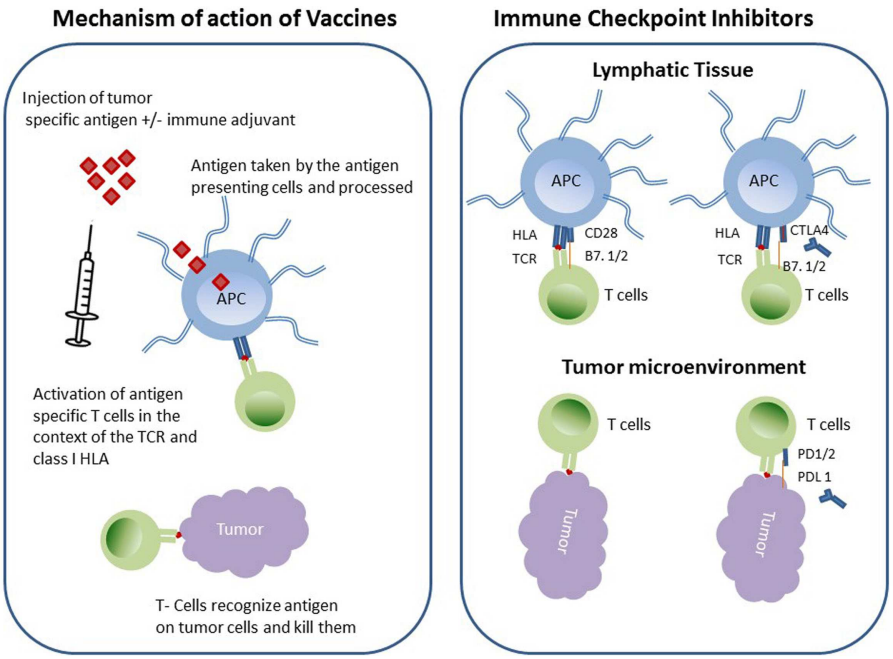


FIGURE 1 | Schematic diagram of immunotherapeutic strategies in NSCLC.

been tested in clinical trials involving NSCLC patients as discussed below.

BELAGENPUMATUCEL-L

Belagenpumatucel-L (Lucanix®) (NovaRx Corporation, San Diego, CA, USA) is an allogeneic tumor cell vaccine prepared from four irradiated human NSCLC cell lines SK-LU-1 (adenocarcinoma), NCI-H 460 (large cell carcinoma), NCI-H 520, and Rh 2 [squamous cell carcinoma, transfected with a transgene plasmid containing an antisense construct against the TGF-β2 gene (12)]. Elevated levels of TGF-β are frequently associated with immunosuppression in cancer through antagonizing the function of natural killer cells (NK) and dendritic cells (DC) (13). Moreover, the prognosis of NSCLC patients has been found to be inversely correlated with the level of TGF-β (14).

In the first of two phase II studies using the intradermal Lucanix vaccine involving stage II–IV NSCLC patients with low-tumor burden who had completed or refused conventional therapy tolerated the treatment well. Those patients with advanced disease who received higher doses ($N = 41, > 2.5 \times 10^7$ cells/intradermal injection monthly) had a significant improved 2-year survival compared to the low-dose cohort [($N = 20, < 2.5 \times 10^7$ cells/injection) (47 versus 18%) ($p = 0.0069$)] (13). A second trial confined to pretreated stage IV NSCLC patients had an OS of 19 months (14). Interestingly, in this trial, the vaccine elicited both a cell mediated and a humoral immune response in the form of high level of cytotoxic cytokines and increased IgG and IgM titers.

The phase III survival, tumor free, overall, and progression-free (STOP) clinical trial, involving advanced NSCLC patients, pretreated with a first-line platinum-based chemotherapy, treated similarly (2.5×10^7 cells/intradermal monthly injection) presented at the European Cancer Congress 2013 revealed a median OS of 20.3 and 17.8 months in Lucanix and placebo groups, respectively [hazard ratio (HR) 0.94; $p = 0.594$]. Although the OS was numerically longer STOP trial did not meet the primary endpoint. On the other hand, this analysis demonstrated improved OS in two subgroups, the non-adenocarcinoma and the stage IIIB/IV patients who started the vaccine therapy within 12 weeks of the finishing the initial chemotherapy.

TG4010

The TG4010 vaccine is a suspension of recombinant modified vaccinia virus of Ankara (MVA strain) vector vaccine that expresses the TAA MUC1 and interleukin (IL)-2 (15). MUC1 is a transmembrane glycoprotein, which is normally expressed on normal duct epithelia, such as those lining the breast, prostate, lung, stomach, bladder epithelium, and sweat glands (16). Its normal function is related to mucin formation; however, in cancer its function is altered, due to excessive glycosylation, which contributes to its immunogenicity. High-MUC1 expression correlates with invasiveness and a poor prognosis for lung cancer (17). Furthermore, MUC1 overexpression activates phosphatidylinositol 3-kinase (PI3K) and the AKT pathways and resultant cell proliferation (18).

The initial randomized phase II study that included 65 patients with stage III/V NSCLC showed that TG4010 (10^8 plaque forming units injected subcutaneously weekly for 6 weeks then every 3 weeks) in combination with chemotherapy (cisplatin/vinorelbine) ($N = 44$) versus TG4010 monotherapy until progression followed by the addition of chemotherapy ($N = 21$) was generally well tolerated. The combination group had a response rate of 30 versus 0% in the TG4010 group, however, a

numerically inferior median and 1-year survival rate (19). Despite this a larger multicenter, open-label randomized phase IIB trial was conducted (20). This study enrolled 148 IIIB (pleural effusion)/IV patients with a 1:1 randomization to the combination therapy of TG4010 plus chemotherapy versus chemotherapy (cisplatin and gemcitabine) alone. The primary end point of the study was achieved with a resultant 6-month progressive free survival (PFS) of the combined group of 43% (95% CI 33.4–53.5) versus 35% (95%CI 25.9–45.3) in the control group. Notably, the objective response rate and median OS of responding patients was higher in the TG4010 group than in the chemotherapy alone group: 41.9 versus 28.4% and 23.3 versus 12.5 months, respectively. In this study, patients who presented with high levels for activation marker for NK cells ($CD16^+CD56^+CD69^+$) at the baseline levels had the worst outcome. Thus, the presences of these markers may act as potential biomarker for the safety and efficiency of TG4010. Of note, FDA approved a phase III study on TG4010 in a subpopulation of patients with advanced NSCLC and normal levels of activated NK cells.

There is an ongoing Phase IIB/III randomized, double-blinded, placebo-controlled study comparing first-line therapy with or without TG4010 immunotherapy product in patients with stage IV NSCLC (TIME trial) currently accruing patients in Europe and the US.

BLP25

BLP25 (Tecemotide®) (also known as L-BLP25 and Stimuvax) is a liposomal vaccine, which is formed from the immunoadjuvant monophosphoryl lipid A, and three lipid components (cholesterol, dimyristoyl phosphatidylglycerol, and dipalmitoyl phosphatidylcholine) (21) that harbors a 25 amino acid synthetic immunodominant core peptide of MUC1 TAA that has been shown to elicit a strong T-cell immune response both in transgenic murine lung cancer models and in patients (21–23). Recently, using a MUC1.Tg lung cancer mouse model, it was demonstrated that pre-administration of cyclophosphamide (CPA) with BLP25 increases the levels of the immune stimulating T-helper 1 (Th1) response [IL-2 and interferon gamma (IFN-γ)], as well as other inflammatory chemokines such as IP-10, MIG, KC, MCP-1, and MIP-1α (22), may enhance immunotherapy by boosting both cellular and humoral mediated antitumor immune responses for the vaccine by inhibiting regulatory T (Treg) cells (24–26).

A phase IIB clinical trial was conducted involving 171 stage IIIB and IV NSCLC patients with stable or better response to first-line chemotherapy or chemo-radiotherapy with a primary objective of OS and toxicity (21). The secondary endpoints investigated the health related quality of life (QQL) and immune response elicited by the vaccine. Patients were randomized to receive BLP25 plus best supportive care (BSC) versus BSC only. BLP25 or placebo was given subcutaneously weekly × 8 then 6 weekly until progression or significant toxicity. All patients in the BLP25 arm received a low dose of CPA prior vaccination. Although, the median survival time was 4.2 months longer in the treatment arm, this result was not statistically significant [17.2 versus 13 months, HR 0.74 (0.53–1.0)]. In addition, the 3-year OS was higher in BLP25 plus BSC group than the BSC group (31 versus 17%, $p = 0.035$). Interestingly, a 17.3-month improved survival as well as improved QQL was observed in those patients with stratified stage IIIB locoregional disease who received the BLP25 plus BSC [30.6 versus 13.3 months, HR 0.54 (0.3–0.99)]. The 3-year OS in this subgroup was also numerically higher in BLP25 plus BSC group than the BSC group (49 versus 27%, $p = 0.07$). Whether or not patients with a lower tumor burden, no metastasis and perhaps patients that receive multimodality treatment may benefit preferentially from this vaccine is unclear. Evidence of T-cell mediated immunity was only detected in only approximately 20% of the patients in the BLP25 arm, thought in part to be due technical problems related to decrease lymphocyte viability during collection and transportation.

On the strength of the above findings, two phase III trials were conducted. The stimulating targeted antigenic responses to NSCLC (START) clinical trial an international, randomized, double-blinded trial evaluated BLP25 as a maintenance therapy in stage III NSCLC patients with stable disease or better response after chemotherapy (27). The study was initiated in 2007 with recruitment of 1513 patients from 264 trial centers in 33 countries worldwide. Unfortunately, as a result of fatal encephalitis reported in a patient with malignant melanoma that was treated with BLP25 on an exploratory trial, the Food and Drug Administration agency placed a hold on the BLP25 clinical trials for approximately 135 days. This hold was suggested to have a negative impact on trial objectives as it resulted in a total of 274 patients from the BLP25 and placebo groups to be excluded from the study. The median OS and the 1–3 year survival rate between the two groups (BLP25 and placebo) were not statistically significant. Interestingly, the median OS for BLP25 compared to placebo arms in the concurrent chemo-radiotherapy subgroup was statistically significant [30.8 versus 20.6 months (HR 0.78, 0.64–0.95)]; however, no differences were noted in patients who had received sequential chemo-radiotherapy. The second phase II trial the INSPIRE trial (BLP25/Stimuvax trial **I**n Asian **NS**CLC **P**atients stimulating **I**mmune **R**esponse) (NCT01015443) is a double-blinded randomized 2:1 (BLP25: placebo) trial and is still ongoing (28). The study will target 420 patients with unrespectable stage III NSCLC from 40 trial sites in Asia (China, Hong Kong, Singapore, South Korea, and Taiwan) excluding Japan.

MAGE-A3

Human melanoma antigen (MAGE)-A, -B, and -C are a family of genes normally expressed during embryogenesis and are also expressed in the immunoprivileged human tissues sites (29). Although, these genes are expressed in testicular germ cells and placenta trophoblasts, the antigens are not presented to the immune cells because of the lack of class I human leukocyte antigen molecules (HLA) (30, 31). For that reason, expression of these antigens on tumor cells that express class I HLA to the immune cells are likely immunogenic. Tumor cells such as melanoma, sarcoma, bladder, liver, esophageal, and lung cancers overexpress these antigens and hence considered tumor-associated antigens (32). MAGE-A3, a subtype of this family of genes, is differentially expressed in early stage (35%) and advanced stage (55%) lung cancer and hence it is theoretically a good target for tumor immunotherapy (33).

The MAGE-A3 vaccine (GlaxoSmithKline) is composed of the recombinant full-length protein MAGE-A3, *Haemophilus influenza* protein D that acts as an immune adjuvant and an immunostimulant AS02B or AS15 (34). The advantage of using the full protein is the production of several immunodominant epitopes that can be presented in the context of HLA class I and II and consequently activate both CD4 and CD8 T-cells. The broad array of T-cell responses can be in the form of Th response, cytotoxic T-cells (CTL), Th17 cells, and memory T-cells that result in immune effector antitumor immune responses (38). Recent findings indicate a beneficial role for MAGE-A3 vaccine in triggering the immune system including a study, which reported 84 genes as a gene expression signature (GS) in melanoma and NSCLC (35). These genes are involved in IFN-γ pathways, adaptive immunity, and specific chemokines that are responsible for T-cell activation and homing. When the MAGE-A3 vaccine was used with these GS-positive NSCLC patients, the disease free interval was in favor of the MAGE-A3 group compared to placebo group. In addition, no effect of the MAGE-A3 vaccine on the OS was noticed when GS was not taking into account, indicating that GS may act as an immune biomarker.

In order to evaluate the clinical benefit of the MAGE-A3 vaccine as an adjuvant treatment in postoperative lung cancer, 182 patients with completely resected MAGE-A3 positive stage IB/II NSCLC were enrolled into randomized (2:1 ratio), double-blinded, placebo-controlled phase II trial (36). Although all patients who received MAGE-A3 developed anti-MAGE-A3 immunoglobulin G antibodies, suggesting that vaccine triggered the immune response, no statistically significant difference were observed between the two groups with regards DFI, DFS, and OS. After applying forest plot analysis for HR (95% CI) to stratification factors, tumor stage, histology, and resection technique, all estimated values favored MAGE-A3 over placebo. Limited sample size and lack of chemotherapy as an adjuvant therapy were the main limitation of this study, which was later modified in the following phase III trial.

MAGRIT (**M**AGE-A3 as **A**djuvant **N**on-Small Cell Lun**G** Cance**R** Immu**noTh**erapy) was the largest ever phase III lung cancer adjuvant trial that aimed in determining the efficiency of MAGE-A3 vaccine as an adjuvant therapy following tumor resection in MAGE-A3 positive stage IB, II, and IIIA NSCLC (37). The other objectives were to study the toxicity. The study started in 2007 and recruited 2270 patient from 400 trial centers in 33 countries. Patients were randomly selected in 2:1 ratio and included patients who undergone surgery with or without adjuvant chemotherapy. Unfortunately, GlaxoSmithKline announced in April 2014 that MAGRIT study was to be discontinued due to failure to meet its primary objective, with no significant difference noted in DFS between MAGE-A3 and placebo group. Subgroup analyses are currently underway to see if there was a subpopulation that may have had more benefit.

OTHER

There are many other vaccination strategies currently in preclinical or early human clinical trial testing. One of these utilizes the antigen PRAME (preferentially expressed in melanoma) involved in retinoic acid receptor repression although expressed in low levels in many normal tissues and is overexpressed in both melanoma and NSCLC and therefore a vaccination target. A dose escalation study of recombinant PRAME protein in a liposomal formulation containing the immune adjuvant AS15 (GSK2302032) is currently recruiting patients with resected early stage NSCLC. Other vaccines directed at epidermal growth factor ligand in combination with cyclophosphamide (CIMAvax) and cell therapy and oncolytic viral strategies containing constructs expressing various antigens or immune stimulating cytokines (GM-CSF) are currently being investigated.

IMMUNE CHECKPOINT REGULATORS

Initiation of adaptive immunity is a complex multifaceted mechanism that takes place between APCs and T-cells. A homeostatic balance between stimulatory and inhibitory signals is required to prevent over/under stimulation of T-cells, which may result in autoimmunity or immunosuppression sequelae, respectively (38, 39). APCs take up foreign antigen, process it, and express the antigen on its surface in the context of class II HLA, which then engages the T-cell receptor on the surface of T-cells. A second signal through the costimulatory molecules facilitated by binding of CD28 on T-cell surface by CD86 (B7-2) on APCs. As a result of these specific interactions, T-cells are activated and secrete cytokines (third signal) such as IL-2 stimulating T-cell clonal proliferation. In order to prevent autoimmunity, T-cell proliferation is negatively regulated by cytotoxic T-lymphocyte antigen 4 (CTLA-4), which is expressed on the surface of activated T-cells. CTLA-4 is a member of immunoglobulin superfamily and binds to B7-2 with much higher affinity than CD28 and therefore when expressed the T-cell response is down regulated. Furthermore, CTLA-4 is expressed by the Tregs thereby enabling them to suppress the effector T-cells. CTLA-4 regulation takes place in the early activation phase of immune induction occurring in the regional lymph nodes at the level of the APC and unprimed T-cell interaction.

Another significant immune check point regulator molecule that has been extensively studied is the programed death-1 (PD-1) molecule (40). PD-1 is expressed on the surface of activated T-cells and its active ligand [PD-L (B7-H1)] is expressed on macrophages and can be also actively induced in endothelial, epithelial, and tumor cells. PD-1 can also binds to PDL-2, which is expressed mainly on APC and some tumor cells. Unlike CTLA-4 negative regulation PD-1/PDL-1 takes place in the peripheral tissue/tumor during the effector phase of T-cell activation. Both CTLA-4 and PD-1 have been targeted by inhibitory antibodies as an adjuvant therapy in cancer in attempt to enhance T-cell activation and tumor immunity (41).

IPILIMUMAB

Ipilimumab also known as MDX-010 and MDX-101 (Yervoy, Bristol-Myers Squibb) is a human monoclonal antibody directed against CTLA-4 molecule. Ipilimumab blocks the interaction of CTLA-4 with its ligand B7-2, resulting in T-cell activation, proliferation, induction of cytotoxic cytokines, and tumor suppression (42). Phase I/II trials have identified the safety and tolerability of CTLA-4 inhibition in several cancers that include the significant risk of colitis and, hepatotoxicity, skin rash, and hypophysitis/hypopituitarism (43). Moreover, they significantly improved OS in patients with malignant melanoma in phase III trials (44).

Two concurrent randomized phase II trials used ipilimumab in combination with chemotherapy (carboplatin/paclitaxel) for extensive stage small cell lung cancer ($n = 130$) and advanced stage NSCLC ($n = 204$) (45, 46). The primary endpoint of these studies was immune-related progression-free survival (irPFS). Secondary endpoints included PFS, best overall response rate (BORR), immune-related BORR (irBORR), OS, and safety. Patients were randomized to three groups (1:1:1), placebo/chemotherapy alone for up to six cycles, concurrent ipilimumab plus chemotherapy (four doses of ipilimumab/chemotherapy followed by two doses of placebo/chemotherapy) or phased ipilimumab (two doses of placebo/chemotherapy followed by four cycles of ipilimumab/chemotherapy). Phased ipilimumab, but not concurrent ipilimumab group, significantly improved irPFS in both the SCLC and NSCLC studies (HR 0.64 $p = 0.03$; HR 0.72, $p = 0.05$, respectively) and PFS in the NSCLC study (HR 0.69, $p = 0.02$) compared to patients who received placebo/chemotherapy alone. This finding was felt to be explained by chemotherapy induced tumor antigen release by chemotherapy trigger T-cell activation thus augmenting the effects of the immune checkpoint blockade (47). Of note, the improved irPFS in the phased ipilimumab NSCLC study was mainly confined to patients who had squamous cell histology. This is consistent with an increase T-cell infiltration found in squamous NSCLC (48). Further, an interesting case report of a patient with metastatic systemic treatment refractory NSCLC who was treated with palliative concurrent radiotherapy and ipilimumab that was associated with both a local and distant tumor complete response. A post-treatment increase in tumor-infiltrating cytotoxic lymphocytes, tumor regression, and normalization of tumor markers was observed. One year after treatment the patient was without evidence of disease based on PET/CT imaging (52). Two phase III trials NCT01450761 (ED-SCLC, etoposide/platinum, $N = 1125$, first data November 2015) and NCT01285609 (advanced NSCLC, carboplatin/paclitaxel, $N = 920$, first data October 2015) are still recruiting participants comparing ipilimumab plus chemotherapy versus chemotherapy alone in patients recently diagnosed ED-SCLC and squamous NSCLC, respectively.

NIVOLUMAB AND MK-3475

Nivolumab (Bristol-Myers Squibb) and MK-3475 (Merck) are fully human antibodies that inhibit PD-1 receptors expressed on activated T-cells (49). Both block the binding of PDL-1/2 with PD-1 on surface of activated T-cells, and consequently increases T-cell activation by removing the inhibitory signaling of PD-1 (50). As PDL-1 is only expressed on selected tumor cells, the adverse effect of the drug is expected to be less than ipilimumab. A Phase I trial ($N = 129$) for nivolumab at three different doses (1, 5, and 10 mg/kg every 2 week) in NSCLC treatment refractory patients reported an overall 2 years survival rate 24% with median OS of 9.9 months with minimal toxicity (51). Interestingly, the 3 mg/kg group did the best with a BORR of 24.3% and a duration of response of 74 weeks and a median OS of 14.9 months. A Phase III trial involving nivolumab compared to docetaxel in second line and beyond is ongoing (NCT01673867) and will recruit 582 patients with metastatic/recurrent non-squamous NSCLC with a primary objective of OS in PD-1 inhibitor versus chemotherapy groups. The secondary objectives will determine PFS and disease related symptom progression, and evaluation of clinical benefit of PD-1 blocker. A second Phase III trial has just started accrual in advanced stage NSCLC PD-1 positive patients in first-line setting randomized to 3 mg/kg nivolumab every 2 week versus investigator choice chemotherapy. It is anticipated that 330 patients will be accrued to the study with a reporting date in 2017.

Merck also announced the result of phase Ib trial with a 24% immune-related response (IRRC), median OS was under a year and with minimal toxicity (49). Interestingly, 6/9 patients who met the IRRC had high levels of PDL-1, suggesting that this could be a predictor of response and survival. There are also six ongoing Phase I and Phase II studies involving PDL-1 blocking antibodies (MPDL3280A) in NSCLC.

SUMMARY

Targeting the immune system as a viable strategy for the treatment of lung cancer was until very recently not felt to be viable. Lung cancer historically was never felt to be a cancer histology that would lend itself to immune manipulation; however, we are now in an era of increased understanding of the complexity of tumor-immune interactions, which has facilitated over the past 5 years an increased interest and application of immune therapeutic strategies. The use of lung cancer directed vaccines and immune checkpoint inhibitors are driving these activities, however, in the future, it remains to be seen if tumor microenvironment cellular populations such as Tregs, myeloid derived suppressor cells (MDSC), tumor-associated macrophages, or soluble tumor immunosuppressive mediators such as indoleamine 2,3-dioxygenase (IDO), arginase, IL-6, IL-10, and other cytokines/chemokines will also be able to be targeted. Further, oncolytic viruses armed with immune stimulating constructs or in combination with immune checkpoint inhibitors, adoptive cellular therapies remain relatively untested in the clinic and are attractive to consider.

REFERENCES

1. World Health Organization. *Cancer* (2013). Available from: http://www.who.int/mediacentre/factsheets/fs297/en/
2. Brodowicz T, Krzakowski M, Zwitter M, Tzekova V, Ramlau R, Ghilezan N, et al. Cisplatin and gemcitabine first-line chemotherapy followed by maintenance gemcitabine or best supportive care in advanced non-small cell lung cancer: a phase III trial. *Lung Cancer* (2006) **52**(2):155–63. doi:10.1016/j.lungcan.2006.01.006
3. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. *Mayo Clin Proc* (2008) **83**(5):584–94. doi:10.4065/83.5.584
4. Katzel JA, Fanucchi MP, Li Z. Recent advances of novel targeted therapy in non-small cell lung cancer. *J Hematol Oncol* (2009) **2**:2. doi:10.1186/1756-8722-2-2
5. Shaw AT, Yeap BY, Solomon BJ, Riely GJ, Gainor J, Engelman JA, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. *Lancet Oncol* (2011) **12**(11):1004–12. doi:10.1016/S1470-2045(11)70232-7
6. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med* (2009) **361**(10):958–67. doi:10.1056/NEJMoa0904554
7. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* (2011) **144**(5):646–74. doi:10.1016/j.cell.2011.02.013
8. Dougan M, Dranoff G. The immune response to tumors. *Curr Protoc Immunol* (2009) **Chapter 20**:Unit 20.11. doi:10.1002/0471142735.im2011s85
9. Dougan M, Dranoff G. Immune therapy for cancer. *Annu Rev Immunol* (2009) **27**:83–117. doi:10.1146/annurev.immunol.021908.132544

10. Kemmler CB, Clambey ET, Kedl RM, Slansky JE. Elevated tumor-associated antigen expression suppresses variant peptide vaccine responses. *J Immunol* (2011) **187**(9):4431–9. doi:10.4049/jimmunol.1101555
11. Drake CG, Lipson EJ, Brahmer JR. Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer. *Nat Rev Clin Oncol* (2014) **11**(1):24–37. doi:10.1038/nrclinonc.2013.208
12. Nemunaitis J, Murray N. Immune-modulating vaccines in non-small cell lung cancer. *J Thorac Oncol* (2006) **1**(7):756–61. doi:10.1097/01243894-200609000-00033
13. Akhurst RJ, Derynck R. TGF-beta signaling in cancer – a double-edged sword. *Trends Cell Biol* (2001) **11**(11):S44–51. doi:10.1016/S0962-8924(01)02130-4
14. Anscher MS, Kong FM, Andrews K, Clough R, Marks LB, Bentel G, et al. Plasma transforming growth factor beta1 as a predictor of radiation pneumonitis. *Int J Radiat Oncol Biol Phys* (1998) **41**(5):1029–35. doi:10.1016/S0360-3016(98)00154-0
15. Rochlitz C, Dreno B, Jantscheff P, Cavalli F, Squiban P, Acres B, et al. Immunotherapy of metastatic melanoma by intratumoral injections of Vero cells producing human IL-2: phase II randomized study comparing two dose levels. *Cancer Gene Ther* (2002) **9**(3):289–95. doi:10.1038/sj.cgt.7700441
16. Peat N, Gendler SJ, Lalani N, Duhig T, Taylor-Papadimitriou J. Tissue-specific expression of a human polymorphic epithelial mucin (MUC1) in transgenic mice. *Cancer Res* (1992) **52**(7):1954–60.
17. Guddo F, Giatromanolaki A, Koukourakis MI, Reina C, Vignola AM, Chlouverakis G, et al. MUC1 (episialin) expression in non-small cell lung cancer is independent of EGFR and c-erbB-2 expression and correlates with poor survival in node positive patients. *J Clin Pathol* (1998) **51**(9):667–71. doi:10.1136/jcp.51.9.667
18. Raina D, Kosugi M, Ahmad R, Panchamoorthy G, Rajabi H, Alam M, et al. Dependence on the MUC1-C oncoprotein in non-small cell lung cancer cells. *Mol Cancer Ther* (2011) **10**(5):806–16. doi:10.1158/1535-7163.MCT-10-1050
19. Ramlau R, Quoix E, Rolski J, Pless M, Lena H, Levy E, et al. A phase II study of Tg4010 (Mva-Muc1-Il2) in association with chemotherapy in patients with stage III/IV Non-small cell lung cancer. *J Thorac Oncol* (2008) **3**(7):735–44. doi:10.1097/JTO.0b013e31817c6b4f
20. Quoix E, Ramlau R, Westeel V, Papai Z, Madroszyk A, Riviere A, et al. Therapeutic vaccination with TG4010 and first-line chemotherapy in advanced non-small-cell lung cancer: a controlled phase 2B trial. *Lancet Oncol* (2011) **12**(12):1125–33. doi:10.1016/S1470-2045(11)70259-5
21. Butts C, Murray N, Maksymiuk A, Goss G, Marshall E, Soulieres D, et al. Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. *J Clin Oncol* (2005) **23**(27):6674–81. doi:10.1200/JCO.2005.13.011
22. Wurz GT, Gutierrez AM, Greenberg BE, Vang DP, Griffey SM, Kao CJ, et al. Antitumor effects of L-BLP25 antigen-specific tumor immunotherapy in a novel human MUC1 transgenic lung cancer mouse model. *J Transl Med* (2013) **11**(1):64. doi:10.1186/1479-5876-11-64
23. Mehta NR, Wurz GT, Burich RA, Greenberg BE, Griffey S, Gutierrez A, et al. L-BLP25 vaccine plus letrozole induces a TH1 immune response and has additive antitumor activity in MUC1-expressing mammary tumors in mice. *Clin Cancer Res* (2012) **18**(10):2861–71. doi:10.1158/1078-0432.CCR-12-0168
24. Clark WH Jr, Mastrangelo MJ, Ainsworth AM, Berd D, Bellet RE, Bernardino EA. Current concepts of the biology of human cutaneous malignant melanoma. *Adv Cancer Res* (1977) **24**:267–338. doi:10.1016/S0065-230X(08)61017-9
25. Berd D, Mastrangelo MJ. Effect of low dose cyclophosphamide on the immune system of cancer patients: reduction of T-suppressor function without depletion of the CD8+ subset. *Cancer Res* (1987) **47**(12):3317–21.
26. Ghiringhelli F, Menard C, Puig PE, Ladoire S, Roux S, Martin F, et al. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. *Cancer Immunol Immunother* (2007) **56**(5):641–8. doi:10.1007/s00262-006-0225-8
27. Butts C, Socinski MA, Mitchell PL, Thatcher N, Havel L, Krzakowski M, et al. Tecemotide (L-BLP25) versus placebo after chemoradiotherapy for stage III non-small-cell lung cancer (START): a randomised, double-blind, phase 3 trial. *Lancet Oncol* (2014) **15**(1):59–68. doi:10.1016/S1470-2045(13)70510-2
28. Wu YL, Park K, Soo RA, Sun Y, Tyroller K, Wages D, et al. INSPIRE: a phase III study of the BLP25 liposome vaccine (L-BLP25) in Asian patients with unresectable stage III non-small cell lung cancer. *BMC Cancer* (2011) **11**:430. doi:10.1186/1471-2407-11-430
29. Jungbluth AA, Silva WA Jr, Iversen K, Frosina D, Zaidi B, Coplan K, et al. Expression of cancer-testis (CT) antigens in placenta. *Cancer Immun* (2007) **7**:15.
30. Juch H, Blaschitz A, Dohr G, Hutter H. HLA class I expression in the human placenta. *Wien Med Wochenschr* (2012) **162**(9–10):196–200. doi:10.1007/s10354-012-0070-7
31. Jassim A, Ollier W, Payne A, Biro A, Oliver RT, Festenstein H. Analysis of HLA antigens on germ cells in human semen. *Eur J Immunol* (1989) **19**(7):1215–20. doi:10.1002/eji.1830190710
32. Jungbluth AA, Busam KJ, Kolb D, Iversen K, Coplan K, Chen YT, et al. Expression of MAGE-antigens in normal tissues and cancer. *Int J Cancer* (2000) **85**(4):460–5. doi:10.1002/(SICI)1097-0215(20000215)85:4<460::AID-IJC3>3.0.CO;2-N
33. Kim SH, Lee S, Lee CH, Lee MK, Kim YD, Shin DH, et al. Expression of cancer-testis antigens MAGE-A3/6 and NY-ESO-1 in non-small-cell lung carcinomas and their relationship with immune cell infiltration. *Lung* (2009) **187**(6):401–11. doi:10.1007/s00408-009-9181-3
34. Marchand M, Punt CJ, Aamdal S, Escudier B, Kruit WH, Keilholz U, et al. Immunisation of metastatic cancer patients with MAGE-3 protein combined with adjuvant SBAS-2: a clinical report. *Eur J Cancer* (2003) **39**(1):70–7. doi:10.1016/S0959-8049(02)00479-3
35. Ulloa-Montoya F, Louahed J, Dizier B, Gruselle O, Spiessens B, Lehmann FF, et al. Predictive gene signature in MAGE-A3 antigen-specific cancer immunotherapy. *J Clin Oncol* (2013) **31**(19):2388–95. doi:10.1200/JCO.2012.44.3762
36. Vansteenkiste J, Zielinski M, Linder A, Dahabreh J, Gonzalez EE, Malinowski W, et al. Adjuvant MAGE-A3 immunotherapy in resected non-small-cell lung cancer: phase II randomized study results. *J Clin Oncol* (2013) **31**(19):2396–403. doi:10.1200/JCO.2012.43.7103
37. Tyagi P, Mirakhur B. MAGRIT: the largest-ever phase III lung cancer trial aims to establish a novel tumor-specific approach to therapy. *Clin Lung Cancer* (2009) **10**(5):371–4. doi:10.3816/CLC.2009.n.052
38. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol* (2005) **23**:515–48. doi:10.1146/annurev.immunol.23.021704.115611
39. Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. *Nat Rev Immunol* (2008) **8**(6):467–77. doi:10.1038/nri2326
40. Riella LV, Paterson AM, Sharpe AH, Chandraker A. Role of the PD-1 pathway in the immune response. *Am J Transplant* (2012) **12**(10):2575–87. doi:10.1111/j.1600-6143.2012.04224.x
41. Ramsay AG. Immune checkpoint blockade immunotherapy to activate anti-tumour T-cell immunity. *Br J Haematol* (2013) **162**(3):313–25. doi:10.1111/bjh.12380
42. Cameron F, Whiteside G, Perry C. Ipilimumab: first global approval. *Drugs* (2011) **71**(8):1093–104. doi:10.2165/11594010-000000000-00000
43. Slovin SF, Higano CS, Hamid O, Tejwani S, Harzstark A, Alumkal JJ, et al. Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. *Ann Oncol* (2013) **24**(7):1813–21. doi:10.1093/annonc/mdt107
44. Ribas A, Kefford R, Marshall MA, Punt CJ, Haanen JB, Marmol M, et al. Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. *J Clin Oncol* (2013) **31**(5):616–22. doi:10.1200/JCO.2012.44.6112
45. Reck M, Bondarenko I, Luft A, Serwatowski P, Barlesi F, Chacko R, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line therapy in extensive-disease-small-cell lung cancer: results from a randomized, double-blind, multicenter phase 2 trial. *Ann Oncol* (2013) **24**(1):75–83. doi:10.1093/annonc/mds213
46. Lynch TJ, Bondarenko I, Luft A, Serwatowski P, Barlesi F, Chacko R, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. *J Clin Oncol* (2012) **30**(17):2046–54. doi:10.1200/JCO.2011.38.4032
47. Ramakrishnan R, Assudani D, Nagaraj S, Hunter T, Cho HI, Antonia S, et al. Chemotherapy enhances tumor cell susceptibility to CTL-mediated killing during cancer immunotherapy in mice. *J Clin Invest* (2010) **120**(4):1111–24. doi:10.1172/JCI40269

