



Lung cancer epidermal growth factor receptor mutations and radiotherapy response: A multicentre clinical study

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ABSTRACT

Purpose: To examine the impact of epidermal growth factor receptor (EGFR) mutations on objective response to palliative lung radiotherapy in patients with metastatic non-small cell lung cancer (NSCLC).

Materials and Methods: A multicentre retrospective study was conducted of patients with metastatic NSCLC diagnosed between March 2010 and June 2012 who received palliative radiotherapy to the chest. Patients included for study had baseline imaging and follow-up imaging 1–3 months after radiotherapy. The primary endpoint was 1–3 month local objective imaging response by the Response Evaluation Criteria in Solid Tumours (RECIST). Patients were divided into EGFR mutation positive (EGFR+) and EGFR wild type (WT) cohorts for analysis.

Results: There were 121 patients for study inclusion: 89 (74%) were EGFR WT and 32 (26%) were EGFR+. The response rate between EGFR WT and EGFR+ cohorts was not significantly different (49 vs. 63%, $p = 0.21$). On multivariate analysis, initiation of a tyrosine kinase inhibitor (TKI) after radiotherapy was associated with a higher rate of response (OR: 5.07, 95%CI: 1.08–23.69, $p = 0.039$) but EGFR mutation status was not. For the EGFR+ cohort, patients with disease progression after initial management on a TKI had a worse response rate compared to patients who were TKI-naïve before starting radiotherapy (30 vs. 77%, $p = 0.018$). Local control was not statistically different between the EGFR cohorts.

Conclusion: The EGFR mutation status alone was not an independent predictor of objective radiographic response to palliative thoracic radiotherapy. Acquired resistance to TKI therapy may be associated with disease cross-resistance to palliative radiotherapy.

Introduction

The epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase that is involved in signal transduction, regulation of DNA synthesis, and cell proliferation. Mutations in the EGFR gene can lead to over-expression of the tyrosine kinase and result in carcinogenesis [1]. Exon 19 deletions and exon 21 mutations account for the large majority of known EGFR mutations. Lung cancers carrying these mutations can be targeted by therapeutic agents such as EGFR tyrosine kinase inhibitors (TKIs).

The effect of EGFR mutations on clinical response to radiation is not well known. Most data comes from laboratory studies examining clonogenic survival in transfected cell lines in response to ionizing radiation [2]. Several pre-clinical studies have suggested a link between EGFR expression level and cellular radioresistance, while EGFR inhibition

enhances radiosensitivity [3–6]. More specifically, there is laboratory evidence asserting that the degree of radioresistance correlates positively with the magnitude of EGFR over-expression [7].

The purpose of this study was to assess the impact of EGFR mutation status on radiotherapy response in a clinical setting. At our institution, EGFR mutation testing is approved for patients with advanced (Stage IIIB or IV) non-small cell lung cancer (NSCLC). With institutional ethics approval, we conducted a retrospective review of NSCLC patients who had palliative radiotherapy to the chest and examined radiographic outcomes.

Materials and methods

Using a Provincial cancer registry, all patients with metastatic (stage IV) non-squamous, non-neuroendocrine, non-small cell lung carcinoma

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diagnosed between March 2010 and June 2012 were identified. The criteria for study inclusion were: measurable disease in the lung (lung parenchyma, luminal airway, pleura, or radiographically enlarged thoracic lymph nodes) treated with palliative radiation, EGFR mutation testing, baseline imaging, and follow-up imaging 1–3 months after radiotherapy completion. Patients with lung collapse or large pleural effusions, which obscure tumour measurement at the time of treatment, were excluded.

The patient's medical records were reviewed for baseline variables including age, sex, performance status, smoking history, and Asian ethnicity. Radiotherapy was delivered at one of six treatment centres in British Columbia, Canada. Treatment characteristics such as radiotherapy dose-fractionation, chemotherapy use, and molecular-targeted therapy use were also included. As per institutional practice, systemic therapy was held during the palliative radiation course.

