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Review

Outcome of EGFR-mutated adenocarcinoma NSCLC patients with changed phenotype to squamous cell carcinoma after tyrosine kinase inhibitors: A pooled analysis with an additional case

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ABSTRACT

The onset of a new histology is a resistant mechanism to tyrosine kinase inhibitors (TKI) in lung adenocarcinoma (ADK), but this phenomenon has not yet been fully clarified. We present a pooled analysis of the outcomes of EGFR-mutated ADK patients with changed phenotype to squamous cell carcinoma (SqCC) following TKI, along with the description of an additional case.

A 67-year-old woman with EGFR-mutated NSCLC received gefitinib and subsequently osimertinib, due to the presence of T790 M at progression. The re-biopsy after third-generation TKI revealed SqCC histology along with the basal EGFR mutation, while T790 M disappeared. The patient rapidly progressed and died despite two chemotherapy cycles. Since this first description of SqCC transformation appearing after treatment with the third-generation TKI osimertinib, other 16 patients, with EGFR-mutated ADK developing a transformation to SqCC histology after treatment with TKIs, were up to now published. From our pooled analysis emerged that most patients were female (82%), 41% were former smokers and no current smokers were identified. Median time to SqCC onset was 11.5 months. In all cases, basal EGFR mutation was maintained, and 11 patients (65%) developed an acquired mutation on exon 20. Interestingly also 790 M mutation appeared in 8 patients (47%). The median survival after SqCC diagnosis was 3.5 months regardless the treatments received.

Therefore, EGFR-mutated lung ADK destined to develop a squamous phenotype were often smokers and maintained the baseline genomic alterations. The prognosis after SqCC diagnosis was extremely poor and current treatments largely inefficacious.

1. Introduction

Lung cancer is the major killer worldwide, however, epidermal growth factor receptor (EGFR) mutation identifies a subgroup of non-small cell lung cancer (NSCLC) patients that differs from the wild-type counterpart, in terms of better outcome and specific responsiveness to tyrosine kinase inhibitors (TKIs) [1]. Indeed, the presence of EGFR-activating mutation makes about 15% of lung adenocarcinoma (ADK) patients eligible to TKI therapy, that had revolutionized NSCLC treatment, being superior to cytotoxic chemotherapy in terms of survival and tolerability [2,3]. Despite these important results, TKI therapy is not curative, and all patients are destined to develop resistance.

Several mechanisms of resistance of lung ADK to TKIs have been identified. Out of them, a single base modification of 790 amino acid on exon 20, defined as T790 M, causing a reduction in the binding of the TKI to EGFR, is responsible for 60% of acquired resistance [4]. In the remaining 40% of patients the disease progression to TKI involves activation of alternative signalling pathways (i.e., MET and HER-2 amplification, KRAS mutation) and also the appearance of a new histologic phenotype, such as small cell lung cancer (SCLC) or squamous cell carcinoma (SqCC) [5]. Noteworthy, while the appearance of the neuroendocrine phenotype could be likely due to transdifferentiation from ADK to SCLC [6], it is actually not clear whether the SqCC phenotype after TKI can be considered the result of a histological transformation or it is simply due to a clonal selection [7].

The innovative third-generation TKI, osimertinib, is destined to

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change in part this scenario. This drug proved to be efficacious in terms of overall survival improvement in patients with T790 M positive ADK when disease progression to first- or second-generation TKIs occurred (AURA trial) [8,9]. Moreover, a recent head-to-head comparison of osimertinib with gefitinib or erlotinib (FLAURA study) demonstrated a clear superiority of this third-generation TKI in the first-line treatment of EGFR mutation-positive advanced NSCLC [10]. With the introduction of osimertinib, the resistance due to T790 M mutation has been overcome, and other mechanisms including histology change should be investigated because are potentially destined to be more relevant.

The few reports published up to now, regarding the onset of SqCC phenotype after TKIs described cases treated with erlotinib, gefitinib and afatinib [11–23]. To our knowledge, no cases have described this transformation after osimertinib.

Herein we present a pooled analysis of published case reports or small case series of SqCC phenotype onset in EGFR-mutated NSCLC patients treated with TKIs. Moreover, we add a new case as a first description of transition from EGFR-mutated lung ADK to SqCC after osimertinib treatment.

