Thai Alesha (Orcid ID: 0000-0001-8966-7677)

TYPE: BRIEF COMMUNICATION

MAIN TEXT:

In Australia, mutations in *EGFR* occur in 15% of patients diagnosed with non-small cell lung cancer (NSCLC) and are found with higher frequency in female, non-smokers of Asian ethnicity.[1] Identification of activating *EGFR* mutations allows the use of targeted therapies with *EGFR* tyrosine kinase inhibitors (TKIs). In contrast, whole genome sequencing of small cell lung cancer (SCLC) has demonstrated mutations commonly in *TP53* and *RB1* but to date, no targetable oncogenes such that chemotherapy remains the standard treatment modality. [2]

Small cell transformation is a well-described phenotypic switch, which occurs as a resistance mechanism to *EGFR* tyrosine kinase inhibitors (TKIs) in less than 5% of patients with *EGFR*-mutant lung adenocarcinoma.[3-5] In these cases, the tumour will often still harbor the original *EGFR* mutation. In addition, there are very few reported cases of de novo *EGFR* mutations occurring in SCLC without mixed SCLC and NSCLC histology or TKI exposure.[6-8]

We report two cases of SCLC in patients of Asian ethnicity and no smoking history that harbor de novo *EGFR* mutations detected in tissue biopsies and subsequently assessed in plasma cell-free DNA (cfDNA) .

Case 1: A 73 year old Taiwanese male never-smoker presented with a dry cough. Computed tomography (CT) revealed a 24x15mm ground glass opacity in the right upper lobe which was avid on fluorodeoxyglucose-positron emission tomography (FDG-PET) (Figure 1). Over a period of two years, the lesion increased in size with the development of a solid component and nodal enlargement. Further investigation with an endobronchial ultrasound guided fine needle aspirate (EBUS FNA) demonstrated many small malignant cells with hyperchromatic nuclei and very little cytoplasm and extensive crush artefact. Immunohistochemical stains showed widespread perinuclear positivity with synaptophysin and patchy perinuclear positivity with Chromogranin A. There was strong diffuse staining for thyroid transcription factor 1 (TTF-1) and Ki-67 was positive in at least 80-90% of tumor nuclei. The combined cytologic and immunohistochemical profile were consistent with SCLC, without any element of adenocarcinoma seen. The patient's non-smoking history prompted molecular testing which demonstrated an L858R mutation in exon 21 of the *EGFR* gene.

The patient completed definitive chemo-radiation with cisplatin and etoposide and post-treatment FDG-PET demonstrated a complete response (Figure 1). Plasma based *EGFR* L858R analysis using droplet digital PCR detected low copy numbers (21 copies/ml) after the patient received one cycle of chemotherapy and was not detected thereafter (Figure 2)

Case 2: A 66 year old ethnically Chinese female never-smoker presented with painless jaundice, Eastern Cooperative Oncology (ECOG) score 2 and was found to have a bilirubin of 122 (<18mmol/L), ALT of 106 (<33u/L), GGT 1557 (<40u/L) and ALP of 617 (35-105u/L). CT staging scans revealed a left hilar mass and extensive liver, bony and cerebral metastases (Figure 1). A liver biopsy showed SCLC with small nests of interconnected islands of small to intermediate sized angulated nuclei without nucleoli and only a small amount of cytoplasm. Immunohistochemical stains for TTF1, synaptophysin, Chromogranin A and p40 were negative. The lack of p40 immunostaining excluded squamous differentiation and because the morphologic features were most in keeping with small cell carcinoma, that diagnosis was strongly favored. No adenocarcinoma histology was seen. Next generation sequencing revealed a L858R mutation in exon 21 of the *EGFR* gene.

Given the elevated ALT, the patient commenced single agent carboplatin with erlotinib 100mg once daily, commencing after the initial cycle with a plan to intercalate TKI therapy with carboplatin. Despite restaging demonstrating some response, she rapidly deteriorated and died one month after commencing treatment. Interestingly, plasma based analysis demonstrated very high levels of L858R DNA (48,000 copies/ml) at the beginning of

treatment which increased to 79,5000 copies/ml, suggesting minimal response to initial therapy (Figure 2).

