

Case Report

The Journey of an EGFR-Mutant Lung Adenocarcinoma through Erlotinib, Osimertinib and ABCP Immunotherapy Regimens: Sensitivity and Resistance

Albina Kibirova^a Malcolm D. Mattes^{b–d} Matthew Smolkin^{c, e}
Patrick C. Ma^f

^aSection of Hematology/Oncology, Department of Medicine, West Virginia University School of Medicine, Morgantown, WV, USA; ^bDepartment of Radiation Oncology, WVU Cancer Institute, West Virginia University, Morgantown, WV, USA; ^cWVU Cancer Institute, West Virginia University, Morgantown, WV, USA; ^dWest Virginia Clinical and Translational Science Institute, Morgantown, WV, USA; ^eSection of Molecular Pathology, Department of Pathology, West Virginia University School of Medicine, Morgantown, WV, USA; ^fPenn State Cancer Institute, Penn State Health Milton S. Hershey Medical Center, Penn State University, Hershey, PA, USA

Keywords

Anti-angiogenesis · Drug resistance · EGFR · Immunotherapy · Non-small cell lung cancer

Abstract

Patients with epidermal growth factor receptor (*EGFR*) mutation positive non-small cell lung cancer (NSCLC) have several *EGFR* targeting tyrosine kinase inhibitors (TKIs) available in front-line management. However, the disease will inevitably progress over time due to acquired resistance. Longitudinal tumor profiling for genomics guided therapy is indicated upon disease

KARGER

Patrick C. Ma, MD, MSc
Penn State Cancer Institute, Penn State Health Milton S. Hershey Medical Center
Penn State University, 500 University Drive, P.O. Box 850, Mail Code CH46
Hershey, PA 17033–0850 (USA)
E-Mail patrickma@pennstatehealth.psu.edu

progression. It is a common scenario yet, when after failure of EGFR-TKIs, potentially actionable genomic alterations are lacking. Management of such patient is challenging with very limited options available. Combination of chemotherapy, anti-vascular/anti-angiogenic and immune-checkpoint inhibitors may become a salvage option for such patients. Here we describe a case of TKI refractory *EGFR*-mutant NSCLC successfully treated with carboplatin, paclitaxel, atezolizumab and bevacizumab combination with remarkable prompt tumor response.

© 2019 The Author(s)
Published by S. Karger AG, Basel

Introduction

Despite excellent initial overall response to targeted EGFR (see [Table 1](#) for glossary) therapy reaching 70–80%, essentially all patients with *EGFR*-mutant NSCLC inevitably progress over time due to acquired drug resistance, thereby limiting their long term survival benefits. Multiple mechanisms of acquired resistance against first-generation EGFR TKIs (erlotinib and gefitinib) have been described, with about half of the cases attributed to the exon 20 gate-keeper *EGFR*-T790M (c.2369C>T) mutation [1–3].

Osimertinib is the only available FDA-approved TKI active against T790M, and recently it has further been approved as first-line therapy in *EGFR* mutant lung cancer patients based on the FLAURA study [4]. In this study, the median duration of response to osimertinib reached 17.6 months (95% CI, 13.8 to 22.0) as compared to 8.5 months (95% CI, 7.3 to 9.8) with first generation TKIs [4]. For patients who develop disease progression on EGFR TKIs, further treatment options are limited. This is particularly true for those who progress on osimertinib, with emerging drug-resistant C797S(c.2390G>C) mutation, which currently is lacking standard therapeutic options [5].

Cytotoxic chemotherapy only yields limited and short-lived responses in general with numerous adverse effects. Attempts to incorporate immune checkpoint inhibitors in patients with *EGFR*-driven NSCLC have initially been also disappointing, especially when used as a single agent programmed death-1 (PD-1) or PD-ligand 1 (PD-L1) antibody [6–8]. Recently, promising data has emerged with overcoming immune checkpoint inhibition resistance by combining it with anti-angiogenic agents. It is believed that anti-vascular endothelial growth factor (VEGF) not only promotes anti-angiogenesis but also acts as a key mediator of the immunosuppressive microenvironment that enables tumor cells to evade immune surveillance [9]. Combining anti-VEGF and immune checkpoint inhibitors has recently been shown to work synergistically in several tumor types.