2. Clinical case

A 67-year-old woman was referred to the Lung Cancer Unit at Spedali Civili Hospital in March 2011, because of dyspnoea and persistent cough. She underwent a CT scan that revealed the presence of a lesion in the apical segment of the left inferior lobe, multiple bilateral peripheral nodules, and pleural thickenings. A diagnostic thoracoscopy with talc pleurodesis was carried out, and a lung EGFR-mutated (L858R on exon 21) ADK was diagnosed.

From April 2011 to February 2016, the patient had received gefitinib, attaining a complete response of right pleural lesions and a partial response of left pleural lesions and parenchymal lung lesions (Fig. 1). In February 2016, disease progression was documented (Fig. 1), and a liquid biopsy revealed the presence of the T790 M mutation. Therefore, the patient was enrolled in the ASTRIS Study and received AZD9291 (osimertinib), as second-line therapy (NCT02474355). Disease stabilization was obtained lasting 10 months, but in November 2016, a re-evaluation CT scan showed a new excavation area of the perihilar lesion in communication with the bronchial system (Fig. 1). This radiological finding was associated with a worsening of clinical conditions with episodes of fever that persisted despite antibiotic therapy. A bronchoscopy with bronchoalveolar lavage and diagnostic biopsy was then performed demonstrating the presence of neoplastic infiltration by SqCC. Interestingly, the driving EGFR mutation (L858R) was maintained in SqCC cells, while T790 M was negative. Further analysis showed high levels of PDL-1 expression on SqCC cells (> 50%). Osimertinib, therefore, was stopped and a third-line-treatment with carboplatin and gemcitabine was introduced. However, a rapid disease progression occurred after 2 cycles and the patient died in January 2017. The overall survival was 70 months from the first diagnosis and 3 months from the SqCC onset, respectively.

3. Systematic literature review

We performed a systematic search of the literature to identify all published cases of SqCC onset in patients with a previous diagnosis of EGFR-mutated ADK and treated with a TKI. We used the PubMed/Medline (US National Library of Medicine National Institutes of Health) database. Search terms were “histologic evolution”, “transformation”, “NSCLC”, “adenocarcinoma”, “EGFR mutated”, “squamous cell”, “tyrosine kinase inhibitors”.

In the selected case reports or case series, ADK and SqCC NSCLC should have been diagnosed in high-quality tumor biopsies or well-preserved cytological samples, according to 2015 WHO classification [24].

As depicted in the PRISMA flow chart (Fig. 2), we initially identified 935 articles, and finally selected 13 published reports that included a total of 16 patients with a documented occurrence of a SqCC phenotype after anti-EGFR TKI therapy [11–23].

Including also the case described in the present report, 17 patients were considered, and their baseline characteristics are summarized in Table 1.

Most of them were female (82%), they were initially treated with gefitinib (47%), erlotinib (35%) or afatinib (18%). Median age was 63 years (range 43–79). Smoking status was available in 15 cases: eight were never-smokers (47%), and seven were former-smokers (41%). No patient was current smoker (Table 2).

The diagnosis of ADK resulted from cytology after CT-guided Fine Needle Aspiration in 1 patient, while histologic samples were available in15 patients. In 3 of them, also pleural fluid was collected. In the remaining patient (#12), the diagnosis of lung ADK was based only on pleural fluid cytological specimens and molecular analysis revealed wild-type EGFR, and exon 21 mutation was documented on the SqCC phenotype at disease progression (Table 1). Since this patient had a significant response to EGFR TKI, the authors concluded that an activating EGFR mutation could have been present also in the tumor at first diagnosis, which was not detected due to the low cellularity of the cytology specimen [19].

Excluding this latter patient, a mutation on exon 19 and exon 21 were found in 9 (53%) and 7 patients (41%), respectively; while no mutation in exon 18 was described. T790 M was detected in two patients before the SqCC onset: it was described at diagnosis in patient #10 and at disease progression after the first-line TKI therapy in patient #17.

