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Article

Potential CRISPR/Cas9 Gene Editing Applications in Reversing Acquired Resistance to EGFR-TKIs due to Secondary and Tertiary Mutations to EGFR in NSCLC

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Simple Summary: Patients with lung adenocarcinoma who have specific gene mutations often receive targeted therapy for treatment. Unfortunately, despite the development of new-generation medications, cancer treatment can become ineffective because the cancer becomes resistant due to idiopathic somatic mutations in the genome. Genome editing is a potential strategy to reverse the single-nucleotide variants that underpin cancer therapy resistance. There are several approaches to how this can be accomplished depending on the location of the tumor.

Abstract: Lung cancer treatment has long been a major field of study in research due to the complexity and variability of cancer. Because drug resistance is a major hurdle for lung cancer treatment, in this article, we will review the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) used for the treatment of Non Small Cell Lung Cancer (NSCLC) and the EGFR mutations that cause resistance to them. Then, we will discuss the applications of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) genome editing in medical treatment. The proposed treatment is specifically for NSCLC patients who have acquired resistance to EGFR TKIs due to mutations to EGFR such as T790M and C797S and would make these TKIs become effective once again. Currently, there is no treatment targeting C797S on the market, but fourth-generation EGFR-TKIs are currently in trial [1,2]. (Figure 1) This proposal will review the potential steps and options available as well as some challenges that may complicate the process.

Keywords: cancer; NSCLC; EGFR mutations; TKIs; CRISPR

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Introduction

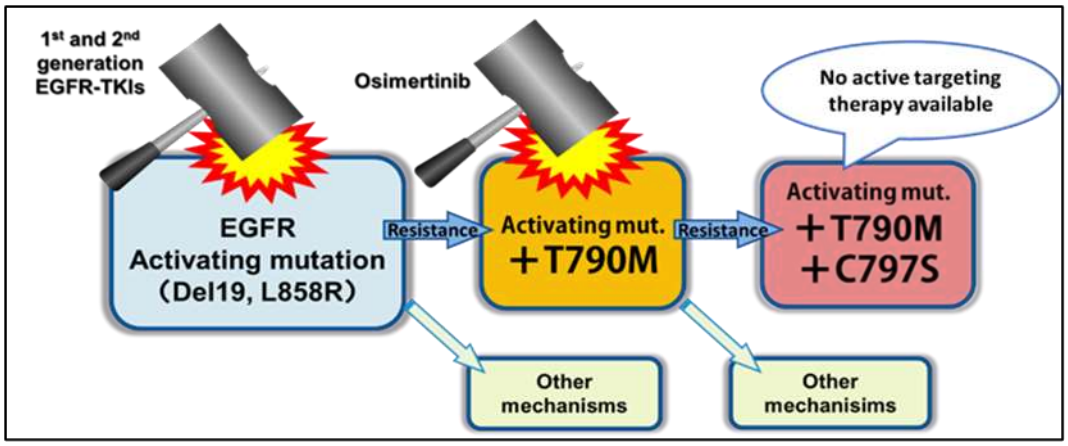

Figure 1. Diagram of EGFR Mutations, TKIs Used to Treat Them, and Resistance Mechanisms [3].

Lung cancer has been the leading cause of cancer deaths worldwide, primarily among smokers. Lung adenocarcinoma is the most common type of NSCLC. Drug resistance is a major issue in cancer treatment and therefore a main focus of cancer research; it re-mains a large problem in the treatment of cancer, as many patients acquire resistance to a treatment after a median of 9.9 to 20.9 months [4,5]. EGFR (also known as human epidermal growth factor receptor [HER1] or ErbB1) is a cell surface protein that regu-lates epithelial tissue development and homeostasis in wild-type organisms and is part of the ErbB family of tyrosine kinases. In wild-type cells, EGFR regulates tissue devel-opment and homeostasis. However, point mutations and amplifications to EGFR may lead to increased cell proliferation and be a driver of tumorigenesis, mostly in breast, lung cancers and glioblastoma [6]. Exon 19 deletions (del-E746-A750) and an exon 21 point mutation (L858R) make up about 85%-90% of all EGFR mutations in NSCLC and are often called EGFR sensitizing mutations, as they confer sensitivity to EGFR TKIs [7]. Furthermore, these EGFR mutations are found in about 10% of white, 19% of Black, and 50% of Asian patients with NSCLC [8]. The group with the highest frequency of EGFR sensitizing mutations are never-smoking Asian women, of which about 63% have these mutations [9]. NSCLC patients with these mutations are usually treated with EGFR TKIs.