The primary endpoint was local objective response by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 at 1–3 months after radiotherapy completion. The 1–3 month follow-up time was chosen because it corresponds with the median time-to-partial response for radiotherapy reported by Imai et al. [8] and because of the short life expectancy in this palliative population. Where patients had multiple chest imaging, the imaging closest to 6–8 weeks post-radiation was used for response measurements. For the purpose of this study, patients were categorized as having a “response” if it was partial or complete according to RECIST. Patients were categorized as having “no response” if they had stable or progressive disease on imaging. Computed tomography (CT) imaging was preferred, but chest x-ray was acceptable as long as the same modality was used at baseline and at follow-up for comparison. A single oncologist measuring objective response was blinded to the patient's EGFR mutation status and treatment history. The secondary endpoint was local control (LC).

Patients were divided into EGFR mutation positive (EGFR +) and EGFR wild type (WT) cohorts for analysis. Baseline characteristics between the two cohorts were compared using *T*-test, Mann-Whitney *U* test, and Chi squared tests. The primary outcome of objective response was evaluated with a multivariate logistic model, using a backwards stepwise selection process. A *p*-value of > 0.10 was used for elimination from the model, while a *p*-value of ≤ 0.5 was used for inclusion in the final model. As EGFR status was the primary variable of interest, it was included in the final model, regardless of significance level. All reported *p*-values are two-sided, with a *p*-value < 0.05 set as the level of significance. Local control was measured from the date of radiotherapy completion to radiographic evidence of local disease progression. Cumulative incidence curves for time-to-recurrence were estimated for each EGFR cohort using the competing risks method. This method of analysis has significant advantages in providing a better estimation of local failure time when there are high death rates from metastatic disease [9,10]. Patient death and initiation of a new systemic therapy were considered competing risk events in this analysis. Differences in the cumulative incidence curves between EGFR cohorts were assessed using Gray's test.

Mutation analysis was conducted at a central laboratory through extraction of the genomic DNA from the submitted specimen. Analysis of EGFR exon 19 (in-frame deletion) and exon 21 (point mutation) was performed by polymerase chain reaction (PCR) using gene specific PCR amplification primers followed by fragment size analysis, and is previously described by Pan et al. [11].

Results

For the period of study, there were 264 patients diagnosed with stage IV NSCLC who had EGFR mutation testing and received palliative radiation to the lung. Of these, 121 patients were eligible for study inclusion. Three patients with anaplastic lymphoma kinase mutations were excluded from this analysis. There were 140 patients excluded due to the lack of follow-up imaging (114 EGFR WT, 26 EGFR +). Of 140

excluded patients, 72 patients had died within 3 months of radiotherapy (64 EGFR WT, 8 EGFR +) and 68 patients were alive at 3 months but did not have follow-up imaging (50 EGFR WT, 18 EGFR +).

For 121 study patients, 89 (74%) were EGFR WT and 32 (26%) were EGFR+ (exon 19 = 19, exon 21 = 13). The median age was 64 years. The EGFR + cohort was different for smoking history, Asian ethnicity and use of systemic therapy. The patient and treatment characteristics by EGFR mutation status are presented in Table 1. The palliative radiotherapy dose was no different between the EGFR cohorts (median = 20 Gy, *p* = 0.61, range 8–50 Gy). The most common dose-fractionation was 20 Gy in 5 fractions (in 62 patients) and 30 Gy in 10 fractions (in 31 patients). The TKIs used were Gefitinib in 32, Erlotinib in 15, and Afatinib in 2 patients.

