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EGFR-tyrosine kinase mutations in non-small cell lung cancer-based responsiveness to anti-cancer therapy

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ABSTRACT

Lung cancer based on physiological appearance and areas of the tissues affected are classified as small cell lung cancer and non-small cell lung cancer. Different types of tumours arise due to varied genetic mutations involved in tumour progression which is specific for cancer subtypes. Non-small cell lung cancer exhibits mutations in the tyrosine kinase domain of epidermal growth factor receptor. Hence, tyrosine kinase inhibitors are used to suppress tumour progression. Due to variation in mutation, responsiveness to drug therapy efficacy is different in different individuals and hence leads to poor prognosis of cancer. This review aims to summarize the EGFR mutations involved in the progression of non-small cell lung cancer and tyrosine kinase inhibitor drugs approved for the treatment.

Keywords: Epidermal Growth Factor Receptor, Tyrosine kinase inhibitors, NSCLC, EAIO45.

1. INTRODUCTION

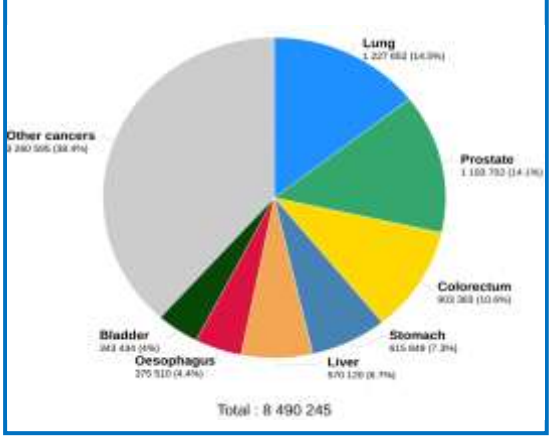
Lung cancer is one of the most common among deadly cancer with approx. 14.5% new cases in males and 8.1% new cases in females with an estimated death cause in approx. 22.2% in males and 13.8% in females [1] [Fig 1A,1B, 2A, 2B]. Due to poor prognosis of lung cancer, around 50% of people diagnosed with lung cancer die within 1 year and 18% among them have 5-year survival rate after diagnosis [2]. One of the major factors in lung progression is smoking and causes among 80% of death worldwide. However, 50% of women with lung cancer and 15-20% of men with lung cancer worldwide are non-smokers [3]. Non-smokers are mainly affected by non-small cell lung cancer [3]. This indicates that lung cancer is also common in non-smokers with genetic history involving tumour development [4]. Based upon the physical appearance of the tumour growth as well as areas been affected by uncontrolled tumour growth, it is further classified into two categories namely, small cell lung cancer and non-small cell lung cancer [5]. Due to heterogeneity in types of lung cancer, various types of mutations occurring among different individuals, generalized treatment, it leads to poor prognosis leading to poor

survival rate. However, mutation-based specific treatment has increased the prognosis rate [6].

ErbB family of tyrosine kinase receptors consist of four different receptors namely, EGFR/HER 1, HER2, HER3 and HER4 [7]. These receptors comprise of an extracellular domain which binds to ligands, hydrophobic transmembrane domain and intracellular cytoplasmic domain possessing tyrosine kinase which are highly conserved as compared to extracellular domain which is lesser conserved [8]. EGFR receptor is activated by binding to ligands such as Transforming Growth Factor (TGF-α) to its extracellular domain which forms homodimer and heterodimer of receptor leading to activation of tyrosine kinase present in intracellular domain of receptor [8-9]. This activation causes phosphorylation of tyrosine residues present in cytoplasmic tail of receptor and provides docking sites for cytoplasmic proteins such as Src homology-2 and phosphotyrosine binding proteins [10]. Binding of these proteins to phosphorylated tyrosine residues triggers activation of intracellular pathways such as Ras/Raf/mitogen activated protein kinase pathway, Phosphatidylinositol 3-kinase /Akt pathway, Phospholipase Cγ, Signal transducers and activators of transcription pathway and Src kinase pathways [11]. These proteins modulate functions of cell such as cell migration, cell adhesion and cell proliferation. The epidermal growth factor receptor protein consists of 486 amino acids, 170 kDa along with having tyrosine kinase domain and binds with an epidermal growth factor [12]. It has been reported that lung cancer is initiated by gene mutation namely Epidermal growth factor receptor which possesses a tyrosine kinase domain [13].

