International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(REVIEW ARTICLE)

A comprehensive review of cancer: CRISPR and its application in lung cancer with a potential in overcoming EGFR mutations

Saamya Yadav [1,*] and Poonam Agrawal [2]

[1] *Santa Clara High School, United States of America.*
[2] *Beckman Center for Molecular and Genetic Medicine, School of Medicine, Stanford University, United States of America.*

International Journal of Science and Research Archive, 2024, 13(01), 3047–3067

Publication history: Received on 13 September 2024; revised on 20 October 2024; accepted on 23 October 2024

Article DOI: https://doi.org/10.30574/ijsra.2024.13.1.2016

Abstract

As a comprehensive review of cancer, oncogenes, and CRISPR, this review explores the transformative potential of CRISPR technology in combating cancer, particularly focusing on lung cancer and oncogenes. CRISPR's precision gene-editing capabilities offer a promising avenue for developing more targeted cancer therapies, overcoming the limitations of current treatments such as chemotherapy and radiation, which often fail due to cancer cell heterogeneity and resistance. Additionally, this review proposes the potential use of CRISPR to target specific oncogenes like EGFR and its significant promise for improving treatment outcomes in lung cancer by directly modifying or regulating gene function. This review synthesizes current research, highlighting the integration of CRISPR with other therapeutic modalities to enhance the effectiveness of cancer treatments, delivery systems that improve targeting and minimize off-target effects, and the future directions in CRISPR-mediated lung cancer therapy.

Keywords: CRISPR; Cancer; Lung Cancer; Targeted Therapy; EGFR

1. Introduction

Cancer is the second leading cause of death in the US, yet there is still no definitive cure or preventative measure for the deadly disease even in the 21st century. In 2024, 2,001,140 new cancer cases and 611,720 cancer deaths are projected to occur in the United States [1]. While, there are options currently available to treat certain cancer types including: surgery, immunotherapy, chemotherapy, and hormone therapy, neither of these treatments provides patients with guaranteed survival and each has its respective side effects that can increase the death toll and decrease quality of life for patients [2]. If cancer is caught before it has metastasized and is in an easily accessible location, surgery would be the best option, but that is unfortunately not always an option, leaving the treatment option of many patients to be cancer drugs. However, it is estimated that approximately 75% of patients do not respond effectively to any given class of cancer drugs [3]. Therefore, finding alternative treatments that can increase the survival rate of cancer patients as well as ensuring a high quality of life is imperative for the progress of medicine and biotechnological research.

1.1. Understanding Cancer

The fundamental idea of cancer is the abnormal and uncontrolled proliferation of mutated cells, which can be classified into two categories: malignant and benign tumors [4,5]. Benign tumors remain at the original site of abnormal proliferation, whereas, malignant tumors have invaded surrounding tissue and spread throughout the body via the bloodstream, resulting in metastasis. The impression most of the population has in regards to cancer comes from malignant tumors, considering their characteristic of invading normal cells is what makes cancer such a difficult disease to treat, unlike benign tumors, which can usually be removed surgically. Lung tumors are one of the few most common types of tumors, which can be both benign or malignant [6]. Generally, benign tumors are rarer in comparison to their

* Corresponding author: Saamya Yadav

counterpart malignant tumors. Specifically in this case, a small percentage of the lung tumors are benign and can be excised surgically if needed, commonly through lobectomies. However, the majority of lung tumors that remain malignant are treated with a combination of radiation therapy, chemotherapy, and immunotherapy [7].

Cancer is developed from a single cell that begins to proliferate, meaning cancer has tumor clonality or is monoclonal. However, merely because a cancerous cell may originate from one mutation, it does not imply that the development of cancer is a one-step process. Tumor progression occurs due to continuous mutations within the cell population and within the cell cycle. The cell cycle is responsible for proliferation, containing multiple checkpoints, tumor suppressors, and growth factors, which are encoded by proto-oncogenes, to regulate cell reproduction [8]. However, when carcinogens, substances that cause cancer, including radiation, viruses, chemicals, and viruses, act by damaging DNA, inducing proto-oncogene mutations, it affects proteins meant to regulate proliferation through cellular differentiation. This causes the damaged cells to continue reproducing, when they should have died through apoptosis [9,10]. Through mice studies cited by Geoffrey M. Cooper, it was determined that tumor promoters, compounds aiding tumor production by stimulating cell proliferation, were required to grow the mutated cells, so both mutations in a cell's DNA and promoters must work simultaneously to result in tumor growth [5,10].

