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# Targeted therapies for non-small cell lung cancer based on CRISPR-Cas9 technology

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**Abstract.** Lung cancer is the leading cause of cancer death worldwide, with non-small cell lung cancer (NSCLC) being the largest subgroup and having very low overall survival rates. This paper will briefly describe the pathogenic mechanism of NSCLC and analyze the pros and cons of several treatments that have been put into the clinic as well as the prospects and potential risks of new gene therapy approaches. Based on these approaches, this article will provide researchable directions for the treatment of NSCLC using CRISPR-Cas9 gene editing technology. The findings of the paper show that targeted drugs rely on the signaling pathway that acts on EGFR to treat cancer, with fewer but still present side effects than conventional therapies. Immunotherapy, which uses a patient's own immune recognition to destroy cancer cells, is subject to problems such as an overactive immune response. CRISPR-Cas9 gene editing technology, which starts with the mutated gene that causes cancer and is intended to eradicate the cancer at its source, is underdeveloped, has no proven clinical protocols and carries the risk of incorrect editing. Nowadays, as cancer strikes globally, research on cancer treatment is of great significance in saving lives, ending cancer, individualizing treatment, promoting scientific progress and fostering socio-economic development.

**Keywords:** NSCLC, CRISPR/Cas9, targeted drugs, chemotherapy.

## 1. Introduction

Lung cancer is the leading cause of cancer deaths globally, with non-small cell lung cancer (NSCLC) being the largest subgroup of lung cancer which accounts for about 85 percent of all cases, and with very low overall survival rates which is at 24 percent at five years [1]. The analysis is based on 977 cases from GDC Data Portal demonstrating a change in survival rate over the years, as can be seen in Figure1, the survival rate after 14 years maintains and stays below 20%, which proves that the probability of an eventual complete cure for lung cancer is less than 20%. This suggests that cancer still causes severe damage to patients and the treatments provided nowadays are not well-developed.

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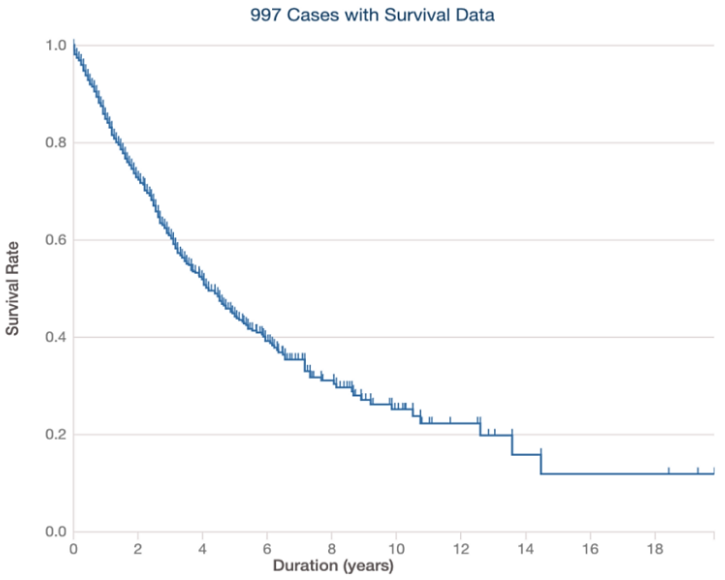



**Figure 1.** Survival rate of 977 cases from GDC [2].

Cancer treatment options are divided into conventional treatment options and targeted therapy. Conventional treatment options are based on one or more treatment modalities consisting of surgery, chemotherapy and radiotherapy [2].

Surgery remains the most direct way of treating cancer nowadays. However, it has several flaws. First, not everyone can undergo surgery. Patients with distant metastases and patients with a combination of other serious internal diseases including kidney disease, endocrine system disease, immune system disease are not suitable for surgeries. Second, lung cancer has a certain recurrence rate after surgery depending on the state before surgical treatment, so surgical treatment cannot guarantee eradication of lung cancer. For example, in early-stage NSCLC, the probability of patients surviving for 5 years or more after undergoing surgical treatment is more than 70%, while the 5-year survival rate of patients with advanced NSCLC is only about 23.3% [1].

Chemotherapy uses chemotherapeutic drugs to kill cancer cells to achieve the therapeutic purpose. As a systemic treatment, although it is effective in treating potentially metastatic lesions (cancer cells that have actually metastasized but cannot be detected clinically because of technological limitations) and cancers that have already metastasized clinically, chemotherapeutic drugs are extremely damaging to the human body. In addition to attacking cancer cells, chemotherapeutic drugs also attack the growth and division of normal cells, which is reflected in the patient's side effects after chemotherapy, such as myelosuppression, nausea and vomiting, oral mucositis, cardiotoxicity, pulmonary toxicity, renal and cytotoxicity, neurotoxicity, gonadal dysfunction, and the second primary tumor and so on. Chemotherapeutic agents for lung cancer include first-line treatment for non-small cell lung cancer such as paclitaxel, gemcitabine and vincristine combined with platinum drugs and doxorubicin and pemetrexed as second-line treatment for non-small cell lung cancer.