In this report, we describe two cases of SCLC with de novo *EGFR* mutations. There are only a few cases in the literature of de novo *EGFR* mutations in SCLC, without an adenocarcinoma component seen.[3-5,9] In our two cases, there was no evidence of adenocarcinoma in either biopsy to suggest mixed type histology. Moreover, neither patient was previously treated with *EGFR*-TKI therapy, ruling out a phenotypic switch secondary to treatment.

SCLC is commonly viewed as a smoker's disease but never smokers account for 2-3% of SCLC cases.[10-12] Two case series reported an incidence of de novo *EGFR* mutations in SCLC of 1-1.18%, rising to 14% in never smokers.[6,7] Another case series found that the accumulated smoking dose in patients with an activating mutation was much lower compared to patients with no activating mutation (median pack-years 30 versus 54 pack years [p=0.020]).[13] These findings suggest that consideration should be given to screening for *EGFR* mutations in patients with SCLC who are light or never-smokers as this is a relatively rare clinical occurrence and evidence so far suggests a higher incidence of an activating mutation.

The significance of Asian ethnicity and the presence of *EGFR* mutations in our two cases of SCLC are unclear. In Australia, the incidence of *EGFR* mutations in NSCLC is reported at 15%, but is up to 50% in the Asian population even when adjusted for sex and non-smoking history.[14,15] Given these differences in frequency based on ethnicity, the molecular pathogenesis of *EGFR*-mutant adenocarcinomas in Asian patients is likely to be different from Caucasian patients and this may also be reflected in patients with SCLC without smoking exposure. Both of the de novo *EGFR*-mutant SCLC cases described here were of Asian ethnicity raising the possibility that other mechanisms of phenotype switching may have resulted in the development of these tumours in the absence of an *EGFR* TKI.

Current available data suggests that a variety of activating *EGFR* mutations can be found in SCLC. A recently published series identified 59 cases in the literature of SCLC with an *EGFR* mutation but only 16 were true de novo cases with no element of adenocarcinoma identified and no prior therapy to TKI. The majority of cases were female non-smokers and the frequency of the type of mutation appears similar to lung adenocarcinoma with Exon 19 deletions and point mutations at L858R accounting for the majority of cases.[9] Panel testing on a cohort of Italian patients with SCLC found two patients out of 113 with an *EGFR* mutation; both were women and never-smokers. Of the two tumours, one contained an exon 19 deletion and the other harbored an

L858R mutation in exon 21.[6] Other cases reported in the literature have also identified a G719A mutation.[9,13]

Genotyping plasma cfDNA is a potential method of detecting and subsequently monitoring treatment in patients who harbour an activating *EGFR* mutation and is an attractive non-invasive option. There are no cases in the literature where patients with SCLC and *EGFR* mutations have had cfDNA levels monitored during treatment. Our first patient had low levels of *EGFR* DNA, which were undetectable after treatment, correlating with the patient's complete response. Our second patient had significantly higher number of plasma copies detected, which increased despite treatment, again, correlating with the patient's clinical progression. Genotyping plasma cfDNA is increasingly used as a predictive biomarker in patients who carry the T790M mutation in NSCLC for third generation TKIs and to monitor treatment response and may have a role in SCLC that also harbour *EGFR* mutations.[16]

Our two cases add to the growing body of evidence suggesting that molecular testing should be considered for patients with certain clinical features such as never or light smoking history and Asian ethnicity. The implications for treatment are unclear with limited reports on response to TKI therapy. However the prognosis appears to be better for non-smokers with SCLC compared to smokers.[7] The prognostic implications may reflect increased

chemosensitivity, more indolent biology or healthier patients. Molecular characterization of SCLC may alter therapeutic strategies and should be considered in select SCLC patients. Furthermore recent advances in plasma testing may facilitate better detection and monitoring of disease status.