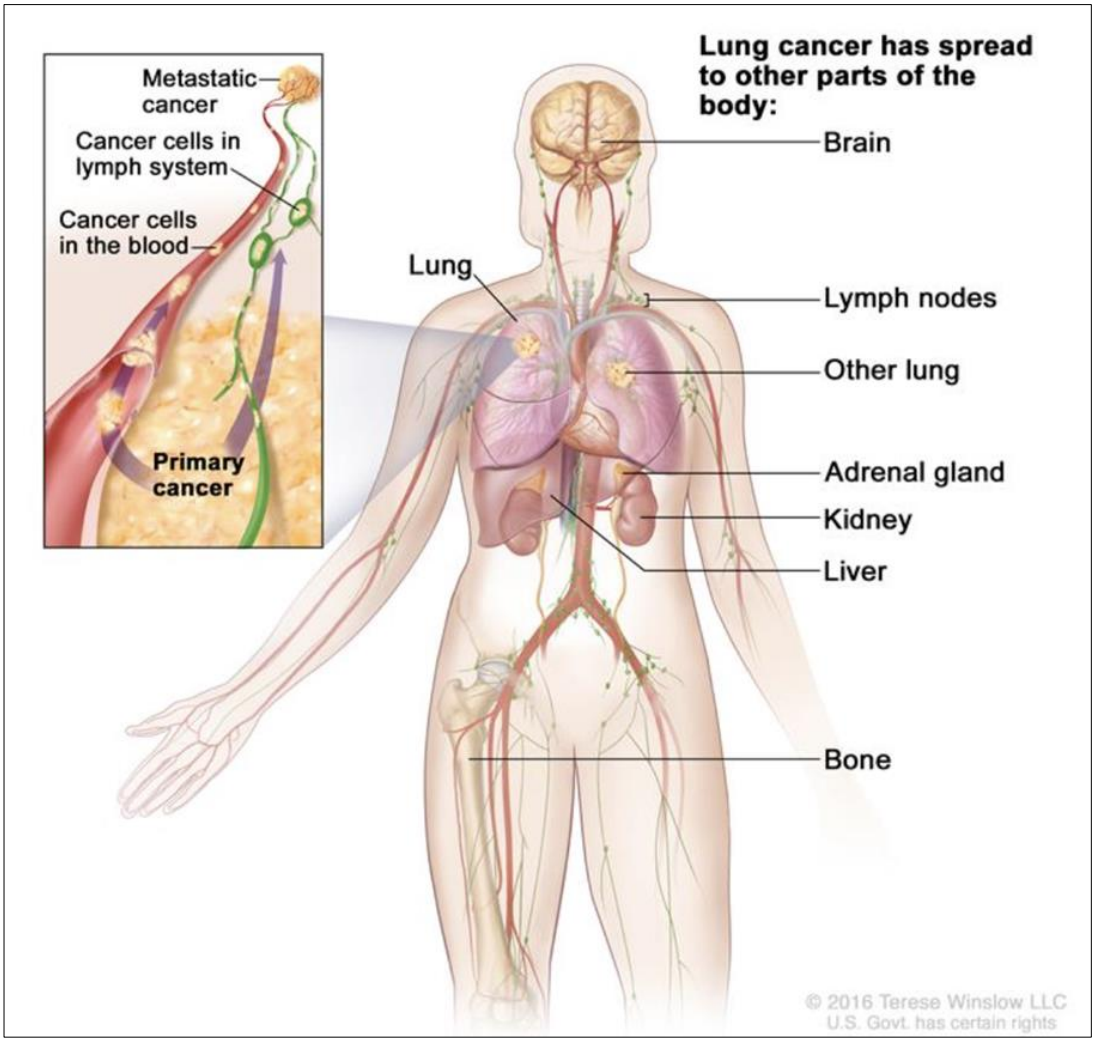

Figure 1 Metastasis. How cancer spreads from the primary tumor site to other parts of the body, focusing on lung cancer. Visualization of the metastatic process, where cancer cells enter the lymphatic system or bloodstream and travel to other organs, spreading cancer cells from the primary cancer site to metastatic tumors. Taken from CancerHelp® Stages of Non-Small Cell Lung Cancer [16]