Radiotherapy refers to the generation of high doses of radiation through special equipment that reaches the tumor cells and prevents them from dividing and growing by breaking the DNA strands within the tumor cells. For intermediate and advanced lung cancer as well as those who refuse early surgery, radiotherapy has become a commonly used tool, with the advantages of good efficacy, high safety and tolerability. Gamma Knife radiotherapy has become the most common treatment for intermediate to advanced lung cancer or early NSCLC that refuses surgery. However, the study shows that as the radiation dose increases, cardiotoxicity also increases, resulting in radiation-induced heart disease (RIHD). The incidence of RIHD, the most common and dangerous complication, was about 42%, which greatly reduced the survival benefit of radiotherapy. As shown in Table1, the varying degrees of health damage caused by different doses of gamma radiation in cancer patients.

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**Table 1.** Relationship between gamma radiation dose and myocardial damage [3].

| Dose | n | ECG or cTnI abnormalities | Myocardial injury rate(%) |
|---|---|---|---|
| <30Gy | 26 | 0 | 0 |
| 30~50Gy | 16 | 2 | 12.5 |
| 50~60Gy | 12 | 2 | 16.7 |
| >60Gy | 10 | 4 | 40 |

Although current medical technology has made great progress in the treatment of NSCLC, the best treatment for non-small cell lung cancer has not yet to be found. Nowadays, based on the advantages of low side effects and high therapeutic efficiency of targeted drugs, it is believed that the research and development of targeted drugs has more space and potential. Thanks to technological advances, this paper will explore the possibility of applying CRISPR-cas9 technology to NSCLC treatment by analysing the pathogenesis of NSCLC and evaluating novel therapeutic approaches.

## 2. Pathogenesis of NSCLC

NSCLC is caused by the accumulation of mutations in multiple genes such as proto-oncogenes, tumor suppressor genes and problems with the immune surveillance function of the immune system. NSCLC is often accompanied by EGFR mutations. The mutations mainly occur in exons 18-21 on gene EGFR. EGFR gene is a kind of proto-oncogenes and the mutation of it account for 50% of patients with non-small cell lung cancer. Among them, substitution at codon 858 of exon 21 (43%) is the main type.

EGFR can be activated by the receptor to form a dimer, which leads to tyrosine autophosphorylation and activation of tyrosine kinases in the cytoplasmic fraction. Downstream signaling pathway proteins can bind to the phosphorylated tyrosine sites on EGFR, causing different intracellular signaling responses, thus triggering a series of biological effects that regulate cell growth, proliferation, differentiation, migration and tumorigenesis. When mammalian epidermal cells overexpress mutant EGFR, especially at exon 19 deletion and exon 21 Condon 858 substitutions, the tensity of tyrosine autophosphorylation of the functional region of the tyrosine kinase is increased. This in turn stimulates the downstream signaling pathways and leads to irregular cell growth, proliferation, differentiation, migration and tumorigenesis.

## 3. New treatments for NSCLC

### *3.1. Targeted drugs*

Currently, advanced NSCLC patients are mostly treated with chemotherapy using cisplatin-based drugs, but the adverse effects caused by chemotherapy, such as a serious decline in immune function, are always an insurmountable gulf for chemotherapy. In particular, lung cancer mostly occurs in the elderly whose immune function is already fragile and weak, resulting in their resistance to chemotherapy. This may even interrupt the chemotherapy program. In recent years, with the continuous development of tumor molecular biology technology, the application of targeted therapeutic agents has become more and more widespread.

The common therapeutic targets of molecular targeted therapy for lung cancer are cell receptors, signal transduction and anti-angiogenesis. Among them, EGFR is currently the most important target [4]. In recent years, molecularly targeted therapeutic agents have achieved remarkable efficacy in clinical treatment, such as gefitinib, erlotinib, cetuximab and bevacizumab [5].

Gefitinib is an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), which has better patients with advanced non-small cell lung cancer. Gefitinib and erlotinib compete for the Mg-ATP binding site of EGFR and inhibit its receptor tyrosine kinase activity and phosphorylation, thereby effectively inhibiting the downstream signaling pathways and thus inhibiting tumor growth, metastasis, angiogenesis and inducing apoptosis of tumor cells with fewer adverse effects [6]. Erlotinib belongs to the quinazoline class of selective epidermal growth factor receptor tyrosine kinase inhibitors. It has the

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same principle of action as gefitinib. Thus it also has been shown to be effective in NSCLC with few adverse effects. Clinical trials have found it to be prolonged and well-tolerated [7].