48. Hiraoka K, Miyamoto M, Cho Y, Suzuoki M, Oshikiri T, Nakakubo Y, et al. Concurrent infiltration by CD8+ T cells and CD4+ T cells is a favourable prognostic factor in non-small-cell lung carcinoma. *Br J Cancer* (2006) **94**(2):275–80. doi:10.1038/sj.bjc.6602934
49. PD-1 inhibitors raise survival in NSCLC. *Cancer Discov* (2014) **4**(1):6. doi:10.1158/2159-8290.CD-NB2013-164
50. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol* (2008) **26**:677–704. doi:10.1146/annurev.immunol.26.021607.090331
51. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* (2012) **366**(26):2443–54. doi:10.1056/NEJMoa1200690
52. Golden EB, Demaria S, Schiff PB, Chachoua A, Formenti SC. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. *Cancer Immunol Res* (2013) **1**(6):365–72.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 July 2014; accepted: 06 October 2014; published online: 22 October 2014.
*Citation: Mostafa AA and Morris DG (2014) Immunotherapy for lung cancer: has it finally arrived? Front. Oncol. **4**:288. doi: 10.3389/fonc.2014.00288*
This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 25 November 2014
doi: 10.3389/fonc.2014.00329

Promising targets and current clinical trials in metastatic non-squamous NSCLC

Alona Zer and Natasha Leighl*

Division of Medical Oncology, Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada

Edited by:
Barbara Melosky, British Columbia Cancer Agency, Canada

Reviewed by:
Marina Chiara Garassino, Istituto Nazionale dei Tumori, Italy
Meng Xu Welliver, The Ohio State University James Cancer Center, USA

****Correspondence:***
Natasha Leighl, Division of Medical Oncology, Princess Margaret Cancer Centre, University of Toronto, 5-105 610 University Avenue, Toronto, ON M5G 2M9, Canada
e-mail: natasha.leighl@uhn.ca

Lung adenocarcinoma is the most common subtype of lung cancer today. With the discovery of epidermal growth factor receptor (*EGFR*) mutations, anaplastic lymphoma kinase (*ALK*) rearrangements, and effective targeted therapy, personalized medicine has become a reality for patients with lung adenocarcinoma. Here, we review potential additional targets and novel therapies of interest in lung adenocarcinoma including targets within the cell surface (receptor tyrosine kinases *EGFR*, human epidermal growth factor receptor 2, *RET*, *ROS1*, mesenchymal–epidermal transition, TRK), targets in intracellular signal transduction (*ALK*, RAS–RAF–*MEK*, PI3K–*AKT*–PTEN, WNT), nuclear targets such as poly-ADP ribose polymerase, heat shock protein 90, and histone deacetylase, and selected pathways in the tumor environment. With the evolving ability to identify specific molecular aberrations in patient tumors in routine practice, our ability to further personalize therapy in lung adenocarcinoma is rapidly expanding.

Keywords: NSCLC, nuclear targets, intracellular pathways, *EGFR*, *ALK*, novel targets, non-squamous

INTRODUCTION

In recent years, we have witnessed a transformation of the treatment paradigm for advanced non-small cell lung cancer (NSCLC). Previously, patients were offered platinum-based chemotherapy, followed by second-line chemotherapy with docetaxel or pemetrexed, and erlotinib after chemotherapy failure, yielding modest benefits in an unselected population (1). Using molecular selection, clinical trials of targeted therapy have demonstrated major improvements in response, quality of life, and progression-free survival compared to chemotherapy, using epidermal growth factor receptor (*EGFR*) TKI in *EGFR* mutant NSCLC and crizotinib in anaplastic lymphoma kinase (*ALK*) rearranged NSCLC (2, 3). Survival is similar in many of these trials, given the high rate of crossover from chemotherapy to the more active agent upon progression.

It is now standard of care to test non-squamous lung carcinoma for the presence of *EGFR* mutation and *ALK* rearrangement upon diagnosis of advanced disease (4), in order to select patients for initial *EGFR* TKI and *ALK* inhibitor therapy. The remarkable activity of these agents in molecularly selected lung cancer patients has led to a rapid increase in studies evaluating new targets and novel targeted agents. These targets include oncogenic driver mutations (genomic alterations that initiate malignant transformation of the normal cell), signal transduction proteins, tumor angiogenesis, and factors in the tumor environment supporting cancer cell proliferation (for example, immune-modulating proteins) (**Figure 1**; **Table 1**). In this review, we discuss selected new and promising targets as well as targeted therapies currently under investigation in non-squamous NSCLC, specifically adenocarcinoma.

TARGETS WITHIN THE CELL SURFACE

EPIDERMAL GROWTH FACTOR RECEPTOR

Targeting *EGFR* has led to a breakthrough in understanding of lung cancer biology, and the NSCLC treatment paradigm. Mutations in *EGFR*, resulting in greater affinity for ATP binding by the *EGFR* tyrosine kinase domain and constitutive activation, are found in ~15% of lung cancers in Caucasians and 40% in Asians (5, 6). Activating mutations are significantly associated with response to *EGFR* TKIs, with erlotinib, gefitinib, and afatinib established as initial standard therapy. However, resistance mutations have been identified, such as T790M in exon 20. There are multiple agents in development with enhanced affinity for T790M mutant lung cancer that may spare wild type *EGFR*, potentially avoiding toxicities like rash and diarrhea. AZD9291 and CO-1686 are examples of such agents, and have reported responses in 58–64% of patients with acquired *EGFR* TKI resistance and documented T790M mutation (7, 8). There are other strategies in development, targeting acquired *EGFR* TKI resistance including chemotherapy with intercalated *EGFR* TKI, combinations with mesenchymal–epidermal transition (*MET*), dual *EGFR* and heat shock protein 90 (HSP90) inhibitors, and more. For example, combination of afatinib and cetuximab has demonstrated activity in patients with acquired *EGFR* TKI resistance and T790M positive and negative tumors (9), and the addition of AUY922 to erlotinib has restored sensitivity in 22% of patients with acquired resistance to erlotinib (10).

HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2

Human epidermal growth factor receptor 2 is a cell surface receptor, and member of the erbB receptor tyrosine kinase family. It is activated by heterodimerization with other ligand-bound members of the erbB family, or by homodimerization. *HER2* is a key oncogene in breast cancer, and is associated with improved outcomes with trastuzumab (anti-*HER2* monoclonal antibody) (11, 12). In NSCLC, *HER2* protein overexpression is found in 6–35% of patients and *HER2* gene amplification is found in 10–20% (13). Trastuzumab has shown minimal activity in lung cancer, both as a

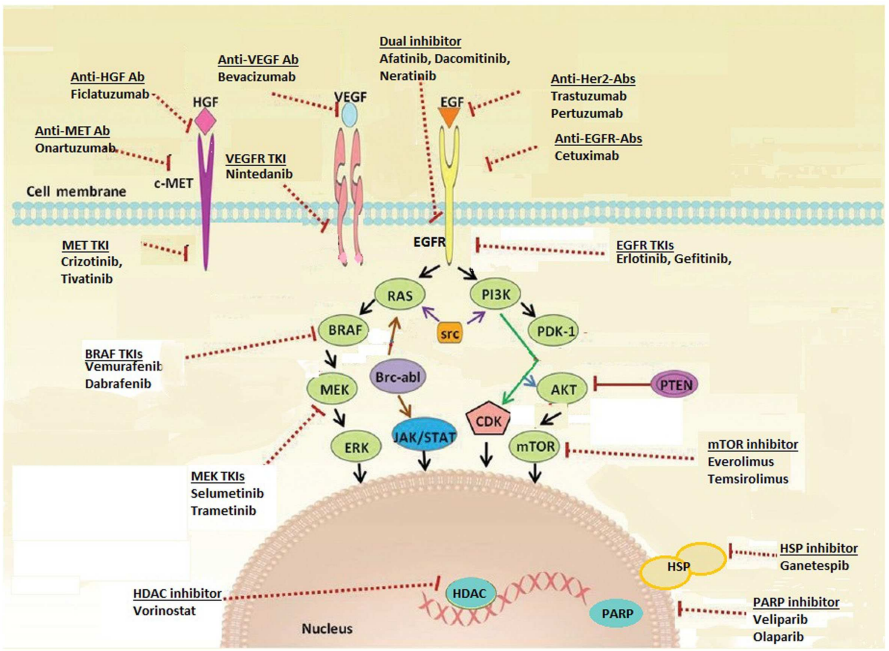


FIGURE 1 | Targetable pathways in the non-squamous NSCLC cell.

single agent and in combination with chemotherapy, particularly in patients with FISH positive or IHC 3+ tumors (14, 15).

HER2 mutations are seen in 2.8–6% of lung adenocarcinomas (16, 17), more commonly in women and non-smokers. These mutations are commonly exon 20 in-frame insertions. Activity has been seen with trastuzumab-based therapy and afatinib (13, 18). A phase I trial of neratinib (an irreversible pan-HER inhibitor) and temsirolimus (mTOR inhibitor) suggested benefit in five patients with *HER2*-mutant NSCLC (19). A phase II trial assessing this combination is underway. Other trials include studies of *HER2*-directed antibodies (trastuzumab, pertuzumab), TKIs (neratinib, dacomitinib, and afatinib), and a peptide vaccine (www.clinicaltrials.gov).

RET

RET (rearranged during transfection), is a known oncogene in thyroid cancer, with both activating mutations and gene rearrangements observed (20). Approximately 1.5% of NSCLC cases have *RET* translocations, typically in younger, non-smoking adenocarcinoma patients (21). Fusion variants include *KIF5B-RET* in adenocarcinoma, CCDC6, NCO4, and TRIM33 also found in thyroid cancer (22, 23).

Vandetanib, sunitinib, sorafenib, lenvatinib, ponatinib, and cabozantinib are all multi-targeted kinase inhibitors that target *RET*. Activity has been seen in *RET*-positive lung cancer patients with cabozantinib and vandetinib, and multiple trials are ongoing in this population with a recent halt in a ponatinib study for safety concerns (24, 25).

ROS1

ROS1 encodes a receptor tyrosine kinase of the insulin receptor super family, with no known ligand and little known about its normal function. *ROS1* fusion genes, with oncogenic transformation potential, have been described in multiple tumor cell lines, including lung cancer. The prevalence of *ROS1* rearrangement in NSCLC is estimated at 1–2%, and can be detected using FISH or IHC. Patients, similar to those with *ALK*-rearranged lung cancer, tend to be younger, never smokers, and have adenocarcinoma histology, although cases in squamous carcinoma have been reported (26). A response rate of 60% has been reported with crizotinib in 35 patients with *ROS-1*-rearranged lung cancer, including two patients with complete response, and median PFS was not reached (27). Multiple other agents are under development including AP26113, foretinib, PF-06463922, ceritinib, and HSP90 inhibitors such as AT13387 (NCT01712217).

MESENCHYMAL–EPIDERMAL TRANSITION RECEPTOR

Mesenchymal–epidermal transition is a receptor tyrosine kinase, which undergoes homodimerization by binding its ligand, hepatocyte growth factor (HGF), to trigger intracellular signaling cascades, including PI3K–*AKT*–mTOR and RAS–RAF–MAPK pathways. In lung cancer, *MET* mutations are rare, but amplification is seen in up to 21%, resulting in constitutive *MET* activation and is believed to be a potential mechanism of acquired *EGFR* TKI resistance (28, 29). *MET* expression is seen in at least one-third of lung cancers, including adenocarcinoma and squamous histology (30).

Targeting *MET* protein-expressing lung cancer has not been successful to date, with negative phase III trials of onartuzumab (anti-*MET* monoclonal antibody), and TKIs including tivantinib (31, 32). Crizotinib activity has been reported in *MET*-amplified tumors (33), with ongoing studies in *EGFR* TKI-resistant lung cancer of *MET* and HGF-targeted agents, such as ficlatuzumab (anti-HGF monoclonal antibody, NCT02034981).

Table 1 | Selected targets and selected targeted agents in lung adenocarcinoma.

Target	Frequency (common clinical features)	Selected agents under study	Current development
CELL SURFACE TARGETS			
EGFR mutations (*EGFR* TKI acquired resistance)	17–43% (female, never smokers, Asian)	AZD9291 CO-1686 HM-61713 Afatinib + cetuximab Erlotinib + AUY922 Gefitinib + INC280 (*MET* TKI)	Phase II/III
HER2 insertion mutation	3–6% (female, never smokers)	Trastuzumab + chemotherapy Afatinib Pertuzumab Neratinib + temsirolimus	Phase II
RET fusion	1–2% (young, never smokers, poorly differentiated tumor)	Vandetanib Cabozantinib Lenvatinib Ponatinib	Phase II
INTRACELLULAR PATHWAYS			
ROS1 fusion	1–2% (young, never smokers)	Crizotinib Ceritinib AP26113 Foretinib PF-06463922 AT13387	Phase I/II
MET amplification	~1%	Crizotinib	Phase I
MET expression	Up to 50%	Ficlatuzumab	Phase II/III
NTRK-1 fusion	~1%	RXDX101	Phase I
KRAS mutations	Up to 30%	Selumetinib + chemotherapy Trametinib + chemotherapy MEK162 + chemotherapy	Phase I–III
BRAF mutation	3%, smokers	Dabrafenib Vemurafenib	Phase I/II
mTOR activation	Up to 90%	Everolimus Temsirolimus Sirolimus	Phase II
NUCLEAR TARGETS			
PARP	n/a	Olaparib + chemotherapy Veliparib + chemotherapy	Phase II/III
HDAC	n/a	Romidepsin Pabinostat Etinostat	Phase II
TUMOR ENVIRONMENT			
RANK-Ligand	n/a	Denosumab + chemotherapy	Phase III
VEGF	n/a	Bevacizumab Nintedanib	Phase II/III
CTLA-4	n/a	Ipilimumab	Phase II/III
PD-1 PD-L1	~40% of lung adenocarcinomas express PD-L1	Nivolumab Lambrolizumab BMS-936559 MPDL-3286A	Phase II/III

n/a, not available; EGFR TKI, epidermal growth factor receptor tyrosine kinase inhibitor; NTRK, neurotrophic tyrosine kinase receptor type; mTOR, mammalian target of rapamycin; PARP, poly-ADP ribose polymerase; HDAC, histone deacetylases; RANKL, receptor activator of nuclear factor kappa-B ligand; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programed cell death protein 1; PD-L1, programed cell death protein 1 ligand.

NTRK1 FUSIONS

These have recently been described in never smokers with adenocarcinoma that is *ALK* and *EGFR* wild type. *NTRK-1* fusions have been identified in 3 of 91 lung adenocarcinoma samples that were *EGFR/KRAS/ALK-1/ROS-1* negative (34). RXDX101 has demonstrated activity in TRK-fusion positive lung cancer in a recent phase I trial (35).

TARGETS WITHIN INTRACELLULAR PATHWAYS

ANAPLASTIC LYMPHOMA KINASE

Anaplastic lymphoma kinase fusion genes, resulting in *ALK* fusion proteins, are present in 3–5% of lung adenocarcinomas, commonly in never smokers and younger patients. The presence of *ALK* fusion strongly predicts response to *ALK* TKIs, such as crizotinib, ceritinib, and others. This topic is discussed in length in a separate review article in this issue.

RAS–RAF–MEK pathway

The *RAS* family of oncogenes includes *H-RAS*, *K-RAS*, and *N-RAS*. RAS proteins encode a membrane-bound GTP-ase that mediates signal transduction from various tyrosine kinase receptors (e.g., *EGFR*, *HER2*) to the RAF/*MEK*/ERK pathway and others, regulating cell growth, proliferation, and apoptosis (36). *KRAS* mutations are seen in ~30% of Western adenocarcinoma cases, fewer in Asian populations, most commonly in codons 12 or 13. *NRAS* and *HRAS* mutations are less common in lung cancer, <1% (37).

K-RAS

The role of mutant *KRAS* (V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) as a prognostic or predictive marker in NSCLC remains controversial. An analysis of LACE-BIO suggests that it is not prognostic in early stage lung cancer, nor does it predict for adjuvant chemotherapy benefit (38). Several studies suggest that it is a potential negative predictor of benefit from *EGFR* TKI (39). While *KRAS* mutations have been identified in patients with and without smoking histories, never smokers are more likely to have transition mutations. Transversion mutations are found almost exclusively in smokers (40).

The most promising agents in development for *KRAS* mutant lung cancer have been *MEK* inhibitors combined with chemotherapy. Selumetinib, a MEK1/2 inhibitor, significantly improved PFS and response when added to docetaxel versus docetaxel plus placebo (HR = 0.58, $p = 0.014$, RR 37 vs. 0%, $p < 0.0001$), with a trend toward greater survival (41); a phase III trial is ongoing. Trametinib, another *MEK* inhibitor, showed activity in combination with docetaxel as well as with pemetrexed (42, 43). The response rate with single agent trametinib is 12%, with similar activity to docetaxel in pre-treated *KRAS* mutant lung cancer patients (44).

BRAF

BRAF, a serine-threonine kinase, lies downstream of *KRAS* and directly activates *MEK* by phosphorylation, which in turn activates ERK. *BRAF* (v-Raf murine sarcoma viral oncogene homolog B) mutations and *BRAF* inhibitors first gained attention in melanoma where 40–60% of tumors harbor activating V600E *BRAF* mutations. Three percent of lung adenocarcinomas harbor *BRAF* mutations, half of the V600E subtype, inducing constitutive kinase activity. These mutations occur more frequently in smokers. Dabrafenib, a *BRAF* kinase inhibitor, demonstrated a 54% RR in 17 *BRAF* V600E-mutated NSCLC patients (45). Vemurafenib is another *BRAF* kinase inhibitor that shown activity in this population. There are ongoing clinical trials assessing *BRAF*, *MEK*, and *AKT* inhibitors in this population.

PI3K–AKT–PTEN pathway

The phosphatidylinositol 3-kinase (PI3K)–*AKT*–mTOR (mammalian target of rapamycin) signaling pathway is one of the most dysregulated pathways in human cancers, including NSCLC. PI3K can be activated by transmembrane receptor tyrosine kinases like *EGFR* or RAS, through phosphorylation of *AKT*. This inhibits pro-apoptotic proteins and promotes cell survival. Activated mTOR complexes (mTORC1), downstream of PI3K–*AKT*, result in increased ribosomal protein synthesis and further *AKT* activation (mTORC2). PI3K-dependent signal transduction can be terminated by PTEN, a tumor suppressor intracellular protein (46).

PIK3CA

PIK3CA encodes the catalytic subunit of PI3K, and mutations and amplification are seen in 2 and 12–17% of NSCLC cases (47, 48). These mutations can co-exist with other known driver mutations in lung adenocarcinoma, including *EGFR* and *KRAS* and in the setting of acquired *EGFR* TKI resistance (49, 50), suggesting that this may not be a driver mutation in itself. Trials of PI3K specific kinase inhibitors are ongoing.

PTEN, *AKT*, mTOR

Loss of PTEN protein expression, with subsequent *AKT* overexpression, occurs in a third of NSCLC cases, and is associated with poor prognosis in lung cancer (51). This may be related to epigenetic silencing, as *PTEN* mutations are rare in NSCLC (52). *AKT* activation and mTOR phosphorylation is found in 51% of NSCLC cases, although *AKT* mutations are rare (<1%). Given the high level of activation and "crosstalk" with the RAS–RAF–*MEK* pathway, studies of mTOR and *AKT* inhibitors are of major interest in lung cancer. Everolimus (RAD001), temsirolimus, and other mTOR inhibitors are being investigated in combination with other targeted agents, including *EGFR* TKIs, although toxicity of these agents remains challenging, with high rates of fatigue and stomatitis (53, 54).

Wnt-beta-catenin pathway

The Wnt signaling pathway is highly active in lung cancer and correlates with metastasis and proliferation, and is believed to maintain cancer stem cells. Activated Wnt signaling inhibits the proteolysis of beta-catenin. Accumulated beta-catenin in cytoplasm moves to the nucleus where it initiates transcription factors promoting cell growth and chemo- and radio-resistance. Down-regulation of Wnt inhibitors is common in NSCLC samples and associated with poor prognosis (55). *WNT* mutations are rare in lung cancer and mutations in Beta-catenin are detected in 2% of lung adenocarcinoma (56). Several targeted therapies against the Wnt pathway are being investigated in early phase trials, including PRI-724, a small molecule beta-catenin inhibitor.

NUCLEAR TARGETS

POLY-ADP RIBOSE POLYMERASE

BRCA1, BRCA2, and PALB2 are proteins responsible for repair of DNA double-strand breaks through the homologous repair pathway; breaks that are not repaired lead to apoptosis. This repair pathway can be disrupted by mutations in *BRCA1*, *BRCA2*, or *ATM* (ataxia telangiectasia mutated), found in 7% of lung adenocarcinomas. High levels of BRCA1 protein expression in lung cancer correlate with poor survival, while decreased expression predicts response to platinum-based chemotherapy (57, 58). The poly-ADP ribose polymerase (PARP) enzyme is key in repairing single-strand DNA breaks, which may lead to double-strand breaks. BRCA deficient or mutated cells are sensitive to PARP inhibition, which may also sensitize cancer cells to alkylator or platinum damage via DNA single- or double-strand breaks. Despite a negative study with iniparib and chemotherapy, veliparib, and olaparib are being evaluated in combination with platinum-based therapy or *EGFR* TKI in NSCLC.

Heat shock protein 90

Heat shock protein 90 is a chaperone protein that assists post-translational folding of several proteins to stabilize and protect them from cellular stresses like heat or hypoxia, including critical proteins in lung cancer such as *EGFR*, *HER2*, *MET*, *ALK*, and others. HSP90 inhibitors have shown activity in *EGFR* mutant lung cancer after the development of resistance, in *ALK*-rearranged tumors and more recently in *EGFR* wild type adenocarcinoma when combined with chemotherapy (59). A phase III clinical trial of docetaxel plus or minus ganetespib in chemo-naïve adenocarcinoma is ongoing. Other HSP90 inhibitors under active investigation in lung cancer include retaspimycin (IPI-504), AUY992, and AT13387.

Histone deacetylase

Histones are a family of proteins bound to DNA strands that maintain the helical structure of DNA. DNA expression is regulated by acetylation and deacetylation of histones. Deacetylation results in condensed DNA and reduced transcription. But histone deacetylase (HDAC), highly expressed in most cancers, may also alter activity of various proteins involved in carcinogenesis including HSP90, STAT3, and p53. HDAC inhibitors have multiple effects on DNA transcription, including induction of HSP90 acetylation (see above), disrupting its function, and resulting in tumor apoptosis. Vorinostat, FDA approved for treatment of cutaneous T-cell lymphoma, showed initial promise when added to chemotherapy in advanced NSCLC, although the subsequent phase III trial was negative (60). Other HDAC inhibitors being studied include etinostat, romidepsin, pabinostat, pivanex, and CI-994.

TARGETS IN THE TUMOR ENVIRONMENT

ANGIOGENESIS

Vascular endothelial growth factor (VEGF) is a pro-angiogenic factor, with a key role in tumor angiogenesis. Its high expression in a variety of tumors, including NSCLC, is associated with poor prognosis (61). Although multiple agents targeting VEGF and VEGF receptors have been studied in lung cancer, only bevacizumab and more recently nintedanib have improved survival in advanced non-squamous NSCLC. Bevacizumab, combined with paclitaxel and carboplatin, improved response, PFS, and survival in the practice-changing ECOG4599 trial (62), although subsequent bevacizumab trials have not improved survival compared to chemotherapy alone. Nintedanib, a multi-targeted VEGF- and FGFR-1 receptor TKI demonstrated greater OS in a subgroup of adenocarcinoma patients when added to docetaxel versus chemotherapy alone (63). Trials of multiple other agents have not demonstrated positive results, although trials with VEGF/R inhibitors, including in different molecular subtype of adenocarcinoma, are ongoing.

Vascular disrupting agents, such as vadimezan, target vasculature directly, not through VEGF/VEGFR. To date, trials of these and multiple other anti-angiogenic agents have not yet yielded benefit.

IMMUNE MODULATION

The immune system plays an active role in eradication of malignant cells. However, the evolution of cancer includes developing mechanisms to escape the immune system. Several approaches are now being investigated to boost anti-cancer immune response, either by inhibiting immune checkpoints (as CTLA-4, PD-1, and PD-L1) or by developing vaccines of cancer antigens. This topic is discussed in length in a separate review article, with the PD-1 checkpoint inhibitors as the most promising current target in immune therapy of lung cancer, with demonstrated single agent activity in both adenocarcinoma and squamous carcinoma (64).

There are multiple other potential targets in lung adenocarcinoma that are not reviewed here, such as the cell surface receptor insulin-like growth factor 1 receptor, apoptotic receptors, and proteins including TRAIL, BCL-1, IAP proteins including survivin, and the proteasome. Additional targets in the tumor environment include adhesion molecules such as integrins, and even osteoclasts, all potentially important targets in lung cancer with ongoing trials of targeted agents.

CONCLUSION

Striking therapeutic advances in metastatic NSCLC have been observed with targeted agents using molecular selection, notable for patients with *EGFR* mutant or *ALK*-rearranged lung cancer. Testing for these oncogenic drivers is now standard of care in advanced lung adenocarcinoma, but they are found in only ~20% of lung adenocarcinomas in Western populations, while remaining patients are eligible only for standard chemotherapy. However, this "success story," as well as improved understanding of molecular pathways of lung carcinogenesis, had led to rapid progress in the identification of novel targets in adenocarcinoma and potential therapies. Despite this enthusiasm, there are still barriers to overcome, including how to approach tumors without single oncogene addiction, i.e., targeting multiple pathways, and also how to overcome primary and secondary resistance to targeted therapies. Finally, the development of accurate, rapid, tissue-, and cost-conserving assays to identify multiple targets simultaneously, including targets beyond genomic sequencing, is urgently needed. In the meantime, drug development and discovery of novel targets in lung adenocarcinoma remain one of the fastest growing areas of research and development in oncology today.

