HHS Public Access

Author manuscript

Clin Lung Cancer. Author manuscript; available in PMC 2017 March 01.

Published in final edited form as:

Clin Lung Cancer. 2016 March ; 17(2): e5–11. doi:10.1016/j.cllc.2015.11.003.

Case Report: Germline Mutation of T790M and Dual/Multiple EGFR Mutations in Patients with Lung Adenocarcinoma

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Abstract

Introduction—The epidermal growth factor receptor (*EGFR)* T790M mutation remains one of the major mechanisms of resistance to *EGFR* tyrosine kinase inhibitors (TKI) treatment. Cases of *de novo EGFR* T790M mutations prior to TKI treatment have been reported, but most of them were somatic mutations. In this study, we report a case of primary *de novo* dual *EGFR* mutations containing a germline T790M mutation in a NSCLC patient. We further describe a case series of NSCLC patients who had primary dual or multiple *EGFR* mutations.

Methods—*EGFR* mutation status was analyzed in 427 patients with lung adenocarcinomas. Clinical, demographic data and sequencing electropherograms were collected on patients with two or more *EGFR* mutations identified prior to *EGFR* TKI treatment. Peripheral blood mononuclear cells were sequenced for germ-line *EGFR* mutation on two patients with primary T790M mutation.

Results—55 out of 427 (13%) patients with lung adenocarcinomas were found to have *EGFR* mutations; twelve of which were identified to have either dual or multiple *EGFR* mutations. Five of these 12 patients (42%) had primary *de novo* T790M mutation and three of them showed similar heights of the mutant and wild-type peaks on sequencing electropherogram, suggesting the possibility of germline mutation. One case of germline *EGFR* T790M mutation was confirmed via sequencing a peripheral blood sample.

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Conclusions—Dual or multiple *EGFR* mutations comprised 2.8% of lung adenocarcinomas in our study. Primary *de novo EGFR* T790M mutation are presented with high frequency (5/12; 42%) in patients carrying dual or multiple *EGFR* mutations.

Keywords

Dual *EGFR* Mutations; T790M germline mutation; Lung Adenocarcinoma

Introduction

Exon 19 deletions and point mutations in L858R are the most common somatic activating mutations in the epidermal growth factor receptor (*EGFR)* gene that confer sensitivity to *EGFR* tyrosine kinase inhibitors (TKI) in lung cancer[1]. However, despite the initial response to *EGFR* TKIs, all patients will eventually develop resistance. One of the most common mechanisms of resistance is acquisition of a second mutation at exon 20 which causes a T790M substitution.[2,3]. Although most of these cases are acquired resistance through somatic mutations, a small number of germline *EGFR* T790M have been reported, and are estimated to occur in 1% of non-small cell lung cancer cases[45,6]. These germline *EGFR* T790M mutations are believed to predispose patients to lung cancer, as preclinical studies have shown the germline T790M mutation to be a weak oncogene that often requires a secondary mutation to potentiate cancer development[5,6].

In Asia, a few cases of dual *EGFR* mutations containing primary *de novo* T790M substitution prior to TKI treatment have been described[7,8], however none were identified to be germline mutations. In contrast, thus far germline *EGFR* T790M mutations have only been described In Caucasian patients with lung cancer[5,6]. Several family members of European descent with hereditary bronchioalveolar carcinoma were identified to have germline T790M mutations[9]. Our group previously reported a case of a 72 year-old patient with a solitary T790M mutation who had a germline T790M mutation in her peripheral blood mononuclear cells (PBMC)[10]. Recently, two USA cases with germline T790M mutations were reported in never smoking female Caucasian patients[5,6]. In this brief report, we describe another case of a Caucasian female patient with lung adenocarcinoma who had a germline *EGFR* T790M mutation and concurrent somatic L858R mutation. We further describe a case series of patient demographics and tumor characteristics associated with primary *EGFR* T790M mutations in NSCLC patients.

Material and methods

Patient Selection and Data Collection

Following Institutional Review Board approval at MD Anderson Cancer Center, clinical and demographic data were collected on all patients with lung adenocarcinomas between May 2005 and Aug 2009 identified to have two or more *EGFR* mutations. Of 2 patients identified to have a primary *de novo* T790M mutation, peripheral blood mononuclear cells were isolated and assessed for germline *EGFR* mutation status.