Cetuximab and bevacizumab are other types of target drug that belongs to monoclonal antibody. Bevacizumab is a humanized monoclonal antibody that inhibits the activation of the vascular endothelial growth factor signaling pathway and normalizes abnormal neovascularisation mainly by binding to VEGF and preventing the interaction between VEGF and EGFR [8]. Bevacizumab is usually given intravenously to patients and is often used in combination with other anti-cancer drugs to enhance the effectiveness of treatment. Cetuximab specifically binds to EGFR on the surface of tumor cells and blocks the dimerization of the receptor, the phosphorylation of tyrosine kinase and signal transduction, and transmits apoptotic signals to the nucleus through the downstream signaling pathway of EGFR, thus exerting the effects of inhibition of cell proliferation and promotion of apoptosis. In addition, cetuximab also prevents the repair of the DNA of the tumor cells that have been damaged by radiotherapy, thus exerting the anti-tumor effect [9].

### *3.2. Immunotherapy*

The immune system plays an important role in maintaining the integrity of the organism by protecting itself from the various exogenous or endogenous aggression to which it is often exposed. The development of cancer is closely related to the immune system. Normal body cells are subject to genetic mutations which, when accumulated, lead to the transformation of normal cells into tumor cells. In the early stages, the immune system is capable of recognizing and removing mutated tumor cells and will effectively identify and eliminate the most immunogenic tumor cells. The survival of poorly immunogenic tumor cells due to low major histocompatibility complex expression, poor antigenic peptide presentation or poor expression of immunogenic tumor associate antigen (TAA) will result in the survival of poorly immunogenic tumor cells, which will result in the phasing of weakly immunogenic tumor cells and the immune system. Therefore, screened tumor mutants escape the recognition of the immune system, and shape the inhibitory environment with the help of intrinsic immune myeloid cells and inflammatory components to avoid being eliminated by the immune system, thus achieving immune escape.

Recently, immunotherapy for tumors has made great strides. Chimeric antigen receptor (CAR) technology has revolutionized the field of cancer therapy. CAR is a synthetic receptor expressed on T-cells that is re-edited to target tumor-associated antigens on the surface of tumor cells. Therefore, it is able to overcome the body's own immune response and immune tolerance and has no MHC restriction. Literature has shown that the application of genetic engineering techniques to splice the scFv gene sequence of EGFR with the sequence of the intracellular region of the T lymphocyte immune receptor, transduction into lymphocytes through the PiggyBac transposon system, and expression of fusion proteins on the surface of the cells can construct EGFR-specific CAR-T cells with antibodies expressing CD47. By targeting EGFR-expressing tumor cells to chemotaxis to the tumor site, CAR-T cell proliferation can be promoted in vivo. Inhibition of CD47/SIPRα pathway and activation of phagocytosis by macrophages in parallel with overt immune cell therapy can also activate the function of residual tumor-specific T cells in situ and increase the anti-tumor effect of endogenous cytotoxic T cells, thus improving the efficacy of specific tumor killing [10].

Other treatments of immunotherapy like immune checkpoint inhibitors, cancer vaccines, cytokine therapy and immune adjuvant therapy also take part in patients' immune systems to help them kill tumor cells.

### *3.3. CRISPR/Cas9 gene editing technology*

CRISPR/Cas9-mediated gene editing is mainly divided into two stages. The first stage is to obtain the exogenous DNA sequence and integrate the protospacers of the exogenous genes into the proximal part of the leading region of the CRISPR sequence of the host chromosome to achieve the tagging function. In the second stage, when the exogenous DNA is invaded again, the CRISPR site is transcribed into RNA precursor (Pre-CRISPR) under the control of the Leader. At the same time, repeats are transcribed

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into trans-activating crRNA (tracrRNA). The pre-crRNA is processed by Cas9 and double-stranded RNA-specific RNase III nuclease to produce a mature crRNA containing only one spacer sequence (invasive exogenous sequence). The crRNA, tracrRNA and Cas9 nuclease form a complex called crRNP, which shears and edits the invading exogenous DNA and then repairs by non-homologous endjoining (NHEJ) or homology-directed repair (HDR) to provide self-immunity [11].

When using CRISPR/Cas9 for genome editing of tissues, cells or embryos, double-stranded cleavage of the target sequences can be achieved by in vitro synthesis of Single guide RNA (sgRNA mixed with Cas9mRNAor Cas9 protein). After repairing by NHEJ and HDR mechanisms, genome targeting can be achieved.