REFERENCES

1. Leighl NB. Treatment paradigms for patients with metastatic non-small-cell lung cancer: first-, second- and third-line. *Curr Oncol* (2012) **19**(Suppl 1):S52–8. doi:10.3747/co.19.1114
2. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* (2009) **361**(10):947–57. doi:10.1056/NEJMoa0810699
3. Shaw AT, Kim DW, Nakagawa K, Seto T, Crinó L, Ahn MJ, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med* (2013) **368**(25):2385–94. doi:10.1056/NEJMoa1214886
4. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *J Thorac Oncol* (2013) **8**(7):823–59. doi:10.1097/JTO.0b013e318290868f
5. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med* (2009) **361**(10):958–67. doi:10.1056/NEJMoa0904554
6. Shi Y, Au JS, Thongprasert S, Srinivasan S, Tsai CM, Khoa MT, et al. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). *J Thorac Oncol* (2014) **9**(2):154–62. doi:10.1097/JTO.0000000000000033
7. Finlay MR, Anderton M, Ashton S, Ballard P, Bethel PA, Box MR, et al. Clinical activity of the mutant-selective EGFR inhibitor AZD9291 in patients (pts) with EGFR inhibitor-resistant non-small cell lung cancer (NSCLC). *J Clin Oncol* (2014) **32**(15_suppl: abstr. 8009):5s. doi:10.1021/jm500973a
8. Sequist LV, Soria J-C, Gadgeel SM, Wakelee HA, Camidge DR, Varga A, et al. First-in-human evaluation of CO-1686, an irreversible, highly selective tyrosine kinase inhibitor of mutations of EGFR (activating and T790M). *J Clin Oncol* (2014) **32**(15_suppl: abstr. 8010):5s.
9. Janjigian YY, Azzoli CG, Krug LM, Pereira LK, Rizvi NA, Pietanza MC, et al. Phase I/II trial of cetuximab and erlotinib in patients with lung adenocarcinoma and acquired resistance to erlotinib. *Clin Cancer Res* (2011) **17**(8):2521–7. doi:10.1158/1078-0432.CCR-10-2662
10. Johnson ML, Hart EM, Rademaker A, Weitner BB, Urman A, Simm HD, et al. A phase II study of HSP90 inhibitor AUY922 and erlotinib for patients with EGFR-mutant lung cancer and acquired resistance to EGFR tyrosine kinase inhibitors. *J Clin Oncol* (2013) **31**(Suppl):abstr 8036.
11. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* (2001) **344**(11):783–92. doi:10.1056/NEJM200103153441101
12. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE Jr, Davidson NE, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* (2005) **353**:1673–84. doi:10.1056/NEJMoa052122
13. Mazières J, Peters S, Lepage B, Cortot AB, Barlesi F, Beau-Faller M, et al. Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives. *J Clin Oncol* (2013) **31**(16):1997–2003. doi:10.1200/JCO.2012.45.6095
14. Clamon G, Herndon J, Kern J, Govindan R, Garst J, Watson D, et al. Lack of trastuzumab activity in nonsmall cell lung carcinoma with overexpression of erb-B2: 39810: a phase II trial of cancer and leukemia group B. *Cancer* (2005) **103**(8):1670–5. doi:10.1002/cncr.20950
15. Gatzemeier U. Randomized phase II trial of gemcitabine-cisplatin with or without trastuzumab in HER2-positive non-small-cell lung cancer. *Ann Oncol* (2004) **15**(1):19–27. doi:10.1093/annonc/mdh031
16. Shigematsu H, Takahashi T, Nomura M, Majmudar K, Suzuki M, Lee H, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. *Cancer Res* (2005) **65**(5):1642–6. doi:10.1158/0008-5472.CAN-04-4235
17. Arcila ME, Chaft JE, Nafa K, Roy-Chowdhuri S, Lau C, Zaidinski M, et al. Prevalence, clinicopathologic associations, and molecular spectrum of ERBB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. *Clin Cancer Res* (2012) **18**(18):4910–8. doi:10.1158/1078-0432.CCR-12-0912
18. Falchook GS, Janku F, Tsao AS, Bastida CC, Stewart DJ, Kurzrock R. Non-small-cell lung cancer with HER2 exon 20 mutation: regression with dual HER2 inhibition and anti-VEGF combination treatment. *J Thorac Oncol* (2013) **8**(2):e19–20. doi:10.1097/JTO.0b013e31827ce38e
19. Gandhi L, Bahleda R, Tolaney SM, Kwak EL, Cleary JM, Pandya SS, et al. Phase I study of neratinib in combination with temsirolimus in patients with human epidermal growth factor receptor 2-dependent and other solid tumors. *J Clin Oncol* (2014) **32**(2):68–75. doi:10.1200/JCO.2012.47.2787
20. Wells SA Jr, Santoro M. Targeting the RET pathway in thyroid cancer. *Clin Cancer Res* (2009) **15**(23):7119–23. doi:10.1158/1078-0432.CCR-08-2742
21. Wang R, Hu H, Pan Y, Li Y, Ye T, Li C, et al. RET fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer. *J Clin Oncol* (2012) **30**(35):4352–9. doi:10.1200/JCO.2012.44.1477
22. Kohno T, Tsuta K, Tsuchihara K, Nakaoku T, Yoh K, Goto K. RET fusion gene: translation to personalized lung cancer therapy. *Cancer Sci* (2013) **104**(11):1396–400. doi:10.1111/cas.12275
23. Borrello MG, Ardini E, Locati LD, Greco A, Licitra L, Pierotti MA. RET inhibition: implications in cancer therapy. *Expert Opin Ther Targets* (2013) **17**(4):403–19. doi:10.1517/14728222.2013.758715
24. Drilon A, Wang L, Hasanovic A, Suehara Y, Lipson D, Stephens P, et al. Response to cabozantinib in patients with RET fusion-positive lung adenocarcinomas. *Cancer Discov* (2013) **3**(6):630–5. doi:10.1158/2159-8290.CD-13-0035
25. Gautschi O, Zander T, Keller FA, Strobel K, Hirschmann A, Aebi S, et al. A patient with lung adenocarcinoma and RET fusion treated with vandetanib. *J Thorac Oncol* (2013) **8**(5):e43–4. doi:10.1097/JTO.0b013e31828a4d07
26. Davies KD, Le AT, Theodoro MF, Skokan MC, Aisner DL, Berge EM, et al. Identifying and targeting ROS1 gene fusions in non-small cell lung cancer. *Clin Cancer Res* (2012) **18**(17):4570–9. doi:10.1158/1078-0432.CCR-12-0550
27. Shaw AT, Ou SH, Bang YJ, Camidge DR, Solomon BJ, Salgia R, et al. Efficacy and safety of crizotinib in patients with advanced ROS1-rearranged non-small cell lung cancer. *J Clin Oncol* (2013) **31**(Suppl):abstr 8032.
28. Beau-Faller M, Ruppert AM, Voegeli AC, Neuville A, Meyer N, Guerin E, et al. MET gene copy number in non-small cell lung cancer: molecular analysis in a targeted tyrosine kinase inhibitor naive cohort. *J Thorac Oncol* (2008) **3**(4):331–9. doi:10.1097/JTO.0b013e318168d9d4
29. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* (2007) **316**(5827):1039–43. doi:10.1126/science.1141478
30. Tsao MS, Liu N, Chen JR, Pappas J, Ho J, To C, et al. Differential expression of Met/hepatocyte growth factor receptor in subtypes of non-small cell lung cancers. *Lung Cancer* (1998) **20**:1–16. doi:10.1016/S0169-5002(98)00007-5
31. Spigel DR, Edelman MJ, O'Byrne K, Paz-Ares L, Shames DS, Yu W, et al. Onartuzumab plus erlotinib versus erlotinib in previously treated stage IIIB or IV NSCLC: results from the pivotal phase III randomized multicenter placebo-controlled METLung (OAM4971g) global trial. *J Clin Oncol* (2014) **32**(Suppl; abstr 8000):5s.
32. Sequist LV, von Pawel J, Garmey EG, Akerley WL, Brugger W, Ferrari D, et al. Randomized phase II study of erlotinib plus tivantinib versus erlotinib plus placebo in previously treated non-small-cell lung cancer. *J Clin Oncol* (2011) **29**(24):3307–15. doi:10.1200/JCO.2010.34.0570
33. Camidge DR, Shapiro G, Otterson GA, Villaruz LC, Villalona-Calero MA, Iafrate AJ, et al. Efficacy and safety of crizotinib in patients with advanced c-MET-amplified non-small cell lung cancer. *J Clin Oncol* (2014) **32**(Suppl; abstr 8001):5s.
34. Vaishnavi A, Capelletti M, Le AT, Kako S, Butaney M, Ercan D, et al. Oncogenic and drug-sensitive NTRK1 rearrangements in lung cancer. *Nat Med* (2013) **19**:1469–72. doi:10.1038/nm.3352
35. De Braud FG, Pilla L, Niger M, Damian S, Bardazza B, Martinetti A, et al. Phase I open label, dose escalation study of RXDX101, an oral pan-trk, ROS1, and ALK inhibitor, in patients with advanced solid tumors with relevant molecular alterations. *J Clin Oncol* (2014) **32**(Suppl; abstr 2502):5s.
36. Montagut C, Settleman J. Targeting the RAF-MEK-ERK pathway in cancer therapy. *Cancer Lett* (2009) **283**(2):125–34. doi:10.1016/j.canlet.2009.01.022
37. Kris MG, Johnson BE, Berry LD, Kwiatkowski DJ, Iafrate AJ, Wistuba II, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. *JAMA* (2014) **311**:1998–2006. doi:10.1001/jama.2014.3741
38. Shepherd FA, Domerg C, Hainaut P, Jänne PA, Pignon JP, Graziano S, et al. Pooled analysis of the prognostic and predictive effects of KRAS mutation status and KRAS mutation subtype in early-stage resected non-small-cell lung cancer in four trials of adjuvant chemotherapy. *J Clin Oncol* (2013) **31**(17):2173–81. doi:10.1200/JCO.2012.48.1390

39. Zhu CQ, da Cunha Santos G, Ding K, Sakurada A, Cutz JC, Liu N, et al. Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada clinical trials group study BR.21. *J Clin Oncol* (2008) **26**(26):4268–75. doi:10.1200/JCO.2007.14.8924
40. Riely GJ, Kris MG, Rosenbaum D, Marks J, Li A, Chitale DA, et al. Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. *Clin Cancer Res* (2008) **14**(18):5731–4. doi:10.1158/1078-0432.CCR-08-0646
41. Jänne PA, Shaw AT, Pereira JR, Jeannin G, Vansteenkiste J, Barrios C, et al. Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. *Lancet Oncol* (2013) **14**(1):38–47. doi:10.1016/S1470-2045(12)70489-8
42. Gandara DR, Hiret S, Blumenschein GR, Barlesi F, Madelaine J, Infante JR, et al. Oral MEK1/MEK2 inhibitor trametinib (GSK1120212) in combination with docetaxel in KRAS-mutant and wild-type (WT) advanced non-small cell lung cancer (NSCLC): a phase I/Ib trial. *J Clin Oncol* (2013) **31**(Suppl): abstr 8028.
43. Kelly K, Mazieres U, Leighl NB, Barlesi F, Zalcman G, Gordon MS, et al. Oral MEK1/MEK2 inhibitor trametinib (GSK1120212) in combination with pemetrexed in KRAS-mutant and wild-type (WT) advanced non-small cell lung cancer (NSCLC): a phase I/Ib trial. *J Clin Oncol* (2013) **31**(Suppl): abstr 8027.
44. Blumenschein GR, Smit EF, Planchard D, Kim D-W, Cadranel J, De Pas T, et al. MEK114653: a randomized, multicenter, phase II study to assess efficacy and safety of trametinib (T) compared with docetaxel (D) in KRAS-mutant advanced non–small cell lung cancer (NSCLC). *J Clin Oncol* (2013) **31**(Suppl):abstr 8027.
45. Planchard D, Mazieres J, Riely GJ, Rudin CM, Barlesi F, Quoix EA, et al. Interim results of phase II study BRF113928 of dabrafenib in BRAF V600E mutation–positive non-small cell lung cancer (NSCLC) patients. *J Clin Oncol* (2013) **31**(Suppl):abstr 8009.
46. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *J Clin Oncol* (2010) **28**(6):1075–83. doi:10.1200/JCO.2009.25.3641
47. Kawano O, Sasaki H, Endo K, Suzuki E, Haneda H, Yukiue H, et al. PIK3CA mutation status in Japanese lung cancer patients. *Lung Cancer* (2006) **54**(2):209–15. doi:10.1016/j.lungcan.2006.07.006
48. Kawano O, Sasaki H, Okuda K, Yukiue H, Yokoyama T, Yano M, et al. PIK3CA gene amplification in Japanese non-small cell lung cancer. *Lung Cancer* (2007) **58**(1):159–60. doi:10.1016/j.lungcan.2007.06.020
49. Chaft JE, Arcila ME, Paik PK, Lau C, Riely GJ, Pietanza MC, et al. Coexistence of PIK3CA and other oncogene mutations in lung adenocarcinoma-rationale for comprehensive mutation profiling. *Mol Cancer Ther* (2012) **11**(2):485–91. doi:10.1158/1535-7163.MCT-11-0692
50. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* (2011) **3**(75):75ra26. doi:10.1126/scitranslmed.3002003
51. Tang JM, He QY, Guo RX, Chang XJ. Phosphorylated Akt overexpression and loss of PTEN expression in non-small cell lung cancer confers poor prognosis. *Lung Cancer* (2006) **51**(2):181–91. doi:10.1016/j.lungcan.2005.10.003
52. Marsit CJ, Zheng S, Aldape K, Hinds PW, Nelson HH, Wiencke JK, et al. PTEN expression in non-small-cell lung cancer: evaluating its relation to tumor characteristics, allelic loss, and epigenetic alteration. *Hum Pathol* (2005) **36**(7):768–76. doi:10.1016/j.humpath.2005.05.006
53. Besse B, Leighl N, Bennouna J, Papadimitrakopoulou VA, Blais N, Traynor AM, et al. Phase II study of everolimus-erlotinib in previously treated patients with advanced non-small-cell lung cancer. *Ann Oncol* (2014) **25**(2):409–15. doi:10.1093/annonc/mdt536
54. Ekman S, Wynes MW, Hirsch FR. The mTOR pathway in lung cancer and implications for therapy and biomarker analysis. *J Thorac Oncol* (2012) **7**(6):947–53. doi:10.1097/JTO.0b013e31825581bd
55. Stewart DJ. Wnt signaling pathway in non-small cell lung cancer. *J Natl Cancer Inst* (2014) **106**(1):djt356. doi:10.1093/jnci/djt356
56. Sequist LV, Heist RS, Shaw AT, Fidias P, Rosovsky R, Temel JS, et al. Implementing multiplexed genotyping of non-small-cell lung cancers into routine clinical practice. *Ann Oncol* (2011) **22**(12):2616–24. doi:10.1093/annonc/mdr489
57. Rosell R, Skrzypski M, Jassem E, Taron M, Bartolucci R, Sanchez JJ, et al. BRCA1: a novel prognostic factor in resected non-small-cell lung cancer. *PLoS One* (2007) **2**(11):e1129. doi:10.1371/journal.pone.0001129
58. Reguart N, Cardona AF, Carrasco E, Gomez P, Taron M, Rosell R. BRCA1: a new genomic marker for non-small-cell lung cancer. *Clin Lung Cancer* (2008) **9**(6):331–9. doi:10.3816/CLC.2008.n.048
59. Ramalingam SS, Goss GD, Andric ZG, Bondarenko I, Zaric B, Ceric T, et al. A randomized study of ganetespib, a heat shock protein 90 inhibitor, in combination with docetaxel versus docetaxel alone for second-line therapy of lung adenocarcinoma (GALAXY-1). *J Clin Oncol* (2013) **31**(Suppl):abstr CRA8007.
60. Ramalingam SS, Maitland ML, Frankel P, Argiris AE, Koczywas M, Gitlitz B, et al. Carboplatin and paclitaxel in combination with either vorinostat or placebo for first-line therapy of advanced non-small-cell lung cancer. *J Clin Oncol* (2010) **28**(1):56–62. doi:10.1200/JCO.2009.24.9094
61. Otrock ZK, Hatoum HA, Musallam KM, Awada AH, Shamseddine AI. Is VEGF a predictive biomarker to anti-angiogenic therapy? *Crit Rev Oncol Hematol* (2011) **79**(2):103–11. doi:10.1016/j.critrevonc.2010.07.008
62. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* (2006) **355**(24):2542–50. doi:10.1056/NEJMoa061884
63. Reck M, Kaiser R, Mellemgaard A, Douillard JY, Orlov S, Krzakowski M, et al. Nintedanib plus docetaxel in NSCLC patients progressing after first-line chemotherapy: LUME Lung 1, a randomized, double-blind phase III trial. *J Clin Oncol* (2013) **31**(Suppl):abstr LBA8011. doi:10.1016/S1470-2045(13)70586-2
64. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* (2012) **366**(26):2455–65. doi:10.1056/NEJMoa1200694

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 17 July 2014; accepted: 31 October 2014; published online: 25 November 2014.

*Citation: Zer A and Leighl N (2014) Promising targets and current clinical trials in metastatic non-squamous NSCLC. Front. Oncol. **4**:329. doi: 10.3389/fonc.2014.00329 This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.*

frontiers in
ONCOLOGY

REVIEW ARTICLE
published: 09 December 2014
doi: 10.3389/fonc.2014.00320

Promising targets and current clinical trials in metastatic squamous cell lung cancer

Mark D. Vincent*

London Regional Cancer Program, Department of Medical Oncology, London Health Sciences Centre, London, ON, Canada

Edited by:
Barbara Melosky, British Columbia Cancer Agency, Canada

Reviewed by:
Sacha I. Rothschild, University Hospital Basel, Switzerland
Gregory Alan Otterson, The Ohio State University, USA

****Correspondence:***
Mark D. Vincent, London Regional Cancer Program, Department of Medical Oncology, 790 Commissioners Road East, London, ON N6A 4L6, Canada
e-mail: mark.vincent@lhsc.on.ca

Squamous cancer of the lung (SQCC), although no longer the premier variant of non-small cell lung cancer, continues to impose a heavy world-wide burden. Advanced SQCC has enjoyed little of the recent progress benefiting patients with adenocarcinoma of the lung, but that has now begun to change. This article reviews the underlying molecular pathology of SQCC, as well as potential new targets and the corresponding novel targeted agents; included are some of which may soon be approvable in this notoriously hard-to-treat indication.

Keywords: squamous, epidermoid, lung cancer, systemic treatment, molecular

INTRODUCTION

Although squamous (epidermoid) lung cancer (SQCC) represents a declining proportion of non-small cell lung cancer (NSCLC), it still represents about 30% of all NSCLC, and as such, accounted for 6300 of the ±24,700 new cases of lung cancer in Canada thought to have occurred in 2013 (1). There is little if any comprehensive data on the histologic subtype distribution by stage, but it is possible that SQCC is somewhat more frequent in earlier stages as evidenced by two large Canadian series of stage III NSCLC, in both of which SQCC was the most frequent histological subtype (2, 3).

Up to, and including the 1990s, histological subtype was not considered to be particularly relevant in determining either the choice of therapy, or its outcomes, in advanced NSCLC. Of course, it had long been realized that SQCC had certain characteristic clinical features, such as a much higher incidence of hypertrophic pulmonary osteoarthropathy (including "clubbing"), non-metastatic paraneoplastic hypercalcemia and proximally situated, cavitating primary lesions, compared to other types of lung cancer. Furthermore, it had also been well understood that SQCC had a stronger association with smoking than adenocarcinoma (ADC), e.g., for current smokers (RR 16.91 vs. 4.21) (4). Unsurprisingly, SQCC is the histological subtype most associated with emphysema (5).

All of these features, while of great interests to diagnostic physicians and respirologists, may also impact directly or indirectly on the management of advanced NSCLC by oncologists. However, shortly after the turn of the century, it became clear that the histological subtyping of lung cancer had a previously unrecognized importance that went way beyond the fine-tuning of management, and even beyond the important distinction between small cell (SCLC) and NSCLC, which had, heretofore, been the major contribution of pathologists. Two types of new molecularly targeted drugs, gefitinib and bevacizumab, and one new chemotherapeutic, pemetrexed, seemed to have dramatically different effects (either with respect to efficacy or toxicity) according to histology, and the increasingly powerful techniques of genetic sequencing and analysis were revealing that SQCC seemed to be a different molecular entity from other types of lung cancer. In an era in which molecular diagnostics is seen increasingly as a way not only to guide the use of existing therapies but also to select patients for clinical trial accrual, and most critically, as a pathway for novel drug design, the traditional "one size fits all" categorization of advanced NSCLC is increasingly seen as obsolete.

That having been said, it is worth noting that often, definitive biopsy material may not be available, not even for histology let alone molecular tests, and the clinician may be forced to rely on a scant, non-specific cytology specimen ("NSCLC-NOS"), and the clinical features may be the only clue to the true histology. Furthermore, novel immunomodulatory drugs are definitely active in both SQCC and ADC, and for these agents, emerging molecular biomarkers may prove to be more predictive such that histological subtyping of NSCLC may, at least in the immunological arena, again become irrelevant.

CONVENTIONAL PATHOLOGY

Because histological subtype now profoundly affects clinical management, and because molecular analysis should be routine, at least in non-SQCC, every patient with advanced NSCLC should, if at all possible, be provided with the opportunity to undergo a professional biopsy procedure. Paradoxically, although patients with advanced NSCLC usually have a higher disease bulk and more potential sites for biopsy, they still may be referred in with a sub-optimal, cytology-only fine needle aspirate (FNA) perhaps motivated by risk-avoidance. In skilled hands, and with an adequately cellular FNA, the diagnostic accuracy and value of cytology and a small biopsy are actually comparable and even complimentary (6). Importantly, both cytology and core biopsy can each be used for immunohistochemistry (IHC) and molecular testing for EGFR and KRAS, providing a cell block-sufficient sample

is obtained. Nonetheless, most pathology departments prefer an adequate core biopsy both for histology (including IHC) and, if indicated, subsequent molecular testing.

It is now generally accepted that only two major types of NSCLC exist, ADC and SQCC, with other types being relatively uncommon (7). SQCC is diagnosed by the presence of keratinization and intercellular bridges, and the absence of features typical of ADC (intracellular mucin and gland formation). If this distinction cannot be rendered by conventional stains, IHC is usually adequate and highly valuable (8). Several investigations have confirmed that cytokeratin 5/6 (CK5/6) and p63 are most useful for SQCC, with a recent study showing for CK 5/6, a sensitivity of 94%, specificity of 97%, PVP of 97%, and PVN of 96%. These values for p63 were 93% (sensitivity), 87% (specificity), 96% (PVP), and 94% (PVN) (9).

Additional criteria include negativity for the ADC markers of CK7, TTF-1, and Napsin A. Although there is some variability in these results, it should be generally noted the SQCC of the lung are likely to be both CK 5/6 and p63 positive, and negative for TTF-1 or CK7. Novel immune panels continue to be developed, and which may prove superior (10).

MOLECULAR PATHOGENESIS AND TARGETED THERAPY

The effort to elucidate the molecular abnormalities in cancer is driven not just by curiosity or technical prowess but by a deeply embedded belief system that mutations drive malignant behavior, and that a description of these "driver mutations" will inevitably lead to the design of targeted drugs that are efficacious, via inhibition of the causal chain, and non-toxic, because of the specificity of the mutation for the cancer. Although this paradigm is overly simplistic, it has proven accurate for ADC in respect to EGFR mutations, and translocation of ALK; in both cases, reliable molecular tests are available, which guide the selection of available targeted drugs, which exhibit marked if temporary activity in subgroups of ADC with these genetic alterations. Unfortunately, EGFR and ALK rearrangements have only been described in rare cases of pure SQCC (11–13), but not at a frequency that would justify testing for either mutation routinely. Furthermore, at least in the case of those rare pure SQCCs with mutated EGFR, the targeted first generation tyrosine kinase inhibitors (EGFR-TKIs) seen to be somewhat less active than in ADC with mutated EGFR (12). Consequently, unless there is pathological evidence of a mixed adeno-squamous pathology (in which case it is worth testing) (14), it is not cost-beneficial to submit SQCCs (nor, probably, "NOS – probably squamous") cases for molecular analysis.

Likewise, KRAS mutations, which are common in (ex-) smokers with ADC, are only occasionally detected in SQCC (6.4% in the West, 1.8% in Asia) (15). KRAS in any event remains directly undruggable, but some believe that NSCLC patients with KRAS mutations may be less responsive to EGFR-TKIs (16). If true, this could explain why SQCCs do relatively well on EGFR-TKIs, despite the absence of EGRF mutations (17).

Squamous (epidermoid) lung cancer, despite the general absence of EGFR, ALK, and KRAS mutations, is genomically speaking, a highly aberrant malignancy (18). This is likely related to its close association with tobacco smoke, which contains over 5000 identified compounds, of which 73 are known carcinogens (19). These compounds form DNA adducts once metabolically activated. Unless repaired, these DNA lesions cause permanent mutations in the complementary strand due to bypass polymerases "inserting the wrong base opposite the adduct" (19). As a result of long-term exposure, thousands of mutations occur in the respiratory cells of smokers, some of which affect the function of growth regulatory genes. A variety of other processes facilitate tumorigenesis, such as inflammatory generation of reactive oxygen species and gene promoter methylation.

The Cancer Genome Atlas Research Network (CGARN) study on lung SQCC revealed many varied DNA alterations (". . . a mean of 360 exonic mutations, 323 altered copy number segments and 165 genomic rearrangements per tumor") (18). Importantly, copy number aberrations do not necessarily imply point mutations in the DNA sequence. The mean rate of exonic somatic mutations was, at 8.1 mutations per megabase, higher than any other cancer type except melanoma. TP53 mutations occurred in at least 81% of 178 samples of SQCC; TP53 is well known as the "guardian of the genome." Other mutations occurred quite often in pathways felt to be important in the mediation of the malignant phenotype, e.g., oxidative stress (KEAP1, 12%, CUL3, 7%, and NFE 2L2, 19%); squamous differentiation (SOX2, 21%; TP63, 16%; NOTCH 1 and 2, 8% and 5%, respectively; ASCL4, 3%; and FOXP 1, 4%). mRNA expression profiling revealed overexpression of SOX2, TP63, and P1K3CA, corresponding to the known 3q26 chromosomal amplicon. The p40 version of p63 may act as an oncogene, expressed by 89% of tumors; RB1 and PTEN (loss-of-function) were also frequent. Amplification or alteration of FGFR 1, 2, and 3 were seen in 7, 3, and 2%, respectively, as well as others involved in PI3K/RTK/RAS signaling (EGFR 9%; ERB B2 4%; ERB B3 2%; PTEN 15%; PIK3CA 16%; AKT3 16%; STK 11, 2%; TSC 1 and 2, 3% each; KRAS 3%; HRAS 3%; NF1 11%; RASA 1 4%; BRAF, 4%).

CDKN2A, a "known tumor suppressor gene," encodes INK 4A/p16 and ARF/p14, which control the cell cycle. This gene is inactivated in 77% of SQCCs (by a variety of mechanisms), often by epigenetic silencing (21%) or homozygous deletions (19%). On the other hand, about 30% overexpress both p16 and p14; often with mutation (see **Tables 1** and **2**).

These changes were seen against a background of a high mutational load in apparently non-contributory genes.

The authors contemplated the totality of this picture in terms of potential therapeutic targets. They felt the location of mutations in key cancer genes, such as a variety of tyrosine kinases, serine/threonine kinases, PI3K regions, proteases, and G-protein coupled receptors, suggested potential therapeutic targets. Unfortunately, however, many of the mutations were inactivations of tumor suppressor genes, which are currently not directly druggable. FGFR alterations, however, are among the most promising, and have recently been extensively reviewed in Ref. (20).

FGFR fibroblast growth factor receptor, is a family of 4 kinase receptors (FGFR 1–4) spanning the cell membrane and involved in signal transduction via downstream RAS/MAPK and PI3K/AKT pathways. In normal physiology, FGFR signaling is involved in angiogenesis and organogenesis. In lung cancer, serum FGF levels

Table 1 | Selected genomic alterations in SqCC.

	Gene	Mutation rate	Normal function	Consequence of alteration	Comment
(a)	KEAP1	12%	Oxidative stress response	Loss-of-function	
(a)	NFE2L2	19%	Oxidative stress response	Activation	
(a)	CUL3	7%	Oxidative stress response	Loss–of-function	
(b)	SOX2	Zero	Squamous differentiation	Activation	Amplified in 21%
(b)	NOTCH1	8%	Squamous differentiation	Mostly loss-of-function	Mutually exclusive with TP63 or SOX2 alterations
(b)	TP63 (p40 isoform)	16%	Squamous differentiation	Activation, oncogene	
(c)	TP53	≥81%	Genomic integrity, apoptosis	Loss-of-function	Disabled in ~90% SqCC
(d)	CDKN2A	15%	Cell cycle control	Loss-of-function	Inactivated in 72% by several mechanisms
(d)	RB1	7%	Cell cycle control	Loss-of-function	Mutually exclusive with CDKN2A alterations
(e)	NF1	11%	RAS inhibitor	Loss-of-function	
(e)	BRAF	4%	Signal transduction	Activation	
(e)	RASA1	4%	RAS inhibitor	Loss-of-function	
(e)	KRAS	<1%	Signal transduction	Activation	Very uncommon in SqCC
(f)	HLA-A	3%	Antigen display	Loss-of-function	May permit avoidance of immune destruction
(g)	PTEN	8%	PI3K/Akt pathway inhibitor	Loss-of-function	
(g)	PIK3CA	16%	PI3K/Akt pathway growth and survival	Activation	AKT3 also activated in 16%
(h)	FGFR1	Few	RTK growth/survival	Activation	Amplified in 21%
(h)	EGFR	±1% L861Q mutation rate	RTK in growth/survival growth function	Activation	Amplified in 9%, rarely mutated
(i)	MLL2	20%	Chromatin regulation	?	

are known to be elevated. Amplification of FGFR 1 may be characteristic of SQCC. The prognostic and predictive significance of these pathway alterations remains under investigation, but the bulk of evidence seems to suggest over-activity correlates with a poorer outcome.

The preclinical data have been sufficiently compelling to warrant the design and trialing of small molecules with FGFR inhibitory activity, including cediranib, nintedanib, pazopanib, and ponatinib. Nintedanib (an inhibitor of VEGFR 1–3, FGFR 1–4, PDGFR, FLT-3, and src), is most advanced, with a positive randomized trial (LUME – Lung 1) in second line (docetaxel ± nintedanib) (21). About 42% of the patients had SQCC; in these, the PFS HR 0.77 was significant ($p = 0.02$), as it was in the ADC subgroup (HR 0.77 $p = 0.0193$). Disease control was superior in the SQCC patients (49.3% vs. 35.5%, $p < 0.0001$). However, the effect on overall survival (OS) (in an exploratory analysis) seemed better in ADC than SQCC. Nintedanib appears tolerable, with GI and liver function abnormalities being most prominent. An excess of 12.3% of the patients required a dose reduction over placebo. LUME-Lung 3 is a first-line phase I/II trial of nintedanib with cisplatin/gemcitabine (NCT01346540).

Ponatinib is undergoing a phase II in SQCC as monotherapy after prior treatment, with FGFR amplification as an eligibility requirement (NCT01761747). A randomized trial of first-line carboplatin/paclitaxel ± cediranib was halted early for futility (22). Cediranib, however, has much less FGFR inhibitory activity than VEGFR blockade.

Pazopanib, another broad-spectrum kinase inhibitor whose actions include FGFR blockade as ancillary to VFGFR inhibition (23), is in a range of trials in A-NSCLC as monotherapy maintenance after first-line chemotherapy (NCT01208064) and with a variety of other traditional drugs in phase II. Other FGFR inhibitors include AZD4547 and BGJ398 and both are in phase I and/or phase II. FP-1039 is a FGF ligand trap also in phase I.

A series from the Massachusetts General Hospital found 16% of 226 SQCCs exhibited FGFR 1 amplifications, but these were not associated with any particular clinical features, suggesting that molecular testing would be required as a biomarker. Furthermore, the amplification was focal (24).

Looking more broadly at the pathway level, the CGARN found, in their 178 SQCC, 69% had an alteration in one of the PI3K/AKT, RTK, or RAS pathways, when considering either mutation in the DNA or amplification. For instance, 26% had either EGFR amplification, an activating BRAF mutation or FGFR 1 amplification, any of which might be targetable by an inhibitory drug. As noted, in the Canadian BR21 trial of erlotinib (an EGFR-TKI) vs. placebo,

Table 2 | Alterations in major pathways in SqCC.