As shown in Table 2, EGFR inhibitors were administered as first, second or third line; and the median duration of TKI therapy before SqCC diagnosis was 11.5 months (range 4–69 months) (Fig. 3).

Of the 13 patients available for response evaluation to initial EGFR

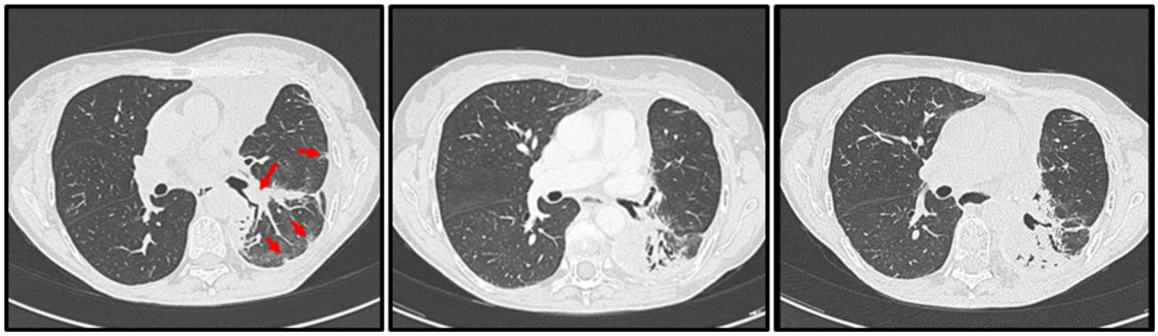

Fig. 1. Follow-up CT lung scans.
Lung CT images assessment performed in June 2011 (image on the left), after two months from the beginning of gefitinib, in February 2011 (image in the middle) showing disease progression after first-line TKI, and in November 2016 (image on the left) when disease progression was described after osimertinib.