Tumor metastasis, an important signal determining the severity of cancer, has become a peaking conversation topic in cancer treatment. The behavior of cancerous cells that distinguishes them from normal cells, and makes controlling cancer so difficult, is centered around cancerous cells surviving for as long as possible, meaning they bypass regulated

proliferation, differentiation, and adapting for peak efficiency [11]. A primary characteristic of non-cancerous cells is that they exhibit density-dependent inhibition of proliferation, a phenomenon where the cell stops proliferation when the finite density of the cell has been reached, dependent on the number of growth factors. However, with cancerous cells, the finite density of the cell is ignored as the growth factor requirement of many tumors is reduced, while some tumors produce their own growth factors, increasing the density of the cell to an infinite level [12,13]. This leads to autocrine growth stimulation, or the automatic stimulation of cell growth, resulting in the cancerous cells being less dependent on growth factors and preventing cell differentiation as growth factors prevent apoptosis, an essential part of cell differentiation. Yet, cancerous cells aren't dependent on growth factors, permitting unregulated proliferation of mutated cells [13]. The goal of cancerous cells is to survive and invade other cells. It does so through cell-cell interactions such as less adhesion, ignorance of contact inhibition, digestion of extracellular matrix, and angiogenesis [14]. Tumor cells are less adhesive to one another, causing a less restrained interaction with other cells, making it easier to invade and metastasize other cells. Normal cells display contact inhibition, which ensures the halt of migration of cells when in contact with one another, resulting in an orderly arrangement of cells, whereas cancerous continue migrating even when in contact with neighboring cells, resulting in tumor cells stacking and becoming disorganized, affecting the growth of neighboring normal cells [15]. More so, to invade and metastasize, cancerous cells release certain proteases that digest the extracellular matrix of other cells, allowing the invasion of the normal cell, as well as angiogenesis, where tumors create their own blood supply to support the growth of a tumor with more than a million cells in addition to metastasizing [5].

1.2. Proto-Oncogene Mutations

There are more than 40 known proto-oncogenes in the human body, and they are imperative for successful cell growth, differentiation, and halting apoptosis, as they encode various intracellular regulatory proteins such as kinases, growth factors, and growth factor receptors [17,18]. Proto-oncogenes are typically most active during embryogenesis due to their function in cell growth and are turned off once the developmental process they regulate is completed. However, when these proto-oncogenes mutate as a result of gene variations, epigenetic changes, chromosome rearrangements, and gene amplification, an oncogene becomes activated, causing uncontrolled cell proliferation, lack of cell differentiation, and bypass of apoptosis, which are all characteristics of cancer [19].

A few of the most common proto-oncogenes, such as cyclin D1 (CCND1) and cyclin E1 (CCNE1), act to continue a cell's process in various stages of the cell cycle in an effort to regulate what cells are being proliferated [18,20]. Similarly, proto-oncogenes, such as EGFR, a receptor for the growth factor EGF in factor-mediated signaling, and KDR, a receptor for the growth factor involved in angiogenesis (VEGF), code for cell surface receptors that aid in the communication between the extracellular environment and the inside of the cell, forming transmembrane receptors [21]. These membranes serve as the cornerstone in cell growth and division, considering cells respond to the external signals conveyed by the extracellular ligand (growth factors that stimulate cell division and growth and bind to transmembrane receptors). When the ligand binds to the receptor, it changes its shape, which activates the intracellular matrix, causing a chain of events that regulate cell growth, proliferation, angiogenesis, or cell death [21,22]. When such receptors turn into oncogenes, the EGFR and KDR proto-oncogenes overexpress their role, resulting in loss of regulatory control due to a continually active receptor without the need for ligand binding or continuous signaling that supports tumor angiogenesis respectively [23]

1.3. Genetic Engineering

Genetic engineering is the process of specifically manipulating an organism's DNA to acquire new genetic features, inactivate target genes, and correct malignant genetic mutations [24]. It has been on the rise since its use was first discovered when Paul Berg created the first recombinant DNA molecules by combining DNA from the monkey virus SV40 with the lambda virus in 1972, establishing the concept of gene therapy [25]. One of the most prevalent discoveries regarding gene editing has been CRISPR-Cas 9. The first discovery of CRISPR, or Clustered regularly interspaced short palindromic repeat, was in 1987 by Yoshizumi Ishino and has now served as the foundation for the most integral genetic engineering technology of the century [26].