Case Report

A 45-years-old Caucasian female, who was a never-smoker and previously healthy, presented with progressive forgetfulness and confusion. MRI of the brain demonstrated multiple metastatic lesions, with the dominant mass in the left frontal lobe measuring 1.5 cm associating with extensive vasogenic edema. She was diagnosed with metastatic lung adenocarcinoma of right upper lung with involvement of mediastinal lymph nodes, brain, liver and bones. She

underwent craniotomy with resection of the largest brain lesion revealing the diagnosis. Histological and immunohistochemical tumor characteristics are illustrated in Fig. 1. Patient then received stereotactic radiosurgery to the 8 largest brain metastatic lesions followed by whole brain radiotherapy. As part of a comprehensive molecular tumor profiling (Caris Molecular Intelligence; Caris Life Sciences, Phoenix, AZ), her tumor was found to be positive for *EGFR* exon 19 deletion (E746-A750del) and a *TP53* mutation (Table 2). It was negative for *ALK* or *ROS1* rearrangements, *KRAS* or *BRAF* mutations and also negative for PD-L1 expression.

Patient was started on erlotinib 150 mg daily as first-line therapy and had a near-complete response on follow-up restaging imaging. Nine months later, she developed oligo-progressive disease with two new skeletal lesions which were treated with local stereotactic body radiotherapy and erlotinib was continued at that time. Over the following 8 months, a similar approach was applied to several other gradually progressive skeletal metastases while further therapeutic options were explored. One of these drug-resistant metastatic skeletal lesion in the pelvis was rebiopsied but yielded no informative molecular insights on repeat molecular profiling. Circulating tumor DNA (ctDNA; also referred to as cell-free DNA, cfDNA) liquid biopsy testing (Guardant360; Guardant Health; Redwood City, CA) was performed and detected the presence of T790M mutation (5.4% of altered cell-free DNA (Fig. 2, Fig. 3A). Based on this finding, patient was started on osimertinib 80 mg daily. She again had an excellent response with a progression-free duration lasting for 12 months. Subsequently she again developed oligoprogressive skeletal metastases, which were successfully managed with local radiotherapy concurrently with osimertinib over the next 6 months. She ultimately progressed on osimertinib more extensively beyond further radiotherapy control on TKI, including multiple mediastinal nodal progression. Mediastinal lymph node rebiopsy was obtained confirming adenocarcinoma histology without transformation, but material was insufficient for broad-based molecular testing. Repeat cfDNA liquid biopsy revealed no *EGFR* mutations (Fig. 3B).

For the third-line of treatment patient was started on a quadruplet combination of carboplatin AUC 6, paclitaxel 200 mg/m², bevacizumab 15 mg/kg and atezolizumab 1,200 mg (ABCP), based on encouraging data from the IMpower 150 study [10]. The first treatment cycle was complicated by subclinical thyroiditis, grade 3 nausea, vomiting and pancytopenia requiring hospital admission. The second cycle was delayed with also a dose reduction on the cytotoxics. Nevertheless, restaging PET/CT scan at week 6 after only one cycle of treatment already demonstrated a near-complete response (Fig. 4). Patient subsequently completed total of 4 cycles of ABCP followed by maintenance bevacizumab and atezolizumab (AB). She remained in radiographic remission for 9.5 months when her repeat restaging PET/CT scan demonstrated enlarging FDG-avid primary RUL lung nodule and several new skeletal lesions and brain MRI revealed new tiny enhancing foci in right frontal and left parietal cerebral cortex. At this time, patient was agreeable for treatment with repeat local radiotherapy to drug-resistant disease lesions while continuing immune checkpoint PD-L1 therapy on atezolizumab maintenance. Bevacizumab was temporarily held during the time of radiotherapy. She has received GKRS to brain lesions and the plan is to continue with focal radiation to skeletal metastases. Repeat cfDNA liquid biopsy profiling at time of ABCP/AB regimen acquired resistant progression revealed re-emergence of *EGFR* exon 19 deletion and new emergence of *CDK6* amplification and *ATM* R3008C mutation (Fig. 3C). Besides, there was a new *CDK12* mutation of unknown significance; and the *TP53* as well as *EGFR* T790M mutations remained undetectable. Overall, it was determined that no new readily targetable alterations were found.