Tumor and Germline Genotyping

DNA sequences for *EGFR* (exons 18–21) extracted from paraffin-embedded tissue (NSCLC tumors) or PBMC (for germline assessment) were amplified using standard PCR primers and sequenced. All sequence variants were confirmed by independent PCR amplifications from at least 2 independent DNA extractions, and sequenced in both directions.

Results

Frequency of primary *de novo* dual or multiple EGFR Mutations in patients with lung adenocarcinomas

We evaluated 427 patients treated at the MD Anderson Cancer Center Thoracic Clinic with lung adenocarcinomas between May 2005 and Aug 2009. Among these NSCLC patients, 55 patients were identified to have *de novo EGFR* mutations in their tumors. Twelve patients (12/427, 2.8%) were found to have either dual or multiple *EGFR* mutations, of whom 5 patients had primary *de novo* T790M mutations. The clinical and demographic information of patients with primary *de novo* T790M mutations are shown in Table 1. The information of patients who had dual or multiple *EGFR* mutations without T790M mutations are included in Supplementary Table 1. All of the mutations were tested in tumor samples, except patient # 4 and # 5 whose peripheral blood samples were also available for germline mutation testing.

Case of a Germline *EGFR T790M* Mutation in a Caucasian Female

A 34 year-old never-smoking Caucasian female presented to local emergency room in July 2009 for a persistent cough. The chest x-ray showed a large right-sided pleural effusion and pathology from the pleural fluid revealed metastatic adenocarcinoma of the lung. A PET/CT revealed extensive hyper-metabolic activity in the pulmonary parenchymal, lymph nodes (right hilar, precarinal, right supraclavicular), and multiple liver and osseous metastases (Figure 1A). At the time of initial treatment, the *EGFR* mutation T790M had not yet been identified, and her poor performance status prevented initial chemotherapy administration. She was therefore started empirically on Erlotinib monotherapy with close monitoring. After one month of erlotinib treatment, her performance status had improved, but the dyspnea was worse and a repeat CT confirmed disease progression (Figure 1B and 1C). She was taken off erlotinib and started on cetuximab, cisplatin and alimta. Unfortunately, she was admitted to hospital after one cycle of treatment due to progressive dyspnea; and was unable to tolerate further treatment and entered into hospice. Sequencing of her tumor specimen obtained prior to treatment revealed dual mutations of both *EGFR* T790M and L858R (Figure 2 A and 2B). Mutation analysis of her peripheral blood mononuclear cells also identified a germline T790M mutation, with equivalent heights of the mutant and wild-type peaks (Figure 2C). The proband's family pedigree is presented (Figure 3).

Discussion

The occurrence of germline *EGFR* T790M mutations is a rare event and has only been reported in case reports.[5,6]. These cases have been predominantly identified in Caucasian females with never-smoking histories and adenocarcinoma histology. Our case report is

consistent with this clinical presentation. From the literature, about 73% of lung cancers that arise in patients with germline *EGFR* T790M mutations appear to also carry second *EGFR* mutations[5]. One hypothesis suggests that germline T790M mutation by itself may be a weak oncogene that requires a secondary mutation to potentiate cancer development[5]. Among the secondary mutations, concurrent L858R mutations are the most common[5]. To evaluate this, we interrogated our lung cancer database of 427 patient samples that had been genotyped for *EGFR* mutations. 3% (12/427) of patients were found to carry dual or multiple *EGFR* mutations before *EGFR* TKI treatment and 5 out of these 12 patients carried primary *de novo* T790M mutation. All of the patients who had a primary *de novo EGFR* T790M mutation had a concurrent second mutation. The clinical and demographic features of these 5 patients who carried primary *EGFR* T790M are summarized in Table 1. The proband patient reported in our case report is listed as patient #5 in the Table. The sequencing electropherogram of other 4 patients were shown in Figure 4. Interestingly, patients #1, #2 and #3 showed almost identical heights of peak in the mutant and non-mutant alleles, suggesting the likelihood of germline mutations. Unfortunately, we are unable to confirm the presence of germline mutations due to the unavailability of blood or normal tissue specimens in these patients. The sequencing data of patient #4 showed different heights of peak in the mutant and non-mutant alleles. The peripheral blood sample from this patient (#4) was available, which did not identify germline T790M mutation (data not shown). Four out of five patients with dual mutations containing primary *de novo* T790M had family histories of lung cancer, although some of the family members were smokers. Among those five patients who had primary *de novo EGFR* T790M mutations in addition to sensitive *EGFR* mutations, three patients received *EGFR* TKI treatments (Table 1). Two patients developed disease progression after 1–1.5 months of *EGFR* TKI treatment. The third patient had multiple wedge resections of her cancers and is stable with good disease control for over 5 years on erlotinib therapy. AlthoughT790M mutations have most commonly developed as a resistance mechanism after TKI treatment, rare cases of *de novo* T790M mutations have been reported in the literature[11–13]. Studies have suggested that *EGFR* T790M mutation might be present in small populations of tumor cells before *EGFR* TKI treatment, and the tumor cells harboring the T790M mutation are likely enriched after drug treatment[12]. Consistent with this suggested theory, our data demonstrated that primary *de novo EGFR* T790M mutation are presented with high frequency (5/12; 42%) in patients carrying dual or multiple *EGFR* mutations.

