Epidermal Growth Factor Receptor (EGFR) Mutation Testing, From Bench to Practice: A Single Institute Experience

Abstract:

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Abstract

Epidermal growth factor receptor (EGFR) gene mutations determine the treatment and prognosis in lung adenocarcinoma. Exon 19 and exon 21 (L858R) deletions represent the most common recognised mutations detected. To date, no figures regarding the prevalence of EGFR mutations in the Irish population have been published. The prevalence of EGFR mutations was retrospectively analysed for all patient samples tested since the introduction of EGFR testing routinely (Mar to Dec 2012) in a single Irish institute. The presence of 41 known treatment linked EGFR mutations in exons 18, 19, 20 and 21 of the EGFR gene was tested in 289 Irish patients. Resection, core biopsy or FNA samples were analysed using a commercially available CE-IVD marked multiplex real-time PCR assay. Samples were included from patients of curative and palliative treatment intent likely to harbour an EGFR mutation.

Introduction

Non-small cell lung cancer (NSCLC) is a heterogeneous disease with vast genomic diversity. Half of all NSCLC tumours harbour somatic mutations in genes like EGFR, HER2, KRAS and BRAF, regardless of smoking status or histological subtype 1,2. Molecular profiling of tumours is of increasing importance in lung cancer as it plays a critical role in treatment decisions. Recent advances in molecular diagnostics have made tumour profiling a reality in clinical practise. Clinically relevant molecular subtypes of lung adenocarcinoma include tumours that harbour activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR). EGFR is a receptor tyrosine kinase receptor which belongs to the EGFR family, which consists of four members: EGFR, ERBB2 (also known as HER2), ERBB3, and ERBB4. Under physiological conditions, EGFR is activated by binding to one of its ligands (like epidermal growth factor). Activated EGFR in turn activates downstream intracellular pathways leading to cellular survival and proliferation. Mutant EGFR in lung cancer is constitutively active, which causes uncontrolled growth and evasion of cell death. The most common mutations of EGFR in NSCLC are exon 19 (L858R) point mutations and exon 19 deletions accounting together for more than 85% of EGFR mutations in the disease 1,2. The prevalence of EGFR mutations in a tumour predicts response to oral EGFR tyrosine kinase inhibitors (EGFR-TKIs) like gefitinib and erlotinib 3,4.

The prevalence of EGFR mutations in Asian patients with lung adenocarcinoma is approximately 40% compared to 15% in Caucasian patients. The prevalence of EGFR mutations has not been previously reported in an Irish population. Here, we present the findings of screening for EGFR mutations in lung adenocarcinoma patients treated in the largest lung cancer service in Ireland. At the end of our report we provide a summary of recent advances and the role of first line TKIs in the treatment of advanced NSCLC.

Methods

The prevalence of EGFR mutations was retrospectively analysed for all patient samples tested since the introduction of EGFR testing as a routine service, (Mar to Dec 2012), in a single Irish institute. Formalin fixed paraffin embedded tissue from resection, core biopsy or FNA samples was analysed for 289 Irish patients using the CE-IVD marked Roche Cobas 4800 mutation detection assay (Roche Diagnostics Limited, UK). The Roche Cobas 4800 assay uses a system of three multiplex real-time PCR reactions for the simultaneous detection of 41 treatment linked mutations in exons 18, 19, 20 and 21 of the EGFR gene. Samples were included from patients of curative and palliative treatment intent.

Results

During the study period 209 patients were tested for an EGFR mutation. Of those tested 51.2% were male (n=107) and 48.8% (n=102) female. The mean age at testing was 67 years. Patients tested had either stage IV disease (88%, n=180) or stage IIIb disease (12%, n=25). Of the study cohort, 29 patients had an EGFR mutation (13.8%, 95% CI 8.4-17.6%). The number of mutations detected 30 in 29 patients. Among the mutations detected relative frequency was as follows: exon 19 mutation disease and if their clinical status allows them to undergo a second biopsy. Our data shows that the frequency of EGFR mutations was higher in females than in males; 63.3% of those with an EGFR mutation were female (n=19, 95% CI 1.2-25.5%) and exon 18 (p.G719X) and exon 20 (p.T790M) both at 3.3% (n=1, 95% CI 0-9.8%) (Figure 1, Table 1). Clinical characteristics of these patients are outlined in Table 1. One patient had two EGFR mutations making the total number of mutations detected 31.