Discussion/Conclusion

Despite the advent of targeted EGFR-TKIs like erlotinib and osimertinib, the development of drug resistance remains a formidable challenge in the management of *EGFR*-mutant NSCLC. Multiple mechanisms of acquired drug resistance have been described in the literature, supporting longitudinal tumor profiling for genomics guided therapy. While T790M mutation represents the dominant mutational resistance mechanism against early generation EGFR-TKIs, there is emerging data describing an acquired mutation in C797 codon of EGFR leading to osimertinib resistance [5]. This mutation has no therapeutic options presently although there is potential 4th generation EGFR-TKI in clinical development [11]. Other possible mechanisms of resistance include activation of alternative bypass pathways (*MET*, *HER2*, *KRAS*, *BRAF*, *PIK3CA*) or oncogenic fusions (*CCDC6-RET*, *FGFR3-TACC3*, *EML4-ALK*), some of which can be exploited with therapeutic interventions [1, 2, 12].

In a real life practice however, as in our presented case, by the time acquired EGFR-TKI resistance develops, commercially available effective therapeutic options are limited and clinical trials are not always readily available. The recent exciting achievement of targeting the immune checkpoint pathway in cancer therapy including NSCLC has not yet significantly impacted patients with most oncogenic-driver mutations, especially in *EGFR* and *ALK*. Most, if not all, of the clinical studies testing immune checkpoint inhibitors excluded patients with known *EGFR* mutations and *ALK*-rearrangements. Studies in second-line immunotherapy trials in *EGFR*-mutated NSCLC patients yielded disappointing response and failed to demonstrate any survival advantage when compared with docetaxel [6–8]. Moreover, combination strategy with addition of immunotherapy to targeted TKIs have also proven to be associated with significant treatment toxicities [13]. Oncogene-driven lung tumors, e.g. *EGFR* mutation and *ALK*-rearrangement, were found to correlate with low tumor mutational burden and often also low PD-L1 expression [14]. It is conceivable that these factors above could account for the relative lack of response and treatment efficacy of immune checkpoint inhibitors in this patient population. Furthermore, even in those with high PD-L1 expression, this was not highly predictive for positive responses either.

The anti-VEGF antibody bevacizumab combined with chemotherapy was approved for the treatment of metastatic NSCLC in combination with carboplatin and paclitaxel based on the ECOG 4599 study in 2006 [16]. The anti-VEGF strategy has been investigated in the IM-power 150 study as a potentiating option in combination with the immune checkpoint PD-L1 inhibitor atezolizumab [10]. The concept behind combining anti-angiogenic agents with immune checkpoint inhibitors is at least partially based on the demonstration that VEGF is not only important for tumor neovascularization, but is also a key immuno-modulating factor produced by solid tumors to evade immune recognition and tumor cell killing [9, 15]. The IM-power 150 study included a small cohort of *EGFR* mutation positive NSCLC patients (35/400 or 8.8%) who progressed on prior EGFR-TKI therapy and were assigned to receive ABCP regimen in comparison with patients who received the same regimen without atezolizumab (BCP). In the subgroup analysis, the median progression-free survival (PFS) in patients with *EGFR* mutation or *ALK*-rearrangement in the ABCP combination arm was 9.7 months which was significantly longer compared to the BCP arm, 6.1 months respectively with unstratified hazard ratio 0.59. The difference was favorably comparable to the entire study cohort of NSCLC patients receiving ABCP combination [10].

