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Case report

Unexpected responses to EGFR inhibition in NSCLC

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ABSTRACT

The presence of activating mutations of the epidermal growth factor receptor (*EGFR*)-gene identifies a distinct and clinically relevant molecular subset of non-small-cell lung cancer. It is now well demonstrated that EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib are superior to standard chemotherapy in this subset of tumors. Nevertheless, in many cases, responses are not durable and last for 6–12 months due to the occurrence of secondary or acquired resistance. Here we present three cases of *EGFR*-mutant lung adenocarcinomas (ADC), that showed an unexpected response to anti-EGFR small molecules. The first patient presented a continued 89 month-long response to erlotinib in a tumor recurred after surgery and conventional chemotherapy. In the other cases, subclinically persistent tumor in the lung tissue was documented histologically in lung resections performed after partial response to TKI treatment. The persistence of interstitial and endolymphatic tumor cells after TKI treatment might explain the common observation of tumor relapse after TKI discontinuation, and sustain the decision to continue treatment in responsive patients as in our first case.

Epidermal growth factor receptor (*EGFR*)-mutant non-small-cell lung cancer (NSCLC) identifies a distinct and clinically relevant molecular subset of lung cancer. Activating *EGFR* somatic mutations have emerged as the most relevant predictor of response to small EGFR tyrosine kinase inhibitors (TKIs) and it is now well demonstrated that in patients whose tumors harbor *EGFR* mutations, EGFR TKIs, geftinib and erlotinib, are superior to chemotherapy in terms of response rates, progression free survival, quality of life and toxicity profile. Nevertheless in many cases responses are not durable, as more often TKI stabilize the disease for 6–12 months followed by the occurrence of secondary or acquired resistance [1].

Here we present three *EGFR*-mutant lung adenocarcinoma (ADC) patients, worth to be reported due to their unusual response to anti-EGFR small molecules. Clinical and molecular details are reported in Tables 1 and 2. The first is a 61-year-old Caucasian, never smoker female who in 2005 underwent left superior lung lobectomy for an early stage (T1N0M0 [2]) ADC. Mutational analysis performed on the resected tumor tissue revealed a deletion affecting exon 19 of the *EGFR* gene, without gene amplification, whereas no mutations were found in *KRAS* exon 2, *PIK3CA* exons 9–20, *BRAF* exon 15 coding sequences; neither *HER2* amplification nor mutation were documented. Molecular analyses were performed as previously described [3]. Based on disease stage adjuvant therapy was not administered. Regular clinical and imaging follow up in 2006 showed at CT scan a mediastinal lymphadenopathy suggestive for disease progression. Subsequent CT-guided biopsy confirmed the diagnosis of lymphnode metastasis of lung ADC. Metastatic cells carried the same genetic profile of the primary tumor. Subsequent analysis demonstrated the absence of *EML4/ALK*

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Table 1
Clinical data on the three patients described.

ID case	Gender	Age at diagnosis (yrs)	Smoking habit (pack/yrs)	TNM at diagnosis	PFS (months)	OS (months)
1	F	61	never smoker (<50)	T1N0M0	89	114
2	F	65	past smoker (<30)	T4N1M0	31	31
3	M	49	past smoker (>70)	T2N1M1	14	16

translocation. A platinum gemcitabine doublet was thus started. CT scan after three cycles showed disease progression with the appearance of a small nodule in the left lung and the coexistence of pathological mediastinal lymphnodes. Based on the mutational profile of both tumor and secondary lesion, erlotinib 150 mg/day was started at the beginning of 2007. The first CT control after three months of treatment revealed a slight reduction of malignant lesion size. A further reduction was documented after 6 months of therapy, in September 2007. Quite unexpectedly, the patient is since then showing a prolonged response with persistent disease control after 89 months of continued therapy, in absence of significant toxicities (mild anemia). Related CT scan images are reported in Fig. 1.