Gene	Direction of dysregulation	Incidence	Normal function	Current targetability
EGFR	↑	9%	Receptor tyrosine kinase: 26%	(Potentially targetable)
ERBB2	↑	4%		
ERBB3	↑	2%		
FGFR1	↑	7%		
FGFR2	↑	3%		
FGFR3	↑	2%		
KRAS	↑	3%	RAS/RAF: 24%	(Potentially targetable)
HRAS	↑	3%		
NRAS	↑	<1%		
RASA1	↓	4%		
NF1	↑	11%		
PIK3CA	↑	16%	PI3K/AKT: 47%	(Potentially targetable)
AKT2	↑	4%		
AKT3	↑	16%		
PTEN	↓	15%		
$CDKN_2A$ methylation	↓	21%	$CDKN_2A$: 72%	(Not currently targetable)
$CDKN_2A$ mutation	↓	18%		
$CDKN_2A$ Exon skip.	↓	4%		
$CDKN_2A$ Hom del	↓	29%		

Table 3 | Molecularly targeted drugs under investigation in Sqcc[a].

FGFR inhibitors	Cediranib; nintedanib; pazopanib; ponatinib; AZD4547; BGJ398; FP-1039
EGFR inhibitor	Afatinib; necitumumab
PIK3CA	Buparlisib; GDC-0032
CDK 4/6	Palbociclib
VEGF-R	Ramucirumab, motesanib
PARP	Veliparib
Clusterin	Custirsen

[a] *Some of these agents have multiple other mechanisms of action in addition.*

the HR in favor of drug was 0.66 in SQCC with a smoking history, almost certainly due to the driver activity of wild-type EGFR (which is quite often overexpressed at the protein level) (25).

Finally, a significant proportion of specimens contained inactivating mutations in the HLA-A gene; this might convey resistance to emerging immunomodulatory regimens. It should also be noted that human papilloma virus, a known carcinogen in the urogenital tract, and in upper respiratory epithelium, has been ruled out as instrumental in lung cancer (26).

Targetable genetic alterations in SQCC were also reviewed recently by Heist et al. from the Massachusetts General Hospital (27), emphasizing that inactivated tumor suppressor genes (tsgs) can only, if at all, be indirectly targeted. They focus on commonly mutated genes (TP53, GRM8, BAI3, ERBB4, RUNX1T1, KEAP1, FBXW7, and KRAS) while noting no currently available agents directed at these mutations. However, they do highlight genomic amplifications and areas of overexpression, which, whether mutated or not, are likely implicated as "drivers," including SOX2 (amplified in 20% of SQCC; and a key stem cell regulator; no drugs in development); PIK3CA, affecting cell survival and proliferation (copy no. gains in >20% SQCCs, mutated in 6.5%) for which several drugs are in development (**Table 3**), especially buparlisib, which is in phase II and GDC-0032; and FGFR 1 (discussed above, and mediating growth, survival, and angiogenesis, for which several drugs are in development e.g., AZ4547 a specific FGFR 1–3 blocker). Although available drugs (apart from AZ4547) inhibit more than just the FGFR system, preclinical work confirms that pure inhibitors of FGFR 1 will inhibit growth of FGFR1 amplified tumor cell lines; IGF1R (insulin-like growth factor receptor) is overexpressed in some SQCCs, acting via the canonical growth and survival pathways, but promising phase II results in SQCC lung were not replicated in two phase III trials of figitumumab (a monoclonal antibody against IGF1R).

Other promising targets include EphA2 (overexpression increases invasiveness, and dasatinib is an available inhibitor); MET (amplified in about 6% of SQCCs, and mediating proliferation and invasion; multiple small molecules and antibodies are in development, but recently a large phase III trial of erlotinib ± onartuzumab failed, despite a prior highly promising randomized phase II in MET overexpressors (28). A platinum/paclitaxel ± onartuzumab randomized phase II is pending (NCT01519804). Rilotumumab, an inhibitor of the MET ligand HGF/SF, is also of interest. Other targets of promise include PDGFRA (amplified in 8–10% of SQCC; sunitinib an available inhibitor); p53/MDM2 (p53 mutations in about 65% of SQCCs, mostly loss-of-function; alternatively, MDM2 overexpression can inactivate p53, as in about 7% of SQCC; no drugs yet in development); AKT(mutated in about 5% of SQCCs; several drugs in development); DDR2 (a RTK promoting migration, proliferation, and survival, and mutated in about 4% of SQCCs; dasatinib may be active); LKB1 (a cell cycle regulator, inactivated in 5–20%; not yet drugged); PTEN (a tsg, and negative regulator of PI3K/AKT, which is then de-repressed when PTEN inactivated, very frequent in both types of NSCLC, especially SQCC; PI3K inhibitors are logical here); NRF2/KEAP1 (an oxidative stress response system; KEAP1 negatively regulates NRF2, and dysregulation of either gene is common in SQCC; no drugs in development yet).

The cyclin-dependent kinases, CDK 4 and 6 are another target of interest and the inhibitor palbociclib (PD-0332991) is in phase II in SQCC.

EGFR is commonly overexpressed (but rarely mutated) in SQCC. The overexpression may be associated with amplification or polysomy (29–31). FLEX, a first-line phase III trial of cisplatin/vinorelbine ± cetuximab, was especially positive in both types of NSCLC if, in an explanatory analysis, EGFR was overexpressed by IHC, independent of mutation status (32, 33), but this remains controversial. As noted, BR.21, a last-line study of erlotinib vs. placebo, showed a beneficial HR

0.67 for SQCC. A 545 patient, first-line phase III trial of cisplatin/gemcitabine ± necitumumab, a novel, fully human lgG1 monoclonal antibody, has just been reported as positive for OS (median survival 11.5 vs. 9.9 months, HR 0.84, $p = 0.012$). One- and 2-year survival also favored the necitumumab arm (34). Patients <70 years seemed to fare better, but the H-score biomarker seemed to exhibit at most a trend for more benefit at higher levels of EGRF expression. Another (phase II) RCT is testing carboplatin/paclitaxel ± necitumumab in first-line SQCC (NCT0176939). Some ±5% patients with SQCC exhibit a truncated form of EGFR known as EGFR-vIII, which is transforming, but which lacks an extra-cellular domain, and may be less amenable to inhibition by antibody. Lux-Lung 8 is an ongoing phase III study of a novel, pan-HER, irreversible inhibitor afatinib, vs. erlotinib, in last-line SQCC, and initial reports indicate a modest superiority over erlotinib (35). It remains an open question as to whether EGFR by IHC or gene copy number, can suffice as a biomarker for benefit from anti-EGFR agents in SQCC.

The VEGF/VEGFR angiogenesis pathway is clearly implicated in SQCC progression (36). Bevacizumab, a VEGF-sequestration antibody, caused an unacceptable rate of fatal hemoptysis in SQCC (37). Whether this relates to the tendency of SQCC to have cavitating primaries close to major airways, or some more complex association with squamous histology, is still unresolved; however, bevacizumab was only approved for use in non-SQCCs, for safety reasons. Bevacizumab may well be active in SQCCs and may be safer in patients with excised primaries; this is not known and would be off-label.

Ramucirumab, a fully human lgG1 monoclonal antibody to VEGRF-2, was recently the subject of a randomized phase III trial (REVEL: docetaxel ± ramucirumab) in second-line NSCLC including SQCC. It was positive for OS; REVEL included 328 SQCC patients, who experienced a modest OS benefit (9.5 vs. 8.2 months, HR 0.88) in a subgroup analysis (38). Motesanib, a small molecule VEGF-R inhibitor, seemed ineffective and prohibitively toxic in a recent randomized trial (39).

It is noteworthy that the successful RCTs in SQCC involve agents not directed at mutated proteins, but at normal components of upregulated pathways in which the specificity arises from contextual, causal dependence rather than biomolecular structural differences. Furthermore, as is usual, the benefits, albeit welcome, appear to be temporary. With the exception of chronic myeloid leukemia and bcr-abl inhibitors, targeted drugs based on the molecular causality principle have uniformly provided only temporary benefit.

RECOGNITION-BASED TARGETING

Interference in the causal chain mediating malignancy is not the only way to target cancer; another way is based on the principle of recognition. The immune system exploits this to protect us very effectively against foreign organisms, and also, cellular transformation; its direct targets are surface-based biomolecular differences ("markers"), irrespective of whether they are causally important drivers or not. Until recently, it was quite erroneously believed that most cancers were not "antigenic enough" to evolve an effective immune response (IR); however, it is now appreciated that the problem is more that the local milieu within the tumor environment is immunosuppressive, partly because of the manipulation of the immune system by negative regulators expressed on the tumor cells, and (still under-appreciated) the highly proteolytic and extremely acidotic extra-cellular milieu in tumors (40), which is likely to damage the three-dimensional structure of extra-cellular recognition peptides on which the IR is utterly dependent.

Squamous (epidermoid) lung cancer cells, by virtue of the very high mutation burden, are likely to express altered proteins as efficient neoantigens in the context of HLA. (The latter, as noted, may be mutated, potentially compromising antigen presentation in SQCC.) New immunomodulatory agents, also known as "immune checkpoint inhibitors," which have proven effective in both forms of lung cancer (as well as melanoma), derive from an understanding of the "immunological synapse" (41), a complex network of positive and negative regulatory interactions that occur among the tumor cells, the dendritic antigen-presenting cells and the T lymphocytes, and which strongly influence whether these effector (cytotoxic) T-cells are activated or not.

So far, useful therapies have been developed to block CTLA4 (an early negative regulator on T-cells, active in draining lymph nodes), with a monoclonal antibody, and the PD-L1/PD1 interaction (with monoclonal antibodies directed against either the PD-1 negative regulator on the T lymphocytes, or the PD-L1 ligand on the tumor cell, including SQCC cells, or dendritic cell). This system acts later in the "cancer-immunity cycle," (42), in the actual tumor milieu. Monoclonal antibodies against all three targets have shown surprising activity in lung cancer, and are in accelerated development; side effects have generally been tolerable, with a range of auto-immune effects, more associated with the anti-CTLA4 monoclonal antibody ipilimumab, and rare but potentially serious pneumonitis with the prominent anti PD-1 agent nivolumab.

The CA184-041 study tested two regimens of combination ipilimumab with chemotherapy (concurrent or phased) vs. chemotherapy only; the phased (but not the concurrent) PFS was significantly superior to the chemotherapy only arm. The SQCC patients seemed to benefit more (HR 0.48 for OS) (43). NCT01285609, an ongoing phase III, tests the combination vs. chemotherapy in first-line SQCC, using the apparently superior phased regimen.

The anti-PD1 agent nivolumab achieved a 6/18 ORR (SQCC) in a phase I study; despite heavy pre-treatment, OS at 2 years was 24% across all 129 NSCLC patients (44). NCT01721759 is an ongoing phase II in SQCC (third line). A phase III in SQCC (nivolumab vs. docetaxel) is underway (NCT01642004), as are phase I trials in A-NSCLC with the various platinum doublets, and also in the highly promising combination with ipilimumab. The role of tumor cell expression of PD-L1 as a biomarker is being investigated in these studies. MK-3475 in another anti-PD1 monoclonal antibody with a 2/6 ORR in SQCC, being moved into phase II/III (NCT01905657), and another pending phase III (NCT0214738), also focused on PD-L1 expressing tumors.

Several anti-PD-L1 antibodies have also shown some activity against SQCC such as BMS-936559 (45). MPDL3280A is

Table 4 | Checkpoint inhibitors under evaluation in advanced SqCC (selected studies).

Clinical Trials.gove Identifier, agent, trial name	Target	Phase	Line	Design	Status
NCT01285609 Ipilimumab	CTLA-4	III	1st	Carboplatin/paclitaxel ± Ipilimumab	Recruiting
NCT01721759 Nivolumab Checkmate 063	PD-1	II	3rd	Single agent nivolumab	Active, not recruiting
NCT01642004 Nivolumab Checkmate 017	PD-1	III	>1st	Nivolumab vs. docetaxel	Active, not recruiting
NCT02041533 Nivolumab Checkmate 026	PD-1	III	1st	Nivolumab vs. investigator's choice chemotherapy	Recruiting
NCT01454102 Nivolumab Checkmate 012	PD-1	I	Multiple	Nivolumab with various platinum doublets and/or biologicals/targeted agents including ipilimumab, erlotinib	Recruiting
NCT01295827 Pembrolizumab KEYNOTE 001	PD-1	I/II	≥1st	Low and high doses, q2 and 3 week schedule	Active, not recruiting
NCT02220894 Pembrolizumab KEYNOTE 042	PD-1	III	1st	Pembrolizumab vs. carboplatin/paclitaxel (or pemetrexed)	Not yet open
NCT02039674 Pembrolizumab KEYNOTE 021	PD-1	I/II	≥1st	Pembrolizumab with various platinum doublets and/or biologicals/targeted agents	Recruiting
NCT02007070 Pembrolizumab KEYNOTE 025	PD-1	II	2nd	Single agent	Recruiting PTO
NCT01905657 Pembrolizumab KEYNOTE 010	PD-1	II/III	≥2nd	2 doses of Pembrolizumab vs. docetaxel	Recruiting
NCT0214738 Pembrolizumab KEYNOTE 024	PD-1	III	1st	Pembrolizumab vs. platinum doublet	Not yet recruiting
NCT01846416 MPDL3280A FIR	PD-L1	II	≥1st	Single agent	Active, not recruiting
NCT02031458 MPDL3280A BIRCH	PD-L1	II	≥1st	Single agent	Recruiting
NCT01903993 MPDL3280A POPLAR	PD-L1	Random II	2nd	MPDL3280A vs. docetaxel	Active, not recruiting
NCT02008227 MPDL3280A OAK	PD-L1	III	2nd	MPDL3280A vs. docetaxel	Recruiting

(Continued)

Table 4 | Continued

Clinical Trials.gove Identifier, agent, trial name	Target	Phase	Line	Design	Status
NCT02087423 MEDI4736	PD-L1	II	3rd	Single agent	Recruiting
NCT02000947 MEDI4736	PD-L1	I	≥1st	MEDI4736 with Tremelimumab	Recruiting
NCT02154490 MEDI4736 LUNG-MAP	PD-L1	II/III	2nd	Multi-arm master protocol vs. docetaxel for various targeted agents including MEDI4726	Recruiting
NCT02087423 MEDI4736 ATLANTIC	PD-L1	II	3rd	Single agent	Recruiting

Ref for this table: Clinical Trials.gov accessed 31 August 2014.

another anti-PD-L1 monoclonal with early demonstrated activity in SQCC (3/20), especially associated with IHC positivity of PD-L1 (46). NCT01903993 will compare MPDL3280A with docetaxel in 2L, followed by the phase III (NCT02008227). MEDI4736 is another anti PD-L1 agent entering phase II in advanced NSCLC (ATLANTIC NCT02087423) and phase III in stage III (PACIFIC), including SQCC.

Readers are referred to two excellent overviews for further details (47, 48), and to **Table 4**.

BONE METASTASES

Bone metastases may occur in any type of lung cancer, and two categories of drugs have shown efficacy in reducing skeletal-related events (SREs). The bisphosphonate zoledronic acid (49–51) is well studied, and patients with elevated osteoclast marker (N-telopeptide of type 1 collagen) appear to experience an OS benefit (52). Denosumab, a monoclonal antibody against RANK-ligand, an osteoclast activator, led to an OS benefit compared with zoledronic acid, in a large NSCLC subgroup analysis (51) (HR 0.78, $p = 0.01$) in which the biggest benefit was experienced by SQCC (HR 0.68 $p = 0.035$), and also which was associated with a lower incidence of SREs than zoledronic acid (53).

CHEMOTHERAPY

No particular third-generation platinum doublet stands out as clearly superior in advanced SQCC, and the decision should be made based on toxicity avoidance (54). In a subgroup analysis of ECOG1594, a large four-armed phase III of taxane and gemcitabine platinum doublets, cisplatin/gemcitabine had the best PFS (4.3 months) and OS (9.4 months) in SQCC, but the differences were not statistically significant. Pemetrexed, however, whether as a single agent or in combination with cisplatin, is inferior and contra-indicated in SQCC, despite its superiority in ADC and large cell (55, 56). Pemetrexed is also ineffective in prolonging either PFS of OS in maintenance in SQCC, in contrast to non-SQCC (57). Pemetrexed may be less efficacious in SQCC because of higher thymidylate synthase levels (58, 59), although differential expression of the folate receptor alpha may also be important (60).

Nab-paclitaxel with carboplatin appears to be superior to paclitaxel/carboplatin in SQCC, with a higher ORR and less grades 3/4 neuropathy and arthralgia in SQCC (61). Neither PFS nor OS were different. The 41% ORR for the nab-paclitaxel arm is notable, as it was independently reviewed in Ref. (62).

In Japan, the LETS phase III study demonstrated the superiority of carboplatin/S-1 over carboplatin/paclitaxel in SQCC (HR 0.713; 14.0 vs. 10.6 months) (63). S1 is an oral fluoropyridine.

Further improvements in chemotherapy are unlikely to emerge without the addition of biologicals, some of which have been detailed above. One additional possibility relates to PARP inhibitors. PARP (poly ADP-ribose polymerase) is an enzyme that participates in several DNA repair pathways (64) and is believed to be important particularly in SQCC (65). An initial randomized phase II experience suggested significant benefit in SQCC with veliparib, a PARP inhibitor (PFS HR 0.50) OS HR 0.72), when added to carboplatin/paclitaxel (66) NCT01560104, a phase III trial of first-line platinum chemotherapy, ±veliparib (ABT-888), is currently underway. An earlier trial of chemotherapy ± iniparib failed, probably because iniparib might not be a sufficiently active PARP inhibitor (67).

The efficacy of chemotherapy may be also be attenuated by anti-apoptotic (pro-survival) proteins like clusterin, expressed in about 70% of NSCLC, apparently unrelated to histological subtype (68, 69). A phase I/II trial of platinum/gemcitabine with clusterin, a 2.0 generation antisense oligonucleotide, achieved an OS of 14.1 months, which was thought sufficient to justify the phase III trial now underway.

CONCLUSION

The proportionate reduction in SQCC is likely to be the result of an ongoing reduction in cigarette smoking (70); however, as long as tobacco products (and, probably, marijuana) (71) are consumed, this disease will be a major public health concern. Although most of the progress in lung cancer in the last decade has occurred in ADC, a recent spate of positive trials has, at last, brightened the prospects for SQCC. OS gains have been shown for ramucirumab, necitumumab, cetuximab, denosumab, and nintedanib

(to be prospectively confirmed); although these results are modest, they do provide a foundation upon which to explore novel biomarkers and potentially synergistic drug combinations. Furthermore, immunomodulators such as anti-PD-1/PD-L1 agents are unquestionably active. The rapidly expanding trove of knowledge on the volatile genome of SQCC has thrown up some further target opportunities, such as the FGFR family. This progress will likely serve to push the 1-year median OS consistently through the 1-year barrier; beyond that, further radical innovation will be necessary.

REFERENCES

1. Canadian Cancer Society's Advisory Committee on Cancer Statistics. *Canadian Cancer Statistics 2013.* Toronto, ON: Canadian Cancer Society (2013).
2. Waters E, Dingle B, Rodrigues G, Vincent M, Ash R, Dar R, et al. Analysis of a novel protocol of combined induction chemotherapy and concurrent chemoradiation in unresected non-small-cell lung cancer: a ten-year experience with vinblastine, cisplatin, and radiation therapy. *Clin Lung Cancer* (2010) **11**:243–50. doi:10.3816/CLC.2010.n.031
3. Vinod SK, Wai E, Alexander C, Tyldesley S, Murray N. Stage III non-small-cell lung cancer: population-based patterns of treatment in British Columbia, Canada. *J Thorac Oncol* (2012) **7**:1155–63. doi:10.1097/JTO.0b013e31824fea07
4. Lee PN, Forey BA, Coombs KJ. Systematic review with meta-analysis of the epidemiological evidence in the 1900s relating smoking to lung cancer. *BMC Cancer* (2012) **12**:385. doi:10.1186/1471-2407-12-385
5. Smith BM, Schwartzman K, Kovacina B, Taylor J, Kasymjanova G, Brandao G, et al. Lung cancer histologies associated with emphysema on computed tomography. *Lung Cancer* (2012) **76**:61–6. doi:10.1016/j.lungcan.2011.09.003
6. Sigel CS, Moreira AL, Travis WD, Zakowski MF, Thornton RH, Riely GJ, et al. Subtyping of non-small cell lung carcinoma: a comparison of small biopsy and cytology specimens. *J Thorac Oncol* (2011) **6**:1849–56. doi:10.1097/JTO.0b013e318227142d
7. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger K, Yatabe Y, et al. International association for the study of lung cancer/American thoracic society/European respiratory society international multidisciplinary classification of lung adenocarcinoma. *J Thorac Oncol* (2011) **6**:244–85. doi:10.1097/JTO.0b013e318206a221
8. Jerome Marson V, Mazieres J, Groussard O, Garcia O, Berjaud J, Dahan M, et al. Expression of TTF-1 and cyto-keratins in primary and secondary epithelial tumors: correlation with histological type and grade. *Histopathology* (2004) **45**(2):125–35. doi:10.1111/j.1365-2559.2004.01893.x
9. Warth A, Muley T, Herpel E, Meister M, Herth FJ, Schirmacher P, et al. Large-scale comparative analyses of immunomarkers for diagnostic subtyping of non-small-cell lung cancer biopsies. *Histopathology* (2012) **61**:1017–25. doi:10.1111/j.1365-2559.2012.04308.x
10. Ring BZ, Seitz RS, Beck RA, Shasteen WJ, Soltermann A, Arbogast S, et al. A novel five-antibody immunohistochemical test for subclassification of lung carcinoma. *Mod Pathol* (2009) **22**:1032–43. doi:10.1038/modpathol.2009.60
11. Fang W, Zhang J, Liang W, Huang Y, Yan Y, Wu X, et al. Efficacy of epidermal growth factor receptor-tyrosine kinase inhibitors for Chinese patients with squamous cell carcinoma of lung harboring EGFR mutation. *J Thorac Dis* (2013) **5**:582–92. doi:10.3978/j.issn.2072-1439.2013.09.15
12. Hata A, Katakami N, Yoshioka H, Kunimasa K, Fujita S, Kaji R, et al. How sensitive are epidermal growth factor receptor-tyrosine kinase inhibitors for squamous cell carcinoma of the lung harboring EGFR gene-sensitive mutations? *J Thorac Oncol* (2013) **8**:89–95. doi:10.1097/JTO.0b013e31827690b5
13. Caliò A, Nottegar A, Gilioli E, Bria E, Pilotto S, Peretti U, et al. ALK/EML4 fusion gene may be found in pure squamous carcinoma of the lung. *J Thorac Oncol* (2013) **9**:729–32. doi:10.1097/JTO.0000000000000109
14. Wang R, Pan Y, Li C, Zhang H, Garfield D, Li Y, et al. Analysis of major known driver mutations and prognosis in resected adenosquamous lung carcinomas. *J Thorac Oncol* (2014). doi:10.1097/JTO.0b013e3182a406d1
15. Dearden S, Stevens J, Wu YL, Blowers D. Mutation incidence and coincidence in non-small cell lung cancer: meta-analyses by ethnicity and histology (mutMap). *Ann Oncol* (2013) **24**:2371–6. doi:10.1093/annonc/mdt205
16. Timar J. The clinical relevance of KRAS gene mutation in non-small-cell lung cancer. *Curr Opin Oncol* (2014) **26**:138–44. doi:10.1097/CCO.0000000000000051
17. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* (2005) **353**:123–32. doi:10.1056/NEJMoa050753
18. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. *Nature* (2012) **489**(7417):519–25. doi:10.1038/nature11404
19. Hecht SS. Lung carcinogenesis by tobacco smoke. *Int J Cancer* (2012) **131**:2724–32. doi:10.1002/ijc.27816
20. Salgia R. Fibroblast growth factor signaling and inhibition in non-small cell lung cancer and their role in squamous cell tumors. *Cancer Med* (2014). doi:10.1002/cam4.238
21. Reck M, Kaiser R, Mellemgaard A, Douillard JY, Orlov S, Krzakowski M, et al. Docetaxel plus nintedanib versus docetaxel plus placebo in patients with previously treated non-small-cell lung cancer (LUME-Lung 1): a phase 3, double-blind, randomised controlled trial. *Lancet Oncol* (2014) **15**:143–55. doi:10.1016/S1470-2045(13)70586-2
22. Laurie SA, Solomon BJ, Seymour L, Ellis PM, Goss GD, Shepherd FA, et al. Randomised, double-blind trial of carboplatin and paclitaxel with daily oral cediranib or placebo in patients with advanced non-small cell lung cancer: NCIC Clinical Trials Group study BR29. *Eur J Cancer* (2014) **50**:706–12. doi:10.1016/j.ejca.2013.11.032
23. Davidson B, Secord AA. Profile of pazopanib and its potential in the treatment of epithelial ovarian cancer. *Int J Womens Health* (2014) **6**:289–300. doi:10.2147/IJWH.S49781
24. Heist RS, Mino-Kenudson M, Sequist LV, Tammireddy S, Morrissey L, Christiani DC, et al. FGFR1 amplification in squamous cell carcinoma of the lung. *J Thorac Oncol* (2012) **7**:1775–80. doi:10.1097/JTO.0b013e31826aed28
25. Clark GM, Zborowski DM, Santabarbara P, Ding K, Whitehead M, Seymour L, et al. Smoking history and epidermal growth factor receptor expression as predictors of survival benefit from erlotinib for patients with non-small-cell lung cancer in the National Cancer Institute of Canada Clinical Trials Group study BR.21. *Clin Lung Cancer* (2006) **7**:389–94. doi:10.3816/CLC.2006.n.022
26. van Boerdonk RA, Daniels JM, Bloemena E, Krijgsman O, Steenbergen RD, Brakenhoff RH, et al. High-risk human papillomavirus-positive lung cancer: molecular evidence for a pattern of pulmonary metastasis. *J Thorac Oncol* (2013) **8**:711–8. doi:10.1097/JTO.0b013e3182897c14
27. Heist RS, Sequist LV, Engelman JA. Genetic changes in squamous cell lung cancer: a review. *J Thorac Oncol* (2012) **7**:924–33. doi:10.1097/JTO.0b013e31824cc334
28. Spigel DR, Ervin TJ, Ramlan R, Daniel DB, Goldschmidt JH, Blumenschein GR, et al. Final efficacy results from OAM558g, a randomized phase II study evaluating MetMAb or placebo in combination with erlotinib in advanced NSCLC. *J Clin Oncol* (2013) **29**(Suppl):Abstr7505. doi:10.1200/JCO.2012.47.4189
29. Zhu CQ, da Cunha Santos G, Ding K, Sakurada A, Cutz JC, Liu N, et al. Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR.21. *J Clin Oncol* (2008) **26**:4268–75. doi:10.1200/JCO.2007.14.8924
30. Ellis PM, Morzycki W, Melosky B, Butts C, Hirsh V, Krasnoshtein F, et al. The role of the epidermal growth factor receptor tyrosine kinase inhibitors as therapy for advanced, metastatic, and recurrent non-small-cell lung cancer: a Canadian national consensus statement. *Curr Oncol* (2009) **16**:27–48. doi:10.3747/co.v16i1.393
31. Kim ES, Hirsh V, Mok T, Socinski MA, Gervais R, Wu YL, et al. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. *Lancet* (2008) **372**:1809–18. doi:10.1016/S0140-6736(08)61758-4
32. Pirker R, Pereira JR, von Pawel J, Krzakowski M, Ramlau R, Park K, et al. EGFR expression as a predictor of survival for first-line chemotherapy plus cetuximab in patients with advanced non-small-cell lung cancer: analysis of data from the phase 3 FLEX study. *Lancet Oncol* (2012) **13**:33–42. doi:10.1016/S1470-2045(11)70318-7
33. Douillard JY, Pirker R, O'Byrne KJ, Kerr KM, Störkel S, von Heydebreck A, et al. Relationship between EGFR expression, EGFR mutation status, and the efficacy of chemotherapy plus cetuximab in FLEX study patients with advanced non-small-cell lung cancer. *J Thorac Oncol* (2014) **9**:717–24. doi:10.1097/JTO.0000000000000141