The median time of imaging follow-up for response evaluation was 1.70 months (EGFR WT = 1.67 vs. EGFR+ = 1.74 months, *p* = 0.40). Treatment responses, according to the RECIST classification, are presented in Table 2. The overall response rate between EGFR WT and EGFR + cohorts were not significantly different (49 vs. 63%, *p* = 0.21). On univariate analysis, the use of chemotherapy before or after radiation, smoking status, and use of TKI prior to radiotherapy were not significant factors associated with response. On multivariate logistic analysis, EGFR mutation status was not a significant factor for radiotherapy response (*p* = 0.79). On the other hand, administration of a new TKI after radiotherapy was significantly associated with response on multivariate analysis (OR: 5.07, 95%CI: 1.08–23.69, *p* = 0.039). There was a trend toward higher dose-fractionation, as measured by equivalent dose in 2 Gy fractions (EQD2), and response but this finding was not statistically significant (OR:1.05, 95%CI: 0.99–1.12, *p* = 0.083). Cumulative incidence curves for local progression for each EGFR cohort are presented in Fig. 1. There was no difference in the cumulative incidence of local progression at 6 months between the EGFR WT and EGFR + cohorts (56.5 vs. 37.5% respectively, *p* = 0.60). In an exploratory subgroup analysis of the EGFR + cohort, we compared patients with disease progression after initial benefit from TKI (*n* = 10) to patients with no history of TKI administration before starting radiotherapy (*n* = 22). For patients who had received a TKI prior to radiotherapy, the median duration of TKI use was 20.1 months (range, 7.7–68.0 months), and the rate of response was worse when compared to patients who were TKI-naïve (30 vs. 77%, *p* = 0.018).

Table 1
Patient and treatment characteristics.

	All n = 121 No. (%)	By EGFR mutation status		<i>p</i>
		EGFR WT n = 89 No. (%)	EGFR+ n = 32 No. (%)	
Median age (years)	64	64	63	0.79
ECOG PS (median)	1	2	1	0.002
Gender				
Female	68 (56)	53 (60)	15 (47)	0.22
Male	53 (44)	36 (40)	17 (53)	
Smoking history				
Never	27 (22)	9 (10)	18 (56)	<0.001
Former	60 (50)	49 (55)	11 (34)	
Current	34 (28)	31 (35)	3 (9)	
Asian ethnicity	23 (19)	8 (9)	15 (47)	<0.001
Systemic treatment before RT				
Chemotherapy	18 (15)	12 (13)	6 (19)	0.48
TKI	14 (12)	4 (4)	10 (31)	<0.001
Systemic treatment after RT*				
Chemotherapy	20 (17)	19 (21)	1 (3)	0.017
TKI	16 (13)	1 (1)	15 (47)	<0.001

EGFR, epidermal growth factor receptor; WT, wild type; +, mutation positive; ECOG PS, Eastern Cooperative Oncology Group performance status; TKI, tyrosine kinase inhibitor; RT, radiotherapy.

* Refers to initiation of a new systemic therapy, not previously administered, after palliative radiation and prior to the follow-up imaging assessment.

Table 2
Treatment response following palliative thoracic radiation.

	All n = 121 No. (%)	By EGFR mutation status	
		EGFR WT n = 89 No. (%)	EGFR+ n = 32 No. (%)
Complete response	1 (1)	1 (1)	0 (0)
Partial response	63 (52)	43 (48)	20 (63)
Stable disease	52 (43)	41 (46)	11 (34)
Progressive disease	5 (4)	4 (4)	1 (3)
Response rate*	53%	49%	63%

EGFR, epidermal growth factor receptor; WT, wild type; +, mutation positive.
* Complete response + Partial response.

Discussion

To our knowledge, this is the first study to evaluate the significance of NSCLC EGFR mutation status on response to palliative thoracic radiotherapy using objective measures. We did not find that EGFR mutation status predicted for radiotherapy response. However, initiation of a TKI in patients with an EGFR mutation was associated with a significantly better rate of response than radiotherapy alone. This underscores the importance of TKI utilization for local disease management even in patients who receive palliative radiotherapy.