The presented case herein illustrates a classic natural history of *EGFR*-mutant NSCLC. It also provides support for the utility of consolidative local radiotherapy against oligo-progressive lesions beyond EGFR TKI acquired drug resistance. After repeated resistance to erlotinib and osimertinib, our patient ultimately achieved a near-complete response after only one cycle of ABCP regimen, demonstrating the possible prompt and remarkable efficacy of the combination strategy in this heavily-pretreated disease scenario. Our patient continued to do well on maintenance AB treatment for 9.5 months of progression-free interval with excellent performance status, and overall >4 years since her initial metastatic cancer diagnosis. Notably, her original tumor stained negative for PD-L1 expression and highlighted rare CD4 and CD8 positive lymphocytes (Fig. 1). However, the 9.5 months duration of response was less than ideal but was also comparable with the outcome in the original IMpower 150 study. She then developed disease progression including CNS involvement. Repeat cfDNA liquid biopsy on ABCP/AB progression demonstrated no readily targetable alterations. Our knowledge on molecular mechanisms in ABCP acquired resistant diseases is very limited at present, but expectedly more studies would continue to emerge in the near future. To our best knowledge, this is the first molecular profiling report of a case of ABCP regimen acquired drug resistant progression. Interestingly, our liquid biopsy molecular profiling at ABCP/AB acquired resistant progression identified novel molecular aberrations, i.e. *CDK6* amplification, *ATM* R3008C, that might represent the genomic driving events behind the drug resistance development on combination of cytotoxic chemotherapy with anti-angiogenic and immune checkpoint inhibitors. While *CDK6* amplification is associated with CDK inhibitor resistance thus negating such therapeutic option for our patient, the *ATM* mutation leading to genomic instability might offer a novel therapeutic opportunity with a PARP and/or an ATM/ATR inhibitor [17].

In conclusion, the PD-L1 immune checkpoint therapy incorporated ABCP regimen provides a promising salvage therapeutic option for patients with *EGFR*-mutation driven NSCLC resistant to targeted TKIs, especially beyond osimertinib. The data from IMpower 150 study provides further support to the development of combinational strategies using chemotherapy, anti-vascular/anti-angiogenic and immune checkpoint inhibitors in these patients. However, clinically validated predictive biomarkers for response to immunotherapy-containing salvage regimens for these patients are still grossly lacking. It is also unclear at present time how the ABCP regimen will compare to secondary targeted therapy approaches if they are uncovered on repeat biopsy and genomic profiling. We propose that randomized clinical studies using this novel combinational strategy should be conducted with a focus on *EGFR*-mutant and other oncogene-addicted NSCLC in order to fully validate this clinical hypothesis and to resolve the related clinical issues outlined above.

Statement of Ethics

This report and related work were conducted in compliance with the guidelines for human studies and Institution Review Board approved study protocol. It is also affirmed that the subject has given her written informed consent to publish her case (including publication of images).

Disclosure Statement

Patrick C. Ma: Speaker Bureau: Takeda, AstraZeneca, Merck, Bristol-Myers Squibb, Bayer. Advisory Board: AstraZeneca, Apollomics, Takeda. Research Funding (to institution): AstraZeneca, Bristol-Myers Squibb, Apollomics, Epicentrix, Merck, Pfizer, Loxo/Bayer.

Albina Kibirova, Malcolm D. Mattes and Matthew Smolkin have no financial relationships to disclose.

Funding Sources

This work was funded by the WVU Cancer Institute, West Virginia University and Penn State Cancer Institute, Penn State Health Milton S. Hershey Medical Center.

Author Contributions

Albina Kibirova and Patrick C Ma conceived the study concept and design. All authors participated in the clinical care of the subject, manuscript writing and revision. All authors approved the final manuscript to be submitted.