34. Thatcher N, Hirsch FR, Szczesna A, Ciuleanu TE, Szafranski W, Dediu M, et al. A randomized, multicenter, open-label, phase III study of gemcitabine, cisplatin (GC) chemotherapy plus necitumumab (IMC-11F8/LY3012211) versus GC alone in the first line treatment of patients with stage IV squamous non-small cell carcinoma. *J Clin Oncol* (2014) **32**(Suppl: Abs 8008):55.
35. Goss G, Felip E, Cobo M, et al. *A Randomized, Open-Label, Phase III Trial of Afatinib vs. Erlotinib as Second-Line Treatment of Patients with Advanced Squamous Cell Carcinoma of the Lung Following First-Line Platinum-Based Chemotherapy: Lux-Lung 8.* ESMO preferred paper section (2014). abstr12220 p.
36. Piperdi B, Merla A, Perez-Soler R. Targeting angiogenesis in squamous non-small cell lung cancer. *Drugs* (2014) **74**:403–13. doi:10.1007/s40265-014-0182-z
37. Johnson DH, Fehrenbacher L, Novotny WF, Herbst RS, Nemunaitis JJ, Jablons DM, et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol* (2004) **22**:2184–91. doi:10.1200/JCO.2004.11.022
38. Garon EB, Ciuleanu TE, Arrieta O, Prabhash K, Syrigos KN, Goksel T, et al. Ramucirumab plus docetaxel versus placebo plus docetaxel for second-line treatment of stage IV non-small-cell lung cancer after disease progression on platinum-based therapy (REVEL): a multicentre, double-blind, randomised phase 3 trial. *Lancet* (2014) **384**(9944):665–73. doi:10.1016/S0140-6736(14)60845-X
39. Novello S, Scagliotti GV, Sydorenko O, Vynnychenko I, Volovat C, Schneider CP, et al. Motesanib plus carboplatin/paclitaxel in patients with advanced squamous non–small-cell lung cancer: results from the randomized controlled MONET1 study. *J Thorac Oncol* (2014) **9**:1143–51. doi:10.1097/JTO.0000000000000227
40. Parkes SK, Chiche J, Pouyssegur J. Disrupting protein dynamics and energy metabolism for cancer therapy. *Nat Rev Cancer* (2013) **13**:611–23. doi:10.1038/nrc3579
41. Topasalian SL, Weiner GJ, Pardoll DM. Cancer immunotherapy comes of age. *J Clin Oncol* (2011) **29**:4828–36. doi:10.1200/JCO.2011.38.0899
42. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* (2013) **39**:1–10. doi:10.1016/j.immuni.2013.07.012
43. Lynch TJ, Bondarenko I, Luft A, Serwatowski P, Barlesi F, Chacko R, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. *J Clin Oncol* (2012) **30**:2046–54. doi:10.1200/JCO.2011.38.4032
44. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* (2012) **366**:2443–54. doi:10.1056/NEJMoa1200690
45. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* (2012) **366**:2455–65. doi:10.1056/NEJMoa1200694
46. Horn L, et al. Abstract MO148.01.2013. Available from: http://www.webges.com/cview/library/wclc/home
47. Stinchcombe TE. Unmet needs in squamous cell carcinoma of the lung: potential role for immunotherapy. *Med Oncol* (2014) **31**(5):960. doi:10.1007/s12032-014-0960-1
48. Langer CJ. Emerging immunotherapies in the treatment of non-small cell lung cancer (NSCLC): the role of immune checkpoint inhibitors. *Am J Clin Oncol* (2014). doi:10.1097/COC.0000000000000059
49. Rosen LS, Gordon D, Tchekmedyian NS, Yanagihara R, Hirsh V, Krzakowski M, et al. Long-term efficacy and safety of zoledronic acid in the treatment of skeletal metastases in patients with non-small cell lung carcinoma and other solid tumors: a randomized, phase III, double-blind, placebo-controlled trial. *Cancer* (2004) **100**:2613–21. doi:10.1002/cncr.20308
50. Hirsh V, Tchekmedyian NS, Rosen LS, Zheng M, Hei YJ. Clinical benefit of zoledronic acid in patients with lung cancer and other solid tumors: analysis based on history of skeletal complications. *Clin Lung Cancer* (2004) **6**:190–4. doi:10.3816/CLC.2004.n.030
51. Hirsh V, Major PP, Lipton A, Cook RJ, Langer CJ, Smith MR, et al. Zoledronic acid and survival in patients with metastatic bone disease from lung cancer and elevated markers of osteoclast activity. *J Thorac Oncol* (2008) **3**:228–36. doi:10.1097/JTO.0b013e3181651c0e
52. Scagliotti GV, Hirsh V, Siena S, Henry DH, Woll PJ, Manegold C, et al. Overall survival improvement in patients with lung cancer and bone metastases treated with denosumab versus zoledronic acid: subgroup analysis from a randomized phase 3 study. *J Thorac Oncol* (2012) **7**:1823–91. doi:10.1097/JTO.0b013e31826aec2b
53. Henry D, Vadhan-Raj S, Hirsh V, von Moos R, Hungria V, Costa L, et al. Delaying skeletal-related events in a randomized phase 3 study of denosumab versus zoledronic acid in patients with advanced cancer: an analysis of data from patients with solid tumors. *Support Care Cancer* (2014) **2**:679–87. doi:10.1007/s00520-013-2022-1
54. Hoang T, Dahlberg SE, Schiller JH, Johnson DH. Does histology predict survival of advanced non-small cell lung cancer patients treated with platin-based chemotherapy? An analysis of the Eastern Cooperative Oncology Group Study E1594. *Lung Cancer* (2012) **81**:47–52. doi:10.1016/j.lungcan.2013.03.018
55. Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* (2008) **26**:3543–51. doi:10.1200/JCO.2007.15.0375
56. Scagliotti G, Hanna N, Fossella F, Sugarman K, Blatter J, Peterson P, et al. The differential efficacy of pemetrexed according to NSCLC histology: a review of two phase III studies. *Oncologist* (2009) **14**:253–63. doi:10.1634/theoncologist.2008-0232
57. Ciuleanu T, Brodowicz T, Zielinski C, Kim JH, Krzakowski M, Laack E, et al. Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomised, double-blind, phase 3 study. *Lancet* (2009) **374**:1432–40. doi:10.1016/S0140-6736(09)61497-5
58. Liu Y, Yin TJ, Zhou R, Zhou S, Fan L, Zhang RG. Expression of thymidylate synthase predicts clinical outcomes of pemetrexed-containing chemotherapy for non-small-cell lung cancer: a systemic review and meta-analysis. *Cancer Chemother Pharmacol* (2013) **72**:1125–32. doi:10.1007/s00280-013-2299-2
59. Ceppi P, Volante M, Saviozzi S, Rapa I, Novello S, Cambieri A, et al. Squamous cell carcinoma of the lung compared with other histotypes shows higher messenger RNA and protein levels for thymidylate synthase. *Cancer* (2006) **107**:1589–96. doi:10.1002/cncr.22208
60. Nunez MI, Behrens C, Woods DM, Lin H, Suraokar M, Kadara H, et al. High expression of folate receptor alpha in lung cancer correlates with adenocarcinoma histology and EGFR [corrected] mutation. *J Thorac Oncol* (2012) **7**:833–60. doi:10.1097/JTO.0b013e31824de09c
61. Socinkski MA, Okamoto I, Hon JK, Hirsh V, Dakhil SR, Page RD, et al. Safety and efficacy analysis by histology of weekly nab-paclitaxel in combination with carboplatin as first-line therapy in patients with advanced non-small-cell lung cancer. *Ann Oncol* (2013) **24**:2390–6. doi:10.1093/annonc/mdt235
62. Hirsh V. Nab-paclitaxel for the management of patients with advanced non-small-cell lung cancer. *Expert Rev Anticancer Ther* (2014) **14**:129–41. doi:10.1586/14737140.2014.881719
63. Yoshioka H, Okamoto I, Morita S, Ando M, Takeda K, Seto T, et al. Efficacy and safety analysis according to histology for S-1 in combination with carboplatin as first-line chemotherapy in patients with advanced non-small-cell lung cancer: updated results of the West Japan Oncology Group LETS study. *Ann Oncol* (2012) **24**:1326–31. doi:10.1093/annonc/mds629
64. O'Sullivan CC, Moon DH, Kohn EC, Lee JM. Beyond breast and ovarian cancers: PARP inhibitors for BRCA mutation-associated and BRCA-like solid tumors. *Front Oncol* (2014) **4**:2–13. doi:10.3389/fonc.2014.00042
65. Levra MG, Olaussen KA, Novello S, Soria JC. PARP inhibitors: an interesting pathway also for non-small cell lung cancer? *Curr Pharm Des* (2013).
66. Ramalingam SS, Blais N, Mazieres J, Reck M, Jones CM, Juhasz E, et al. *A Randomized, Double Blind, Phase 2 Trial of Veliparib (ABT-888) with Carboplatin and Paclitaxel in Previously Untreated Metastatic (or Advanced Non-Small Cell Lung Cancers.* Poster Display Session, ESMO (2014). Abstr1234 p.
67. Sinha GJ. Downfall of iniparib: a PARP inhibitor that doesn't inhibit PARP after all. *J Natl Cancer Inst* (2014) **106**:1. doi:10.1093/jnci/djt447
68. July LV, Beraldi E, So A, Fazli L, Evans K, English JC, et al. Nucleotide-based therapies targeting clusterin chemosensitize human lung adenocarcinoma cells both in vitro and in vivo. *Mol Cancer Ther* (2004) **3**:228–32.
69. Niu L, Zang J, Cai L, Li C, Cao H. Relationship of clusterin expression with Bax and p53 expression in non-small cell lung cancer. *Zhongguo Fei Ai Za Zhi* (2007) **10**:284–7. doi:10.3779/j.issn.1009-3419.2007.04.05
70. Dwyer-Lindgren L, Mokdad AH, Srebotnjak T, Flaxman AD, Hansen GM, Murray CJ. Cigarette smoking prevalence in US counties: 1996-2012. *Popul Health Metr* (2014) **12**:5. doi:10.1186/1478-7954-12-5

71. Callaghan RC, Allebeck P, Sidorchuk A. Marijuana use and risk of lung cancer: a 40-year cohort study. *Cancer Causes Control* (2013) **24**:1811–20. doi:10.1007/s10552-013-0259-0

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 02 June 2014; accepted: 26 October 2014; published online: 09 December 2014.

*Citation: Vincent MD (2014) Promising targets and current clinical trials in metastatic squamous cell lung cancer. Front. Oncol. **4**:320. doi: 10.3389/fonc.2014.00320*
This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 13 October 2014
doi: 10.3389/fonc.2014.00229

Role of radiotherapy in metastatic non-small cell lung cancer

Sergio L. Faria*

Department of Radiation Oncology, McGill University Health Centre, Montreal General Hospital, Montreal, QC, Canada

Edited by:
Vera Hirsh, McGill University Health Centre, Canada

Reviewed by:
Heloisa De Andrade Carvalho, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brazil
Bo H. Chao, The Ohio State University, USA

****Correspondence:***
Sergio L. Faria, Department of Radiation Oncology, MGH, McGill University Health Centre, 1650 Cedar Av, room D5.400, Montreal, QC H3G1A4, Canada
e-mail: sergio.faria@muhc.mcgill.ca

Radiotherapy has had important role in the palliation of NSCLC. Randomized trials tend to suggest that, in general, short regimens give similar palliation and toxicity compared to longer regimens. The benefit of combining chemotherapy to radiosensitize the palliative radiation treatment is an open question, but so far it has not been proved to be very useful in NSCLC. The addition of molecular targeted drugs to radiotherapy outside of approved regimens or clinical trials warrants careful consideration for every single case and probably should not be used as a routine management. Stereotactic radiosurgery (SRS) and stereotactic body radiation therapy (SBRT) are modern techniques being used each time more frequently in the treatment of single or oligometastases. In general, they offer good tumor control with little toxicity (with a more expensive cost) compared to the traditionally fractionated radiotherapy regimens.

Keywords: palliative radiotherapy, metastatic NSCLC, stereotactic body radiosurgery, radiosensitizers

INTRODUCTION

Good palliative local treatment should be simple, fast, generally efficient, and not very expensive. Radiotherapy has largely been used to palliate NSCLC for all these reasons (1). A good example is the fact that one simple treatment of external beam radiation treatment (EBRT) may stop a hemoptysis. Multiple prospective randomized trials using different dose/fractionation schedules have shown that palliative radiotherapy can often alleviate thoracic and extra-thoracic symptoms in patients with locally advanced or metastatic NSCLC (1–3).

Indications for thoracic EBRT include, but are not limited to: hemoptysis, cough, chest pain, dyspnea, obstructive pneumonia, dysphagia related to esophageal compression, superior vena cava syndrome, hoarseness, or stridor. Symptoms caused by malignant pleural effusion, lymphangitic carcinomatosis, and multiple parenchymal diseases typically are not suitable for palliative thoracic EBRT.

Indications for extra-thoracic EBRT include, but are not limited to: brain, adrenal, bone, and liver metastases.

REVIEWS

In 2009, a comprehensive review involving 14 randomized clinical trials, all related to different dose schedules to palliate the symptomatic primary lung cancer, was performed by the Cochrane Collaboration (4). In general, the results of those trials suggest that there are not significant differences among short compared to long radiotherapy regimens in terms of palliation, but higher-dose regimens were associated with mild increased acute toxicity, particularly esophagitis (**Table 1**). However, the studies are not homogeneous, the end points and assessments were different, and the reviewers did not make a clear conclusion on the ideal regimen of palliative radiation treatment. In fact, in clinical practice, depending on the institution, we have seen different doses and fractionations regimens being used for similar clinical situations. It is very well possible that, at least in part, remuneration directs practical management. The case for bone metastases is a good example. Despite a considerable body of evidence from randomized trials supporting the use of a single fraction of 8 Gy for radiation therapy, there is still considerable use of longer regimens such as 30 Gy in 10 fractions (5).

TYPICAL PALLIATIVE RADIOTHERAPY DOSE AND FRACTIONATION IN NSCLC

At the McGill University Health Centre (MUHC), we practice evidence-based medicine preferring short and simple treatments. Our typical palliative dose is 17 Gy in two fractions (1 week apart) after randomized trials concluded that this regimen is simple, well tolerated, and efficient compared to other regimens (6, 7).

SVC SYNDROME

In the past, SVC syndrome was considered a potentially life-threatening medical emergency requiring immediate radiotherapy as the quickest way to relieve the obstruction. Emergency radiotherapy is no longer considered necessary for most patients (8). Patients who present with stridor due to central airway obstruction or severe laryngeal edema represent a true medical emergency, and these patients require immediate treatment (stent placement and/or radiotherapy) to decrease the risk of sudden respiratory failure and death. Evidence-based guidelines for management of SVC syndrome are not available. Most of the malignancies causing SVC syndrome, including NSCLC, are radiation-sensitive, and symptomatic improvement is usually apparent within 72 h, associated with complete relief of symptoms of SVC obstruction in 63% of patients with NSCLC (9). Radiotherapy treatments are typically

Table 1 | Randomized controlled trials assessing palliative lung radiotherapy fractionation [from Ref. (3)].

Study	Year	Radiotherapy schedules compared	Evaluable patients (*n*)	Survival[a] by regimen (*P* = NS unless specified)	Symptom control by regimen (*P* = NS unless specified)
Simpson	1985	40 Gy/20 F daily continuous/4 weeks vs. 30 Gy/10 F/2 weeks vs. 40 Gy/10 F/4 weeks, split course	316	6.2 vs. 6.9 vs. 6.4 months	No difference
Teo	1988	45 Gy/18F/3.5 weeks vs. 31.2 Gy/4 F/4 weeks	273	20 vs. 20 weeks	Better with 45 Gy, $P = 0.012$
MRC	1991	30 Gy/10 F/2 weeks or 27 Gy/6 F/2 weeks or 17 Gy/2 F/8 days	369	177 vs. 179 days	No difference
MRC	1992	17 Gy/2 F/8 days vs. 10 Gy/1 fraction	235	100 vs. 122 days	No difference
Abratt	1995	35 Gy/10 F/2.5 weeks vs. 45 Gy/15F/3.75 weeks	84	8.5 vs. 8.5 months	No difference
MRC	1996	36 or 39 Gy/12 or 13 F/2.5 weeks vs. 17 Gy/2 F/8 days	509	1. 9 vs. 2.7 months , $P = 0.03$	No difference
Rees	1997	17 Gy/2 F/8 days vs. 22.5 Gy/5 F/5 days	216	23 vs. 18% (1 year)	No difference
Reinfuss	1999	50 Gy/25 F/5 weeks (conventional) vs. 40 Gy/10 F daily (split course with 4 weeks gap) vs. delayed radiotherapy (20 25 Gy/4 or 5 F when symptomatic).	240	18 vs. 6 vs. 0%, $P < 0.05$ (2 years)	No assessment of symptoms
Nestle	2000	32 Gy/16 F twice daily/10 days vs. 60 Gy/30 F/6 weeks	152	36 vs. 38% (1 year)	No difference
Bezjak	2002	20 Gy/5 F/1 weeks vs. 10 Gy/1 F	230	6 vs. 4.2 months, $P = 0.03$	Better for 20 Gy on Lung Cancer Symptom Scale, $P = 0.009$
Sundstrom	2004	17 Gy/2 F/8 days vs. 42 Gy/15 F/3 weeks vs. 50 Gy/25 F/5 weeks	407	6.8 vs. 7.0 vs. 8.2 months	No difference
Erridge	2005	30 Gy/10 F/2 weeks vs. 10 Gy/1 F	148	23 vs. 28 weeks	Better for 30-Gy arm, $P = 0.05$
Kramer	2005	30 Gy/10 F/2 weeks vs. 16 Gy/2 F/8 days	297	20 vs. 11%, $P = 0.03$ (1 year)	No difference
Senkus-Konefka	2005	20 Gy/5 F/l weeks vs. 16 Gy/2 F/8 days	100	5.3 vs. 8.0 months, $P = 0.016$	No difference

F, fraction; Gy, gray; NS, non-significant.

[a] *Survival given as median value or percentage at specific timepoint.*

administered over a course of 1–2 weeks with larger fraction sizes of 3–8 Gy (e.g., 17 Gy in 2 fractions, 20 Gy in 5 fractions, and 30 Gy in 10 fractions), with the goal of achieving a more rapid response by using larger daily doses (10).

BRACHYTHERAPY

In our Department at MUHC, we have the facilities to use high dose rate (HDR) endobronchial brachytherapy for palliation of hemoptysis or obstruction (we use doses between 6 and 10 Gy at 1 cm). Brachytherapy has been used sporadically. Comparing brachytherapy to EBRT is difficult. There is currently no randomized or meta-analysis based evidence to recommend endobronchial brachytherapy as the routine initial palliative management of endobronchial obstruction resulting from lung cancer (11).

The use of concurrent chemotherapy with palliative irradiation in lung cancer.

The question if some systemic treatment should be used as a radiosensitizer of palliative radiotherapy in NSCLC is open for discussion. The question is pertinent because several randomized studies have demonstrated that, when compared with best supportive care, chemotherapy not only significantly improves survival but also reduces symptoms and enhances quality of life in stage IV NSCLC. However, in palliative radiotherapy the total dose is usually not very high (to avoid risk of radiation induced toxicity). In general, in the group of metastatic NSCLC patients that need local radiotherapy palliation, the addition of chemotherapy may increase toxicity, cost, and may complicate the delivery of the whole treatment without significant improved palliation. At this time, it seems that there is no added benefit for the use

of chemotherapy concurrently with radiation therapy in the palliation of thoracic symptoms in lung cancer patients (3, 4), but this is of course an open topic and indications may be discussed in a case by case basis. There is only one randomized clinical trial addressing this issue showing that the use of continuous-infusion fluorouracil showed only a discrete better response but with increased toxicity in palliation of NSCLC (12). However, fluorouracil is not an agent currently used in NSCLC, and the available data of palliative radiotherapy with the use of other agents commonly used today as systemic treatment in NSCLC such as platinum based chemotherapy, vinorelbine, or gemcitabine, are not very persuasive (13, 14).

THE USE OF TARGET AGENTS WITH PALLIATIVE RADIOTHERAPY

When used in combination with radiotherapy, molecularly targeted agents aim to increase the effect of the radiation on the tumor. Substantial preclinical data have accumulated to show that these agents can potentially enhance the tumor response to radiotherapy through a variety of mechanisms (15). They offer new but challenging possibilities for clinical practice. There is a growing number of publications and reviews on the topic of combination of radiotherapy and targeted therapies in many cancers, including NSCLC (16, 17). The addition of targeted agents to thoracic radiation so far has not improved outcomes in patients with locally advanced NSCLC (18, 19).

The combination of radiotherapy and molecular agents targeting vascular endothelial growth factor (VEGF) mediated angiogenesis may evolve synergistic effects leading to enhanced tumor cell killing on the one hand, but to enhanced normal tissue damage on the other hand (20). To date, there are only limited data on the efficacy and toxicity of anti-angiogenic agents given in combination with radiotherapy in lung cancer.

Given the strong preclinical rationale for combining EGFR inhibitors (Cetuximab, Panitumumab, Erlotinib, Gefitinib, Lapatinib, and Trastuzumab) with radiation, additional studies are crucial. Phase I/II data and lack of long-term experience suggest that physicians should consider combined modality approaches with caution, considering the possibility of uncommon but potentially severe toxicity (21).

With high-precision irradiation techniques (such as "stereotactic body radiation therapy"), the combination with targeted agents is feasible with apparent no increase in severe adverse events. Nevertheless, the addition of molecular targeted drugs to radiotherapy outside of approved regimens or clinical trials warrants careful consideration for every single case.

The problem of timing is particular to radiotherapy and molecularly targeted agent combination research. It cannot be assumed that giving the drug concurrently with radiation (as it happens with chemotherapy) is always the optimal treatment strategy. Indeed, drugs that cause cell cycle arrest or prolong cells in the radio-resistant phase of the cell cycle may jeopardize the radiation effect (17).

BRAIN METASTASES

When brain metastases occur in patients with NSCLC, there is often also active disease at the primary site or elsewhere in the body. In few cases, the brain is the only site with active disease (22).

There are many guidelines on the treatment of brain metastases showing that therapeutic intervention (radiotherapy or surgery) is associated with improved brain control (23).

Stereotactic radiosurgery (SRS) to the brain involves a single shot of high dose radiotherapy and can control very efficiently one to few metastases either close to the surface or deep in the brain (24). No randomized trials compared SRS with traditional surgical resection. The traditional whole brain radiotherapy (WBRT) (that covers the whole brain) treats the metastases and may also prevent the growth of new metastases, but may cause side effects such as memory loss. Recent Cochrane review shows that there is low quality evidence that adding upfront WBRT to surgery or to SRS decreases any intracranial disease progression at 1 year. There is also no clear evidence of an effect on overall and progression free survival (25).

Stereotactic radiosurgery has become increasingly important treatment technique in the management of brain metastases, but it is not available everywhere and it is more expensive than WBRT. An approach of SRS alone as initial treatment of brain metastases has allowed patients to delay or avoid WBRT and its associated side effects. "One of the most critical questions on this topic is how "benefit" is defined and from who's perspective – patient, provider, payer, or society" (26). Whether the cost of SRS in multiple brain metastases versus just WBRT approach is justified has yet to be defined.

STEREOTACTIC BODY RADIATION THERAPY

Stereotactic body radiation therapy or "stereotactic ablative radiotherapy" (SART) is a technique similar to SRS, but used in tumors outside the brain. It utilizes precisely targeted high dose radiation to the tumor while minimizing radiation to adjacent normal tissue. It has the luxury of using 4D CT scans to manage the pulmonary motion during treatments. This technique allows treatment of small to moderate sized tumors, in either a single or limited number of high daily dose fractions, with high chances of local control and little toxicity. SBRT has a role in treating selected patients with painful bone metastases or with oligometastases in lungs, liver, or other sites. In spine metastases, for example, non-randomized data show good results with this technique (27). Radiation Therapy Oncology Group (RTOG) study number 0631 is an open Phase II/III Study of Image-Guided SBRT for localized spine metastasis comparing one treatment of 16 Gy delivered with SBRT versus a single fraction of 8 Gy (28).

In patients with NSCLC and with oligometastases, there is a trend to treat them with SBRT, although there is no evidence-based data to show that SBRT is better than traditional palliative radiotherapy. Recently, a proposal submitted to RTOG was not approved because many participants would consider abusive not to offer SBRT in those cases. There is no prospective randomized trial to answer this question. The COMET study (stereotactic ablative radiotherapy for comprehensive treatment of oligometastatic tumors (SABR-COMET) is a randomized Phase II Trial (PIs: David Palma, and Suresh Senan) open in Europe and Canada, comparing patients with up to five metastatic lesions from any primary tumor site who can receive SABR. Eligible patients are randomized to

either standard palliative radiotherapy versus SABR (with further chemotherapy at discretion of medical oncologist).

REFERENCES

1. Okawara G, Mackay JA, Evans WK, Ung YC, Lung Cancer Disease Site Group of Cancer Care Ontario's Program in Evidence-based Care. Lung Cancer Disease Site Group of Cancer Care Ontario's program in evidence-based care. Management of unresected stage III non-small cell lung cancer: a systematic review. *J Thorac Oncol* (2006) **1**:377–93. doi:10.1097/01243894-200605000-00019
2. National Comprehensive Cancer Network. *Treatment Guidelines – NCCN Clinical Practice Guidelines in Oncology Non-Small Cell Lung Cancer V.2.* (2010). Available from: http://www.nccn.org/professionals/physician_gls/PDF/nscl.pdf
3. Rodrigues G, Videtic GM, Sur R, Bezjak A, Bradley J, Hahn CA, et al. Palliative thoracic radiotherapy in lung cancer: an American Society for Radiation Oncology evidence-based clinical practice guideline. *Pract Radiat Oncol* (2011) **1**:60–71. doi:10.1016/j.prro.2011.01.005
4. Lester JF, Macbeth FR, Toy E, Coles B. Palliative radiotherapy regimens for non-small cell lung cancer. *Cochrane Database Syst Rev* (2006) (4):CD002143. doi:10.1002/14651858.CD002143.pub2
5. Kachnic L, Berk L. Palliative single-fraction radiation therapy: how much more evidence is needed? *J Natl Cancer Inst* (2005) **97**:11. doi:10.1093/jnci/dji166
6. Sundstrøm S, Bremnes R, Aasebø U, Aamdal S, Hatlevoll R, Brunsvig P, et al. Hypofractionated palliative thoracic RT (17 Gy/2) in advanced NSCLC is comparable to standard fractionation for symptom control and survival. A national phase III trial. *J Clin Oncol* (2004) **22**:801–10. doi:10.1200/JCO.2004.06.123
7. Cross CK, Berman S, Buswell L, Johnson B, Baldini EH. A prospective study of palliative hypofractionated radiation therapy (8.5 Gy x 2) for patients with symptomatic NSCLC. *Int J Radiat Oncol Biol Phys* (2004) **58**(4):1098–105. doi:10.1016/j.ijrobp.2003.08.005
8. Schraufnagel DE, Hill R, Leech JA, Pare JA. Superior vena caval obstruction. Is it a medical emergency? *Am J Med* (1981) **70**:1169. doi:10.1016/0002-9343(81)90823-8
9. Rowell NP, Gleeson FV. Steroids, radiotherapy, chemotherapy and stents for superior vena caval obstruction in carcinoma of the bronchus: a systematic review. *Clin Oncol (R Coll Radiol)* (2002) **14**:338. doi:10.1053/clon.2002.0095
10. Jonathan F, Wan AB. Superior vena cava syndrome. Review article. *Hematol Oncol Clin North Am* (2010) **24**(3):501–13. doi:10.1016/j.hoc.2010.03.003
11. Ung YC, Yu E, Falkson C, Haynes AE, Stys-Norman D, Evans WK, et al. The role of high-dose-rate brachytherapy in the palliation of symptoms in patients with non-small cell lung cancer: a systematic review. *Brachytherapy* (2006) **5**:189–202. doi:10.1016/j.brachy.2006.05.001
12. Ball D, Smith J, Bishop J, Olver I, Davis S, O'Brien P, et al. A phase III study of radiotherapy with and without continuous-infusion fluorouracil as palliation for non-small cell lung cancer. *Br J Cancer* (1997) **75**:690–7. doi:10.1038/bjc.1997.123
13. Hoffman PC, Cohen EE, Masters GA, Haraf DJ, Mauer AM, Rudin CM, et al. Carboplatin plus vinorelbine with concomitant radiation therapy in advanced nonsmall cell lung cancer. *Lung Cancer* (2002) **38**:65–71. doi:10.1016/S0169-5002(02)00144-7
14. Choi B, Robins HI, Schiller J, Mehta M. A phase I study of gemcitabine plus palliative radiation therapy for advanced lung cancer. *Cancer Chemother Pharmacol* (2008) **63**:175–9. doi:10.1007/s00280-008-0708-8
15. Lin SH, George TJ, Ben-Josef E, Bradley J, Choe KS, Edelman MJ, et al. Opportunities and challenges in the era of molecularly targeted agents and radiation therapy. *J Nat Cancer Inst* (2013) **105**(10):686–93. doi:10.1093/jnci/djt055
16. Niyazi M, Maihoefer C, Krause M, Rödel C, Budach W, Belka C. Radiotherapy and "new" drugs-new side effects? *Radiat Oncol* (2011) **6**:177. doi:10.1186/1748-717X-6-177
17. Searle EJ, Illidge TM, Stratford IJ. Emerging opportunities for the combination of molecularly targeted drugs with radiotherapy. *Clin Oncol (R Coll Radiol)* (2014) **26**(5):266–76. doi:10.1016/j.clon.2014.02.006
18. Spigel DR, Hainsworth JD, Yardley DA, Raefsky E, Patton J, Peacock N, et al. Tracheoesophageal fistula formation in patients with lung cancer treated with chemoradiation and bevacizumab. *J Clin Oncol* (2010) **28**:43–8. doi:10.1200/JCO.2009.24.7353
19. Sacco PC, Maione P, Rossi A, Bareschino MA, Schettino C, Guida C, et al. Combination of radiotherapy and targeted therapies in the treatment of locally advanced non-small cell lung cancer. *Target Oncol* (2011) **6**(3):171–80. doi:10.1007/s11523-011-0169-6
20. Mazeron R, Anderson B, Supiot S, Paris F, Deutsch E. Current state of knowledge regarding the use of antiangiogenic agents with radiation therapy. *Cancer Treat Rev* (2011) **37**:476–86. doi:10.1016/j.ctrv.2011.03.004
21. Xu Y, Zhang Y, Ma S. EGFR inhibitors with concurrent thoracic radiation therapy for locally advanced non-small cell lung cancer. *Lung Cancer* (2011) **73**(3):249–55. doi:10.1016/j.lungcan.2011.04.017
22. Ashworth A, Rodrigues G, Boldt G, Palma D. Is there an oligometastatic state in non-small cell lung cancer? A systematic review of the literature. *Lung Cancer* (2013) **82**(2):197–203. doi:10.1016/j.lungcan.2013.07.026
23. Tsao MN, Rades D, Wirth A, Lo SS, Danielson BL, Gaspar LE, et al. Radiotherapeutic and surgical management for newly diagnosed brain metastasis(es): an American Society for Radiation Oncology evidence-based guideline. *Pract Radiat Oncol* (2012) **2**:210–25. doi:10.1016/j.prro.2011.12.004
24. Khalsa SS, Chinn M, Krucoff M, Sherman JH. The role of stereotactic radiosurgery for multiple brain metastases in stable systemic disease: a review of the literature. *Acta Neurochir (Wien)* (2013) **155**(7):1321–7. doi:10.1007/s00701-013-1701-5
25. Soon YY, Tham IW, Lim KH, Koh WY, Lu JJ. Surgery or radiosurgery plus whole brain radiotherapy versus surgery or radiosurgery alone for brain metastases. *Cochrane Database Syst Rev* (2014) **3**:CD009454. doi:10.1002/14651858.CD009454.pub2 [Epub ahead of print].
26. Halasz LM, Rockhill JK. Stereotactic radiosurgery and stereotactic radiotherapy for brain metastases. *Surg Neurol Int* (2013) **4**(Suppl 4):S185–91. doi:10.4103/2152-7806.111295
27. Wang XS, Rhines LD, Shiu AS, Yang JN, Selek U, Gning I, et al. Stereotactic body radiation therapy for management of spinal metastases in patients without spinal cord compression: a phase 1-2 trial. *Lancet Oncol* (2012) **13**:395. doi:10.1016/S1470-2045(11)70384-9
28. Available from: http://www.rtog.org/ClinicalTrials/ProtocolTable/StudyDetails.aspx?study=0631

Conflict of Interest Statement: The Guest Associate Editor Vera Hirsh declares that, despite being affiliated to the same institution as author Sergio Faria, the review process was handled objectively and no conflict of interest exists. The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 08 May 2014; accepted: 08 August 2014; published online: 13 October 2014.
*Citation: Faria SL (2014) Role of radiotherapy in metastatic non-small cell lung cancer. Front. Oncol. **4**:229. doi: 10.3389/fonc.2014.00229*
This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 30 July 2014
doi: 10.3389/fonc.2014.00192

Collaborative care in NSCLC; the role of early palliative care

Marnie Howe*[1] *and Ronald L. Burkes*[2] *

[1] *Department of Family and Community Medicine, Division of Palliative Medicine, University of Toronto, Toronto, ON, Canada*
[2] *Department of Medicine, Division of Medical Oncology, University of Toronto, Toronto, ON, Canada*

Edited by:
Vera Hirsh, McGill University Health Centre, Canada

Reviewed by:
Veerle Surmont, Ghent University, Belgium
Shahab Babakoohi, Medstar Good Samaritan Hospital, USA

***Correspondence:**
Ronald L. Burkes, Mount Sinai Hospital, 600 University Avenue, Suite 1221, Toronto, ON M5G 1X5, Canada
e-mail: rburkes@mtsinai.on.ca

The management of non-small cell lung cancer (NSCLC) has evolved into a multidisciplinary team approach that traditionally has involved medical oncology, radiation oncology, and thoracic surgery. However, in the era of personalized medicine the importance of molecular diagnostics requires adequate tissue for histologic subtyping and molecular testing and thus requires the engagement of other subspecialties such as pathology, respirology, and interventional radiology. Unfortunately in 2014, the majority of patients presenting with NSCLC will succumb to their disease and the early integration of palliative care into the treatment strategy will improve the quality of life and end-of-life care of our patients and may in fact improve their overall survival.