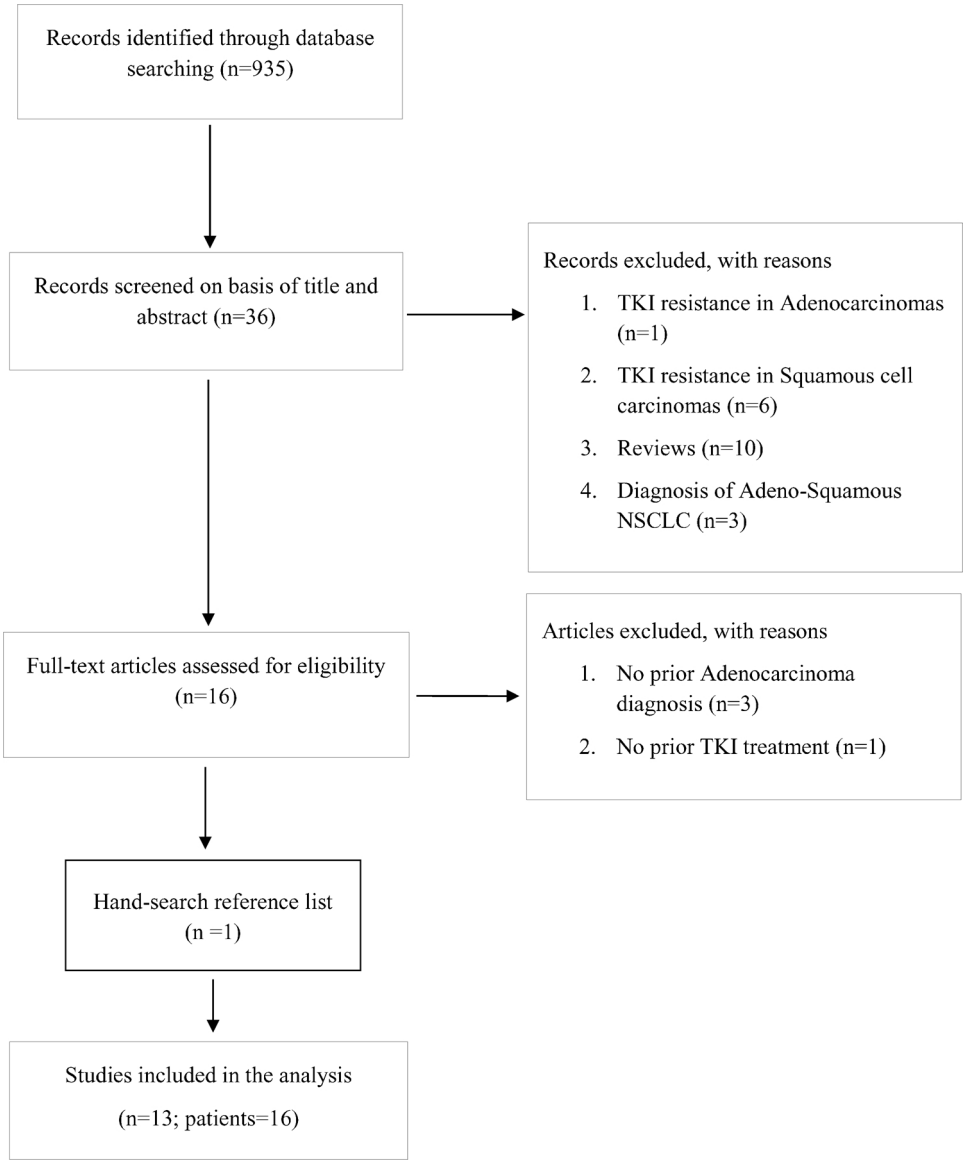

Fig. 2. PRISMA Flow Chart. Flow Chart of studies selection.

TKI therapy, 11 (65%) achieved a partial response (PR), and 2 (12%) had stable disease (SD).

The diagnosis of the SqCC phenotype onset was based on biopsy samples in 16 patients, whereas in one case it was performed on a cytological specimen. All activating EGFR mutations presented at diagnosis were maintained at progression in the SqCC.

Noteworthy, when SqCC was detected, newly acquired mutations were proved in 11 patients (Table 1), including S768I (1 patient), PIK3CA (2 patients) and T790 M (8 patients). The basal T790 M found in patient #10 was maintained when SqCC phenotype occurred, whereas in patient #17 the T790 M found at liquid biopsy was not detected at the biopsy performed after osimertinib (Table 1). In addition PDL-1 expression was observed in > 50% of tumor cells.

Treatment after SqCC phenotype onset was described for only 12 patients and included chemotherapy (24%), TKI (41%), or a combined approach (6%). Two patients did not benefit from any therapy as they died shortly after rapidly progressive disease. The antitumor activity of systemic therapies administered after SqCC detection was low: a partial response was observed in only 4 patients submitted to third-generation TKI (Table 2).

Information on survival status after SqCC carcinoma was available in 13 patients (76%) and the estimated median overall survival, including also our patient, was 3.5 months (Fig. 2).

4. Discussion

Change in tumor histology with the appearance of either small cell or squamous cell phenotype is repeatedly observed in EGFR-mutated lung ADK after TKI therapy. The biological mechanisms associated with this transformation are unclear, and either clonal selection or trans-differentiation can be considered. Herein, we provided a pooled analysis of published data regarding SqCC onset from EGFR-mutated NSCLC ADK after TKI, and described the first case occurred in a patient treated with the third-generation TKI, osimertinib.

In our analysis of 17 patients, females (82%) seemed to be more represented as compared with the general population diagnosed with EGFR-mutated lung ADK(ranging from 57% to 79.5%). Median age (63 years) was older than EGFR-mutated NSCLC patients enrolled in clinical trials testing TKIs [25–28]. Since EGFR mutations are usually more frequent among non-smoking patients, the percentage of former smokers in this series (41%) appears relevant. Initial biopsies could have been inadequate to detect a possible adeno-squamous histology and

Table 1
Patients characteristics before and after SqCC onset and outcomes. (ADK adenocarcinoma; SqCC: squamous cell carcinoma; PL: pleural fluid analysis; RT: Radiation Therapy; WT; Wild Type; TKI: Tyrosine Kinase Inhibitor; cbdca: Carboplatin; cddp: Cisplatin; pem: Pemetrexed; gem: Gemcitabine; vnr: Vinorelbine).