Several groups in Asia have reported small subsets of NSCLC patients with dual *EGFR* mutations[7,8,14]. It was estimated that complex mutations occurred in 13–18% of Asian patients with *EGFR* mutations[15,16]. Our study found similar findings, demonstrating that 12 of 55 patients (22%) carried dual or multiple *EGFR* mutations. It has been hypothesized that since these dual mutations are found in-*cis* that it confers a ‘second-hit’ growth advantage to the cancer cells[14]. In murine models, the expression of *EGFR* T790M has been reported to induce lung adenocarcinomas, suggesting that this mutation alone can be tumorigenic, although a control with wild-type *EGFR* overexpression is missing[17]. However, mice expressing the *EGFR* T790M transgene alone developed tumors with much longer latency than mice expressing either *EGFR* L858R+T790M or *EGFR* L858R alone[17]. Although more definitive studies are required, this is highly suggestive that primary de novo *EGFR* T790M

mutations, whether somatic or germline, can lead to lung adenocarcinomas and that a secondary EGFR mutation further potentiates this and leads to an earlier onset of malignancy[18].

From a clinical perspective, there are several interesting features observed in the cases of germline T790M mutations. To date, all cases have been reported in Caucasian patients except for two cases that were found in patients of East Indian descent[19]. From the cases where family histories were reported, the onset of lung cancer appears to occur at a younger age in each subsequent generation and appears to be inherited in an autosomal dominant pattern[5,6]. Our proband patient was diagnosed with lung cancer at age of 34 and the other case was reported in a 29 years old female, representing the two youngest patients in the literature[5]. This acceleration of disease onset in patients with younger ages in subsequent generations might due to anticipation; a phenomenon that has been described in inherited diseases such as Huntington disease and Fragile X syndrome, in which the disease arises at younger ages or with augmented severity in subsequent generations. DNA instability in subsequent generations is likely cause of anticipation. In addition, environmental factors may also play a role. Similar to sporadic *EGFR* mutations, germline T790M mutations are predominantly found in women. This might be explained by a growing body of evidence showing functional interactions between the estrogen and *EGFR* pathways[20]. Studies suggest that women have more altered activity in DNA repair compared to men and it is well documented that women that smoke are more prone to develop KRAS mutations[21]. Further studies are necessary to understand the roles of gender and biology of germline T790M mutation in lung cancer development.

EGFR T790M mutation was previously considered to be an acquired resistant mutation to EGFR TKI therapy. However our study along with previously published cases suggested that primary *de novo EGFR* T790M mutations may occur as high a frequency as 35–42% in patients with dual or multiple *EGFR* mutations, which support the theory that patients with *EGFR* mutation might harbor unseen T790M mutations at presentation and that the resistance mutation is only identified after clonal selection occurs after *EGFR* TKI therapy[4,11,12].

In a separate issue, germline *EGFR* T790M mutations were reported to be present in approximately 50% of all patients with primary *EGFR* T790M, although this statistic was generated from a very limited number of patients due to the rarity of disease[4]. However, it is reasonable to consider performing germline testing in all patients with primary de novo *EGFR* T790M mutations. This would subsequently lead to potentially screening first-degree relatives of lung cancer patients with germline *EGFR* T790M mutations and monitoring them, given the increased risk of developing lung cancer.