The incidence of mutations was higher in females than in males; 63.3% of those with an EGFR mutation were female (n=19, 95% CI 46.1-80.6%). Reciprocally, 36.7% of the EGFR mutations were detected in males (n=11, 95% CI 19.4-53.9). All samples tested were from a fixed paraffin embedded tissue or cell preparations. Turn around time (TAT) for EGFR mutation processing substantially improved over ten month period, from over five weeks to less than four working days (Figure 2).

Discussion

Lung cancer is the most common cause of cancer related death in Ireland. It is estimated that lung cancer rates will continue to increase over the next 10 years, especially in females due to rising numbers of smoking females. Prior to the discovery of EGFR mutations, platinum based chemotherapy was the standard of care in metastatic NSCLC, with modest improvements in overall survival, but at the expense of significant toxicities. Treatment of lung cancer continues to be a major health concern, requiring more research and novel therapeutic approaches.

Clinical features that suggest the presence of an EGFR mutation include adenocarcinoma histology, Asian ethnicity, female sex, never and light smokers. Our findings are by large consistent with these clinical features. Of the patients tested, 29 patients (12%) were females. We found that 62% (n=18) of patients with mutant EGFR never smoked. Although clinical criteria may be useful in patients selection for screening, international guidelines recommend routine screening for EGFR mutations in all metastatic lung adenocarcinomas, as this will have important therapeutic implications. EGFR-based targeted therapy is recommended in patients harbouring EGFR activating mutations. We propose to screen all patients with metastatic lung adenocarcinoma for EGFR activating mutations regardless of their clinical characteristics. However, in some cases screening is not possible due to the small volume of diagnostic samples, in these instances we recommend repeating the biopsy only if patients clinical features are suggestive of an EGFR mutation disease and if their clinical status allows them to undergo a second biopsy. Our data shows that the frequency of EGFR mutations in patients with metastatic lung adenocarcinoma treated in our centre is 13.8%. This rate is comparable to the rate of 13% reported in other Caucasian populations. There are no reported studies of the prevalence of EGFR mutations in an Irish population. One potential source of bias in our study is the fact that data were collected from a single institute. The number of samples tested in our centre between August 2012 and November 2012 was...
The role of TKIs is not limited to EGFR mutant NSCLC. Two phase III clinical trials have demonstrated the superiority of TKIs over best supportive care in patients with heavily pre-treated metastatic wild-type EGFR NSCLC. The success of TKIs in producing clinically significant results in patients with mutant EGFR lung cancer has generated an accelerated interest in identifying additional molecular targets. ALK gene rearrangements in lung cancer were discovered in 2007. Subsequently, crizotinib, an ALK inhibitor, received accelerated approval by the US Food and Drug Administration. This in part can be explained by the lower toxicity profile of EGFR TKIs, with the commonest side effect being acne-like skin rash. The rates of grade 3 or 4 neutropenia were as low as 0.54%. It is important to note that no treatment has yet been approved for resistant disease.

In conclusion, we have shown the results of screening for EGFR mutations in patients with lung adenocarcinoma in a tertiary institute in Ireland. Our practice, in accordance with international guidelines, is to screen all patients with metastatic lung adenocarcinoma for EGFR mutations. We found that 13.8% of patients with metastatic lung adenocarcinoma treated at our centre have an EGFR activating mutation. A rate that is similar to that reported in international trials in Caucasian patients. NSCLC patients with EGFR mutations represent only a small percentage of patients with metastatic lung adenocarcinoma. Therefore, it is important to screen all patients with metastatic lung adenocarcinoma for EGFR mutations.

References