REFERENCES

1. Peters MJ, Bowden JJ, Carpenter P, et al: Outcomes of an Australian testing programme for epidermal growth factor receptor mutations in non-small cell lung cancer. Intern Med J 44:575-80, 2014
2. George J, Lim JS, Jang SJ, et al: Comprehensive genomic profiles of small cell lung cancer. Nature 524:47-53, 2015
3. Oser MG, Niederst MJ, Sequist LV, et al: Transformation from non-small-cell lung cancer to small-cell lung cancer: molecular drivers and cells of origin. The Lancet Oncology 16:e165-e172
4. Watanabe S, Sone T, Matsui T, et al: Transformation to small-cell lung cancer following treatment with EGFR tyrosine kinase inhibitors in a patient with lung adenocarcinoma. Lung Cancer 82:370-2, 2013
5. Suda K, Murakami I, Sakai K, et al: Small cell lung cancer transformation and T790M mutation: complimentary roles in acquired resistance to kinase inhibitors in lung cancer. Sci Rep 5:14447, 2015
6. Bordi P, Tiseo M, Barbieri F, et al: Gene mutations in small-cell lung cancer (SCLC): results of a panel of 6 genes in a cohort of Italian patients. Lung Cancer 86:324-8, 2014
7. Sun JM, Choi YL, Ji JH, et al: Small-cell lung cancer detection in never-smokers: clinical characteristics and multigene mutation profiling using targeted next-generation sequencing. Ann Oncol 26:161-6, 2015
8. Shiao TH, Chang YL, Yu CJ, et al: Epidermal growth factor receptor mutations in small cell lung cancer: a brief report. J Thorac Oncol 6:195-8, 2011
9. Siegele BJ, Shilo K, Chao BH, et al: Epidermal growth factor receptor (EGFR) mutations in small cell lung cancers: Two cases and a review of the literature. Lung Cancer 95:65-72, 2016
10. Ou SH, Ziogas A, Zell JA: Prognostic factors for survival in extensive stage small cell lung cancer (ED-SCLC): the importance of smoking history, socioeconomic and marital statuses, and ethnicity. J Thorac Oncol 4:37-43, 2009
11. Wolf M, Holle R, Hans K, et al: Analysis of prognostic factors in 766 patients with small cell lung cancer (SCLC): the role of sex as a predictor for survival. Br J Cancer 63:986-92, 1991
12. Morabia A, Wynder EL: Cigarette smoking and lung cancer cell types. Cancer 68:2074-8, 1991
13. Tatematsu A, Shimizu J, Murakami Y, et al: Epidermal growth factor receptor mutations in small cell lung cancer. Clin Cancer Res 14:6092-6, 2008
14. Shi Y, Au JS, Thongprasert S, 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 9:154-62, 2014

15. Shigematsu H, Lin L, Takahashi T, et al: Clinical and Biological Features Associated With Epidermal Growth Factor Receptor Gene Mutations in Lung Cancers. Journal of the National Cancer Institute 97:339-346, 2005
16. Oxnard GR, Thress KS, Alden RS, et al: Association Between Plasma Genotyping and Outcomes of Treatment With Osimertinib (AZD9291) in Advanced Non-Small-Cell Lung Cancer. J Clin Oncol 34:3375-82, 2016