Before SqCC onset								Post SqCC onset						
n.	Gender, Age	Smoking status	TKI	TKI line	Sampling method	EGFR mutational status	TKI treatment time (m)	Sampling method	EGFR mutational status	Additional mutations	Treatment post SqCC onset	OS* (m)	OS** (m)	Reference n.
1.	F, 66	Never	Erlotinib	II	Biopsy and PL	19 p.E746_A750del	8	Biopsy	19 p.E746_A750del	–	–	14	0	11
2.	F, 63	Never	Erlotinib	I	Biopsy	21 p.L858R	5	Biopsy	21 p.L858R	PIK3CA	Gefitinib and cbdca/gem	14	7	12
3.	F, 58	Former	Erlotinib	II	CT-guided Biopsy	19 p.E746_A750del	11	CT-guided Biopsy	19 p.E746_A750del	T790 M (only on SNC lesion)	–	20 †	–	13
4.	F, 74	Former	Gefitinib	I	Biopsy	21 p.L858R	10	Biopsy	21 p.L858R	T790 M	Cbdca/vnr	21 †	11	14
5.	F, 79	Never	Gefitinib	I	Biopsy and PL	19 p.E746_A750del	15	Biopsy	19 p.E746_A750del	T790 M	Gefitinib	26 †	7	14
6.	F,43	Former	Gefitinib	I	Biopsy	21 p.L858R	8	Biopsy	21 p.L858R	S7681	Gefitinib	11	1	15
7.	F, 51	–	Gefitinib	I	Biopsy	19 p.E746_A750del	4	Biopsy	19 p.E746_A750del	–	Cddp/Gem	10 †	6	16
8.	F, 61	Never	Gefitinib	I	Biopsy	21 p.L858R	12	Biopsy	21 p.L858R	–	Erlotinib	24	0	16
9.	F, 48	Never	Gefitinib	I	Biopsy	19 p.E746_A750del	24	Biopsy	19 p.E746_A750del	–	–	30	0	17
10.	F, 64	Never	Gefitinib	I	Biopsy	21 p.L858R and p.T790 M	–	Biopsy	21 p.L858R and p.T790 M	–	Rociletinib	- †	10	17
11.	F, 67	–	Afatinib	I	CT-FNA	19 p.E746_A750del	6	US-FNA	19 p.E746_A750del	PIK3CA	Cbdca/vnr	10 †	4	18
12.	F, 63	Never	Erlotinib	III	PL	WT	22	Biopsy	21 p.L858R	T790 M	–	43	–	19
13.	M, 68	Former	Erlotinib	I	Biopsy	21 p.L858R	9	Biopsy	21 p.L858R	T790 M	Osimertinib	17	3	20
14.	F, 44	Former	Afatinib	I	Biopsy and PL	19 p.E746_A750del	18	Biopsy	19 p.E746_A750del	T790 M	Osimertinib	20 †	2	21
15.	M, 40	Former	Afatinib	I	Biopsy	19 p.E746_A750del	24	Biopsy	19 p.E746_A750del	T790 M	–	24 †	–	22
16.	M, 69	Former	Erlotinib	I	Biopsy	19 p.E746_A750del	12	Biopsy	19 p.E746_A750del	T790 M	Osimertinib	15 †	–	23
17.	F, 67	Never	Gefitinib osimertinib	I-II	Biopsy	21 p.L858R	69	Biopsy	21 p.L858R	T790 M negative	Cbdca/gem	70	1	Current article

* From ADC diagnosis.
** From SqCC onset.
† Alive at the last follow-up.

Table 2
Pooled data of our case plus published cases.(ADK: adenocarcinoma NSCLC; SqCC: Squamous cell carcinoma NSCLC; TKI: Tyrosine Kinase Inhibitor; na: not available).