1.3.1. CRISPR Cas Systems

CRISPR-Cas9 is an adaptive immune process found in many prokaryotic organisms as an attempt to prevent infection from foreign elements. The main components of CRISPR are the RNA-guided Cas9 endonuclease, which is responsible for cleaving foreign DNA, and single-guide RNA (sgRNA), which is transcribed as CRISPR RNA (crRNA) and trans-activating CRISPR RNA (transcrRNA) [27]. Functional CRISPR systems process in 3 stages: (1) adaptation, (2) crRNA biogenesis, and (3) interference. The first step is when the prokaryote is attacked by foreign invaders, the foreign DNA is cut, or cleaved, into short fragments by the Cas protein, which is then integrated into a series of repeating DNA

sequences in the prokaryote, forming a CRISPR array, which is separated by the spacers created by the cleaved DNA. In the second step, the CRISPR array is transcribed to form precursor CRISPR RNA (pre-crRNA), which, through biogenesis, manufactures mature crRNAs. In the last step, a complex is formed between the crRNA and the Cas proteins, which enables the recognition of protospacers on the invader's nucleic sequence. When the same invader attacks again, RNA segments from the CRISPR arrays attach to specific regions of the invader's DNA and use Cas9 to cut the DNA apart, thereby disabling the invader [28,29] However, in some cases, a short protospacer adjacent motif (PAM) is required to complete both the adaptation and interference stages, allowing self and nonself discrimination, the ability to differentiate between and invader DNA and own DNA, a fundamental aspect of its function in bacterial immunity. In comparison, PAM-independent systems have evolved to use strategies including using a protospacer flanking site and lack of a base-pairing potential between the target RNA and the crRNA 5'- repeat handle and 3'-flanking region [30].