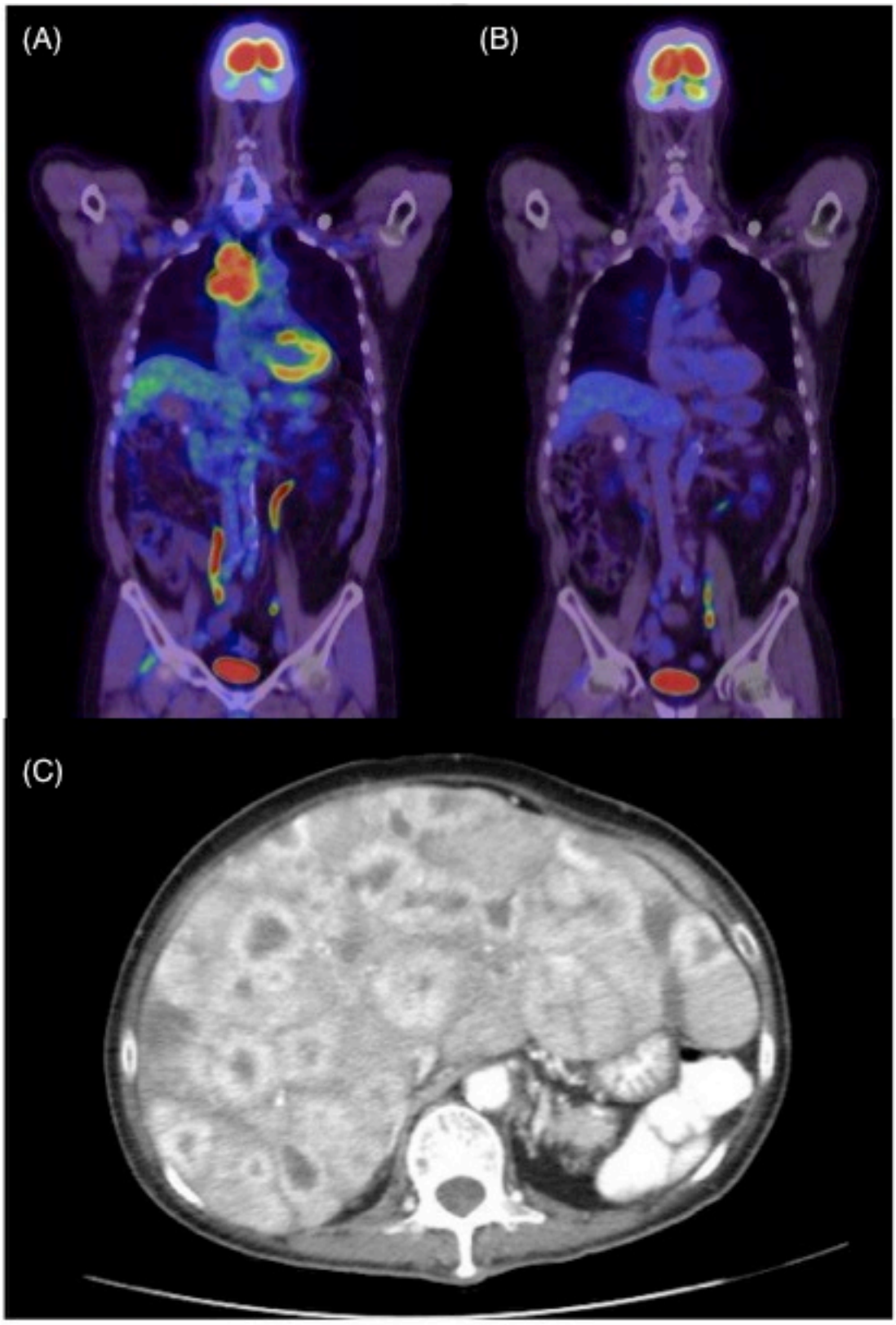

Figure 1

(a) Patient 1: FDG-PET prior to definitive chemo-radiotherapy
(b) Patient 1: FDG-PET post completion of definitive chemo-radiotherapy
(c) Patient 2: CT abdomen at diagnosis