References

- Westover D, Zugazagoitia J, Cho BC, Lovly CM, Paz-Ares L. Mechanisms of acquired resistance to first- and second-generation EGFR tyrosine kinase inhibitors. *Ann Oncol*. 2018 Jan;29 suppl_1:i10–9.
- Morgillo F, Della Corte CM, Fasano M, Ciardiello F. Mechanisms of resistance to EGFR-targeted drugs: lung cancer. *ESMO Open*. 2016 May;1(3):e000060.
- Mok TS, Wu YL, Ahn MJ, Garassino MC, Kim HR, Ramalingam SS, et al.; AURA3 Investigators. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. *N Engl J Med*. 2017 Feb;376(7):629–40.
- Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al.; FLAURA Investigators. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N Engl J Med*. 2018 Jan;378(2):113–25.
- Oxnard GR, Hu Y, Mileham KF, Husain H, Costa DB, Tracy P, et al. Assessment of resistance mechanisms and clinical implications in patients with EGFR T790M-positive lung cancer and acquired resistance to osimertinib. *JAMA Oncol*. 2018 Nov;4(11):1527–34.
- Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med*. 2015 Oct;373(17):1627–39.
- Lee CK, Man J, Lord S, Cooper W, Links M, GebSKI V, et al. Clinical and molecular characteristics associated with survival among patients treated with checkpoint inhibitors for advanced non-small cell lung carcinoma: A systematic review and meta-analysis. *JAMA Oncol*. 2018 Feb;4(2):210–6.
- Lee CK, Man J, Lord S, Links M, GebSKI V, Mok T, et al. Checkpoint inhibitors in metastatic EGFR-mutated non-small cell lung cancer-A meta-analysis. *J Thorac Oncol*. 2017 Feb;12(2):403–7.
- Manegold C, Dingemans AC, Gray JE, Nakagawa K, Nicolson M, Peters S, et al. The Potential of combined immunotherapy and antiangiogenesis for the synergistic treatment of advanced NSCLC. *J Thorac Oncol*. 2017 Feb;12(2):194–207.
- Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al.; IMpower150 Study Group. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. *N Engl J Med*. 2018 Jun;378(24):2288–301.
- Wang S, Song Y, Liu D. EA1045: the fourth-generation EGFR inhibitor overcoming T790M and C797S resistance. *Cancer Lett*. 2017 Jan;385:51–4.

- 12 Ou SI, Horn L, Cruz M, Vafai D, Lovly CM, Spradlin A, et al. Emergence of FGFR3-TACC3 fusions as a potential by-pass resistance mechanism to EGFR tyrosine kinase inhibitors in EGFR mutated NSCLC patients. *Lung Cancer*. 2017 Sep;111:61–4.
- 13 Ahn MJ, Sun JM, Lee SH, Ahn JS, Park K. EGFR TKI combination with immunotherapy in non-small cell lung cancer. *Expert Opin Drug Saf*. 2017 Apr;16(4):465–9.
- 14 Dong ZY, Zhang JT, Liu SY, Su J, Zhang C, Xie Z, et al. EGFR mutation correlates with uninflamed phenotype and weak immunogenicity, causing impaired response to PD-1 blockade in non-small cell lung cancer. *OncoImmunology*. 2017 Jul;6(11):e1356145.
- 15 Ohm JE, Carbone DP. VEGF as a mediator of tumor-associated immunodeficiency. *Immunol Res*. 2001;23(2-3):263–72.
- 16 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 Dec;355(24):2542–50.
- 17 Perkhofor L, Schmitt A, Romero Carrasco MC, Ihle M, Hampp S, Ruess DA, et al. ATM deficiency generating genomic instability sensitizes pancreatic ductal adenocarcinoma cells to therapy-induced DNA damage. *Cancer Res*. 2017 Oct;77(20):5576–90.