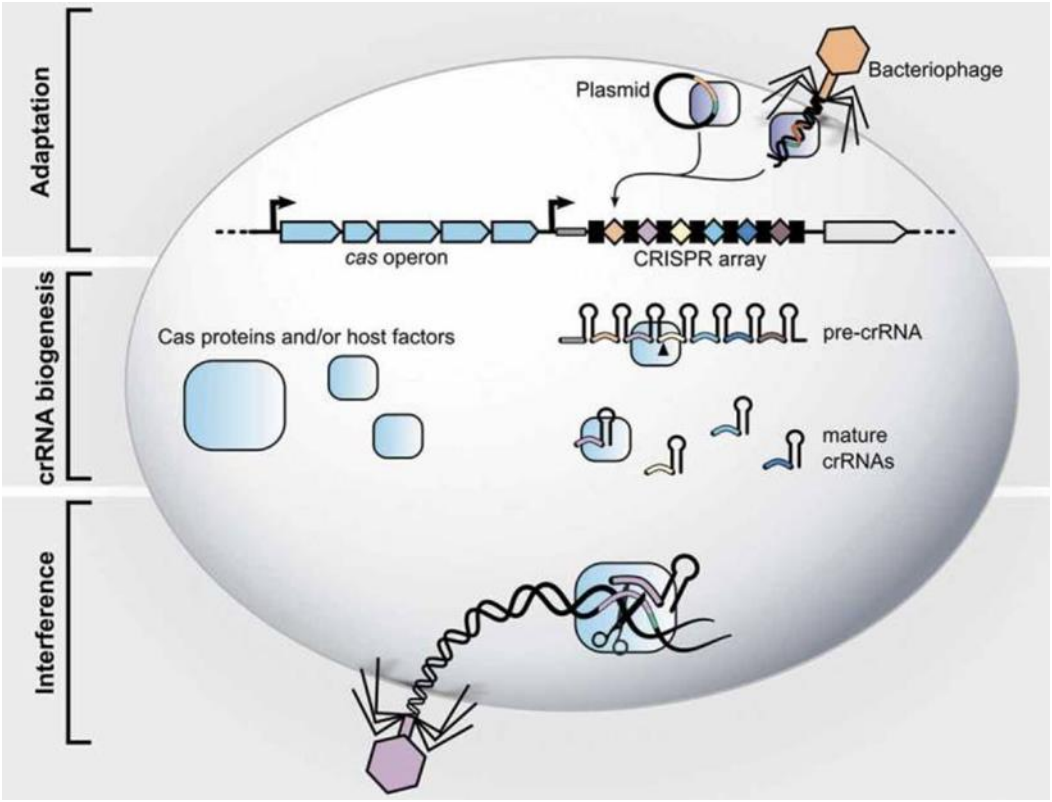

Figure 2 General mechanism of the CRISPR/Cas9 system, including the stages of adaptation, crRNA biogenesis, and interference. How foreign DNA is incorporated into the CRISPR array, and later, crRNA guides Cas proteins to target and cleave specific DNA sequences. Taken from: Rhun et al. [30]

CRISPR-Cas systems can be divided into 2 classifications: class 1, with multi-Cas protein effector complexes, and class 2, with single effector proteins. The first discovered and most commonly used of all the categories is the Type II CRISPR system derived from Streptococcus pyogenes called SpCas9. Since SpCas9 is a PAM-dependent Cas system, an 'NGG' PAM is required to create a double-stranded break (DSB), which can later be resolved with NHEJ or HDR repair pathways [24,31].

Specifically in the case of genome editing, adapted from the naturally occurring CRISPR processes found in prokaryotic cells, sgRNA uses Cas9 endoneucleic properties to create a double-stranded break (DSB) at the targeted location, which is repaired by either non-homologous end joining (NHEJ) pathway or the homology-directed repair (HDR) pathway [24, 32]. While NHEJ is efficient, it is an error-prone process that rejoins the DBS without using a template with homologous sequences, resulting in random insertions or deletions (indels). Whereas, HDR has the precision lacking in NHEJ, by utilizing homologous regions as templates for the repair, but is more time-consuming [33].

1.3.2. Diverse Applications of Cas Variants

Since the discovery of SpCas9 (derived from Streptococcus pyogenes), more variants of the Cas enzyme have evolved from the implementation of CRISPR systems in eukaryotic cells, broadening the usage of CRISPR from originally editing specific genomic loci via a programmable guide RNA (gRNA) molecule that mediates complementary DNA to RNA base

pairing to now having options for customized editing based on the properties of the cell being altered. Cas9 orthologs such as Staphylococcus aureus Cas9 (SaCas9) allow for alterations of different genetic loci due to recognizing different PAM sequences. Smaller enzymes such as CjCas9 and NmeCas9 can be used for more concise packaging of smaller delivery vectors, or vehicles designed to deliver therapeutic genetic material [31,34]. While there are a limited number of naturally occurring Cas enzymes, lab-generated variants are always being developed. Scientists such as Jennifer Doudna and Emmanuelle Charpentier, who both discovered CRISPR-Cas9 technology and have been involved in refining Cas9 variants to enhance their precision and versatility. Their dedication to discovery has been recognized to the highest level as proclaimed Nobel Laureates; Feng Zhang, who has worked on developing smaller Cas9 variants, such as Cas9n, eSpCas9, with improved specificity and editing efficiency; and David Liu who is known for developing base editing technologies based on Cas9 variants (BE3, ABE) that enable precise nucleotide changes in the genome without creating double-strand breaks, have been pioneering figures in the world of Cas system discovery [35,36]. With their discoveries and many others, countless outlets for the usage of CRISPR has become a possibility. The potential CRISPR holds in regard to genetic engineering is enormous, which is why this review will acknowledge the fundamentals of cancer and the usage of CRISPR as a method for treating cancers that result from proto-oncogenic mutations.