A 65-year-old former smoker Caucasian woman was diagnosed in 2012 with an ADC of left inferior lobe, associated with mediastinal lymphoadenopathy and pleural secondary lesions. Based on the detection of the L858R *EGFR* mutation, therapy with gefitinib was started. CT scan after six months of therapy showed a partial response with shrinkage of the tumor primary lesion, complete resolution of the pleural effusion, and stability of hilar nodes. After a multidisciplinary evaluation, the patient underwent surgical lobectomy. The histological examination of the surgical sample showed a fibroelastotic area corresponding to the lesion documented on CT, associated with diffuse interstitial and lymphatic spread of minute tumor aggregates in subpleural, perivascular and peribronchial areas. No evidence of interstitial lung disease was documented. Treatment with gefitinib was thus resumed and continued until now (months) in absence of clinically detectable disease recurrence. The last patient was a 49-year-old former smoker Caucasian, who was diagnosed in 2012 with stage IV lung ADC, metastatic to the brain (single lesion). The tumor carried a deletion of the exon 19 of the *EGFR* coding sequence. Whole brain radiotherapy (30 Gy) was started in association to gefitinib. CT scan after six months of therapy demonstrated a single lung nodule, in absence of brain and abdominal disease. After a multidisciplinary evaluation, lung tumor was resected. On histological examination, focal fibroelastosis was identified, and minute tumor aggregates were observed in the perivascular and peribronchial interstitium and lymphatics with a micronodular appearance. Interstitial lung disease was not observed. Based on these data, gefitinib was continued until disease progression 8 months after surgery. Notably, in both case 2 and 3 known molecular mechanisms of genetic resistance to EGFR inhibitors were tested on bioptic samples at diagnosis and on subsequent available surgical specimens: no *EGFR* T790M mutation were documented and tumors DNA harbored a wild type *KRAS* and *MET* coding sequences, no *EGFR* and *MET* gene amplifications were found as well. Case 2 and 3 histological and CT scan images are presented in Fig. 1.

The EGFR TKIs, gefitinib and erlotinib, act as reversible competitive inhibitors of the tyrosine kinase domain of EGFR that bind to its adenosine-5′ triphosphate-binding site. Somatic *EGFR* activating mutations in NSCLC have been associated with dramatic tumor responses and favorable clinical outcomes with these molecules. However, most patients who initially respond to gefitinib and erlotinib eventually become resistant and experience disease progression. We present here the case of a really continued response - nearly a complete disease remission - induced by erlotinib in an advanced *EGFR*-mutant lung ADC, which recurred after surgery and the failure of conventional chemotherapy. This is to our knowledge among the most impressive documented responses to EGFR TKIs in NSCLC, deserving some considerations. A major point concerns the therapeutic context in which such a prolonged treatment must be placed. It is known that maintenance therapy with erlotinib or gefitinib produces a significant increase of progression-free survival (PFS) and overall survival (OS) in NSCLC patients [4]. Nevertheless, it is conceivable that the persistently responsive phenotype observed here might be driven by a tumor and/or host genetic asset which cooperates in sustaining addiction [5] to the small TKI. In order to verify this hypothesis we checked the molecular profile of a panel of genes involved in the EGFR signaling pathway, which are known to play a major role in lung malignant transformation, and no mutation was documented. Altered expression of the *ERCC1* gene was not identified as well (data not shown). Another relevant issue is related to if and when TKI should be discontinued in responsive patients, mainly when, after prolonged treatment, imaging data continue to be negative for disease evidence. In other words, in such unexpected setting (as in the first case reported) is it allowed to stop anti EGFR therapy? In order to answer to this question, we present two other cases which are not relevant for the duration of response to small EGFR inhibitors, but really because for both of them large surgical specimens of tumors exposed to TKIs are available for histo-patological

Table 2
Molecular profile of the analyzed cases. For case 2 and 3, in green data red data obtained on biopsy at diagnosis and confirmed on subsequent surgical specimens; in blue data evaluated in only surgical specimen to analyze the status of transducers involved in acquired resistance to anti EGFR agents.