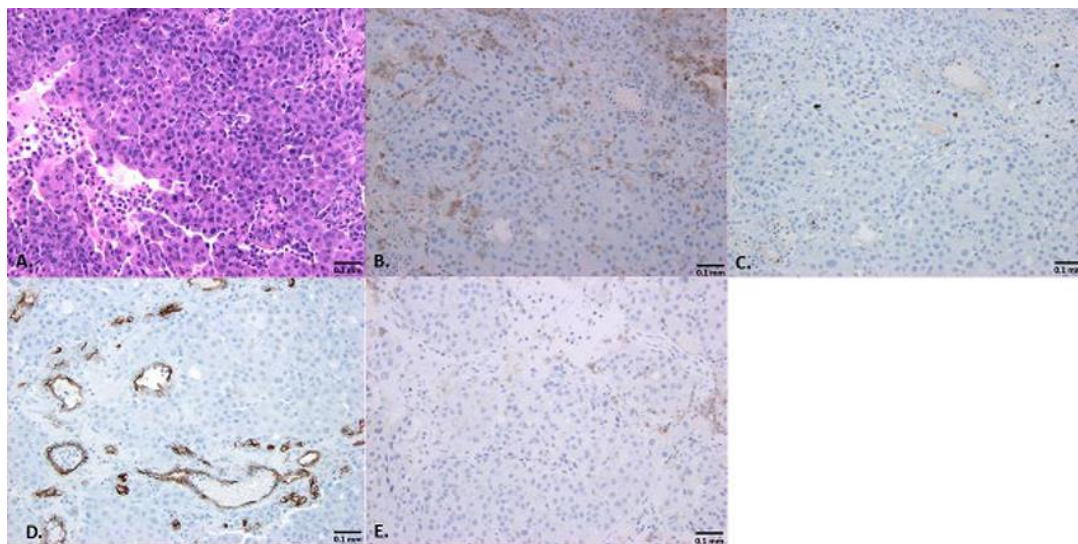


Fig. 1. Histological and immunohistochemical characteristics of the metastatic tumor. **A.** Metastatic adenocarcinoma to the brain (H&E at 200×); **B.** An immunohistochemical stain for CD4 at 200× highlights rare lymphocytes and non-specific staining; **C.** An immunohistochemical stain for CD8 at 200× highlights rare lymphocytes; **D.** A CD34 immunohistochemical stain at 200× highlights endothelium; **E.** An immunohistochemical stain for PD-L1 (22C3) highlights rare weak tumor cell membranous staining, rare positive staining in lymphocytes.

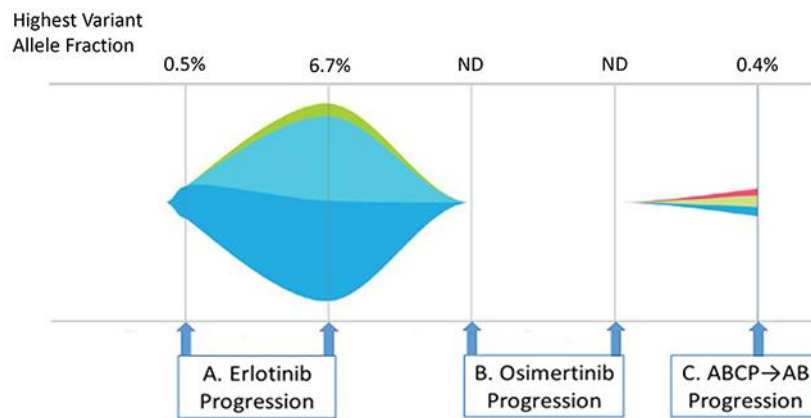


Fig. 2. Summary longitudinal liquid cfDNA profiling (Guardant360) with the tumor response map.