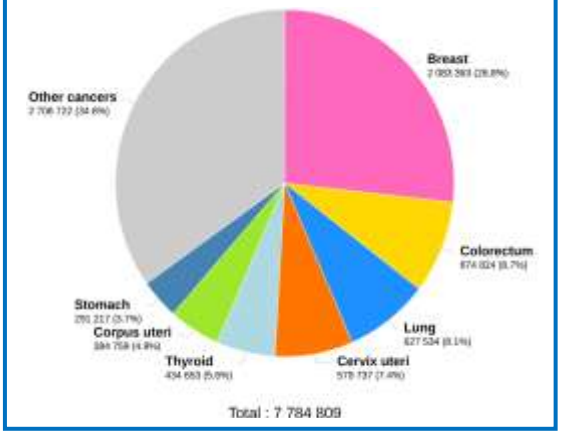
This mutation is mostly observed in patients harbouring non-small cell lung cancer [14-16]. Due to the findings that EGFR mutation leads to lung cancer progression; it is possible to treat these

Figure 1A: Estimated number of new cases in 2020, worldwide, males, ages 10-79 [59]



patients with inhibitors of tyrosine kinase as previously discussed that EGFR gene possesses tyrosine kinase domain. It has also been reported that patients treated with drugs which are inhibitors of tyrosine kinase, tumour regression was found to be highly efficient and good survival rate [17] However, not all non-small cell lung cancer patients were effectively treated by the common drug as compared to some of the patients. This finding led to further investigation by the screening of gene sequence of the patients wherein it has been reported that there are many different genes involved in EGFR signaling mechanisms in the progression of lung cancer [18]. These genes belong to the EGFR family and possess tyrosine kinase domain. The most frequent mutation

Figure 1B: Estimated number of new cases in 2020, worldwide, females, ages 10-79. [59]



in the tyrosine kinase domain is the deletion mutation in Exon 19 and point mutation in exon 21 of NSCLC patients [19-21]. These mutations lead to activation in the tyrosine kinase domain of Epidermal growth factor receptor which eventually destabilizes the conformation of tyrosine kinase which is originally maintained in the absence of ligand [21]. Hence, accordingly, these findings led to advancement in drugs which are specific for specific mutation as mutation increases sensitivity towards inhibitor drugs such as gefitinib, which was the first selective EGFR inhibitor drug approved by FDA as third-line therapy when platinum and docetaxel chemotherapy fails to treat local and metastatic NSCLC's [22]. However, in July 2015, FDA approved gefitinib drug as first-line therapy in patients harbouring EGFR mutation [23]. Hence, any patient suffering from non-small cell lung cancer needs to be first screened for a genetic mutation that is involved in the cancer progression. Once the mutation has been identified accordingly cancer can be treated by anti-cancer drug therapy involving specific mutational based targeted drug therapy in association with chemotherapy. Treatment also depends on the stage of cancer since, stage of cancer is an important factor in determining whether the treatment would be effective. Despite mutational specific treatment, still, it has been observed that many cases which were previously responding to the drug therapy and exhibited good survival rate, after certain time of exposure exhibited a decrease in drug response [24]. Half of the tumour developed acquired resistance to gefitinib drug due to a secondary T790M mutation among Japanese patients [24-26]. This is a major challenge yet, that as the exposure of drug therapy increases, it leads to building up in bodily resistance mechanisms to the therapy. This leads to a major challenge suggesting that there is a continuous need for modification in drugs according to resistance targeted drug therapy to compete with acquired resistance mechanism in non-small cell lung cancer therapy. However, to develop drugs, it is essential to study the mutational resistance mechanisms involved in activation of genes which are involved in resistance mechanisms. Also, these genes lead to an altered pathway which even in the response of tyrosine kinase inhibitor drugs, leads to cancer progression. Hence, simultaneous screening of mutation should be carried on at different stages of cancer to effectively detect resistance pathway followed by the treatment and future drug development for advanced mutation. This review aims to summarize mutations within tyrosine kinase domain of EGFR responsible towards progression of non-small cell lung cancer and drug therapy that targets tyrosine kinase domain including resistance

Figure 2B: Estimated number of deaths in 2020, worldwide, females, ages 10-79.[59]