Table 1 Different Cas9 variants, each with distinct properties such as PAM sequence and location, derivation, and CRISPR/Cas Type. It highlights how these variants can be used in different contexts, depending on the gene-editing needs. For example, some Cas9 variants may be smaller, making them easier to package into delivery systems like viral vectors, while others have improved specificity to minimize off-target effects. Modified from Zhang et al [37]

Name	Cas	Resources	CRISPR/ Cas	PAM*	PAM location
SpCas9	Cas9	*Streptococcus pyogenes*	Type II	NGG	3′
SaCas9	Cas9	*Streptococcus aureus*	Type II	NNGRRT	3′
FnCas9	Cas9	*Francisella Novicida*	Type II	NGG	3′
NmCas9	Cas9	*Neisseria meningitidis*	Type II	NNNNGATT	3′
CjCas9	Cas9	*Campylobacter jejuni*	Type II	NNNNRYAC	3′
St1Cas9	Cas9	*Streptococcus thermophilus*	Type II	NNAGAAW	3′
St1Cas9	Cas9	*Streptococcus thermophilus*	Type II	NGGNG	3′
AsCas12a	Cas12a(cpf1)	*Acidaminococcus sp.*	Type II	TTTV	5′
LbCas12a	Cas12a(cpf1)	*Lachnospiraceae bacterium*	Type II	TTTV	5′
FnCas12a	Cas12a(cpf1)	*Francisella Novicida*	Type II	TTTN or YTN	5′
LsCas13#	Cas13 (C2c2)	*Leptotrichia shahii*	Type VI		
Cas14[&]	Cas14	Archaea			
FnCas9 variant	Cas9	Modified FnCas9	Type II	YG	3′
Modified SpCas9	Cas9	Engineered SpCas9	Type II	NGA or NAG	3′
SaCas9-KKH	Cas9	Engineered SaCas9	Type II	NNNRRT	3′
SpCas9-HF	Cas9	Engineered SpCas9	Type II	NGG	3′
eSpCas9	Cas9	Engineered SpCas9	Type II	NGG	3′
SpCas9-NG	Cas9	Engineered SpCas9	Type II	NG	3′
xCas9	Cas9	Engineered SpCas9	Type II	NG	3′
Sniper-Cas9	Cas9	Engineered SpCas9	Type II	NGG	3′
evoCas9	Cas9	Mutated SpCas9	Type II	NGG	3′
HypaCas9	Cas9	Mutated SpCas9-HF	Type II	NGG	3′
Cas9-NRNH	Cas9	Engineered SpCas9	Type II	NRNH	3′
SpG	Cas9	Engineered SpCas9	Type II	NGN	3′
SpRY	Cas9	Engineered SpCas9	Type II	NRN or NYN	3′

2. Utilizing CRISPR Technology

2.1. CRISPR's Application in Various Cancer Types

CRISPR has been developing for the past 2.5 decades, leading to many advances in its delivery and specificity, allowing its usage to broaden throughout the years. Understanding the underlying cause of cancer and how CRISPR works, has enabled scientists to apply CRISPR technology to combat different cancers [38]. Due to the versatility and capacity of CRISPR tools, there are multiple applications of CRISPR in cancer research ranging from the establishment of simultaneous insertion of genetic constructs via HDR and gene knockout via NHEJ in a single zygote injection, providing

T-cell receptor (TCR) knockout (KO) CAR-T cells to target B-cell malignancies like leukemia and lymphoma, elimination of oncogenic virus-like HPV, and diagnostic screening systems, models, and gene therapy for lung cancer [39]. Since this review is focused on lung cancer treatment, it will only evaluate the use of CRISPR in various other cancer types to provide further background on cutting-edge technology.