First to Third-Generation EGFR-TKIs and Their Uses and Limitations

First-generation EGFR TKIs (such as gefitinib and erlotinib) and second-generation EGFR TKIs (such as afatinib and dacomitinib) are used in clinical practice as first-line treatment on patients with EGFR-sensitizing mutations (although osimertinib, a third-generation TKI, is recommended by the National Comprehensive Cancer Net-work® [NCCN®] as a treatment option when the mutation is discovered prior to first line therapy) [8]. First-generation TKIs inhibit the receptors of EGFR by reversibly binding to the intracellular kinase domain of EGFR, inhibiting autophosphorylation and therefore reducing intracellular signaling cascades that predispose cells to en-hanced proliferation. However, most patients acquire resistance to these agents after a median of 10.9 to 14.7 months [10,11,12]. The most common secondary resistance mechanism is the EGFR T790M mutation, which is the cause of about 50-60% of ac-quired resistance to first-generation and second-generation TKIs [13,14]. The T790M mutation causes resistance by increasing EGFR's affinity for ATP back to wild-type levels, causing EGFR-TKIs to be outcompeted by ATP and their efficacy decreased [15]. Second-generation TKIs were designed in response to acquired resistance to first gen-eration TKIs due to alternate signaling pathways through other members of the ErbB family. These second-generation TKIs irreversibly inhibit EGFR, HER2, HER3, and HER4 (all members of the ErbB family), overcoming a factor of resistance that was present in their first-generation counterparts; however, T790M still renders sec-ond-generation TKIs ineffective [16].

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Osimertinib is a third-generation EGFR TKI used as first-line or adjuvant treatment in patients with locally advanced or metastatic NSCLC with EGFR sensitizing mutations and T790M; however, it has also been approved for first-line use for patents with just EGFR-sensitizing mutations [17]. It has proven to be superior in terms of progression free survival (PFS) over first and second generation TKIs in most populations [18,19,20]. However, as with its predecessors, resistance eventually develops. Mech-anisms of resistance (in order of frequency) include EGFR exon 20 C797S mutations (more frequent in second-line setting) or due to the development of MET amplification (more common in first line setting. C797S prevents osimertinib from forming covalent bonds in the ATP-binding domain of EGFR kinase, resulting in EGFR-TKI dysfunction [21].

CRISPR/Cas9 Based Treatment

One compelling solution to the problem of acquired drug resistance in cancer treat-ment—particularly since resistance is acquired through mutations— is to correct the mutation with a genome editing approach. The CRISPR/Cas9 system has allowed the ability to specifically target and edit mammalian genome with precision. Furthermore, recent FDA approval of CRISPR-based therapies targeting germline-caused sickle cell diseases has underscored the clinical relevance of this approach. Casgevy (ex-agamglogene autotemcel) is the first FDA approved CRISPR based treatment and has had positive outcomes with Sickle Cell Disease (SCD) in patients with recurrent vaso-occlusive crises and are twelve years of age and older. Patients' hematopoietic stem cells are extracted and edited using a CRISPR/Cas9 system and a lentiviral vector. The edited stem cells are reintroduced to the patient and increase the production of fe-tal hemoglobin, which replaces sickled blood cells [22].

In the past decade, there have been various studies on the use of CRISPR-based gene editing for treatment of a multitude of diseases and disorders. Heart-1 is a Phase 1b clinical trial utilizing VERVE-101, a CRISPR-based gene editing therapy that is deliv-ered in vivo to hepatocytes via a lipid nanoparticle vehicle to inactivate PCSK9, a gene that causes low density lipoprotein-cholesterol (LDL-C) to raise to dangerously high levels, resulting in plaque buildup in the arteries. This treatment was administered by a single intravenous infusion in patients with Heterozygous familial hypercholester-olemia. There was durable 39%-55% reduction of LDL-C and no evidence of off-target editing in liver cells. This demonstrated the first proof-of-concept for in vivo DNA base editing in humans [23].

The strategies used in these treatments can be applied to the reversal of T790M and C797S. To reverse a point mutation, a homology-directed repair (HDR) pathway ap-pears to be the desired mechanism to achieve precise editing. In comparison to the nonhomologous end joining (NHEJ) pathway, HDR tends to be less efficient due to competition with the NHEJ pathway. However, there have been numerous strategies found to increase the efficiency of the HDR pathway such as inhibiting the NHEJ pathway, regulating HDR-related factors, cell cycle synchronization, optimally de-signing the donor DNA template, and optimizing the proximity of the CRISPR and donor DNA components [24].