ID CASE	EGFR		HER2		KRAS	PIK3CA	BRAF	MET		EML/ALK
	mutation	amplification	mutation	amplification	ex 2	ex 9-20	ex 15	mutation	amplification	translocation
1	del ex 19	euploid	wt	euploid	wt	wt	wt			no
2	L858R	euploid			wt			wt	euploid	
3	del ex 19	euploid			wt			wt	euploid	

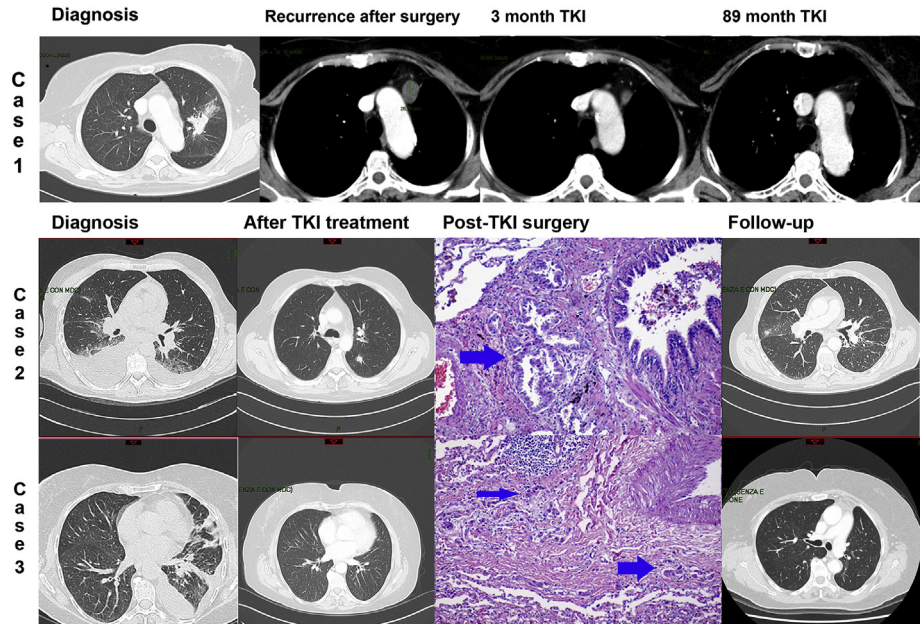

Fig. 1. Patient 1 CT scans obtained at the time of first diagnosis, at tumor recurrence after surgery, after the first 6 months of TKI therapy, documenting a reduction of the lesion size, and at 89 months follow-up, showing persistent response to TKI. Patient 2 and 3 CT scan at diagnosis and after TKI treatment, showing almost complete response; electron micrographs of the resected lung specimen, with interstitial infiltration and microembolic diffusion of tumor cells (arrow), in the absence of an obvious tumor mass, in both cases (hematoxylin and eosin, 20x); follow-up CT scan, showing tumor recurrence in patient 2, 13 months after diagnosis, and absence of disease in patient 3, 19 months after diagnosis.

analysis. Thus, the other two cases reported provide helpful insights on how TKIs behave at the microscopic level. Indeed, while it is well documented that genetic variation in *EGFR* pathway genes may confer susceptibility to interstitial lung diseases (ILDs) [6,7] very few morphological data are available on the tumor histological response to small EGFR inhibitors. We observed in both cases the disappearance of the initial tumor mass, however associated with the diffuse persistence of tumor cell aggregates in the lymphatic vessel. This observation has so far not been reported in the few studies analyzing post-TKI lung resections [8–10], most of which focused on early tumor response to neoadjuvant treatment. The subclinical persistence of interstitial and endolymphatic tumor cells after prolonged TKI treatment might explain the common observation of tumor relapse after TKI discontinuation, and sustain the decision to continue treatment in responsive patients as we did in our first case. Clearly, further investigations are required to define the molecular profile of these persistently responsive patients and to distinguish them from the vast majority of those that develop acquired resistance.

Written informed consent was obtained from the patient for publication of this Case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

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