Keywords: collaborative care, NSCLC, early palliative care

INTRODUCTION

Lung cancer is globally the leading cause of cancer death for both men and women, more so than colorectal, breast and prostate cancer combined (1). According to the Canadian Cancer Society's statistics 2013 report, lung cancer accounts for approximately 25% of cancer deaths each year (2). Histologically, the majority of lung cancers diagnosed are non-small cell lung cancer (NSCLC). The most important risk factor for developing lung cancer remains tobacco use, accounting for an estimated 86% of lung cancer cases in high-income countries like Canada and the median age at diagnosis is 70. However, the incidence of lung cancer in non-smoking young women is increasing (2).

Although there is no data available specifically on NSCLC in Canada, the National Cancer Institute's surveillance, epidemiology, and end results (SEER) database in the United States has reported 5-year survival rates across all stages being only 16%, with stage IIIA, IIIB, and IV at 14, 5, and 1%, respectively (3). Although post-operative adjuvant treatment, combined-modality treatment approaches and the newer targeted therapies have improved outcomes in a subset of patients, NSCLC continues to be diagnosed late with the majority of patients presenting with advanced stage IV disease with an associated significant symptom burden. Thus, the goal of systemic therapy in this setting is to prolong survival and improve quality of life (QoL) by treating cancer related symptoms.

Dr. Ellis has previously written an excellent review of the importance of the multidisciplinary team management of patients with NSCLC (4). In this review, he addressed such things as diagnostic assessment clinics, multidisciplinary case conferencing, and involvement of the patient as part of the team as well as psychosocial and nutritional support. Thus, the primary focus of this review will be on the early integration of palliative care. As well we will briefly address the rationale for an increased awareness of recent advances in lung cancer care among the various specialties involved in the assessment of patients with lung cancer and the need to establish a cohesive network for ongoing communication and collaboration.

ONE EXAMPLE OF THE IMPORTANCE OF A COLLABORATIVE CARE APPROACH TO NON-SMALL CELL LUNG CANCER

Although chemotherapy remains the standard of care for most patients, there has clearly been a shift toward personalized therapy with the understanding of molecular diagnosis and treatment of lung cancer. This has come about by the identification of a number of actionable mutations including the EGFR and ALK fusion genes, which have revolutionized therapy in those patients who harbor these mutations (5–7). As demonstrated by the Lung Cancer Mutation Consortium, two-thirds of NSCLC patients have an oncogenic driver and those patients with these drivers have been shown to live longer if they receive the corresponding targeted agent (8).

Unfortunately, the majority of patients present to the medical oncologist without knowledge of the EGFR or ALK mutation status, which has the potential for delaying the start of treatment. Furthermore, a significant number of patients will be found to have insufficient tissue to do the appropriate molecular analysis, which will further delay treatment and often requires commencing chemotherapy in patients in whom it might be unnecessary thus exposing them to increased toxicity and an inferior treatment. Therefore, it is important for physicians involved in the diagnosis and treatment of lung cancer to be aware that maximizing tissue yield for histologic subtyping and molecular testing is not only crucial but also essential to be able to offer patients a personalized treatment approach.

Thus, an increased awareness of recent advances in lung cancer care by engaging multidisciplinary teams in discussions about innovative knowledge transfer strategies would hopefully lead to more effective practice. It is also important for professionals

including respirologists, thoracic surgeons, internal medicine, and interventional radiologists working with lung cancer patients to establish a cohesive network for ongoing communication and collaboration.

INTEGRATION OF EARLY PALLIATIVE CARE

A study examining inoperable NSCLC patients with a good performance status found an average of 14.3 symptoms reported by these patients at presentation. Even patients with a WHO Performance Status of 0 (fully active) reported an average of 11.6 symptoms. The most commonly reported symptoms included fatigue, lack of energy, shortness of breath, cough, worrying, and chest pain (9). Similarly, a Canadian study conducted in the ambulatory setting found newly diagnosed lung cancer patients had a greater symptom burden compared to other cancer sites (10). As expected, as lung cancer progresses, so does the symptom burden. A group of patients in a community radiation oncology program were found to have increased symptom severity during the last few months of their life, compared to the 3-months prior (11). These studies highlight the importance of early palliative care (EPC) involvement to help guide symptom management.

Palliative care has also been shown to assist with goals of care discussions and address psychosocial supports. The WHO now defines palliative care as "an approach that improves the quality of life of patients and their families facing the problem associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain and other problems including physical, psychosocial, and spiritual." As well they state "is applicable early in the course of illness, in conjunction with other therapies that are intended to prolong life, such as chemotherapy or radiation therapy, and includes those investigations needed to better understand and manage distressing clinical complications" (12). Patients report inadequate communication with providers about shared decision-making at the end-of-life (EOL). This better understanding of their illness through important conversations can help patients and families prepare for EOL, which they have reported as a valuable aspect of their care. In fact, patients rely on their physicians to discuss hospice, advanced directives, and other EOL care options. Studies have shown deficiencies in the area of communication surrounding EOL (13, 14) and palliative care can assist in facilitating these important discussions.

The American Society of Clinical Oncology published a provisional clinical opinion in 2012 recommending the integration of palliative care into standard oncology care for patients with metastatic cancer. This recommendation was based on a phase III clinical trial conducted by Temel and colleagues of 151 ambulatory patients newly diagnosed with NSCLC. Patients were randomly assigned to standard oncology care or EPC. The primary outcome was QoL at 12 weeks, with secondary outcomes including mood, understanding of illness, and aggressiveness of care at the EOL. Both QoL and depression had significantly improved with EPC. In addition, patients in the intervention group had higher documentation rates of resuscitation preferences, as well as less aggressive care at EOL, including intravenous chemotherapy in the last 2 weeks of life. Despite this, patients who received EPC had a significantly prolonged survival, by approximately 3 months (15).

A recent review by Irwin et al. postulated the possible mechanisms for prolonged survival attributed to EPC in patients with metastatic NSCLC. They identified four randomized controlled trials, including the Temel study above, which studied palliative care interventions' effects on QoL and EOL care in cancer patients. They all found improved QoL in patients who received EPC, and two of the studies showed increased survival in the intervention groups. Irwin and colleagues hypothesized several variables are associated with increased survival in this population based on current literature (16). The first being palliative care's focus on relieving suffering and improving symptom distress. Both health-related QoL and physical symptoms have been associated with prognosis (17, 18). Secondly, they identified treating depression as a potential mechanism for improved survival. The relationship between depression and survival may be due to multiple effects. Improving depression may have a beneficial impact on health behaviors linked to treatment adherence and well-being. Depression is known to activate the hypothalamic–pituitary axis and thus increase levels of cortisol, affecting the immune system and specifically, helper T cells (19, 20). A third mechanism identified is EPC's focus on disease/prognosis understanding and goals of care discussions. When patients had a better understanding of their prognosis, they were less likely to receive chemotherapy at EOL. Many believe that there is a point at EOL where the toxicity of chemotherapy may hasten death. Temel et al. found patients assigned to EPC were less likely to receive intravenous chemotherapy at EOL and the interval between last chemotherapy infusion and time of death was longer for patients who received EPC compared to those who did not (16). Finally, the focus on increasing social support for both the patient and their caregivers may also be a factor in extending length of survival. Many studies have demonstrated that marital status is associated with a survival advantage for lung cancer patients, which may translate into increased social support (21, 22).

In a more recent retrospective study, Hui and colleagues (23) looked at the impact, timing, and setting of palliative care referrals on the quality of end-of-life in cancer patients. A total of 366 adult cancer patients at a tertiary care center who between September 1, 2009 and February 28, 2010 who received a palliative care referral and who had contact with the cancer center within the last 3 months of life were included in the study. Outpatient referrals were associated with significantly fewer emergency room visits, hospital admissions, hospital deaths, ICU admissions, and a shorter duration of hospital stay, when compared to inpatient referrals. Outpatient palliative care referral remained an independent factor for improved end-of-life care in multivariate analysis. It is noteworthy that 20% of patients in each group had a diagnosis of lung cancer. Early outpatient involvement with palliative care allows patients to develop a longitudinal therapeutic relationship. Through the course of multiple clinic visits the palliative care team can help facilitate goals of care discussions and advanced care planning, with the hope of reducing aggressive interventions at EOL. The palliative care team also has access to community resources and supports for both the patient and their families as their disease progresses. Finally, the palliative care focus on symptom assessments not only improves QoL but can also minimize unnecessary ER or hospital admissions with patient education and routine follow-up for symptom management (23).

CONCLUSION

Clearly, our ultimate goal is to improve the diagnosis and care of lung cancer patients. The treatment of NSCLC has evolved considerably including the introduction of post-operative adjuvant therapy for early stage disease and the use of combined-modality therapy for stage III disease that requires the collaboration of medical oncology, radiation oncology, and thoracic surgery. The management of advanced disease has also evolved significantly and a personalized approach to treatment in advanced stage IV disease is a reality. Although traditionally, collaboration has primarily been between thoracic surgery, radiation oncology, and medical oncology, in the era of personalized medicine it is important for all physicians involved in the diagnosis and treatment of lung cancer to be aware that maximizing tissue yield for histologic subtyping and molecular testing is not only crucial but also essential to be able to offer patients the appropriate therapy in a timely fashion. Unfortunately in 2014, the majority of patients presenting with NSCLC will succumb to their disease and studies have shown that the early integration of palliative care into the management strategy will improve the QoL and EOL care of our patients.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* (2011) **61**(2):69–90. doi:10.3322/caac.20107
2. Canadian Cancer Society. *Lung Cancer Statistics* (2014). Available from: http://www.cancer.ca/en/cancer-information/cancer-type/lung/statistics/?region=on
3. American Cancer Society. *Non-Small Cell Lung Cancer Survival Rates by Stage* (2014). Available from: http://www.cancer.org/cancer/lungcancer-non-smallcell/detailedguide/non-small-cell-lung-cancer-survival-rates
4. Ellis PM. The importance of multidisciplinary team management of patients with non-small-cell lung cancer. *Curr Oncol* (2012) **19**:S7–15. doi:10.3747/co.19.1069
5. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* (2004) **350**:2129–39. doi:10.1056/NEJMoa040938
6. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* (2004) **304**:1497–500. doi:10.1126/science.1099314
7. Kwak EL, Bang YJ, Camidge DR, Shaw A, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* (2010) **363**(18):1693–703. doi:10.1056/NEJMoa1006448
8. Kris M, Johnson B, Berry L, Kwiatkowski D, Iafrate AJ, Wistuba I, et al. Treatment with therapies matched to oncogenic drivers improves survival in patients with lung cancers: results from the lung cancer mutation consortium (LCMC). *J Thorac Oncol* (2013) **8**(Suppl 2):S3. doi:10.1001/jama.2014.3741
9. Hopwood P, Stephens RJ. Symptoms at presentation for treatment in patients with lung cancer: implications for the evaluation of palliative treatment. The Medical Research Council (MRC) lung cancer working party. *Br J Cancer* (1995) **71**:633–6. doi:10.1038/bjc.1995.124
10. Degner LF. Symptom distress in newly diagnosed ambulatory cancer patients and as a predictor of survival in lung cancer. *J Pain Symptom Manage* (1995) **10**:423–31. doi:10.1016/0885-3924(95)00056-5
11. Lutz S. Symptom frequency and severity in patients with metastatic or locally recurrent lung cancer: a prospective study using the lung cancer symptom scale in a community hospital. *J Palliat Med* (2001) **4**:157–65. doi:10.1089/109662101750290191
12. World Health Organization. *WHO Definition of Palliative Care* (2014). Available from: http://www.who.int/cancer/palliative/definition/en/
13. Gysels M, Richardson A, Higginson IJ. Communication training for health professionals who care for patients with cancer: a systematic review of effectiveness. *Support Care Cancer* (2004) **12**(10):692–700. doi:10.1007/s00520-004-0666-6
14. Huskamp HA, Keating NL, Malin JL, Zaslavsky AM, Weeks JC, Earle CC, et al. Discussions with physicians about hospice among patients with metastatic lung cancer. *Arch Intern Med* (2009) **169**(10):954–962. doi:10.1001/archinternmed.2009.127
15. Temel JS, Greer JA, Muzikansky A, Muzikansky A, Gallagher ER, Admane S, et al. Early palliative care for patients with metastatic non-small-cell lung cancer. *N Engl J Med* (2010) **363**:733–42. doi:10.1056/NEJMoa1000678
16. Irwin KE, Greer JA, Khatib J, Temel JS, Pirl WF. Early palliative care and metastatic non-small cell lung cancer: potential mechanisms of prolonged survival. *Chron Respir Dis* (2012) **10**(1):35–47. doi:10.1177/1479972312471549
17. Gotay CC, Kawamoto CT, Bottomley A, Efficace F. The prognostic significance of patient-reported outcomes in cancer clinical trials. *J Clin Oncol* (2008) **26**(8):1355–62. doi:10.1200/JCO.2007.13.3439
18. Quinten C, Coens C, Mauer M, Comte S, Sprangers MAG, Cleeland C, et al. Baseline quality of life as a prognostic indicator of survival: a met-analysis of individual patient data from EORTC clinical trials. *Lancet Oncol* (2009) **10**:865–71. doi:10.1016/S1470-2045(09)70200-1
19. Nelson EL, Wenzel LB, Osann K, Dogan-Ates A, Chantana N, Reina-Patton A, et al. Stress, immunity, and cervical cancer: biobehavioral outcomes of a randomized controlled trial. *Clin Cancer Res* (2008) **14**(7):2111–8. doi:10.1158/1078-0432.CCR-07-1632
20. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcomes. *Science* (2006) **313**:1960–4. doi:10.1126/science.1129139
21. Lai H, Lai S, Krongrad A. The effect of marital status on survival in late-stage cancer patients: an analysis based on surveillance, epidemiology, and end results (SEER) data, in the United States. *Int J Behav Med* (1999) **6**:150–76. doi:10.1207/s15327558ijbm0602_4
22. Kravdal O. The impact of marital status on cancer survival. *Soc Sci Med* (2001) **52**(3):357–68. doi:10.1016/S0277-9536(00)00139-8
23. Hui D, Kim SH, Roquemore J, Dev R, Chisholm G, Bruera E. Impact of timing and setting of palliative care referral on quality of end-of-life care in cancer patients. *Cancer* (2014) **120**(11):1743–9. doi:10.1002/cncr.28628

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 16 May 2014; accepted: 07 July 2014; published online: 30 July 2014.
*Citation: Howe M and Burkes RL (2014) Collaborative care in NSCLC; the role of early palliative care. Front. Oncol. **4**:192. doi: 10.3389/fonc.2014.00192*
This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

REVIEW ARTICLE
published: 16 September 2014
doi: 10.3389/fonc.2014.00238

Management of common toxicities in metastatic NSCLC related to anti-lung cancer therapies with EGFR–TKIs

Barbara Melosky [1] **** and Vera Hirsh*** [2]

[1] *Medical Oncology, British Columbia Cancer Agency – Vancouver Centre, Vancouver, BC, Canada*
[2] *Department of Medical Oncology, McGill University Health Centre, Royal Victoria Hospital, Montreal, QC, Canada*

Edited by:
Martin J. Edelman, University of Maryland Greenebaum Cancer Center, USA

Reviewed by:
Rabab Mohamed Gaafar, Cairo University, Egypt
Bo H. Chao, The Ohio State University, USA

****Correspondence:***
Barbara Melosky, British Columbia Cancer Agency, 600-10th Avenue West, Vancouver, BC V5Z 4E6, Canada
e-mail: bmelosky@bccancer.bc.ca

Tyrosine kinase inhibitors (TKIs) against the epidermal growth factor receptor (EGFR) are the standard of care treatment in non-small cell lung cancer (NSCLC). TKIs are used first line in EGFR mutation-positive NSCLC; erlotinib is the only TKI approved for subsequent lines of treatment in EGFR wild-type NSCLC. As promising as TKIs are in helping patients avoid some of the side effects of traditional cytotoxic chemotherapy, they do come with a variety of side effects. This article will describe the most common adverse events associated with the epidermal EGFR family of TKIs including diarrhea, rash, mucositis, and paronychia. The objective of this paper is to provide simple guidelines to assist oncologists in managing these common toxicities. As patient survival is often directly correlated with successful therapeutic drug delivery, the management of TKI-induced adverse events ensures proper treatment and may avoid discontinuation or reduction of the therapeutic.

Keywords: tyrosine kinase inhibitor, EGFR, adverse event management, diarrhea, rash, stomatitis/mucositis, paronychia

INTRODUCTION

Tyrosine kinase inhibitors (TKI) are a relatively new class of targeted therapeutics used to treat a range of diseases and disorders, primarily cancers. The first TKIs, described in 1988, specifically inhibited the epidermal growth factor receptor (EGFR) cascade (1). This unique class of orally administered small molecule therapeutics has found their way into the standard of care treatment in almost all types of malignancy including non-small cell lung cancer (NSCLC). Many new EGFR–TKIs will continue to emerge, and the number of TKIs in use will continue to expand.

Tyrosine kinase inhibitors may help patients avoid some of the side effects of traditional cytotoxic chemotherapy, where toxicities usually involve bone marrow involvement. However, as promising as the TKIs are, they do come with a variety of side effects. These gastroenterologic side effects, such as diarrhea and mucositis, and cutaneous side effects, such as rash and paronychia, deserve attention as successful management can extend the time that the patient is on therapy. To date, there have been few clinical studies conducted to study these side effects. The objective of this paper is to provide simple guidelines to assist oncologists in managing these common toxicities. While the use of EGFR–TKIs can also have more severe adverse events (SAE) including ocular disorders, interstitial lung disease, or hepatotoxicity, these SAE are uncommon and fall beyond the scope of this paper.

DIARRHEA

Diarrhea is one of the most frequent adverse events of EGFR–TKI therapy. The gastrointestinal tract expresses EGFR on cells of epithelial origin.

Abbreviations: BCCA, BC cancer agency; EGFR, epidermal growth factor receptor; NCI, National Cancer Institute; NSCLC, non-small cell lung cancer; QOL, quality of life; RCT, randomized clinical trial; SAE, severe adverse event; TKI, tyrosine kinase inhibitor.

CAUSES AND INCIDENCE

Epidermal growth factor receptor–tyrosine kinase inhibitors induced diarrhea is thought to result from excess chloride secretion that causes a secretory form of diarrhea (2). Severe diarrhea can result in fluid and electrolyte loss, which then can lead to dehydration, electrolyte imbalances, and renal insufficiency. Alterations in gastrointestinal transit and digestion can lead to nutritional deficiencies that can negatively impact the quality of life (QOL) of patients (3).

The incidence of diarrhea with EGFR–TKI treatment in phase III clinical trials ranges from 27 to 87% with up to 25% of patients experiencing SAEs (**Table 1**).

GRADING AND ASSESSMENT OF DIARRHEA

The severity of diarrhea is graded using the National Cancer Institute's CTCAE (**Table 2**). These criteria (grades) do not provide a complete assessment, and additional information should be obtained from the patient evaluation. It is important to rule out other possible causes of diarrhea. These include medications such as laxatives, stool softeners, antibiotics, or antacids; dietary factors such as excess consumption of fiber or dairy products, greasy foods; comorbid infections such as intestinal obstruction, fecal impaction, and surgeries (short bowel or gastrectomy); or radiation toxicity.

Laboratory investigations include a complete blood count and differential to rule out neutropenia, blood tests to assess renal function, and electrolyte abnormalities and a stool culture or *Clostridium difficile* toxin screen to check for bacterial pathogens. To rule out co-existing disorders such as bowel obstruction or perforation, abdominal radiography, endograph endoscopy, or biopsy might need to be performed. Duration of diarrhea, stool characteristics, and co-existing symptoms should also be obtained from the patient (3, 4).

MANAGEMENT RECOMMENDATIONS FOR DIARRHEA

Patients should be advised to immediately report any symptoms of diarrhea, so they can be managed early and effectively. Patients have to understand the importance of avoiding/preventing dose reductions or discontinuation of EGFR–TKIs.

Dietary changes and over-the-counter anti-diarrheal medications can generally be used to manage EGFR–TKI induced diarrhea. This management is identical to that of chemotherapy-induced diarrhea (3–5).

Patients who experience diarrhea should avoid greasy, spicy, and fried foods as they can exacerbate the symptoms. Until symptoms start to resolve, patients can eat a diet of bananas, rice, apple sauce, and toast (BRAT) and avoid foods that may increase abdominal cramping and bloating such as Brussels sprouts, cabbage, and broccoli. When symptoms start to improve, foods such as eggs, pasta, and skinless chicken can be added. Patients should drink 3–4 l of fluid to prevent dehydration. Prolonged diarrhea may cause diminished lactase activity resulting in lactose intolerance thus milk products should be avoided for about a week following diarrhea (3–5).

The pharmacologic management of diarrhea is generally limited to over-the-counter loperamide (**Figure 1**). After the first diarrhea, patients should start 4 mg of loperamide followed by 2 mg after each loose stool or every 4 h to a maximum daily dose of 20 mg. If symptoms persist for more than 24 h, the dose of loperamide can be increased to 4 mg followed by 2 mg every 2 h. If 12 h have passed without diarrhea, loperamide can be stopped (3–6).

Epidermal growth factor receptor–tyrosine kinase inhibitors cessation is required for grade 3 or 4 diarrhea and then restart EGFR–TKI at a lower dose once the severe symptoms have subsided. Use of octreotide is generally for chemotherapy-induced diarrhea and there is no evidence to support its use in the EGFR–TKI setting.

Table 1 | Incidence of diarrhea with EGFR–TKIs in NSCLC clinical trials.

EGFR–TKI	Description	Grade (%) All ≥3
Erlotinib 150 mg	All studies	(10–69) (0–17)
	Phase III studies	(40–68) (2–12)
Gefitinib 250 and 500 mg	All studies	(27–75) (0–25)
	• 250 mg	(27–58) (0–10)
	• 500 mg	(51–75) (5–25)
	Phase III studies	(27–69) (3–25)
	• 250 mg	(27–58) (3–10)
	• 500 mg	(51–69) (12–25)
Afatinib 40 and 50 mg	All studies	(67–100) (0–33)
	• 40 mg	(67–97) (0–7)
	• 50 mg	(87–100) (17–33)
	Phase III studies	
	• 50 mg	(87–17)
Dacomitinib 15, 30, and 45 mg	All studies (phase II)	(77–97) (0–15)
	• 30 mg	(77–0)
	• 45 mg	(81–97) (13–15)

Adapted from Hirsh (10).

RASH

Cutaneous side effects of EGFT–TKIs can include skin rashes of many different types and severity. The rash is very acne-like in appearance, and is accurately described as a papulopustular eruption. Other descriptions include the terms acneiform skin reaction, acneiform rash, acneiform follicular rash, acne-like rash, maculopapular skin rash, and amonomorphic pustular lesions. The EGFR–TKI induced rash most often appears on the face and chest, but can be more widespread. The rash may be triggered by sun exposure (7). Dry skin, pruritus, ocular, and hair changes are also common.

CAUSES AND INCIDENCE

Epidermal growth factor receptor is expressed in the basal layer of the epidermis, and its normal physiological roles include stimulation of epidermal growth, inhibition of differentiation, and acceleration of wound healing. As the name of this receptor is "epidermal," it is no surprise that inhibitor toxicity may include the epidermis. Pathophysiological effects of EGFR inhibition include impaired growth and migration of keratinocytes, and the expression of inflammatory chemokines by these cells, which results in inflammatory cell recruitment (8). Not surprisingly, a histologic analysis demonstrates a mixed inflammatory infiltrate in the upper areas of the skin. This inflammation and subsequent cutaneous injury accounts for many of the symptoms observed in patients being treated with this class of TKI, including tenderness, papulopustules, and periungual inflammation (9).

Table 2 | US National Cancer Institute grading for diarrhea.

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
An increase of <4 stools over baseline, per day	An increase of 4–6 stools over baseline, per day	An increase of 7 or more stools over baseline per day	Life-threatening consequences	Death
		Incontinence	Urgent intervention indicated	
		Hospitalization indicated		
		Limits self-care activities of daily living		

Adapted from the Common Terminology Criteria for Adverse Events (15).

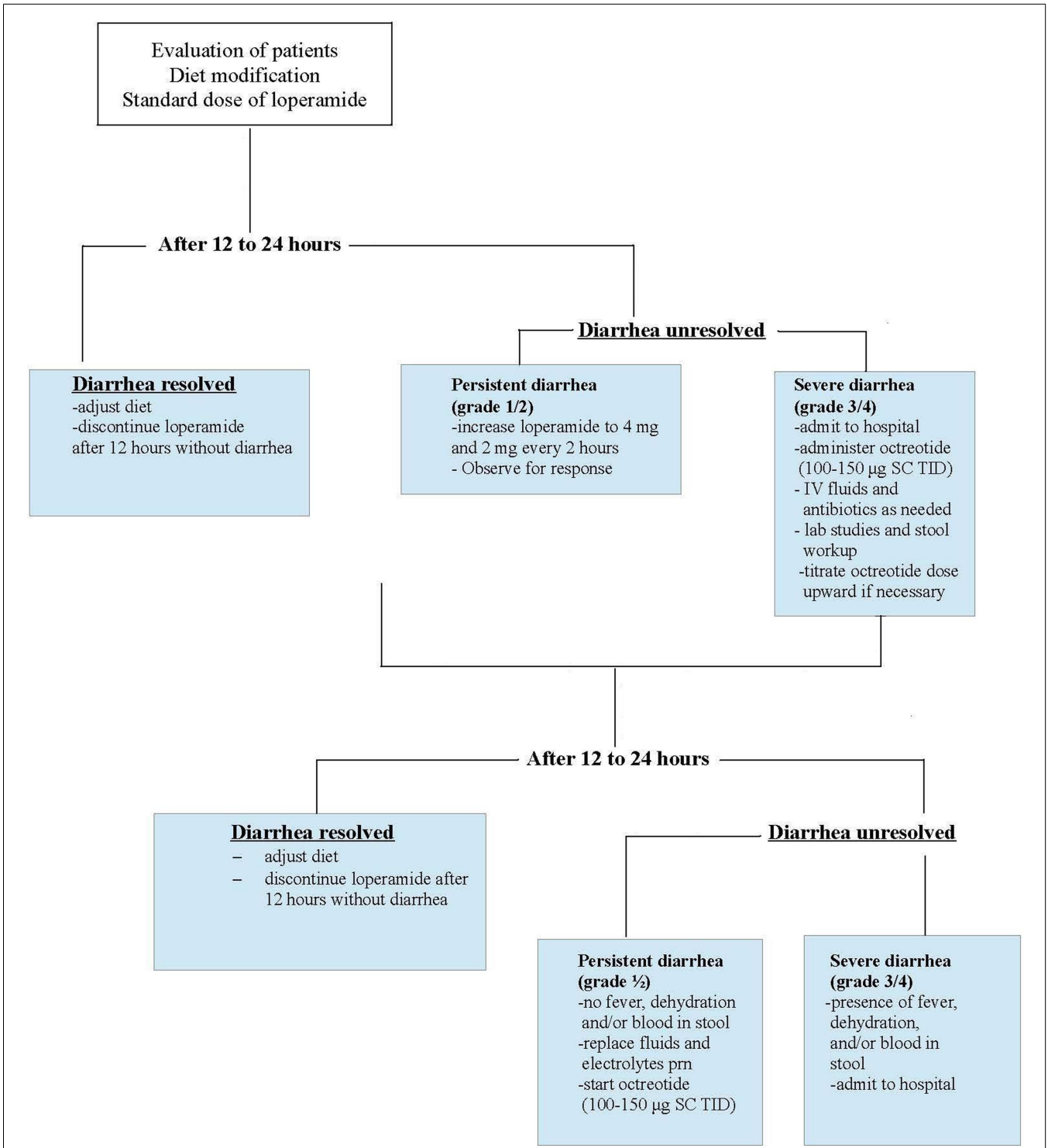


FIGURE 1 | Management of diarrhea induced by chemotherapy or EGFR–TKIs. IV, intravenous, SC, subcutaneous, TID, three times daily. Adapted from Hirsh (10).

There are several phases to the cutaneous manifestations. In the first week of TKI treatment, patients often experience sensory disturbances, erythema, and edema. In the second week of TKI treatment, patients experience papulopustular eruptions, followed by crusting in week 4. In the 4–6 weeks following, a background of erythema and dry skin can be seen in areas previously affected by the papulopustular eruption (8).

Table 3 | Rate of grade 3 rash, *Paronychia* and *Stomatitis/mucositis* observed in trials.

Grade 3/4 adverse events	EGFR–TKI and trial			
	Gefitinib IPASS (3) (*N* = 607) (%)	**Erlotinib EURTAC (4) (*N* = 84) (%)**	**Afatinib LUX-Lung 3 (5) (*N* = 229) (%)**	**Afatinib LUX-Lung 6 (6) (*N* = 239) (%)**
Rash/acne	3.1	13	16.2	14.6
Stomatitis/mucositis	0.2	NR	8.7	5.4
Paronychia	0.3	NR	11.4	NR

NR: not reported.

Incidence of all grades of rash in phase 3 clinical trials varies from 37 to 78% (10). Grade 3 rash ranges from 3.1 to 16.2% depending on the trial (**Table 3**) (11–14).