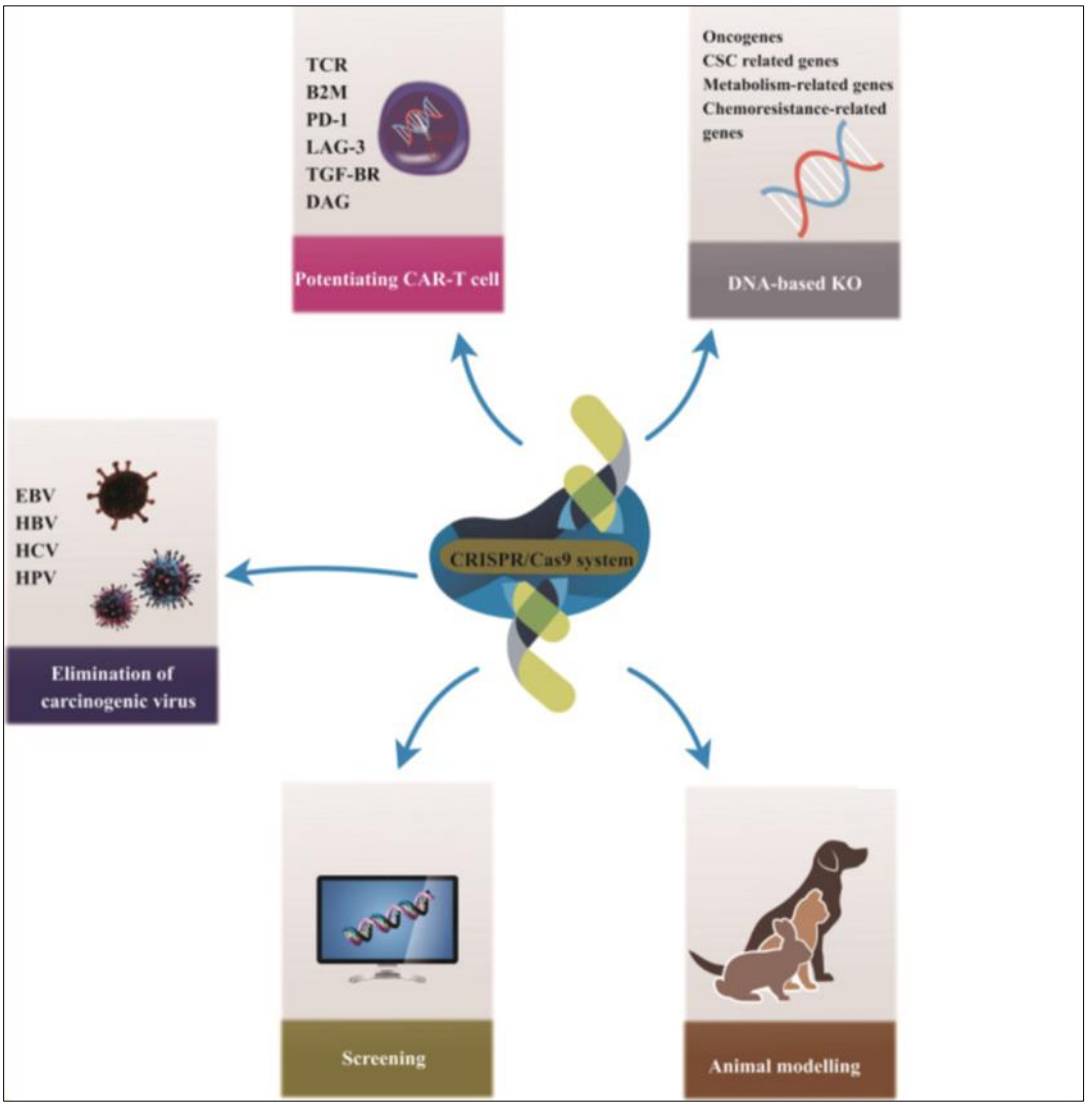

Figure 3 Schematic indicating the CRISPR/Cas9 system across different domains of cancer research. CRISPR can potentiate CAR-T cell therapies by modifying various immune checkpoints and T-cell receptors, used to knock out oncogenes, such as those involved in chemoresistance and cancer stem cell-related pathways, in diagnostics, and to eliminate oncogenic viruses like HPV and HBV, which are known to trigger carcinogenesis. Adapted from Baghini et al [39]

In precision medicine, particularly in cancer treatment, the goal is to focus on identifying driver mutations that contribute to tumor progression while ignoring passenger mutations. When first analyzing cancer genomes, researchers found hundreds to thousands of somatic mutations in each tumor, but distinguishing driver mutations was challenging due to an incomplete catalog of cancer-related genes [40,41]. Traditional techniques like siRNA, mutant cDNA overexpression, and random genome engineering in cell and animal models, though valuable, had challenges regarding precision and efficiency when studying tumorigenesis [42,43]. The introduction of CRISPR technology revolutionized cancer research by enabling precise genetic knockouts (KOs) without off-target effects, allowing researchers to study gene function more accurately [31,44]. CRISPR's ability to create clean KOs has advanced the development of organoid cultures and animal models, such as KO mice, eliminating the labor-intensive screening of embryonic stem cells (ESCs) [31,45]. This efficiency has allowed the simultaneous creation of multiple *in vivo* models and combinations of genetic modifications within the same organism, significantly enhancing zygote targeting strategies [24]. These improvements

have enabled the introduction of specific genetic changes into zygotes and germ cells, which can be inherited by future generations, providing a more efficient approach than traditional microinjection techniques in cancer research [46].