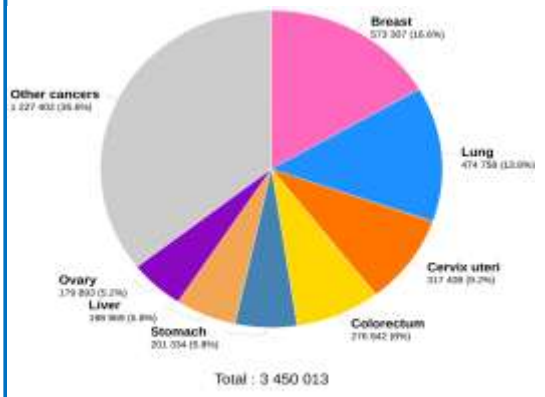
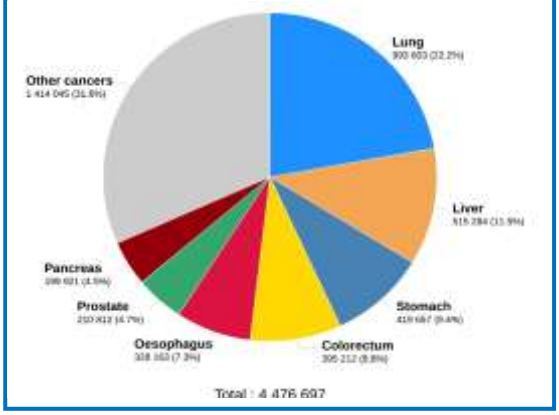


Figure 1A: Estimated number of deaths in 2020, worldwide, males, ages 10-79.[59]

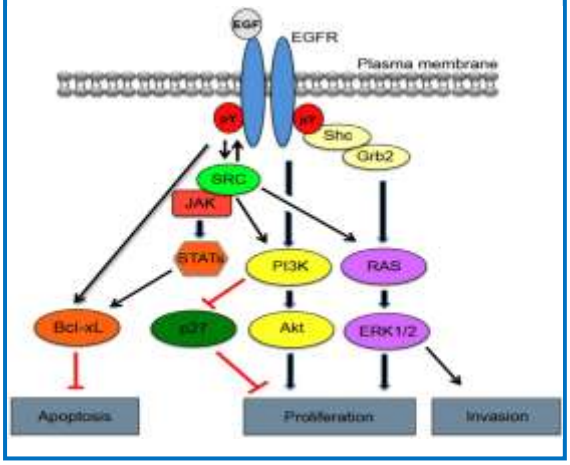


mechanisms acquired in response to tyrosine kinase inhibitor drugs.

2. EGFR mutation:

Epidermal growth factor receptor protein possesses an affinity for members of Epidermal growth factor family and has tyrosine kinase domain which can be inhibited by tyrosine kinase inhibitors such as gefitinib. In non-small cell lung cancer, EGFR mutations were the first molecular alteration to be reported. Due to the prevalence of high frequency of mutation in EGFR in non-small cell lung cancer, leads to genetic alteration in earlier stages in the development of lung cancer [27]. The mutations in EGFR are associated with the ATP-binding site of

Figure 3: Epidermal growth factor receptor downstream signaling pathways in cancer [60]



tyrosine kinase domain which is targeted by tyrosine kinase inhibitors Gefitinib and erlotinib. These mutations are also referred to as activating mutations and are found in exon 18,19,20,21 of EGFR gene [22][23][24-25]. These mutations are further classified into three classes. Class 1 mutation involves in-frame deletions in exon 19 which includes amino acid residue leucine-747 to glutamic acid- 749 [28]. Class 2 mutation involves single nucleotide substitution leading to an amino acid alteration in Exon 21 which substitutes leucine to arginine at codon 858, glycine substituted by serine at codon number 719. Class 3 mutations include in-frame insertion or duplication in exon 20 and accounts for 5 % of activation of Tyrosine kinase mutation. Deletion of amino acid in Exon 19 and point mutation substitution of amino acid in exon 21 are referred to as classical mutations as they account for 90% of EGFR activating mutations [29]. These activating mutations are responsible for resistance to tyrosine kinase inhibitors [30].

EGFR mutation response to TKI: It has been reported that NSCLC tumour harbouring patients who initially responded to tyrosine kinase inhibitor therapy is effective for a limited period. This is due to acquired drug resistance due to secondary mutations. Research studies reported that around 50% of the patients carried secondary mutation threonine to methionine at codon 790 at the time when they developed acquired resistance towards to Tyrosine kinase inhibitor therapy [31-33]. These mutations were reported from patients being treated with TKI-therapy as they were not reported in tumours from patients not treated by TKI-therapy indicating that this mutation is acquired due to selective pressure during treatment [34].

