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 209 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 that belongs to the EGFR family, which consists of four members: EGFR, ERBB2 (also known as HER2), ERBB3, and ERBB4[3]. 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[3]. The most common mutations of EGFR in NSCLC are exon 21 (L858R) point mutations and exon 19 deletions accounting together for more than 85% of EGFR mutations in the disease[3,4]. The presence of EGFR mutations in a tumour predicts response to oral EGFR tyrosine kinase inhibitors (EGFR-TKIs) like gefitinib and erlotinib[4].

The prevalence of EGFR mutations in Asian patients with lung adenocarcinoma is approximately 40% compared to 15% in Caucasian patients[5]. 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 209 Irish patients using the CE-IVD marked Roche Cobasfi 4800 EGFR mutation detection assay (Roche Diagnostics Limited, UK). The Roche Cobasfi 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%). Clinical characteristics of these patients are outlined in Table 1. One patient had two EGFR mutations making the total number of mutations detected 30 in 29 patients. Among the mutations detected relative frequency was as follows: exon 19 deletion 63.3% (n=19, 95% CI 46.1-80.6%), exon 21 (p.L858R) 16.7% (n=5, 95% CI 3.3-30.0%), exon 20 insertions 13.3% (n=4, 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 2).

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 formalin 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[6]. 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[6]. 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[1,5]. Our findings are by large consistent with these clinical features. Of the patients who harbour an EGFR mutation, 19 (65.5%) 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. Routine screening is not recommended in pure squamous histology[7,8]. In our institute we do 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 patientâ s 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 15% reported in other Caucasian populations[5]. 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

in the range of 35 to 39 samples per month. Prior to August 2012 we tested less than 20 samples per month. That does not reflect implementation of selection criteria as much as it reflects that the service became well established and fully funded only after August 2012 in our centre.

Presence of an EGFR mutation predicts response to tyrosine kinase inhibitors (TKIs). There are two classes of oral EGFR TKIs in clinical use; reversible EGFR TKIs like geftinib and erlotinib, and irreversible TKIs like afatinib. Six phase III clinical trials have compared first line platinum based chemotherapy with gefitinib or erlotinib in patients with EGFR mutant lung adenocarcinoma[9-14]. All these trials have consistently demonstrated superiority of EGFR TKIs in achieving longer progression free survival and greater response rates. In the four trials where mature overall survival data is available, there was no significant difference between EGFR TKIs and chemotherapy[9-12]. This is likely because cross over was allowed between the control arm and the experimental arm. Quality of life which is crucial in the palliative setting was assessed in four of these trials using various assessment tools, with results showing that patients who received EGFR TKIs had a better quality of life than patients who received platinum based chemotherapy[9-11,13]. 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%[15]. Based on this compelling evidence we treat all patients with mutant EGFR stage IV lung adenocarcinoma with a TKI. The role of oral TKIs in mutant EGFR NSCLC in the neoadjuvant and adjuvant settings is currently under investigation in a number of clinical trials[16-19].

Similarly, phase III data on the irreversible TKI, afatinib, has shown a longer progression free survival in an EGFR mutant population when compared to standard chemotherapy[20]. A phase IIb trial comparing first line afatinib to gefitinib in patients with mutant EGFR lung adenocarcinoma is recruiting patients at present. Another phase III trial comparing first line afatinib to erlotinib is also in the process of recruiting patients[21]. Lapatinib, a HER2/EGFR TKI, used commonly in the treatment of breast cancer, and dacomitinib, a pan HER TKI, are currently being evaluated in phase III clinical trials[22]. Unfortunately, 30% of patients with EGFR mutant lung adenocarcinoma do not respond to TKIs, and the vast majority of those who do respond eventually go on to develop resistance[23]. Several mechanisms have been described to illustrate this phenomenon. Second-site EGFR mutations is the most common resistance mechanism, with T790M mutation being the most reported. The T790M mutation blocks the binding of TKIs to the ATP pocket in the EGFR kinase domain. Activating downstream mutations in the EGFR pathway such as PIK3CA, BRAF and ERK, are also known to cause resistance to TKIs. Met overexpression has been reported in up to 10% of resistant cases.

Finally, 5% of patients show histological transformation to small cell lung cancer with resultant resistance to TKIs. Several drugs have been developed to overcome this resistance. Second generation TKIs like neratinib and pelitinib are being tested in phase II clinical trials[23]. It is important to note that no treatment has yet been approved for resistant disease.

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[5,8]. ISEL and BR.21 showed that treatment with a TKI led to an increase in the progression free survival and a better quality of life. A survival benefit was shown in BR.21 but not ISEL. However, the data available is less clear when it comes to comparing TKIs with chemotherapy. INTEREST and TITAN are two trials that compared gefitinib and erlotinib, respectively, with conventional chemotherapy in patients with metastatic, EGFR wild-type NSCLC, who were previously treated with platinum-based chemotherapy. The authors of both trials reported that TKIs were not inferior to conventional chemotherapy. In contrast to these findings, the TAILOR study found that conventional chemotherapy produced a better response rate and a longer progression free survival when compared to erlotinib in a similar patient population. Finally, the SATURN trial examined the benefit of maintenance TKIs following treatment with platinum based chemotherapy in patients with EGFR wild-type NSCLC. Results showed a prolongation in the progression free survival[8]. 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 (FDA) in 2012 for ALK positive patients[24]. Insertion mutations in HER2 are seen in 2% to 4% of NSCLC patients. Studies are ongoing to assess the role of TKIs (afatinib and neratinib) in the treatment of this subtype[25].

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 lung adenocarcinomas overall. The war on lung cancer is far from over. Basing treatment options on the molecular profile of individual tumours, in other words personalized medicine, is an invaluable weapon in this war.

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