The proposed process to implement this would consist of an initial biopsy and Next Generation Sequencing (NGS), the designation of a CRISPR/Cas9 to directly target a specific somatic mutation, the delivery to the cancer, and another biopsy to determine if the treatment was successful. This proposed treatment is specifically for patients with lung adenocarcinoma who have acquired resistance to the TKIs used for treat-ment due to T790M or C797S mutations. This should be determined through NGS of EGFR exon 20 (where both T790M and C797S occur), which would likely be tested in tandem with other commonly mutated regions of EGFR, exons 18 to 21 [3]. Knowing exactly which mutations exist in the cancer will allow for the proper usage of CRISPR/Cas9 and the genome editing would be applied in vivo. In order to design a single guide RNA (sgRNA) for a CRISPR/Cas9 system, you must identify a protospac-er-adjacent motif (PAM) adjacent to the target site (NGG for Cas9). The sample used for NGS would be acquired through a biopsy of the tumor via bronchoscopy or in the case of metastasis to pleura, via thoracentesis.

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Delivery of the CRISPR/Cas9 System

The CRISPR/Cas9 system may be delivered in the following ways: 1) directly to the tumor via intra-tumoral injection delivery utilizing robotic bronchoscopy, 2) to the pulmonary vasculature via a pulmonary artery catheter and/or selective bronchial embolization, 3) intrapleural administration via chest tube in the case of pleural me-tastasis. Itra-tumoral injection delivery via bronchoscopy is performed under general anesthesia and requires endotracheal intubation. The bronchoscope is directed manu-ally or by robotic platform into the edge of the lung and a needle would be advanced into the tumor to deliver the CRISPR/Cas9 system. Complications may be anesthesia related (central nervous system toxicity, hemodynamic instability, methemoglo-binemia, malignant hyperthermia), bleeding, pneumothorax (1% to 3%), cardiac ar-rhythmias, vocal cord injury, pneumomediastinum, hypoxia and rarely death [25].

Because there is dual blood supply to the lung— arterial blood via the bronchial arter-ies from the aorta and the pulmonary artery from the right ventricle— depending on where the blood comes from to the tumor, delivery to the pulmonary vasculature via a pulmonary artery (PA) catheter and/or selective bronchial embolization is possible. A PA catheter is inserted into a large vein (eg. femoral or brachial) and advanced to the right atrium, right ventricle and floated to the pulmonary artery and the CRISPR/Cas9 system would be delivered to the branch leading to the tumor. For bronchial emboliza-tion, a catheter is inserted into the femoral artery and advanced to the bronchial ar-tery. Potential complications may include pneumothorax, hemothorax, arrhythmias, valve rupture, cardiac perforation, pulmonary infarction [26].

In the case of pleural metastasis, intrapleural administration via chest tube may be ap-plicable. Chest tube placement (chest tube thoracostomy) is placed with local anesthe-sia and can be used to deliver the CRISPR/Cas9 system into the pleural cavity. Alterna-tively, an indwelling pleural catheter can be inserted in a similar fashion and can also be used for persistent pleural effusions and also deliver CRISPR to the pleural cavity. Both can be performed as an outpatient however, pain at the insertion site is common [27]. Potential complications may include bleeding, infection, injury to spleen, liver, heart or aorta [28].

To assess the efficacy of the treatment a re-biopsy should be performed with NGS and if successful, TKI therapy should be continued.

The CRISPR/Cas9 system that is encoded by plasmid will be encapsulated by a lipid nanoparticle. Lipid nanoparticles are one of the most common non viral nucleic acid delivery methods and can protect the CRISPR/Cas9 system from destruction from nu-clases and enter target cells via endocytosis [29].

Conclusion

When patients acquire resistance to all of the EGFR-TKIs on the market, often their next best option is chemotherapy. However, osimertinib has shown to be the better treatment for these patients– the median progression-free survival (PFS) of osimertinib is 18.9 months [19] compared to 5.2 months on platinum-based chemotherapy and the objective response rate is about 70% with osimertinib and 30% with chemotherapy [30]. Because of this, many researchers are working on a fourth-generation TKI that specifically targets C797S, however, it appears that inevitably, there will be acquired resistance to those new TKIs through various, unpredictable means. Therefore, rather than developing a new TKI when resistance is discovered, it seems reasonable to sug-gest a treatment that re-allows osimertinib to be effective.

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