Figure 1 Final.jpg

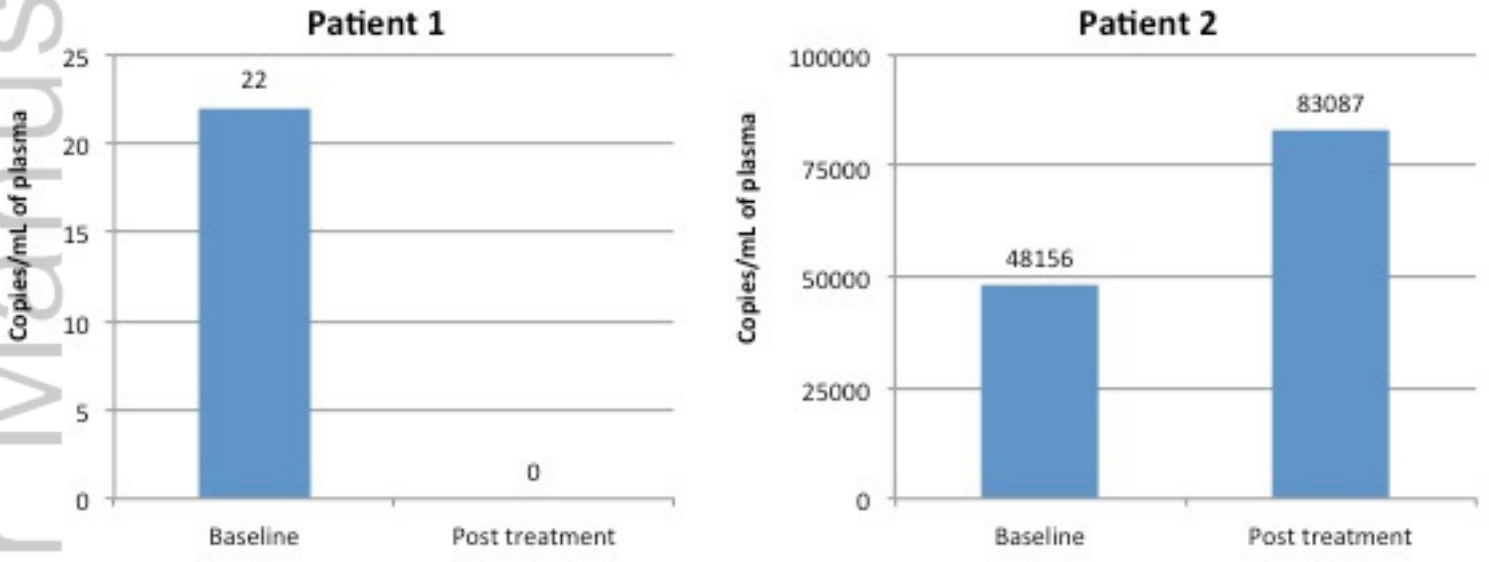

Figure 2.

L858R mutation copies per millilitre of plasma at baseline and post treatment

Figure 2 Final.jpg

De novo activating epidermal growth factor mutations in small cell lung cancer

Authors
Dr Alesha Thai, MBBS (Hons)
- Affiliation: Department of Medical Oncology, Austin Health

Dr Puey Ling Chia. MBBS, FRACP
- Affiliations: Department of Medical Oncology, Austin Health
  Olivia Newton-John Clinical Research Institute, Austin Health

Associate Professor Prudence A. Russell, MBBS (Hons), FRCPA[1]
- Title: Senior Anatomical Pathologist
- Affiliation: Department of Anatomical Pathology, St Vincent's Hospital, University of Melbourne

Hongdo Do, PhD
- Title: Postdoctoral Research Fellow
- Affiliation: Olivia Newton-John Clinical Research Institute, Austin Health

Dr Alex Dobrovic. B.Sc (Hons) PhD
- Title: Group Leader, Translational Genomics and Epigenomics
- Affiliation: Olivia Newton-John Clinical Research Institute, Austin Health

Associate Professor Paul Mitchell
- Title: Director of North Eastern Metropolitan Intergrated Cancer Services
- Affiliations: Department of Medical Oncology, Austin Health
  Olivia Newton-John Clinical Research Institute, Austin Health

Associate Professor Thomas John. MBBS PhD, FRACP
- Title: Head of Lung Oncology
- Affiliation: Department of Medical Oncology, Austin Health
  Olivia Newton-John Clinical Research Institute, Austin Health

Postal Address
Department of Medical Oncology, Austin Hospital
145 Studley Road, Heidelberg, Victoria 3084
AUSTRALIA
Fax: 9457 6698

Email: Alesha_thai@hotmail.com OR Alesha.thai@austin.org.au
Phone: 0416 33 5678

Acknowledgements
Nil

Word count
Abstract: 99
Main text: 1220

[1] A/Prof Prudence A. Russell
Address: Department of Anatomical Pathology, St Vincent's Hospital. 41 Victoria St, Fitzroy, Victoria 3065. Australia

Abstract

De novo activating epidermal growth factor mutations (*EGFR*) in small cell lung cancer (SCLC)

A.Thai[1], PL Chia[1,2], P. A. Russell[3], H.Do[2], A. Dobrovic[2], P.Mitchell[1,2], T.John[1,2]

[1]Department of Medical Oncology, Austin Health, [2]Olivia Newton-John Clinical Research Institute, Austin Health, [3]Department of Anatomical Pathology, St Vincent's Hospital, University

In Australia, mutations in *EGFR* occur in 15% of patients diagnosed with non-small cell lung cancer (NSCLC) and are found with higher frequency in female, non-smokers of Asian ethnicity. Activating mutations in the *EGFR* gene are rarely described in SCLC. We present two cases of de novo *EGFR* mutations in patients with SCLC detected in tissue and in plasma cell free DNA (cfDNA), both of whom were of Asian ethnicity and never-smokers. These two cases add to the growing body of evidence suggesting that screening for *EGFR* mutations in SCLC should be considered in patients with specific clinical features.

Key Words

Small cell lung cancer, *EGFR* mutation, de novo, cfDNA, cell free DNA