Baseline characteristics	**n**	**%**	**Time to SqCC onset from TKI start**	**m (months)**	
Gender			**Median time**	11.5 m	
M	3	18			
F	14	82	**Erlotinib**	9 m	
Median Age 63 years (range 43-79)			**Gefitinib**	12 m	
Smoking Status			**Afatinib**	18 m	
Never	8	47			
Former	7	41			
na	2	12	**TKI I line**	11 m	
EGFR Exon			**TKI II line**	9.5 m	
19	9	53	**TKI III line**	22 m	
21	7	41	**TKI I and II line**	69 m	
Wild type	1	6			
ADK treatments	**n**	**%**	**SqCC treatments**	**n**	**%**
TKI administered			**Chemotherapy**	4	24
Erlotinib	6	35	**TKI**	7	41
Gefitinib	7	41	**Chemotherapy plus TKI**	1	6
Afatinib	3	18	**Na**	5	29
Gefitinib then osimertinib	1	6	SqCC Best Overall Response	n	%
TKI treatment line			**Partial responses**	4	24
I line	13	76	**Stable disease**	4	24
II line	2	12	**Progression disease**	5	29
III line	1	6	**Died for other reasons**	1	6
I and II line	1	6	**na**	3	18
ADK Best Overall Response to TKI	**n**	**%**	**Survival status**	**n**	**%**
Partial responses	11	64	**Patients alive at last follow up**	9	53
Stable disease	2	12			
Progression disease	0	0	**Overall Survival from NSCLC diagnosis**	20 months	
na	4	24	**Overall Survival from SqCC onset**	3,5 months	

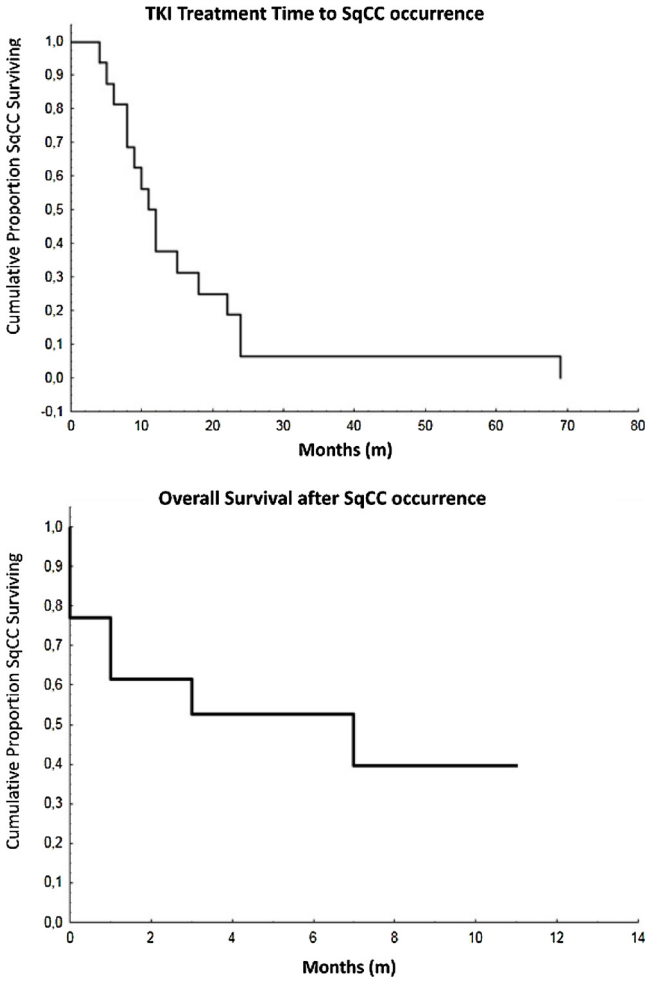

Fig. 3. TKI treatment time before SqCC onset and Overall Survival after SqCC occurrence.
Kaplan-Meier estimates of TKI treatment time from initial diagnosis of lung adenocarcinoma to SqCC diagnosis and Kaplan-Meier estimates of overall survival after SqCC diagnosis.
(SqCC = Squamous Cell Carcinoma, NSCLC).

therefore we cannot exclude a proportion of squamous cell at diagnosis that could have progressed through clonal selection of previous mixed neoplasms. EGFR activating mutations on primary lung ADK included E746_A750 deletion in exons 19 (53%) or L858R on exon 21 (41%); no mutation on exons 18 or 20 was described. Of note, all SqCC maintained the EGFR mutation (E746_A750 deletion or L858R mutation) described on ADK biopsies at the time of first diagnosis. Since the prevalence of activating EGFR mutations in squamous cell carcinomas is reportedly less than 5% [29–31], these data support the hypothesis that the new SqCC phenotype detected in biopsies carried out at disease progression could arise from primary adenocarcinoma cells. A recent review paper summarized the in vitro and in vivo findings supporting the evidence on the adenocarcinoma to squamous cell transdifferentiation in EGFR-mutated lung cancer after TKI administration [32]. The data we have collected, however, are insufficient to support this interesting hypothesis.