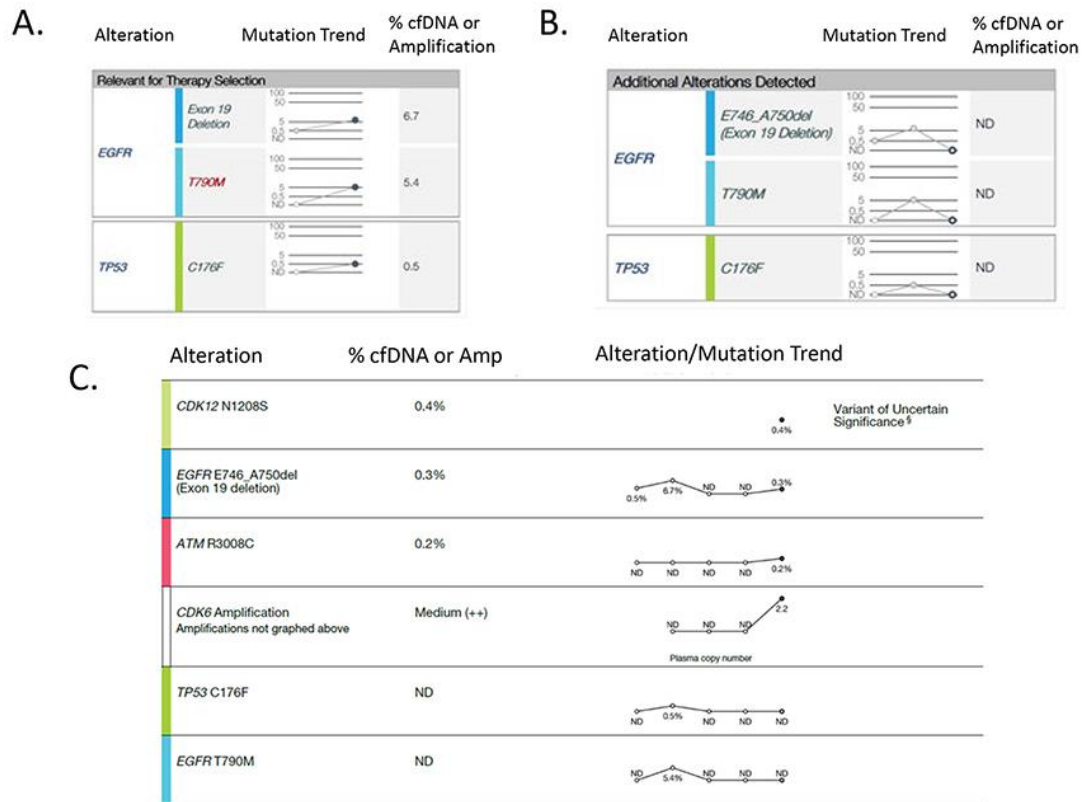


Fig. 3. Longitudinal liquid cfDNA profiling (Guardant360) results. **(A)** New emergence of acquired *EGFR* T790M mutation with 5.4% allele frequency of altered circulating cell-free DNA (% cfDNA) demonstrated on erlotinib progression, which disappeared in the following 2 serial liquid biopsies while on osimertinib, during profiling upon drug resistance to osimertinib **(B)**. Subsequent profiling on ABCP progression revealed the presence of initial driver *EGFR* exon 19 deletion (*E746-A750del specific*) without *EGFR* T790M mutation, and new additional alterations (*CDK12* N1208S, *ATM* R3008C and *CDK6* amplification) **(C)**.