Other retrospective studies report better clinical outcomes with radiotherapy for patients with EGFR driver mutations but to a varying degree. Tanaka et al. [12] reported on 104 patients with locally advanced lung adenocarcinoma who were treated with concurrent chemoradiation and found no difference in overall response rate between EGFR WT and EGFR + patients. While LC was better in patients with an EGFR mutation, EGFR + patients had higher rates of distant metastases and worse progression free survival. Similarly, Yagishita et al. [13] found no significant difference in rates of response based on EGFR mutation status. However, both LC and progression free survival were better in EGFR+ patients. The latter, in contrast to Tanaka et al., is likely because a majority of patients with EGFR mutations in this study subsequently received a TKI. In a comparative outcome analysis of patients with locally advanced NSCLC treated with chemoradiation, Lim

et al. [14] reported that the overall response rate and LC were higher in the EGFR+ group compared with EGFR WT. Finally, Mak et al. [15] studied 123 patients with locally advanced NSCLC, reporting better LC and overall survival in EGFR+ patients. In this study distant recurrence and relapse free survival were no different.

The better clinical response to radiotherapy in EGFR+ patients observed in these studies are discrepant with laboratory studies reporting that EGFR over-expression confers radioresistance [3–7]. One explanation for the more favorable finding in clinical studies could be from the concurrent use of systemic therapy and its radiosensitizing and anti-tumor effects, which may be enhanced in EGFR mutants [4,16]. In our study, none of the patients received concurrent systemic therapy, as chemotherapy or TKIs were stopped during the radiotherapy course. We found that better outcomes were attributed to treatment with TKI in patients with an EGFR mutation rather than the presence of an EGFR mutation alone. Further, we did not find a significant difference in LC between our EGFR cohorts, possibly because of the high rate of subsequent treatment with a new systemic therapy and high death rates in this palliative population which were considered as competing risk events in our analysis. Another explanation is that some EGFR cell lines do not demonstrate enhanced radioresistance. Das et al. [17] reported that clonogenic survival of mutant EGFR NSCLC in response to radiation was reduced compared to EGFR WT, indicating a radiosensitive biology for EGFR mutants in contrast to other laboratory studies. Possible mechanisms for radiosensitivity include delayed DNA repair kinetics, defective radiation induced arrest in DNA synthesis or mitosis, and pronounced increases in apoptosis [17].

Little is known about how EGFR + NSCLC with acquired resistance to TKIs respond to radiotherapy. In a study of 47 patients, Hirata et al. [18] examined the response rate for brain metastases following radiotherapy for patients who were EGFR + TKI-resistant, EGFR + TKI-naïve, and EGFR WT. They reported that EGFR + TKI-resistance was associated with a low efficacy of brain radiotherapy and was an independent predictor of worse survival. Using the same definitions for TKI-resistance (disease progression after initial benefit from TKI) and for TKI-naïve (no history of TKI administration before starting radiotherapy) our study also found worse response rates in EGFR+ patients with progressive disease after TKI use. Our data supports the assertion that acquired