Conclusion

We report a case of germline *EGFR* T790M mutation along with a case series of NSCLC patients with dual/multiple EGFR mutations. Primary de novo *EGFR* T790M mutation is found at a higher frequency in patients who carry dual or multiple *EGFR* mutations. Sensitive detection method such as next generation sequencing will be helpful in identifying

these less frequent mutations. Although this occurs in a rare population of patients, it is important to identify these patients rapidly and enroll them onto clinical trials with 3rd generation TKIs or *EGFR* T790M specific targeted therapy. Further study is essential to further our understanding of this disease and its resistance mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant support: This study is supported by NIH–T32 Research Training in Academic Medical Oncology (Y.L. and C.V.P).

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Clinical Practice Points

- We report a confirmed case of germline *EGFR* T790M mutation along with a case series of dual/multiple EGFR mutations in NSCLC patients with lung adenocarcinoma.
- Primary or *de novo EGFR* T790M mutations are found at a higher frequency in patients who carry dual or multiple EGFR mutations.
- Germline *EGFR* T790M mutations are rare but increase lung cancer susceptibility.
- Screening relatives of lung cancer patients with germline *EGFR* T790M mutation maybe important given the increased risk of developing lung cancer.

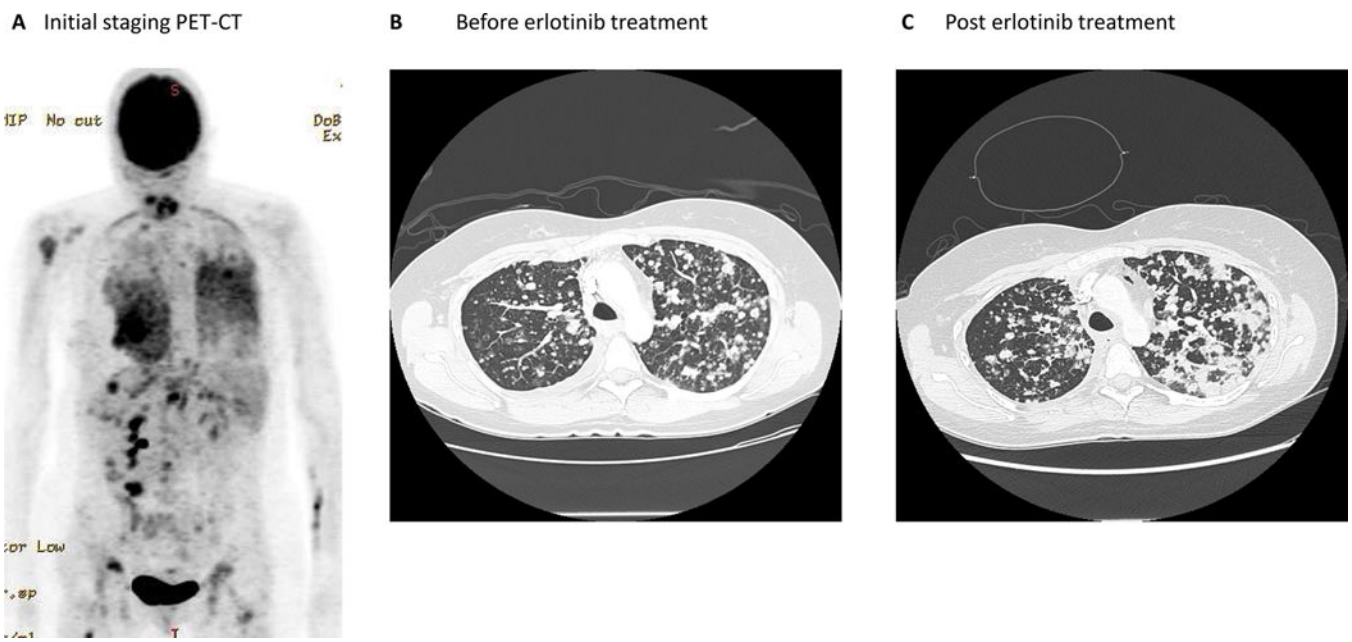

Figure 1.
PET-CT and CT scans of proband patient with germline EGFR T790M mutation.