The substitution at exon 21 codon 858 is the main cause of NSCLC. Table 2 (data from Benchling) presents three scenarios to fix this mutant site by applying the CRISPR/Cas9 technology.

**Table 2.** Three scenarios designed by the Benchling website.

| Cut Position | Strand | Guide Sequence | PAM | On-Target Score | Off-Target Score |
|---|---|---|---|---|---|
| 55191831 | + | TTTTGGGCGGGCCAAA | TGG | 39.4 | 84.0 |
| 55191832 | + | TTTGGGCGGGCCAAACTGCT | GGG | 48.4 | 81.4 |
| 55191837 | + | GCGGGCCAAACTGCTG | CGG | 51.2 | 62.4 |

Of these three scenarios, the first one is the most appropriate to apply to cancer treatment. First, it meets the requirement that the cutting distance should be within 10 nucleotides of the PAM site. Second, though the on-target score is not high, the off-target score is relatively highest among the scores of the three scenarios which suggests that the first scenario is the most conserved one. It has the potential to become the safest method when it is applied to human body someday.

## 4. Comparison between new treatments

Targeted drugs and gene editing are two different types of cancer treatments with different principles, applications and effects.

### *4.1. Principles and mechanisms*

Targeted drugs are drugs designed to target specific molecules or signaling pathways that are overexpressed or abnormally activated in cancer cells. These drugs can interfere with the growth, division or other key biological processes of cancer cells, thereby inhibiting the growth or spread of tumors. Gene editing technologies (especially CRISPR/Cas9) allow direct intervention and modification of a cell's genome to correct, delete, or insert specific genes. In cancer therapy, gene editing can be used to repair or adjust cancer-related genes, inhibit tumor growth or enhance the immune system's attack on cancer cells.

### *4.2. Scope of application*

Targeted drugs have been widely used in the clinic for a variety of cancer types, such as breast, lung, and colorectal cancers. Each targeted drug is usually designed to target a molecule or pathway in a specific cancer. Gene editing technology is still in the research and clinical trial stage, but it has a wide range of potential applications, including altering the properties of cancer cells, treating cancer, preventing tumor recurrence, and enhancing the effects of the immune system.

### *4.3. Specificity and safety*

Targeted drugs have a high degree of specificity and are able to selectively target specific molecules or signaling pathways, thereby reducing their impact on normal cells. However, long-term use may lead to drug resistance. The specificity of gene editing depends on the designed target and the precision of the technology. Advances in technology and precision have improved specificity, but there is still a risk of mis-editing or inaccurate editing.

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### *4.4. Side effects and safety*

Targeted drugs usually have a better safety profile than traditional chemotherapy drugs, but may still cause side effects such as skin reactions, nausea, diarrhea and fatigue. The safety and side effects of gene editing are still being studied. Possible side effects include unanticipated genomic alterations, immune responses, effects beyond the targeted gene, and cytotoxicity.

## 5. Conclusion

New advances have been made in the field of cancer treatment with the gradual deepening of the exploration of natural sciences and the gradual development of science and technology. However, so far, both treatments that have already been put into clinical care, such as the use of targeted drugs, and gene-editing technology, which has not yet been put into clinical use, have their potential risks and hazards.

Targeted drugs are usually more selective than conventional chemotherapy drugs and therefore have relatively fewer side effects, but they can still cause a range of different side effects that vary depending on the type of drug, individual differences and treatment regimen. For example, skin reactions such as rash, dryness, itching, redness and flaking. Certain drugs, such as cetuximab, are especially likely to cause these skin reactions. Immunotherapy drugs such as immune checkpoint inhibitors such as pembrolizumab and natalizumab may trigger immune system reactions such as skin rashes, gastrointestinal problems, and thyroid problems. Plus, targeted drugs also have side effects like nausea and vomiting, diarrhea, high blood pressure, heart and neurological problems, kidney problems, fatigue and abnormal liver function.

Gene editing technology has potential applications in cancer treatment and research, but there are also some risks and challenges. For example, inaccurate editing. Gene editing techniques may introduce inaccurate editing, such as mismatch repair or incomplete repair on the target gene, resulting in undesired mutations or genomic instability. This may increase the risk of cancer, as genomic damage is a key factor in cancer development. Side effects and an immune response is also an obstacle that need to be overcome. The use of gene editing techniques, particularly the CRISPR-Cas9 system, may trigger an unwanted cellular immune response leading to cell damage or death, thereby increasing the potential risk of cancer development. What's more, target identification, target specificity, and therapeutic resistance from the technical side, ethical and moral issues from the social side have to be taken into consideration as well. Plus, unknown Long-Term effects also needed to be considered. As the use of gene editing technologies in medicine is relatively new, the long-term effects and risks are unknown. It may take years or even decades to assess the long-term effects, including potential effects on cancer.

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