3. EGFR-TKIs:

Epidermal growth factor receptor belongs to ErbB family and consists of four receptor tyrosine kinase namely Her 1, Her 2, Her 3, Her 4. EGFR activation leads to initiation of signal cascades including RAS/RAF/MAPK pathway/STAT pathway/MEK/ERK/AKT/mTOR/P13K [29][31]. Till date, four generations of EGFR-TKIs have been developed for the treatment of NSCLC patient harbouring EGFR-mutation [34-35].

4. First-generation EGFR-TKIs:

Gefitinib, erlotinib, icotinib are the first-generation EGFR-TKIs that inhibit the binding of ATP with tyrosine kinase domain by reversibly binding to EGFR and blocking the cell proliferation which ultimately leads to death of the cell [36].

Gefitinib:

Gefitinib drug was the first tyrosine kinase inhibitor drug against EGFR developed by AstraZeneca, London, UK for the treatment of NSCLC and was available even before the discovery of activating mutations in EGFR kinase domain [37]. Gefitinib received US Food and Drug Administration approval in May 2003 as monotherapy for the treatment of advanced-stage non-small-cell lung cancer tumour if platinum-based and docetaxel therapies do not respond [38-39]. Later, in June 2005, the use of Gefitinib drug was restricted by FDA to only NSCLC patients who were already being treated by gefitinib and exhibited positive outcome as well as new patients who were included in clinical trials after the approval of Institutional Review board. Finally, gefitinib received FDA approval on 13th July 2015 as first-line treatment of EGFR -mutation harbouring patients after the discovery of activating mutations in EGFR tyrosine kinase domain in 2004 [19-23,40-41].

Erlotinib:

Erlotinib which was originally discovered in 1997 was approved by U.S Food and Drug Administration in November 2004 for metastatic NSCLC tumour treatment if any one of the prior chemotherapy regimens fails. In 2010, Erlotinib was approved for treatment in unselected NSCLC patients who had previously exhibited significant improvement from platinum-based chemotherapy [8][34][36][10,35,37]. Based on EURTAC (European Randomized Trial of Tarceva versus chemotherapy) study, erlotinib exhibited better response as compared to platinum-based chemotherapy in phase 3 clinical trials and hence was received FDA approval on May 2013 as first-line therapy for EGFR mutant non-small cell lung cancer patients [42-43].

Icotinib:

Icotinib hydrochloride is a small molecular drug developed in 2002 in China and exhibits structural similarities with Gefitinib and Erlotinib. When compared with gefitinib in phase 3 ICOGEN trials, icotinib exhibited equivalent efficacy and lesser toxic than gefitinib. Also, the overall incidence of adverse conditions was 61% in icotinib and 70% in gefitinib [39][34][35, 40]. Hence, based on ICOGEN study results, icotinib received China FDA approval in June 2011 for second- or third-line treatment of metastatic NSCLC patients. Later, on 13th November 2014. Icotinib was approved in China for the first line treatment of EGFR-mutation harbouring patients [44].

5. Second-generation EGFR TKIs:

Afatinib and Dacomitinib are second generation EGFR Tyrosine kinase inhibitor drugs that irreversibly bind to EGFR.

Afatinib:

Afatinib is an anilinoquinazoline derivative possessing an acrylamide group and functions to inhibit kinase activity by binding to ATP competitively. It blocks the enzymatic activity of receptors of members of ErbB family [45]. Afatinib received approval in U.S in July 2013 for treatment of EGFR-mutant NSCLC patients as first-line therapy. In January 2018, afatinib received supplemental new drug application from FDA as first-line treatment for metastatic NSCLC patient tumours exhibiting non-

EGFR mutations along with other EGFR mutations namely, G719X, S768I, L861Q [46].