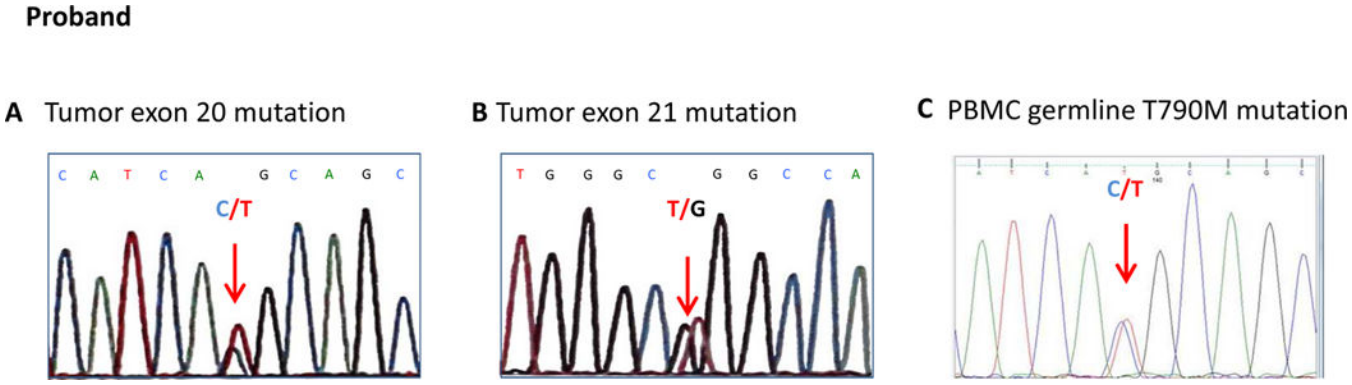

Figure 2.
The results from PCR sequencing on proband's tumor sample (A and B) and peripheral blood cells (C)