It is well known, that the adeno squamous phenotype could harbour the EGFR mutations, and these mutations occur in both ADK and SqCC components [33]. These patients are frequently treated with TKI, however, the reported PFS (8.08 months) seems to be shorter than the median time to SqCC onset from TKI start, described in this paper (11 months) [34].

Baseline activated mutations, especially L858R EGFR mutations, may be lost at re-biopsy performed disease progression to TKI [35]. This phenomenon was not seen in this series. As reported in Table 2, all the 7 tumors harbouring the L858R EGFR mutation at baseline maintained the same mutation at disease progression. In addition 1 case diagnosed as wild type became positive for L858R afterwards.

The overall survival observed from TKI start (20 months) in this series seems to be at lower limits to that expected in this population [30] suggesting that patients destined to SqCC onset may potentially have a poorer survival than those who did not.

The prognosis of EGFR-mutated NSCLC patients with disease progression to TKIs due to SqCC onset was extremely poor with a median OS of 3.5 months, although for some patients the OS was longer. Despite a 24% objective response rate, the treatments adopted (chemotherapy and targeted therapies) after SqCC onset was substantially inefficacious in terms of survival prolongation.

We recently published a pooled analysis on patients with small cell (SCLC) neuroendocrine transformation after TKI administered for EGFR mutated or ALK re-arranged ADK [6]. Some similarities and discrepancies are evident between the 2 series.

Among discrepancies, the time from TKI start to the histological transformation was shorter in patients developing SqCC than that of patients with SCLC (11.5 vs. 18 months) and this observation suggest that the occurrence of the SCLC phenotype after TKI could be more delayed than the SqCC phenotype [6]. In addition, the survival outcome of SqCC transformed patients was worse than that of patients with SCLC transformation (median OS, 3.5 vs 6 months). Moreover, the T790 M mutation was detected in a higher proportion of SqCC cases (47%) than SCLC counterpart (7.9%) and more former smokers (41%) were observed among patients developing SqCC histology comparison to those with SCLC (17%) [6]. How the smoking status could contribute to SqCC

transformation is a matter for future research.

With respect to similarities, both SqCC and SCLC phenotypes occurring after TKI maintained the same EGFR mutation present in the former adenocarcinomas.

A peculiarity of the transformed SqCC is the high frequency of T790 M. In our case report, however, the SqCC at re-biopsy was negative for this mutation after osimertinib. Moreover, since in our case T790 M before osimertinib was detected by liquid biopsy, we cannot exclude that a SqCC phenotype could have been present at that time. If this is true, then we can hypothesize that osimertinib could be active in transformed SqCC harbouring T790 M.

The adoption of 2 different methods of EGFR detection (liquid biopsy versus tissue sample) before osimertinib therapy and at disease progression, respectively, is admittedly a limitation. Discrepant results between the 2 methods have been described in literature [36]. The absence of T790 M in squamous histology after osimertinib therapy, therefore, need confirmation in a patient series. The FLAURA study [10] demonstrated the efficacy of osimertinib as first-line treatment in NSCLC patients with EGFR mutation. Based on these data, osimertinib is destined soon to replace first and second generation TKIs in this setting. If our observation is true then we could expect in the near future changes in the mutational profile of SqCC occurring after TKI, with disappearance of T790 M due to the introduction of osimertinib.

In conclusion, our pooled analysis shows that the appearance of the SqCC phenotype in EGFR-mutated lung adenocarcinoma is associated with poor prognosis. Our data are unable to provide evidence on mechanism underlying this phenomenon (i.e. whether it is due to clonal selection or transdifferentiation). Current treatment strategies, adopted in the management of primary SqCC, appear to be largely inefficacious in the SqCC observed after TKI therapy, and novel treatment approaches are needed.

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