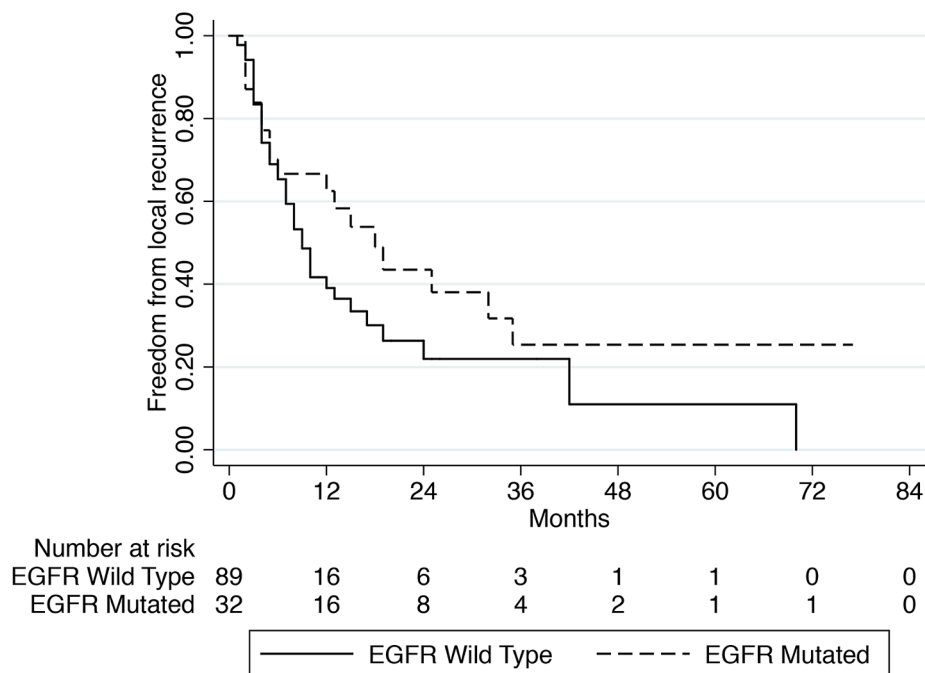


Fig. 1. Cumulative incidence of local progression. The cumulative incidence was estimated using a competing risk model, with death and new systemic therapy intervention as competing risk events.

resistance to EGFR TKIs are associated with cross-resistance to radiotherapy [18]. However, given the small patient numbers in our sub-analysis, our data should be interpreted with caution and larger studies are needed. A possible mechanism of cross-resistance was put forth by Huang et al. [19], who found a loss of p53 in TKI-resistant cells and anti-EGFR antibody-resistant cells, suggesting a central role of p53 in regulating acquired resistance through regulation of cell-cycle arrest, apoptosis, and DNA damage repair.

As with all retrospective analyses, interpretation of these results is limited by bias. The study size was relatively small and the indications for palliative radiotherapy were diverse, while recognizing that many patients will have symptom relief in the absence of a radiologic response. There were a greater proportion of EGFR WT patients who were excluded from this study due to the lack of follow-up imaging from early death. These patients may have had more aggressive disease behaviour or worse treatment response, which could have resulted in an over-estimation of the response rate for the EGFR WT cohort. Other limitations of our study include the small size of some subgroups. The relative infrequency of the EGFR mutation in our NSCLC population likely limited our ability to detect small differences in response rates between EGFR cohorts. Our study size was comparable or larger to other EGFR mutation studies in locally advanced lung cancer treated with chemoradiation [12,14,15]. Larger randomized controlled studies are necessary to corroborate these findings. Many of the patients in this study had several lines of systemic therapy before and after radiotherapy, which could have affected the results. To account for this, we used a competing risk analysis, where death and initiation of a new systemic therapy were competing risk events. Our data represents the real-world situation where patients often undergo multiple lines of therapy throughout their course of disease. Finally, the radiotherapy dose was not uniform in this study, but we did not find a correlation between EGFR mutation status and radiotherapy dose prescribed.

With a better knowledge of how genetic mutations affect disease behaviour in NSCLC, we may be able to better anticipate disease response and better sequence treatment modalities. Our study adds to the current literature that the EGFR pathway may serve as an important mediator of radiation resistance. However, it is likely that radiation response and EGFR signaling are influenced by many factors, such as the application of systemic therapies, so future prospective studies are needed.

Conclusions

EGFR mutation status alone is not an independent predictor of objective radiographic response to palliative thoracic radiotherapy in patients with NSCLC. Administration of an EGFR TKI in patients with an EGFR mutation remains important in achieving a better response even in patients who receive palliative radiotherapy. For patients with progressive disease after initial benefits from a TKI, further studies are needed to assess the degree of cross-resistance with radiotherapy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The first and second author contributed equally to this manuscript.

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