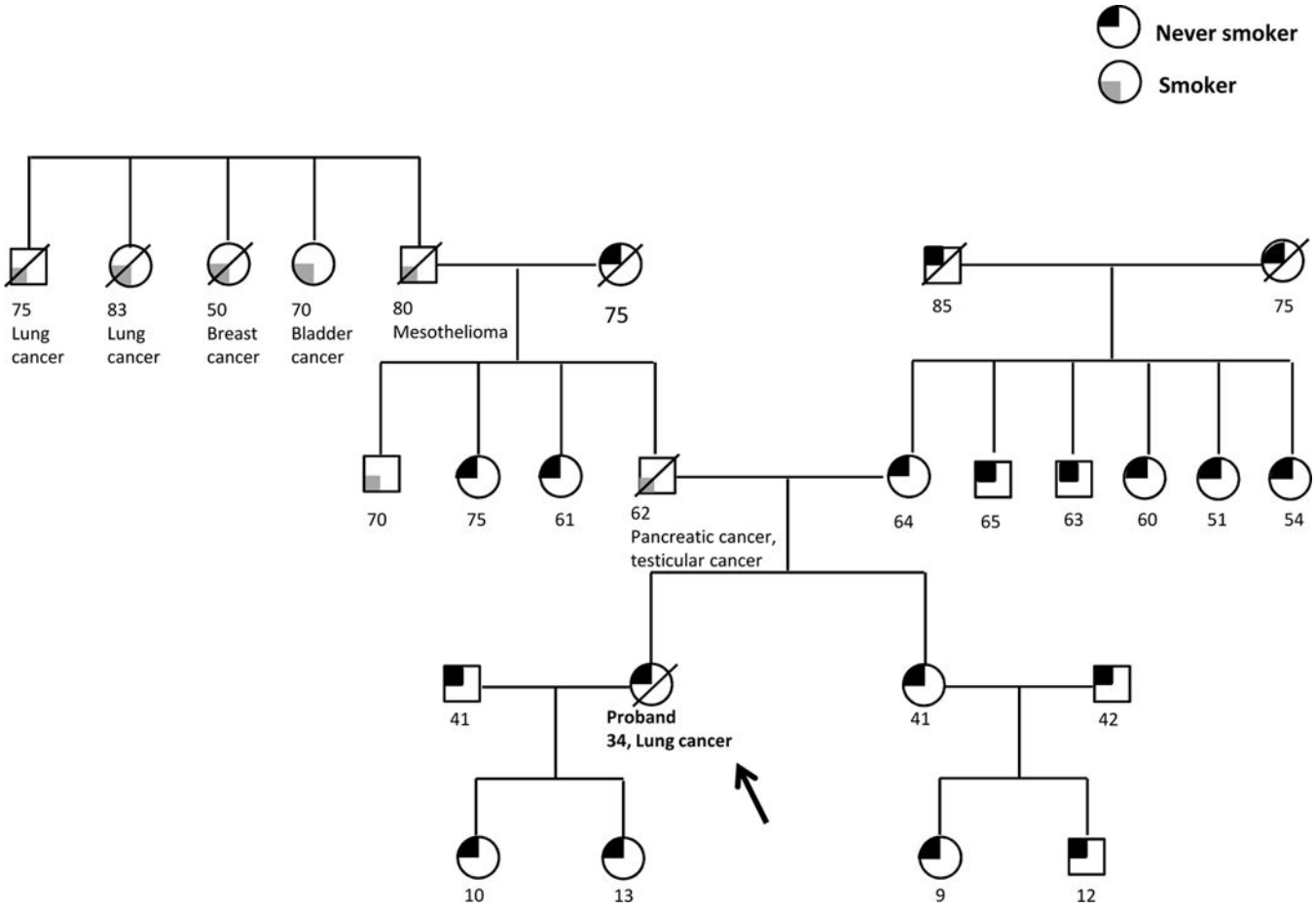

Figure 3.

Pedigree of family with germline T790M mutation. Age, smoking history and other cancer history are recorded.