MANAGEMENT RECOMMENDATIONS FOR SKIN RASH

Patient education is a very important aspect of management, and several important points need to be communicated and emphasized. First of all, the rash is not contagious; the skin toxicity is not infectious, but inflammatory. Secondly, this skin rash is not acne, and so patients should be strongly discouraged from treating the rash with over-the-counter acne medications, as acne medication is very drying and will exacerbate the pruritus. Third, as dry skin is almost universally experienced by patients taking this medication, they should be instructed to use an alcohol-free emollient cream applied twice daily, preferably to their entire body. Finally, since sun exposure may aggravate the pruritus, patients are advised to avoid sun exposure, and a broad spectrum sunscreen is strongly recommended (9).

The specific treatment algorithms for rashes caused by EGFR inhibitors vary widely throughout the different centers that use these agents in their clinics. Nonetheless, some basic principles may apply to all situations. Early treatment of rash can prevent symptoms from becoming worse, so clinicians are advised to assess patients weekly, and intervene when the first symptoms of rash appear. Management strategies for EGFR–TKI induced rash are shown in **Table 4**.

Mild reactions (NCI-CTC grade 1) (15) are generally localized with no associated physical symptoms. Treatment options include topical low–medium potency corticosteroids. Other options include the addition of clindamycin 1% gel to hydrocortisone 1% and the use of oral semi-synthetic tetracyclines (i.e., doxycycline or minocycline). The EGFR inhibitor should be continued while the rash is being treated.

Moderate reactions (NCI-CTC grade 2) (15) are more severe and can include symptoms such as tenderness or itching. The recommended treatment is hydrocortisone 1 or 2.5% cream ± clindamycin 1% cream, as well as a 4-week course of an oral tetracycline antibiotic, such as doxycycline 100 mg daily or minocycline 100 mg twice daily. Minocyline can cause nausea in a small percentage of patients, and reduction to 100 mg daily may be better tolerated. As the rash from the EGFR–TKI may wax and wane, the treatment may need to be repeated at several intervals.

Table 4 | BCCA management guidelines for EGFR–TKI induced rash.

Grade	Toxicity	EGFR inhibitor
1	Macular or papular eruption or erythema with no associated symptoms	Maintain dose level of TKI Consider clindamycin 2% and hydrocortisone 1% in a lotion to be applied topically BID as needed
2	Macular or papulopustular eruption or erythema with pruritus or other symptoms that are tolerable or interfere with daily life	Maintain dose level of TKI Consider clindamycin 2% and hydrocortisone 1% in a lotion to be applied topically BID as needed +minocycline 100 mg PO BID for 1–2 weeks or longer as needed
3	Severe, generalized erythroderma, or macular, popular or vesicular eruption	Withhold EGFR TKI for 10–14 days When improvement to grade 2 or less, continue at 50% of original dose If toxicities do not worsen, escalate by 25% increments of original dose until starting dose is reached If no improvement, discontinue Continue treatment with clindamycin 2% and hydrocortisone 1% in a lotion to be applied topically BID as needed +minocycline 100 mg PO BID for 1 to 2 weeks or longer as needed
4	Generalized exfoliative, ulcerative, or blistering skin toxicity	Discontinue treatment

Adapted from the management guidelines utilized in the BC Cancer Agency (BCCA) Oncology Department.

Severe reactions (NCl-CTC grade 3) (15) are generalized with major symptoms affecting activities of daily living and are intolerable to the patient. Though histological findings suggest that the papulopustular eruption has an inflammatory component, the use of topical oral corticosteroids is based on empirical data. A temporary 7–10 days discontinuation of the TKI involved is recommended, with subsequent reintroduction at a lower dose according to the product monograph. Treatment with both a steroid cream and oral tetracycline as per moderate rash is encouraged during the interruption period. When treatment is

reintroduced, dose escalation of the TKI being used is often possible.

Some guidelines, including BC Cancer Agency guidelines, include drug dose reduction to alleviate severe drug reactions. While side effects of the TKIs are often unpleasant, effort must be made to maintain patients on their cancer therapies. If a TKI is administered in only one dose, for example, in the case of gefitinib where only one dose exists, switching the patient to another TKI that has more flexible dosing is strongly recommended.

Dry skin in the trunk and extremities is very common in patients being treated with EGFR–TKIs. Fragrance free creams and ointments are recommended over lotions, which may contain alcohol. For scaling and hyperkeratosis, ammonium lactate and urea-containing preparations are also useful, but they should be used with care because of greater skin sensitivity in these patients.

A scalp rash may be successfully treated with the basic principles above, however, a gel can be formulated as cream and lotion treatment can be unappealing in the hair or hairline area of the neck. Patients may often develop lesions and plaques on the scalp, which can be treated with topical clindamycin 2% plus triamcinolone acetonide 0.1% in equal parts of propylene glycol and water until resolution.

MUSOSITIS/STOMATITIS

In addition to diarrhea, patients taking EGFR–TKIs often experience other gastrointestinal side effects. Although used interchangeably, mucositis refers to inflammation of the gastrointestinal tract while stomatitis refers to inflammation of the mouth (15, 16). Symptoms can also include tingling in the mouth or on the tongue, ulcers or cracks on the side of the mouth (B. Melosky, unpublished observation). This side effect is rarely seen with the first generation TKIs, but is observed with the second generation TKIs. In the original IPASS trial, 0.2% of patients treated with gefitinib reported grade 3 mucositis/stomatitis (11), while 8.7% of patients treated with afatinib in LUX-Lung 3 experienced grade 3 mucositis/stomatitis (**Table 3**) (13). As this is a new finding, the true incidence of mucositis/stomatitis has not often been evaluated in a trial setting.

MANAGEMENT RECOMMENDATIONS FOR MUCOSITIS/STOMATITIS

There have not been any randomized control trials to determine the best ways to manage EGFR–TKI induced mucositis/stomatitis. A careful examination of patients prior to treatment to determine baseline oral health status and inflammation is recommended, as well as observation during treatment (17). Patients are encouraged to practice good oral hygiene including frequent brushing with a soft brush, flossing, and rinsing with saline. Avoid commercial mouthwashes as they often contain alcohol, which can exacerbate the situation. As symptoms become more severe, oral care should become more frequent (16).

The following strategies for treating stomatitis/mucositis are recommended (Dr. Kim Papp, personal communication). The recommended treatment for grade 1 stomatitis/mucositis is kenalog in Orabase®, applied two or three times a day. The recommended treatment for grade 2 stomatitis/mucositis is kenalog in Orabase®, with the addition of 250–350 mg of erythromycin a day. The recommended treatment for grade 3 stomatitis/mucositis is clobetasol ointment instead of kenalog in Orabase®, with an increase in erythromycin dose to 500 mg daily. EGFR–TKI is maintained for grades 1 and 2, and temporarily discontinued for grade 3, until the stomatitis/mucositis improves to grade 2, at which point it is resumed at 50% of the original dose and then increased if symptoms do not get worse.

PARONYCHIA

In addition to the skin rash, other cutaneous manifestations may be observed in patients treated with EGFR–TKIs. Paronychia inflammation or infections associated with the lateral nail folds of the toes and fingers can become a concern after a longer period of treatment. Although EGFR–TKI related nail changes are usually mild, they can also be severe and symptomatic, especially with the newer generation of TKIs (8, 18). Of note, paronychia is almost never seen with first generation EGFR–TKIs, erlotinib, and gefitinib (**Table 3**).

MANAGEMENT RECOMMENDATIONS FOR PARONYCHIA

A challenge in managing paronychia is that there have not been any randomized control trials testing out treatment/management options. Patients are advised to use emollient lotions, to wear gloves during chores or cleaning, and to avoid impacts on fingers and toes.

Table 5 | Management guidelines for paronychia (Hirsh, personal communication).

Grade	Toxicity	EGFR inhibitor
1	• Nail fold edema or erythema • Disruption of the cuticle	• Topical antibiotics/antiseptics[a] • Vinegar soaks[b] • Topical ultrapotent steroids (clobetasol priopionate) applied twice daily
2	• Nail fold edema or erythema with pain • Associated with discharge or nail plate separation • Limiting instrumental activities of daily living	• Same as in grade 1 • Silver nitrate application weekly
3	• Limiting self-care activities of daily living	**Interrupt afatinib; refer to a dermatologist and resume afatinib at a reduced dose (10 mg) if patient recovers to grade ≤1** • Same as in grade 2 • Consider nail avulsion and systematic antibiotics[c]

[a] *Examples of topical antibiotics/antiseptics: clindamycin 1%, erythromycin 1%, tetracycline 1%, or chloramphenicol 1%, iodine ointment.*

[b] *Vinegar soaks consist of soaking fingers or toes in a solution of white vinegar or water 1:1 for 15 min every day.*

[c] *Systemic antibiotics include tetracyclines and antimicrobials (erythromycin should be avoided).*

The following strategies for treating the inflammation and infection are recommended (**Table 5**, Dr. Hirsh). The recommended treatment for grade 1 paronychia is topical antibiotics and antiseptics including clindamycin 1%, erythromycin 1%, tetracycline 1%, or chloramphenicol 1%, iodine ointment. Vinegar soaks, whereby fingers or toes are soaked in a 1:1 solution of white vinegar and water for 15 min a day are recommended. In addition, oral doxycycline may be effective along with a high potency corticosteroid such as clobetasol propionate applied to nail beds twice a day. For more severe cases (grade 2), silver nitrate may be applied weekly. Patients with splinter hemorrhages can be treated with liquid bandage. In severe cases (grade 3), the EGFT–TKI should be discontinued until symptoms improve. Additionally, intralesional corticosteroid injections or removal of the nail plate may be beneficial.

CONCLUSION

Tyrosine kinase inhibitors against the epidermal growth factor have become standard of care in cancers such as NSCLC. Therapy with EGFR–TKIs has improved clinical outcomes, but they are accompanied by a number of adverse events that can be effectively managed, especially diarrhea, rash, mucositis, and paronychia. Strategies for early and ongoing management of rash and diarrhea are essential to patient compliance and treatment outcome.

ACKNOWLEDGMENTS

The authors wish to thank Stavroula Kalantzis and Chrystal Palaty for their assistance in the preparation of the manuscript.

REFERENCES

1. Yaish P, Gazit A, Gilon C, Levitzki A. Blocking of EGF-dependent cell proliferation by EGF receptor kinase inhibitors. *Science* (1988) **242**(4880):933–5. doi:10.1126/science.3263702
2. Harandi A, Zaidi AS, Stocker AM, Laber DA. Clinical efficacy and toxicity of anti-EGFR therapy in common cancers. *J Oncol* (2009) **567486**(10):6. doi:10.1155/2009/567486
3. Maroun JA, Anthony LB, Blais N, Burkes R, Dowden SD, Dranitsaris G, et al. Prevention and management of chemotherapy-induced diarrhea in patients with colorectal cancer: a consensus statement by the Canadian Working Group on Chemotherapy-Induced Diarrhea. *Curr Oncol* (2007) **14**(1):13–20. doi:10.3747/co.2007.96
4. BC Cancer Agency (BCCA). *BCCA Guidelines for Management of Chemotherapy-Induced Diarrhea.* (2004). Available from: http://www.bccancer.bc.ca/NR/rdonlyres/4E7EF86A-EAA5-4F3C-B147-B2512799F6B3/7371/GuidelinesforManagementofCID.pdf
5. Saltz LB. Understanding and managing chemotherapy-induced diarrhea. *J Support Oncol* (2003) **1**(1):35–46.
6. Healthcare MC. *Imodium Product Monograph.* Guelph, ON: McNeil Consumer Healthcare (2008).
7. Lacouture ME, Lai SE. The PRIDE (papulopustules and/or paronychia, regulatory abnormalities of hair growth, itching, and dryness due to epidermal growth factor receptor inhibitors) syndrome. *Br J Dermatol* (2006) **155**(4):852–4. doi:10.1111/j.1365-2133.2006.07452.x
8. Lacouture ME, Melosky BL. Cutaneous reactions to anticancer agents targeting the epidermal growth factor receptor: a dermatology-oncology perspective. *Skin Therapy Lett* (2007) **12**(6):1–5.
9. Lacouture ME. Mechanisms of cutaneous toxicities to EGFR inhibitors. *Nat Rev Cancer* (2006) **6**(10):803–12. doi:10.1038/nrc1970
10. Hirsh V. Managing treatment-related adverse events associated with EGFR tyrosine kinase inhibitors in advanced non-small-cell lung cancer. *Curr Oncol* (2011) **18**(3):126–38. doi:10.3747/co.v18i3.877
11. Mok TSWY, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, et al. Gefitinib or carboplatin–paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* (2009) **361**(10):947–57. doi:10.1056/NEJMoa0810699
12. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* (2012) **13**(3):239–46. doi:10.1016/S1470-2045(11)70393-X
13. Yang JC, Hirsh V, Schuler M, Yamamoto N, O'Byrne KJ, Mok TS, et al. Symptom control and quality of life in LUX-Lung 3: a phase III study of afatinib or cisplatin/pemetrexed in patients with advanced lung adenocarcinoma with EGFR mutations. *J Clin Oncol* (2013) **31**(27):3342–50. doi:10.1200/JCO.2012.46.1764
14. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. *Lancet Oncol* (2014) **15**(2):213–22. doi:10.1016/S1470-2045(13)70604-1
15. Common Terminology Criteria for Adverse Events (CTCAE). *Version 4.03.* U.S. Department of Health and Human Services NIoH, National Cancer Institute (2010). Available from: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf
16. Brown CG, Yoder LH. Stomatitis: an overview: protecting the oral cavity during cancer treatment. *Am J Nurs* (2002) **4**:20–3. doi:10.1097/00000446-200204001-00005
17. Lacouture ME, Anadkat MJ, Bensadoun R-J, Bryce J, Chan A, Epstein JB, et al. Clinical practice guidelines for the prevention and treatment of EGFR inhibitor-associated dermatologic toxicities. *Support Care Cancer* (2011) **19**(8):1079–95. doi:10.1007/s00520-011-1197-6
18. Melosky B. Supportive care treatments for toxicities of anti-EGFR and other targeted agents. *Curr Oncol* (2012) **19**(Suppl):1. doi:10.3747/co.19.1054

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

*Received: 02 June 2014; accepted: 19 August 2014; published online: 16 September 2014. Citation: Melosky B and Hirsh V (2014) Management of common toxicities in metastatic NSCLC related to anti-lung cancer therapies with EGFR–TKIs. Front. Oncol. **4**:238. doi: 10.3389/fonc.2014.00238*

This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 03 July 2014
doi: 10.3389/fonc.2014.00173

Is the evaluation of quality of life in NSCLC trials important? Are the results to be trusted?

Vera Hirsh*

Department of Medical Oncology, McGill University Health Centre, Royal Victoria Hospital, Montreal, QC, Canada

Edited by:
Karen L. Reckamp, City of Hope Comprehensive Cancer Center, USA

Reviewed by:
Jae Yul Kim, City of Hope Comprehensive Cancer Center, USA
Jhanelle Elaine Gray, H. Lee Moffitt Cancer Center and Research Institute, USA

***Correspondence:**
Vera Hirsh, Department of Medical Oncology, McGill University Health Centre (MUHC), Royal Victoria Hospital, 687 Pine Avenue West, Room E3.53, Montreal, QC, H3A 1A1, Canada
e-mail: vera.hirsh@muhc.mcgill.ca

The majority of patients with non-small cell lung cancer present at the time of diagnosis with stage IV metastatic disease and they experience 2 or more disease-related symptoms. These symptoms may have a negative impact on their health-related quality of life (HR QOL). Data has shown many of these patients prefer a therapy to improve their symptoms rather than receive a therapy which slightly prolongs their survival without improving their symptoms. The improvement of disease-related symptoms on a specific drug or regimen augments the significance of prolongation of the progression-free survival or the response rate as well as symptom worsening. The choice of the questionnaires to evaluate patients' reported outcomes and HR QOL benefits and the methods of collecting the data and their interpretations are very important. Only if the data are collected and analyzed properly will they be meaningful and can then be viewed as components that add the total value to a treatment and provide a comprehensive picture of the benefits and risks of a certain anticancer therapy.

Keywords: HR QOL, symptoms, data collection, metastatic NSCLC, clinical trials

INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide for both men and women (1). A majority of these patients present at the time of diagnosis with metastatic disease. Approximately 90% of patients with advanced non-small cell lung cancer (NSCLC) experience two or more disease-related symptoms such as cough, dyspnea, pain, anorexia, or fatigue (2). These symptoms in turn can cause psychological distress and may have a negative impact on a patient's health-related quality of life (HR QOL). High degrees of psychological distress influence the emotional well-being in both patients and their families. In one survey, 68% of patients preferred a therapy that would improve disease-related symptoms without prolonging their life as opposed to treatment(s) that slightly prolonged their survival without improving symptoms (3).

A patient's well-being can be affected both through symptom-control, treatment-related toxicity, and treatment efficacy. Therefore, treatments which can decrease the tumor burden and growth, and be less toxic, are very important for patients with advanced NSCLC (4, 5). It is of the utmost importance for these patients to preserve their independence and not be dependent on their loved ones feeling like a burden at the end of their lives (6–8).

Some studies suggest a link between tumor response and improvement of symptoms such as cough, dyspnea, chest pain, and also systemic symptoms such as fever, anorexia, and weight loss (9–11). The improvements in symptoms further augment the significance of good response rates or prolonged PFS. As the median OS of most of the patients with metastatic NSCLC is modest (around 1 year), with specific new targeted agents it approaches 2 years, therefore HR QOL and patients' reported outcomes (PROs) carry high importance and thus will be reviewed here.

COLLECTION OF THE DATA

Patients' reported outcomes and HR QOL benefits are usually assessed during clinical trials using the self-administered cancer-specific European Organization for Research and Treatment of Cancer (EORTC) questionnaires QLQ C30 (12), the lung cancer-specific EORTC QLQ LC13 (13), and the Euro QOL EQ-5D (14) questionnaire (in afatinib LUX LUNG phase 3 trials or crizotinib phase 3 trials) or functional assessment of cancer therapy-lung (FACT-L) (15) (functional assessment of cancer treatment in lung cancer) questionnaire (i.e., in IPASS phase 3 trial with gefitinib). The QLQ C30 questionnaire consists of five functional scales (physical, role, cognitive, emotional, and social functioning), three symptom scales (fatigue, pain, and nausea/vomiting), a global health status/QOL scale, and single items, i.e., dyspnea, loss of appetite, constipation, diarrhea, sleep disturbance, and financial impact. The QLQ LC13 questionnaire incorporates one multi-item scale to assess dyspnea and a series of single items assessing cough, pain, sore mouth, dysphagia, peripheral neuropathy, alopecia, and use of pain medication.

For each scale/item, a linear transformation is applied to standardize the raw score on a range from 0 to 100 with 100 representing the best possible function/QOL for functional scales, and the highest burden of symptoms for symptom scales and symptom items. A 10-point change in an item or domain is perceived to be clinically meaningful (16). The percentage of patients who are classified as improved ($\geq$10-point increase for functioning scales and $\geq$10-point reduction for symptom domains or items from baseline scores) with respect to each of the questionnaires is examined (16). In addition, the time-to-deterioration of an item/domain score is defined as the item from randomization to the first appearance of a score that is 10 points or more lower or higher than the baseline

score (≥10-point reduction for functioning scales and ≥10-point increase for symptom scales or items).

The EQ-5D is a disease-generic questionnaire that comprises the EQ-5D and EQ-visual analog scale (VAS). The EQ-5D measures five dimensions of health (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression). Each dimension comprises three levels (no problems, some/moderate problems, and extreme problems). Utility scores range from 0 to 1 and are calculated from the five EQ-5D items scores using the United Kingdom preference weights (17). The EQ-VAS records the patient's self-rated health status on a vertical, graduated (0–100) VAS.

Functional assessment of cancer therapy-lung questionnaire (version 4) comprises 36 items across 5 domains/categories: physical, social, family, emotional, and functional well-being. The Lung cancer subscale consists of symptoms, cognitive function, and regret of smoking. Scores range from 0 (not at all) to 4 (very much) (15).

Each protocol specifies a schedule for questionnaires to be completed (at baseline, every 2–4 weeks, at the end of the treatment visit, and during the first follow-up visit). The use of concomitant medications is assessed at the baseline and during the trial, especially analgesic use, anti-anxiety/depression medications, O_2 use, etc.

INTERPRETATION OF THE RESULTS

Patients must answer the questionnaires prior to learning the results of their tests (scans) from their physicians in order to obtain reliable results. Help with the questionnaires should be available by knowledgeable staff in the clinic or hospital. Since patients must fill out the questionnaire by themselves, supervision of this procedure in order to ensure objectivity is important. Attention should be paid to the baseline scores. In randomized trials, are they well-balanced? Are they low (i.e., low burden of symptoms) or high (i.e., high burden of symptoms)? If the baseline scores are low, the percentage of patients with improved symptoms on certain anticancer treatments might be difficult to find. Delay of the symptom deterioration is usually of high importance. The longitudinal analysis which looks at symptoms and HR QOL over time (at different visit intervals) might be informative.

The compliance of patients a propos to the completion of their questionnaires must be reported at the baseline and also during the study. The compliance during the study should remain at ≥80% in order to interpret the results appropriately. In the case of EORTC questionnaires, both EORTC QLQ LC13 and QLQ C30 have to be analyzed to get a complete picture *not* only of lung cancer-related symptoms, but also of symptoms related to cancer treatment toxicities. The patients' symptoms are treated by analgesics, cough suppressants, O_2, anti-depressants, appetite stimulating agents, etc., and they all have to be incorporated in the final analysis. Other factors such as patient's performance status (improving or deteriorating), weight loss, and special emotional counseling are of great value and can influence patients' HR QOL.

CONCLUSION

Patients' reported outcomes and health-related quality of life outcomes are important parameters of the evaluation of new drugs or regimens of patients in advanced NSCLC, but only if the data are collected and analyzed correctly. They should be viewed as components of the total value of a treatment. They should provide, together with the other primary and secondary endpoints, a comprehensive picture of the benefits and risks of anticancer therapies for patients with metastatic NSCLC. This is the position taken by the Food and Drug Administration (18) and the European Medicine Agency (19, 20).

Dedicated personnel are required for this time-consuming process of collecting and analyzing the PROs and HR QOL data. The delivery of reliable results from these questionnaires requires the team work of knowledgeable and devoted workers. Consequently enabling patients with advanced NSCLC to feel more comfortable and independent during the last months or years of their life becomes a very important task in their treatment.

REFERENCES

1. The National Cancer Institute. *Stage IV NSCLC Treatment.* The National Institute (2012). Available from: http://www.cancer.gov/cancertopics/pdq/treatment/non-small-cell-lung//healthprofessional/page11
2. Socinski MA, Morris DE, Masters GA, Lilenbaum R; American College of Chest Physicians. Chemotherapeutic management of stage IV non-small cell lung cancer. *Chest* (2003) **123**:226S–43S. doi:10.1378/chest.123.1_suppl.226S
3. Cella D, Peterman A, Hudgens S, Webster K, Socinski MA. Measuring the side effects of taxane therapy in oncology: the functional assessment of cancer therapy-taxane (FACT-taxane). *Cancer* (2003) **98**:822–31. doi:10.1002/cncr.11578
4. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* (2000) **92**:205–16. doi:10.1093/jnci/92.3.205
5. Silvestri G, Pritchard R, Welch HG. Preferences for chemotherapy in patients with advanced non-small cell lung cancer: descriptive study based on scripted interviews. *BMJ* (1998) **317**:771–5. doi:10.1136/bmj.317.7161.771
6. McPherson CJ, Wilson KG, Lobchuk MM, Brajtman S. Self-perceived burden to others: patient and family caregiver correlates. *J Palliat Care* (2007) **23**:135–42.
7. McPherson CJ, Wilson KG, Murray MA. Feeling like a burden to others: a systematic review focusing on the end of life. *Palliat Med* (2007) **21**:115–28. doi:10.1177/0269216307076345
8. Johnson JO, Sulmasy DP, Nolan MT. Patients' experiences of being a burden on family in terminal illness. *J Hosp Palliat Nurs* (2007) **9**:264–9. doi:10.1097/01.NJH.0000289656.91880.f2
9. de Marinis F, Pereira JR, Fossella F, Perry MC, Reck M, Salzberg M, et al. Lung Cancer Symptom Scale outcomes in relation to standard efficacy measures: an analysis of the phase III study of pemetrexed versus docetaxel in advanced non-small cell lung cancer. *J Thorac Oncol* (2008) **3**:30–6. doi:10.1097/JTO.0b013e31815e8b48
10. Mayer RL. Hunting accidents. *South Med J* (1991) **84**:1284. doi:10.1097/00007611-199110000-00035
11. Tanvetyanon T, Soares HP, Djulbegovic B, Jacobsen PB, Bepler G. A systematic review of quality of life associated with standard chemotherapy regimens for advanced non-small cell lung cancer. *J Thorac Oncol* (2007) **2**:1091–7. doi:10.1097/JTO.0b013e31815cff64
12. Catravas GN, Takenaga J, McHale CG. Effect of chronic administration of morphine on monoamine oxidase activity in discrete regions of the brain of rats. *Biochem Pharmacol* (1977) **26**:211–4. doi:10.1016/0006-2952(77)90305-7
13. Bergman B, Aaronson NK, Ahmedzai S, Kaasa S, Sullivan M. The EORTC QLQ-LC13: a modular supplement to the EORTC core quality of life questionnaire (QLQ-C30) for use in lung cancer clinical trials. EORTC study group on quality of life. *Eur J Cancer* (1994) **30A**:635–42. doi:10.1016/0959-8049(94)90535-5
14. Hancock E. Crisis intervention in a newborn nursery intensive care unit. *Soc Work Health Care* (1976) **1**:421–32. doi:10.1300/J010v01n04_03
15. Cella DF, Tulsky DS, Gray G, Sarafian B, Linn E, Bonomi A, et al. The Functional Assessment of Cancer Therapy Scale: development and validation of the general measure. *J Clin Oncol* (1993) **11**:570–9.

16. Osoba D, Rodrigues G, Myles J, Zee B, Pater J. Interpreting the significance of changes in health-related quality of life scores. *J Clin Oncol* (1998) **16**: 139–44.
17. Hirsh V, Cadranel J, Cong XJ, Fairclough D, Finnern HW, Lorence RM, et al. Symptom and quality of life benefit of afatinib in advanced non-small cell lung cancer patients previously treated with erlotinib or gefitinib: results of a randomized phase IIb/III trial (Lux-Lung 1). *J Thorac Oncol* (2013) **8**:229–37. doi:10.1097/JTO.0b013e3182773fce
18. ASCO/FDA Lung Cancer Endpoints Workshop. *Executive Summary of Workshop Discussion: Workshop Summary on Endpoints for Approval of Cancer Drugs for Lung Cancer* (2003). Available from: http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DevelopmentResources/CancerDrugs/ucm094744.pdf
19. (EMEA) EMA. *Guideline on the Evaluation of Anticancer Medicinal Products in Man* (2005). Available from: http://www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/12/WC500017748.pdf
20. (EMEA) EMA. *Reflection Paper on the Regulatory Guidance for the Use of Health-Related Quality of Life (HRQL) Measures in the Evaluation of Medicinal Products* (2005). Available from: http://www.ispor.org/workpaper/emea-hrql-guidance.pdf

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 April 2014; accepted: 18 June 2014; published online: 03 July 2014.
Citation: Hirsh V (2014) Is the evaluation of quality of life in NSCLC trials important? Are the results to be trusted? Front. Oncol. ***4****:173. doi: 10.3389/fonc.2014.00173*
This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

frontiers in ONCOLOGY

MINI REVIEW ARTICLE
published: 22 September 2014
doi: 10.3389/fonc.2014.00258

Economic impact of tissue testing and treatments of metastatic NSCLC in the era of personalized medicine

Donna M. Graham and Natasha B. Leighl*

Department of Medicine, Division of Hematology and Oncology, Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada

Edited by:
Barbara Melosky, British Columbia Cancer Agency, Canada

Reviewed by:
Alex Zhavoronkov, The Biogerontology Research Foundation, UK
Meng Xu Welliver, The Ohio State University James Cancer Center, USA

****Correspondence:***
Natasha B. Leighl, Department of Medicine, Division of Hematology and Oncology, Princess Margaret Cancer Centre, University of Toronto, 610 University Avenue, Toronto, Ontario M5G 2M9, Canada
e-mail: natasha.leighl@uhn.ca

A paradigm-shift in the management of non-small cell lung cancer (NSCLC) has resulted in many new therapies becoming available for patients with advanced disease. Stratification of treatment by histologic and molecular subtype is recommended to obtain the greatest clinical benefit for patients while minimizing adverse effects of treatment. However, these advances in diagnosis and treatment of NSCLC have come at a financial cost. This review highlights the economic impact of screening for molecular abnormalities and targeted treatment for advanced NSCLC. Major determinants of cost are drug acquisition and molecular testing. As technologies advance, molecular testing costs may reduce. However, we must collaborate with payers and manufacturers to ensure that high drug costs do not limit patient accessibility to potentially beneficial treatment.

Keywords: metastatic NSCLC, economic impact, tissue testing, personalized medicine, medical economics

INTRODUCTION

Increasing understanding of the biology of cancer has resulted in strategies to personalize therapy for patients. In advanced non-small cell lung cancer (NSCLC), these advances have led to stratification of treatment by histological and molecular subtype to obtain the greatest clinical benefit, while minimizing adverse effects of treatment (1–3). However, these innovations in diagnosis and treatment of NSCLC have come at a financial cost. Where cure is not an option, the impact of cost is a significant consideration in provision of cancer care.

TREATMENT FOR NSCLC

The SWOG 9509 (4) and ECOG 1594 (5) studies established platinum-based doublet chemotherapy as the treatment of choice in advanced NSCLC. These studies did not demonstrate benefit between treatment regimens for any subgroup analyzed. However, comparison of pemetrexed with docetaxel as second-line therapy (6), and a subsequent randomized trial comparing the combination of pemetrexed/cisplatin with gemcitabine/cisplatin as first-line treatment (7), highlighted a clinical benefit for pemetrexed in patients with non-squamous histology, giving the first suggestion that NSCLC can no longer be treated as one disease. Further attempts to improve outcomes included the addition of bevacizumab to platinum-doublet chemotherapy. This combination resulted in hemoptysis when used to treat patients with squamous cell carcinoma (8), resulting in selective treatment of patients with non-squamous histology only. A modest survival benefit of two months was seen in the bevacizumab arm with overall survival of 12.3 months compared with 10.3 months for the chemotherapy alone arm (9) and 4 months in the adenocarcinoma subgroup.

Further therapeutic options became available with the emergence of epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitors (TKIs). The presence of activating mutations in exons 18, 19, 20, and 21 of *EGFR* in NSCLC [in 15% of adenocarcinoma (10)] predicts for improvements in progression-free survival, response, and quality of life with the use of EGFR TKIs for this subpopulation of patients compared to traditional chemotherapy (10–12). In addition, the presence of the echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (*EML4-ALK*) fusion gene in 2–7% of cases of NSCLC (13) is a target for therapy with crizotinib with enhanced response rates and progression-free survival when compared to second-line chemotherapy in pre-treated patients, and more recently first-line treatment (3). These targeted therapies have dramatically changed the diagnosis and treatment of NSCLC.