Dacomitinib:

Dacomitinib is an irreversible tyrosine kinase inhibitor marketed by Pfizer. Several studies show that dacomitinib failed to show better clinical efficacy as compared to first-generation TKIs and exhibits higher toxicity as compared to erlotinib in unselected NSCLC patients [47]. Based on ARCHER 1050; NCT01774721 study, in which safety and efficacy of both dacomitinib and gefitinib in 452 patients fostering unresectable and metastatic NSCLC were compared and the trial revealed significant improvement in survival. Hence, dacomitinib received Food and Drug Administration approval on 27th September 2018 as first-line therapy for metastatic NSCLC patients with EGFR exon 19 deletions of leucine -747 to glutamic acid -749 and exon 21 L858R amino acid substitution as first-line therapy [48].

6. Acquired Resistance mechanism to first and second-generation TKI therapy:

Tyrosine kinase receptor that binds to tyrosine kinase domain in EGFR mutation gets activated in the event of genetic alterations leading to the progression of cancer. Inhibition of this receptor by tyrosine kinase inhibitor drugs leads to suppression of signalling of EGFR downstream pathway involved in the progression of cancer which results in cell growth arrest. Niederst.et.al in his study reported the identification of various molecular mechanisms that develop as the exposure of tyrosine kinase receptor inhibition drug increases in non-small cell lung cancer type patients. This development of resistance to the drug led to a decrease in drug effectiveness and poor survival of cancer patients. This is due to activation of different tyrosine kinase receptor that despite tyrosine kinase inhibitor drugs that target the tyrosine kinase domain of EGFR genes at a specific position, leads to activation of different RTKs that continue the downstream signalling pathway maintaining the proliferation of tumour growth. Despite significant response towards first and second-generation TKIs initially, it has been observed that patients on EGFR-TKIs acquire resistance to treatment [49-51]. One of the common mechanisms for acquired resistance is T790M mutation which accounts for 50-60% of secondary resistance towards primary EGFR-TKI therapy [52]. This led to the development of third-generation Epidermal growth factor receptor-tyrosine kinase inhibitors.

7. Osimertinib:

It is a third-generation EGFR-TKI which selectively and irreversibly targets sensitive EGFR mutation including T790M. Osimertinib is a mono-anilino-pyrimidine compound and has 200 times higher potency against T790M and L858R as compared to wild-type EGFR tyrosine kinase [53-54]. Osimertinib was approved by U.S Food and Drug Administration for treatment against T790M mutation on 30th March 2017 based on AURA3 trials conducted to determine the safety and efficacy of Osimertinib drug [55].

Despite the good response of Osimertinib initially, resistance to Osimertinib was reported. Research studies reported a point mutation C797S on exon 20 responsible for this resistance as covalent bonding of Osimertinib to EGFR was hampered in the presence of this mutation [56]. Also, the occurrence of this mutation was found in 22 out of 99 NSCLC patients receiving Osimertinib for treatment [57]. Hence, to overcome this resistance posed by C797S mutation, fourth-generation EGFR-TKI was developed.

8. EAIO45:

EAIO45 is an allosteric non-ATP competitive inhibitor of EGFR harbouring mutation and posed high selectivity to mainly L858R and T790M mutation. Researchers confirmed that EAIO45 in combination with cetuximab significantly inhibited the proliferation of Ba/F3 cells harbouring L858R/T790M mutation [56-58]. In a study carried in a genetically modified mouse model of L858R/T790M driven lung cancer, the effect of EAIO45 was observed in combination with cetuximab as well as alone without cetuximab. No response in tumour reduction was observed with EAIO45 alone. This confirmed that EAIO45 could overcome the resistance from T790M and C797S mutation [56-58].

9. CONCLUSION

Most of the mutations involved in NSCLC progression arises from mutations in EGFR tyrosine kinase domain that plays important role in the downstream signalling pathway leading to cancer progression. Therefore, current treatment involves targeting the tyrosine kinase receptor gene that can lead to the suppression of cell growth and improve the survival rate in patients. However, not all members of the EGFR family are activated simultaneously in a specific case, which indicates that different gene mutation leads to differences in gene activation and that the targeted drug therapy is specific for mutations. Members of EGFR family despite playing role in the similar signalling pathway, specific drug therapy is essential for efficient response. However, increased drug treatment has also led to an increase in resistance mechanisms ultimately leading to cancer progression and no response to targeted drug therapy. Hence further studies are essential to keep a check on molecular changes by the screening of gene mutation at every stage to avoid any shortcomings. This approach may lead to overcoming major challenges in the treatment of lung cancer despite various molecular resistance mechanisms modifications in future.

10. ACKNOWLEDGEMENT

NA

11. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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13. REFERENCES

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