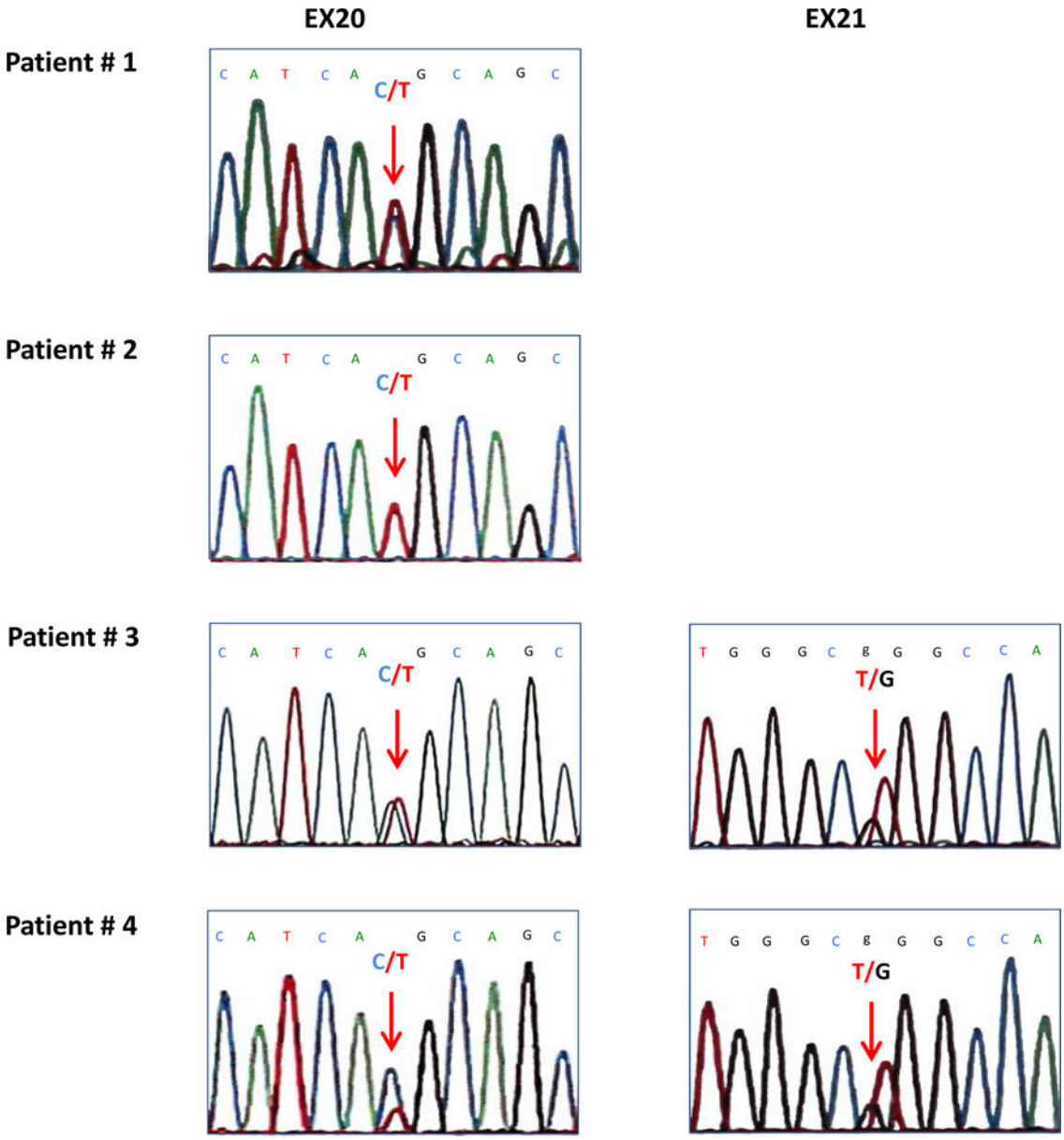

Figure 4.

The results from PCR sequencing on additional patients who carried primary EGFR T790M mutation on patients' tumor samples.

Table 1

Summary of lung cancer patients with dual or multiple EGFR mutations containing primary EGFR T790M mutations.

Patient #	Mutations	Age	Sex	Ethnicity	Pk Yrs/quit Yrs	Histology	Stage at Diagnosis	Metastasis	Family History	Other Cancers	Germline Mutation	Treatment History	TTP on EGFR TKI
1	G719S [18]; **T790M [20]**	51	F	Caucasian	0	Adenocarcinoma	IV/T4N2M1	Lungs, Skeletal	**F: Lung Ca (nS), pA: Lung Ca (nS), pU: Lung Ca (nS)**	None	**NA**	Palliative radiation, then lost to f/u, EGFR TKI treatment history unavailable	Unavailable
2	[del 19]; **T790M [20]**	71	M	Caucasian	0	Adenocarcinoma	IV/T2N3M1	Left choroid, Skeletal, Adrenals	B: “Bone cancer”	None	**NA**	Taxotere/Carboplatin × 2 cycles, discontinued due to presumed Taxotere reaction → Alimta/ Carboplatin × 1 discontinued due to Carboplatin reaction→ Alimta × 3 cycles →Erlotinib × 1.5 months → Gemzar × 1	1.5 months
3	**T790M [20]**; L858R [21]	62	F	Caucasian	0	Bronchioalveolar Carcinoma	IV/T2N0M1	Bilateral lungs	**S: Lung Ca (nS), M: Lung Ca (S), F: Lung Ca (S)**	SCC of skin	**NA**	Multiple wedge resections → Erlotinib for more than 5 years by now	> 5 years
4	**T790M [20]**; L858R [21]; [del 19]	53	F	Caucasian	0	Adenocarcinoma	IIIA/T4N0M0	None	**F: Lung Ca (S)**, B: Glioblastoma, M: Uterine Ca, pU: H+N Ca (S), mGF: Colon Ca, mA: Breast Ca, mC: Breast Ca	Papillary thyroid carcinom a	**No**	Carbo/Taxotere × 6 cycles, stereotactic XRT, no evidence of disease to date	No TKI initiated to date
5 **(proband)**	**T790M [20]**; L858R [21]	34	F	Caucasian	0	Adenocarcinoma	IV/T4N3M1	Skeletal, Liver, Pulmonary	**See family Pedigree**	None	**Yes**	Erlotinib × 1 month → Cetuximab/Cis/Alimta × 1 month	1 month

Abbreviation Legend: F – Father, M – Mother, B – Brother, S – Sister, GF – Grand Father, GM – Grandmother, A – Aunt, U – Uncle, p – Paternal, m – Maternal, Pk-Pack, TTP-Time to Progression, TKI-Tyrosine Kinase Inhibitor, nS – Never Smoker, S – Smoker