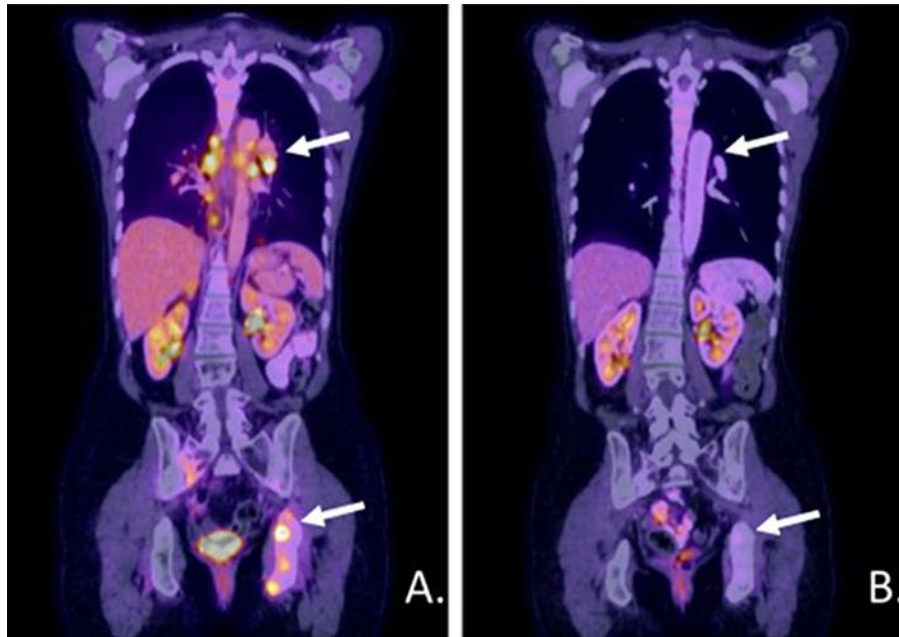


Fig. 4. PET/CT scans prior to initiation of ABCP therapy (**A**) and after one cycle of treatment (**B**), shown. Remarkable and prompt near-complete response with radiographic and metabolic resolution of extensive mediastinal lymphadenopathy and left pelvis bony metastases in resistant progression against osimertinib was noted after the 1st cycle of ABCP salvage treatment (arrows).

Table 1. Glossary of genomic terms and nomenclature

| | |
|------------------------------------|---|
| EGFR | Epidermal growth factor receptor, transmembrane receptor tyrosine kinase |
| T790M (c.2369C>T) | This pathogenic mutation occurs within exon 20 of EGFR kinase domain and results in an amino acid substitution at position 790 from a threonine (T) to a methionine (M) |
| C797S (c.2390G>C) | This pathogenic mutation occurs within exon 20 of EGFR kinase domain and results in an amino acid substitution at position 797 in EGFR, from a cysteine (C) to a serine (S) |
| PD-1 | Programmed cell death protein 1, is a receptor expressed on T-cells, activation of which causes suppression of immune response |
| PD-L1 | Programmed death ligand 1, transmembrane protein, regulating immune response by interacting with PD-1 receptor |
| Exon 19 deletion (E746-A750del) | In-frame deletions occurring within exon 19 of the EGFR kinase domain, one of the most common EGFR mutations in NSCLC |
| TP53 | Tumor protein p53 is a gene that codes for a tumor suppressor protein |
| ALK | Anaplastic lymphoma kinase, transmembrane receptor tyrosine kinase |
| ROS1 | ROS proto-oncogene 1, integral membrane protein receptor tyrosine kinase |
| KRAS | KRAS proto-oncogene, GTPase |
| BRAF | B-Raf proto-oncogene, serine/threonine kinase |
| TPS | Tumor proportion score |
| MET | c-Met protooncogene |
| FISH | Fluorescence In Situ Hybridization |
| CDK12 | Cyclin dependent arginine-serine kinase 12, regulating cell cycle progression and transcription |
| ATM | Ataxia-Telangiectasia serine/threonine kinase, involved in double-strand DNA damage repair |
| CDK6 | Cyclin dependent serine/threonine protein kinase 6, regulating cell cycle progression and transcription |
| ATR | Ataxia telangiectasia and Rad3-related serine/threonine-protein kinase, involved in single-strand DNA damage repair |

Table 2. Comprehensive tumor molecular profiling analysis of case tumor specimen by Caris Molecular Intelligence (CMI)

| Genes tested with alterations | | | | | |
|--|--------------|------------------|--------------|---------------------|------|
| Gene | Alteration | Frequency, % | Exon | Result | |
| EGFR | E746_A750del | 71 | 19 | Mutated, pathogenic | |
| TP53 | C176F | 42 | 5 | Mutated, pathogenic | |
| Genes tested with no mutations detected | | | | | |
| ABL1 | BRCA2 | Her2/neu (ERBB2) | IDH1 | NOTCH1 | ROS1 |
| AKT1 | c-KIT | ERBB3 | IDH2 | NRAS | SMO |
| ALK | CDK4 | FGFR1 | JAK2 | NTRK1 | SRC |
| Androgen receptor | CDKN2A | FGFR2 | KDR (VEGFR2) | PDGFRA | VHL |
| APC | CHEK1 | FGFR3 | KRAS | PDGFRB | WT1 |
| ARAF | CHEK2 | FLT3 | MEK1 | PIK3CA | |
| ATM | c-MET | GNA11 | MEK2 | PTCH1 | |
| BAP1 | CSF1R | GNAQ | MLH1 | PTEN | |
| BRAF | CTNNB1 | GNAS | MPL | RAF1 | |
| BRCA1 | DDR2 | HRAS | NF1 | RET | |