COSTS OF LUNG CANCER MANAGEMENT

Increasing costs of cancer management are a global issue. The estimated cost of cancer care in the United States (US) was $124.57 billion in 2010, with a minimum estimated cost of lung cancer care being $12.12 billion. Using the most conservative estimates, this cost was predicted to increase by 25% to $15.19 billion by 2020 (14). However, this does not account for changes in treatment strategy and the introduction of novel agents. Canadian data have shown that the proportion of patients receiving systemic therapy has doubled from 18.1 to 37.5% from 1997 to 2007 but that the treatment costs tripled during this interval (15). Cost is of major concern to patients and payors, with medical debt being the most common cause of personal bankruptcy in the US (16). Molecularly targeted agents, while providing clinical benefit, carry a high price tag. The monthly cost of the EGFR TKI erlotinib is $2,847CAD (Canadian dollars), and the ALK TKI crizotinib costs $10,400CAD for a month's supply (17). Additional expenses may also apply, including overhead costs, within certain countries.

With the expanding use of targeted therapies in this population, even greater increases in the cost of lung cancer treatment are

anticipated. In addition, the cost of further diagnostic testing to aid treatment selection will escalate costs in the management of lung cancer. Obtaining value for money when prescribing expensive medications is critical for patients, payors, and society.

DIAGNOSTIC TESTING

As histology and molecular subtype are such critical determinants of cancer treatment, adequate tissue sampling is vital. Approximately 70% of NSCLC is diagnosed at an advanced stage, usually by small biopsy sampling rather than surgical resection. International guidelines have recommended routine immunohistochemical staining (IHC) of all NSCLC for diagnosis, histologic subtype and molecular testing for *EGFR* mutation, and *EML4-ALK* fusion for patients with advanced NSCLC (18–20). With small tissue or cytology samples, the diagnostic yield may be compromised, resulting in a requirement for re-biopsy to obtain more tissue to accurately provide a diagnosis. In the IPASS study, 44% of patients did not have available tissue for molecular testing (12), similar to 55% of patients in the BR.21 study (2). In addition, the tumor content may be insufficient for molecular testing (21). Amount of tissue required and labor intensiveness depend on the techniques employed, e.g., IHC requires less tissue and is less costly than fluorescent *in situ* hybridization (FISH) or sequencing, costing $40CAD compared with $388CAD for FISH (22). Therefore, the availability of tissue and method of testing are of clinical and economic importance.

Standardized IHC is recommended for diagnosis of NSCLC and the determination of histologic subtype. The current gold standard for *EML4-ALK* testing, used in initial clinical studies as a companion diagnostic tool, is the use of a break-apart FISH assay (Vysis ALK Break Apart FISH Probe, Abbott Molecular Inc., Des Plaines, IL, USA). However, reverse-transcriptase polymerase chain reaction, IHC, chromogenic *in situ* hybridization (CISH), and other techniques may also be used. The most reliable of these alternative methods is IHC, due to improved sensitivity and specificity of the antibodies (23). IHC has been shown to correlate with FISH in several studies (24, 25), providing a far less costly and more easily accessible method for preliminary detection of *EML4-ALK* fusion, which may subsequently be confirmed with FISH (26). *EGFR* mutation testing can be performed using Sanger sequencing, and other less labor-intensive methods of *EGFR* mutation testing have been developed, which may have even greater sensitivity (27, 28). Multiplex assays and next-generation sequencing in lung cancer samples are tested for several genomic aberrations simultaneously and usually include *EGFR* genotyping.

Personalized therapy relies on the presence of a predefined clinical, pathological, or molecular biomarker. Biomarkers can be incorporated into drug development by different methods. Where a biomarker is integral to the drug development process, the population are screened and pre-selected for treatment on the presence of this biomarker. In order for this to be a valid strategy, robust preclinical data must strongly support this methodology. Crizotinib (an ALK, ROS-1, and MET inhibitor) is an example of a drug that was developed using an integral approach (29). An *a priori* hypothesis of efficacy in patients with *EML4-ALK*+, *ROS-1*+, and *MET* amplified tumors was used to enroll only these subpopulations to the study. This trial design led to accelerated approval for this agent, where the relatively low frequency of *EML4-ALK* in the population of NSCLC may have otherwise resulted in a negative outcome. However, there is a concern that this approach may miss activity in biomarker-negative patients who may potentially benefit from an agent, if they are excluded from clinical trials. In addition, the cost of identifying the target population in this type of study is not accounted for.

Alternatively, a biomarker may be integrated into trial design, allowing both biomarker-positive and -negative patients to receive treatment, thereby enabling assessment of benefit in both groups. In this case, all patients are tested for the presence of the biomarker, and analysis of the subpopulation of interest occurs retrospectively. This was the case with the EGFR TKIs, where the biomarker of interest was initially thought to be EGFR protein expression (30) but pre-specified subgroup analysis confirmed a greater benefit for this therapy in patients with the presence of *EGFR* mutation in the tumor (12, 31–33).

ECONOMIC ANALYSES

Economic analyses aim to contextualize the cost of healthcare services by providing a measure of the cost and consequences for different treatments. The gold standard for oncology is the cost–utility analysis. Results are commonly presented as incremental cost-effectiveness ratios (ICER) and cost per quality-adjusted life year (QALY) to give a measure of the value of the intervention based on clinical benefit and costs (34). The quality of an economic assessment is often driven by the existing clinical data to support the intervention (35).

Different paths of drug development become important in economic analyses when considering the methods by which the intervention in the target population and the comparator is defined. There are different approaches to evaluate the cost of personalized medicine. It is possible to focus only on the target population and compare the intervention with other comparators in that group. However, the cost of identifying the target population through molecular testing will not be incorporated in this design, thereby potentially underestimating total cost of therapy. An alternative approach would be to compare a strategy of testing for the target biomarker in the entire population followed by treatment of the target population, with a strategy involving no biomarker testing and standard of care therapy. However, this relies on availability of an accurate assay for biomarker assessment in order to identify the true target population as a proportion of the population as a whole. An effect on small target populations may have minimal change in outcome for the population as a whole, especially if the target population is very small, e.g., 1–2%. Also, improvements in technology may result in a change of testing strategy and modified costs in the future. Another approach may be to separate the test and treat component of the analysis (26).

EGFR TKIs

These agents were developed before the optimal target population was defined. Thus, early trials in lung cancer involved unselected advanced NSCLC patients.

An economic analysis of erlotinib in previously treated otherwise unselected patients with NSCLC was performed by the NCIC clinical trials group (NCIC CTG) based on data from the NCIC

CTG BR.21 study. An ICER of $94,638CAD per life-year gained (LYG) (95% confidence interval of $52,359–429,148CAD) was identified (36). Exploratory analysis identified that treating never-smokers and patients with high tumoral *EGFR* gene copy number were the most cost-effective strategies. Interestingly, in patients with sensitizing *EGFR* mutations, treatment was associated with an ICER of $138,168CAD/LYG compared with $87,994CAD/LYG for patients with *EGFR* wild-type tumors. This likely reflects the small survival benefit noted in this study for both groups and the shorter duration of therapy in patients with *EGFR* wild-type tumors.

Over time, we have learned that patients with *EGFR*-mutated advanced NSCLC derive the greatest benefit from EGFR TKI therapy, which is superior to chemotherapy in terms of response rate, quality of life, and progression-free survival, although not in overall survival due to crossover in clinical trials, with a recent exception (35). Given this clinical benefit, a number of analyses have been performed to assess cost-effectiveness in this setting (**Table 1**). Using platinum-doublet chemotherapy as a comparator, a CE estimate of £59,216–70,390/QALY was calculated for first-line gefitinib in a British study (37), but was not considered cost effective at standard willingness-to-pay thresholds. A number of studies have also investigated the cost-effectiveness of EGFR TKI treatment with *EGFR* mutation testing included. Based on the IPASS study (12), a Singaporean study suggested that first-line treatment of *EGFR*-mutated NSCLC with gefitinib was a cost-effective strategy with a CE estimate of $77,160 Singaporean dollars/QALY (38) compared with carboplatin/paclitaxel, carboplatin/pemetrexed, or carboplatin/pemetrexed/bevacizumab. Of note, their model included second-line gefitinib for patients treated with initial chemotherapy irrespective of *EGFR* genotype.

The potential for insufficient diagnostic tissue available for *EGFR* mutation testing (2, 12) prompted a study in which either no lung adenocarcinoma patient samples were tested (all received first-line chemotherapy), a second testing scenario where half of the patients had sufficient tissue for *EGFR* testing, or a third scenario where half of the patients had repeat tumor biopsy for *EGFR* testing (although 15% still had insufficient tissue after re-biopsy). First-line erlotinib therapy resulted in an ICER of $110,658/QALY gain compared with carboplatin/paclitaxel with the testing strategy and $122,234/QALY using the re-biopsy strategy. With carboplatin/pemetrexed as a comparator, the ICER for the repeat biopsy strategy was $180,665/QALY; adding bevacizumab increased the ICER significantly to $359,619/QALY, in excess of commonly accepted thresholds for cost-effectiveness (39). A recent study from the perspective of the Chinese healthcare system investigated the cost-effectiveness of first-line erlotinib compared with platinum-doublet chemotherapy in advanced *EGFR* mutation positive lung cancer patients based on outcomes from the OPTIMAL trial (42). Treatment with upfront erlotinib was deemed cost effective with an ICER of $85,927.41USD/QALY gained. Of note, this analysis assumed that after the first 5 months (seven cycles) of therapy, subsequent erlotinib would be donated by Roche China.

A U.S. study demonstrated a modest budget impact of *EGFR* mutation testing and erlotinib as first-line therapy for patients with *EGFR* mutation positive advanced disease compared with platinum-doublet based chemotherapy regimens, from a U.S. health plan perspective (43). Increasing *EGFR* testing rates from 50 to 100% increased overall health plan expenditures by $0.013 per member per month (PMPM). Treatment costs contributed $0.012 PMPM with extended duration of treatment giving the greatest contribution. The cost of *EGFR* mutation testing was estimated at $0.002 PMPM, but was offset by the cost-savings associated with treatment of chemotherapy-related adverse events (−$0.002 PMPM).

Recent clinical data suggest an improvement in progression-free and overall survival for patients with *EGFR*-mutant NSCLC when treated with afatinib compared with platinum-based chemotherapy (44, 45). Although unable to estimate a plausible ICER based on the manufacturer's submission, afatinib was considered to be a reasonable option for first or second-line treatment for patients with *EGFR*-mutant NSCLC, with exploratory estimates from the Evidence Review Committee of an ICER of £39,300/QALY gained with afatinib compared to pemetrexed/cisplatin in the overall population, and an ICER of £23,700/QALY gained in the non-Asian population based on trial data provided (46).

ALK INHIBITORS

The cost-effectiveness of testing methodology for *EML4-ALK* fusion-positive tumors has been assessed using differing techniques, from a societal perspective using the US healthcare system (26). By varying *ALK* testing methods and population tested, the CE of FISH testing for all patients was estimated at $106,707USD/QALY, compared with $57,165USD/QALY for IHC. In a clinically selected population of non-smokers with *EGFR*- and *KRAS*-wild type adenocarcinoma, the CE estimates were $4,756USD/QALY and $2,548USD/QALY for FISH and IHC, respectively. One cost-effectiveness analysis has explored the use of crizotinib for the first-line treatment of patients with *EML4-ALK* fusion-positive tumors from the Canadian public healthcare perspective (22). The comparator was a platinum-doublet chemotherapy regimen in patients with non-squamous NSCLC, and the model incorporated subsequent treatment with pemetrexed and erlotinib. A re-biopsy strategy was employed in case of inadequate tissue. The method of assessment for EML4-ALK positive tumor was by initial IHC and, if positive, confirmatory testing with FISH. The incremental cost of crizotinib therapy for a gain of 0.11 QALYs was $2,725CAD/patient, with an ICER of $255,970/QALY gained. For patients with confirmed EML4-ALK positive tumors, first-line therapy with crizotinib produced an ICER of $250,632CAD/QALY, in excess of commonly accepted cost-effectiveness thresholds. Sensitivity analysis highlighted the major driver of cost as the price of crizotinib therapy. Despite FISH testing costs exceeding those of IHC, the relative cost of crizotinib was so great that use of the cost of initial FISH testing instead of IHC had minimal impact on the overall ICER.

HISTOPATHOLOGY

Patients with non-squamous NSCLC derive benefit from pemetrexed-based chemotherapy and from the addition of bevacizumab to a platinum-doublet. Although histologic subtype is not a recognized biomarker, these data have led to treatment selection

Table 1 | Cost-effectiveness studies of first-line EGFR TKI therapy.

Author	Type of study	EGFR TKI and comparator	Model	Cost of testing	Perspective	ICER per QALY	Cost-effective?	Remarks
Brown et al. (37)	Cost-effectiveness analysis	Gefitinib compared with platinum-doublet chemotherapy	Decision model comparing gefitinib with carbo/tax in patients with *EGFR* mutation positive disease	No	National Health Service	£59,216–70,390	No	Clinical data from IPASS: (12)
de Lima Lopes et al. (38)	Cost–utility analysis	Gefitinib compared with carbo/gem Subset analysis of gefitinib as second-line Assumed 60% with *EGFR* mutation	Decision tree with testing versus no testing and multiple lines of treatment. Test positive: gefitinib, carbo/gem, BSC Test negative: Carbo/gem, BSC No testing: Carbo/gem, gefitinib, BSC	Included $380	Singaporean health care system, 2010 Singapore dollars	$77,160	Yes	Clinical data from 3 trials: IPASS: (12);WJTOG 345: (32); C000000376: (33)
Handorf et al. (39)	Cost-effectiveness analysis	Erlotinib compared with carbo/tax, carbo/pem, and carbo/pem/bev	Decision analytic model with testing versus no testing and re-biopsy included Test positive: erlotinib Test negative: platinum-based chemotherapy No testing or insufficient tissue on repeat biopsy: platinum-based chemotherapy	Yes	Payer's perspective	$110,658 for carbo/tax test and treat $122,234 for carbo/tax re-biopsy $180,665 for carbo/pem $359,619 for carbo/pem/bev	Yes	Re-biopsy strategy included: assumed 15% yielded insufficient tissue
Brown et al. (40)	Cost-effectiveness analysis	Gefitinib compared with platinum-doublet chemotherapy	Decision model comparing gefitinib with cis/tax, carbo/tax or cis/doc	No	UK National Health Service and Personal Social Services	Mean £35,700 (range £59,216–70,390)	No	Clinical data from IPASS: (12, 31); WJTOG 345: (32); C000000376: (33); Mean negotiated NHS costs included
Wang et al. (41)	Cost-effectiveness analysis	Erlotinib compared with carbo/gem	Markov model comparing carbo/gem for 4 cycles with erlotinib until progression	No	Chinese health care system, 2010 US dollars	$85927.41 (range $58,584.57–336,404.20)	Yes	Clinical data from OPTIMAL trial: (42) Cost of erlotinib included only for first 7 cycles (from cycle 8 or month 5 onward cost is zero due to donations from Roche China)

EGFR TKI, epidermal growth factor tyrosine kinase inhibitor; ICER, incremental cost-effectiveness ratio; LY, life years; QALY, quality of life year; carbo/gem, carboplatin and gemcitabine chemotherapy; BSC, best supportive care; carbo/tax, carboplatin and paclitaxel; carbo/pem, carboplatin and pemetrexed; carbo/pem/bev, carboplatin, pemetrexed and bevacizumab; cis/tax, cisplatin and paclitaxel; cis/doc, cisplatin and docetaxel.

for patients based on histologic subtype. Given the significant cost of these agents when compared with other standard chemotherapy regimens, several economic assessments have been performed. A cost–utility analysis of the addition of bevacizumab to platinum-based chemotherapy compared with chemotherapy alone (9) in patients with non-squamous NSCLC estimated an increase of 0.13 QALYs with the addition of bevacizumab, at a cost of $72,000USD per patient. The incremental cost–utility ratio for the addition of bevacizumab was $560,000USD/QALY (47), exceeding accepted thresholds for cost-effectiveness.

In the first-line setting, pemetrexed/cisplatin improved median overall survival by 1 month in advanced non-squamous lung carcinoma patients, when compared with gemcitabine/cisplatin, and an ICER estimated at £17,000–25,000/QALY (48). When pemetrexed is used as maintenance, the median survival gain compared to observation is 5 months, with an ICER of $122, 371USD/LYG.

CONCLUSION

The management of lung cancer has transformed in recent years, due to increasing stratification of treatment based on pathologic and molecular characteristics. Optimizing treatment by using personalized therapy has resulted in improved treatment responses, quality of life, and progression-free survival of patients with NSCLC, with some evidence of survival benefit. However, this comes at a price, and, acknowledging that these actionable mutations are present only in a small subset of NSCLC tumors, we must act in the best interests of all our patients to ensure that this is affordable for the benefit gained.

In order to focus on the relevant population for a molecularly targeted therapy, tissue must be available and the testing method must be accurate. However, as in the case of *EML4-ALK*, there may be methods to select the target population with lower cost, and these technologies will continue to evolve. Further evolution of next-generation sequencing and multiplex platforms may also improve the cost-effectiveness of testing, where multiple abnormalities can be evaluated with a single test. While molecular testing beyond *EGFR* and *ALK* is not currently recommended as standard of care in NSCLC (18), more comprehensive genomic testing will likely become cheaper and more accessible in the future, minimizing time and tissue requirements in efforts to better personalize therapy (49, 50).

Cost-effective, -accurate, and -efficient methods of diagnosis must be employed, which allow equal accessibility to therapy for all patients. However, the major cost determinant in most economic evaluations of targeted treatment in NSCLC is drug price. Economic evaluations are integral to assessment of value for a given therapy as these may be used to enable funding decisions by policy-makers, and to negotiate pricing strategies with manufacturers where possible. There has been a paradigm-shift in the treatment of NSCLC with exciting new therapies revolutionizing treatment for patients with a previously dismal prognosis. As clinicians, we must ensure that as many patients as possible derive benefit from a personalized approach. Collaboration with payers and manufacturers is a key to ensure that cost of treatment is not prohibitive for patients and permitting further advances in lung cancer therapy.

REFERENCES

1. Mantripragada K, Khurshid H. Targeting genomic alterations in squamous cell lung cancer. *Front Oncol* (2013) **3**:195. doi:10.3389/fonc.2013.00195
2. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* (2005) **353**(2):123–32. doi:10.1056/NEJMoa050753
3. Camidge DR, Bang YJ, Kwak EL, Iafrate AJ, Varella-Garcia M, Fox SB, et al. Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a phase 1 study. *Lancet Oncol* (2012) **13**(10):1011–9. doi:10.1016/S1470-2045(12)70344-3
4. Kelly K, Crowley J, Bunn PA Jr, Presant CA, Grevstad PK, Moinpour CM, et al. Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non – small-cell lung cancer: a southwest oncology group trial. *J Clin Oncol* (2001) **19**(13):3210–8.
5. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* (2002) **346**(2):92–8. doi:10.1056/NEJMoa011954
6. Hanna N, Shepherd FA, Fossella FV, Pereira JR, De Marinis F, von Pawel J, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *J Clin Oncol* (2004) **22**(9):1589–97. doi:10.1200/JCO.2004.08.163
7. Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* (2008) **26**(21):3543–51. doi:10.1200/JCO.2007.15.0375
8. Johnson DH, Fehrenbacher L, Novotny WF, Herbst RS, Nemunaitis JJ, Jablons DM, et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol* (2004) **22**(11):2184–91. doi:10.1200/JCO.2004.11.022
9. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* (2006) **355**(24):2542–50. doi:10.1056/NEJMoa061884
10. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med* (2009) **361**(10):958–67. doi:10.1056/NEJMoa0904554
11. Douillard JY, Shepherd FA, Hirsh V, Mok T, Socinski MA, Gervais R, et al. Molecular predictors of outcome with gefitinib and docetaxel in previously treated non-small-cell lung cancer: data from the randomized phase III interest trial. *J Clin Oncol* (2010) **28**(5):744–52. doi:10.1200/JCO.2009.24.3030
12. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* (2009) **361**(10):947–57. doi:10.1056/NEJMoa0810699
13. Crystal AS, Shaw AT. New targets in advanced NSCLC: EML4-ALK. *Clin Adv Hematol Oncol* (2011) **9**(3):207–14.
14. Mariotto AB, Yabroff KR, Shao Y, Feuer EJ, Brown ML. Projections of the cost of cancer care in the United States: 2010-2020. *J Natl Cancer Inst* (2011) **103**(2):117–28. doi:10.1093/jnci/djq495
15. de Oliveira C, Bremner KE, Pataky R, Gunraj N, Haq M, Chan K, et al. Trends in use and cost of initial cancer treatment in Ontario: a population-based descriptive study. *CMAJ Open.* (2013) **1**(4):E151–8. doi:10.9778/cmajo.20130041
16. Himmelstein DU, Thorne D, Warren E, Woolhandler S. Medical bankruptcy in the United States, 2007: results of a national study. *Am J Med* (2009) **122**(8):741–6. doi:10.1016/j.amjmed.2009.04.012
17. Truven Health Analytics. *Red Book online.* (2014). Available from: http://www.redbook.com/redbook/index.html
18. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the college of American pathologists, international association for the study of lung cancer, and association for molecular pathology. *J Mol Diagn: JMD.* (2013) **15**(4):415–53.
19. Ellis PM, Blais N, Soulieres D, Ionescu DN, Kashyap M, Liu G, et al. A systematic review and Canadian consensus recommendations on the use of biomarkers in the treatment of non-small cell lung cancer. *J Thorac Oncol* (2011) **6**(8):1379–91. doi:10.1097/JTO.0b013e318220cb8e
20. Kerr KM, Bubendorf L, Edelman MJ, Marchetti A, Mok T, Novello S, et al. 2nd ESMO consensus conference on lung cancer: pathology and molecular biomarkers for non-small-cell lung cancer. *Ann oncol* (2014;) **25**(9):1681–90. doi:10.1093/annonc/mdu145

21. Thunnissen E, Kerr KM, Herth FJ, Lantuejoul S, Papotti M, Rintoul RC, et al. The challenge of NSCLC diagnosis and predictive analysis on small samples. Practical approach of a working group. *Lung Cancer* (2012) **76**(1):1–18. doi:10.1016/j.lungcan.2011.10.017
22. Djalalov S, Beca J, Hoch JS, Krahn M, Tsao MS, Cutz JC, et al. Cost effectiveness of EML4-ALK fusion testing and first-line crizotinib treatment for patients with advanced ALK-positive non-small-cell lung cancer. *J Clin Oncol* (2014) **32**(10):1012–9. doi:10.1200/JCO.2013.53.1186
23. Mino-Kenudson M, Chirieac LR, Law K, Hornick JL, Lindeman N, Mark EJ, et al. A novel, highly sensitive antibody allows for the routine detection of ALK-rearranged lung adenocarcinomas by standard immunohistochemistry. *Clin Cancer Res* (2010) **16**(5):1561–71. doi:10.1158/1078-0432.CCR-09-2845
24. Paik JH, Choe G, Kim H, Choe JY, Lee HJ, Lee CT, et al. Screening of anaplastic lymphoma kinase rearrangement by immunohistochemistry in non-small cell lung cancer: correlation with fluorescence in situ hybridization. *J Thorac Oncol* (2011) **6**(3):466–72. doi:10.1097/JTO.0b013e31820b82e8
25. Yi ES, Boland JM, Maleszewski JJ, Roden AC, Oliveira AM, Aubry MC, et al. Correlation of IHC and FISH for ALK gene rearrangement in non-small cell lung carcinoma: IHC score algorithm for FISH. *J Thorac Oncol* (2011) **6**(3):459–65. doi:10.1097/JTO.0b013e318209edb9
26. Atherly AJ, Camidge DR. The cost-effectiveness of screening lung cancer patients for targeted drug sensitivity markers. *Br J Cancer* (2012) **106**(6):1100–6. doi:10.1038/bjc.2012.60
27. Kamel-Reid S, Chong G, Ionescu DN, Magliocco AM, Spatz A, Tsao M, et al. EGFR tyrosine kinase mutation testing in the treatment of non-small-cell lung cancer. *Curr Oncol* (2012) **19**(2):e67–74. doi:10.3747/co.19.862
28. Beasley MB, Milton DT. ASCO provisional clinical opinion: epidermal growth factor receptor mutation testing in practice. *J Oncol Pract* (2011) **7**(3):202–4. doi:10.1200/JOP.2010.000166
29. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* (2010) **363**(18):1693–703. doi:10.1056/NEJMoa1006448
30. Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin Cancer Res* (2001) **7**(10): 2958–70.
31. Fukuoka M, Wu YL, Thongprasert S, Sunpaweravong P, Leong SS, Sriuranpong V, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). *J Clin Oncol* (2011) **29**(21):2866–74. doi:10.1200/JCO.2010.33.4235
32. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol* (2010) **11**(2):121–8. doi:10.1016/S1470-2045(09)70364-X
33. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* (2010) **362**(25):2380–8. doi:10.1056/NEJMoa0909530
34. Drummond MF, Sculpher MJ, Torrance GW, O'Brien BJ, Stoddart GL. *Methods for the Economic Evaluation of Health Care Programmes*. 3rd ed. New York: Oxford University Press (2005).
35. Mittmann N, Evans WK, Rocchi A, Longo CJ, Au HJ, Husereau D, et al. Guidelines for health technologies: specific guidance for oncology products in Canada. *Value Health* (2012) **15**(3):580–5. doi:10.1016/j.jval.2011.12.006
36. Bradbury PA, Tu D, Seymour L, Isogai PK, Zhu L, Ng R, et al. Economic analysis: randomized placebo-controlled clinical trial of erlotinib in advanced non-small cell lung cancer. *J Natl Cancer Inst* (2010) **102**(5):298–306. doi:10.1093/jnci/djp518
37. Brown T, Boland A, Bagust A, Oyee J, Hockenhull J, Dundar Y, et al. Gefitinib for the first-line treatment of locally advanced or metastatic non-small cell lung cancer. *Health Technol Assess* (2010) **14**(Suppl 2):71–9. doi:10.3310/hta14Suppl2/10
38. de Lima Lopes G Jr, Segel JE, Tan DS, Do YK, Mok T, Finkelstein EA. Cost-effectiveness of epidermal growth factor receptor mutation testing and first-line treatment with gefitinib for patients with advanced adenocarcinoma of the lung. *Cancer* (2012) **118**(4):1032–9. doi:10.1002/cncr.26372
39. Handorf EA, McElligott S, Vachani A, Langer CJ, Bristol Demeter M, Armstrong K, et al. Cost effectiveness of personalized therapy for first-line treatment of stage IV and recurrent incurable adenocarcinoma of the lung. *J Oncol Pract* (2012) **8**(5):267–74. doi:10.1200/JOP.2011.000502
40. Brown T, Pilkington G, Bagust A, Boland A, Oyee J, Tudur-Smith C, et al. Clinical effectiveness and cost-effectiveness of first-line chemotherapy for adult patients with locally advanced or metastatic non-small cell lung cancer: a systematic review and economic evaluation. *Health Technol Assess* (2013) **17**(31). doi:10.3310/hta17310
41. Wang S, Peng L, Li J, Zeng X, Ouyang L, Tan C, et al. A trial-based cost-effectiveness analysis of erlotinib alone versus platinum-based doublet chemotherapy as first-line therapy for Eastern Asian nonsquamous non-small-cell lung cancer. *PLoS One* (2013) **8**(3):e55917. doi:10.1371/journal.pone.0055917
42. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* (2011) **12**(8):735–42. doi:10.1016/S1470-2045(11)70184-X
43. Bajaj PS, Veenstra DL, Goertz HP, Carlson JJ. Targeted erlotinib for first-line treatment of advanced non-small cell lung cancer: a budget impact analysis. *J Med Econ* (2014) **12**:1–9. doi:10.3111/13696998.2014.912987
44. Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol* (2013) **31**(27):3327–34. doi:10.1200/JCO.2012.44.2806
45. Wu YL, Zhou C, Hu CP, Feng J, Lu S, Huang Y, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. *Lancet Oncol* (2014) **15**(2):213–22. doi:10.1016/S1470-2045(13)70604-1
46. National Institute for Health and Care Excellence (NICE). *Afatinib for Treating Epidermal Growth Factor Receptor Mutationpositive Locally Advanced or Metastatic Non-small-cell Lung Cancer*. London: NICE (2014).
47. Goulart B, Ramsey S. A trial-based assessment of the cost-utility of bevacizumab and chemotherapy versus chemotherapy alone for advanced non-small cell lung cancer. *Value Health* (2011) **14**(6):836–45. doi:10.1016/j.jval.2011.04.004
48. Fleeman N, Bagust A, McLeod C, Greenhalgh J, Boland A, Dundar Y, et al. Pemetrexed for the first-line treatment of locally advanced or metastatic non-small cell lung cancer. *Health Technol Assess* (2010) **14**(Suppl 1):47–53. doi:10.3310/hta14Suppl1/07
49. Sequist LV, Heist RS, Shaw AT, Fidias P, Rosovsky R, Temel JS, et al. Implementing multiplexed genotyping of non-small-cell lung cancers into routine clinical practice. *Ann Oncol* (2011) **22**(12):2616–24. doi:10.1093/annonc/mdr489
50. Buzdin AA, Zhavoronkov AA, Korzinkin MB, Venkova LS, Zenin AA, Smirnov PY, et al. Oncofinder, a new method for the analysis of intracellular signaling pathway activation using transcriptomic data. *Front genet.* (2014) **5**:55. doi:10.3389/fgene.2014.00055

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 16 June 2014; accepted: 05 September 2014; published online: 22 September 2014.

*Citation: Graham DM and Leighl NB (2014) Economic impact of tissue testing and treatments of metastatic NSCLC in the era of personalized medicine. Front. Oncol. **4**:258. doi: 10.3389/fonc.2014.00258*

This article was submitted to Thoracic Oncology, a section of the journal Frontiers in Oncology.

ADVANTAGES OF PUBLISHING IN FRONTIERS

FAST PUBLICATION
Average 90 days from submission to publication

COLLABORATIVE PEER-REVIEW
Designed to be rigorous – yet also collaborative, fair and constructive

RESEARCH NETWORK
Our network increases readership for your article

OPEN ACCESS
Articles are free to read, for greatest visibility

TRANSPARENT
Editors and reviewers acknowledged by name on published articles

GLOBAL SPREAD
Six million monthly page views worldwide

COPYRIGHT TO AUTHORS
No limit to article distribution and re-use

IMPACT METRICS
Advanced metrics track your article's impact

SUPPORT
By our Swiss-based editorial team

frontiers

EPFL Innovation Park · Building I · 1015 Lausanne · Switzerland
T +41 21 510 17 00 · info@frontiersin.org · frontiersin.org