Chris Jung et al.'s [47] study optimized genome editing in mouse zygotes by using mouse embryonic stem cells to identify the most effective parameters for HDR and gene knockout. They found that Cas9 and Cas9nickase were the most efficient for precise genetic modifications, and their approach allowed for simultaneous insertion of genetic constructs via HDR and gene knockout in a single zygote injection, marking a significant improvement over traditional methods [47].

CRISPR technology has been pivotal in cancer research, particularly in developing T-cell receptor (TCR) knockout strategies combined with anti-CD19 chimeric antigen receptor (CAR) T-cell therapy, which can enhance T-cell therapies for treating relapsed and refractory B-cell acute lymphoblastic leukemia (B-ALL)[48,49]. Stenger et al's [50] approach involves using CRISPR-Cas9 to knock out the TCR in T cells, which reduces the risk of graft-versus-host disease (GvHD), a major challenge in allogeneic treatments [51]. By trying to prevent GvHD, Stenger et al [50] employed CRISPR Cas9 systems to KO the TCRβ chain, one of the two polypeptide chains that form the TCR, essential for recognizing antigens presented by the major histocompatibility complex (MHC) on antigen-presenting cells, and plays a crucial role in T-cell activation and immune response. In primary T cells while also introducing a second-generation retroviral CAR with a specific signaling component used in chimeric CAR T-cell therapy to enhance T-cell activation, proliferation, and survival, led to improved antitumor efficacy and CD19-specific killing capacity [52,53]. Compared to TCR-expressing T cells, these modified TCR-KO-CAR-T cells showed decreased alloreactivity and a balanced phenotype of effector and central memory T cells. In an in vivo model of childhood B-ALL obtained from patients, TCR-KO-CAR-T cells enhanced survival by efficiently controlling CD19+ leukemia. Despite the possibility of alloreactivity, the production of endogenous TCR with CAR improved T cell life and extended leukemia control. Despite these advances, the study found that TCR-KO-CAR-T cells did not persist as long as CAR T cells with an intact TCR, emphasizing that while CRISPR-Cas9-mediated TCR knockout offers promising avenues for reducing immune rejection and improving the safety of CAR T-cell therapies, maintaining long-term treatment efficacy remains a challenge [50].

These 2 studies: optimizing CRISPR/Cas9 for targeting constructs in mouse zygotes and the earlier experiment on TCR knockout in CAR T cells highlight the versatile and powerful role CRISPR technology plays in cancer research and potential therapies. Both studies focus on optimizing CRISPR systems for precise, targeted interventions. This work represents a significant leap forward in genome editing, enabling simultaneous insertion and knockout in a single step, which is crucial for modeling complex genetic diseases. A major theme across both studies is the ability of CRISPR to achieve precise genetic alterations, yet a notable challenge is maintaining long-term efficacy, as seen in the reduced persistence of TCR-KO-CAR-T cells compared to T cells with intact TCRs. This limitation points to a broader gap in understanding the long-term dynamics of CRISPR-modified cells in therapeutic contexts. While both studies highlight CRISPR's promise in gene editing and cancer treatment, they also emphasize the need for further research to address issues of treatment durability and the complex interplay of genetic modifications in live organisms, which are critical for translating these advances into sustained real-world clinical outcomes.

Carcinogenic viruses such as HPV, HBV, HCV, EBV, KSHV, and SV40, along with other less significant viruses, are the cause of approximately 10-15% of cancer [39,54]. The two main categories of infectious agents that cause cancer are direct carcinogens: viral oncogenes and indirect carcinogens [55]. However, this review will only evaluate CRISPR's application in the direct carcinogenic infections, which are listed above.

In this study, Raviteja Inturie and Per Jemth [56], used CRISPR/Cas9 to target and disrupt the E6 and E7 oncogenes of HPV16 and HPV18 in HeLa cells. Specific gRNAs were designed to target these oncogenes, and the CRISPR/Cas9 constructs were introduced into the cells using a transfection reagent. The targeted regions were amplified and sequenced to confirm CRISPR-induced mutations [56]. Senescence-associated β-galactosidase assays, showed that cells had entered a state of growth arrest, a natural defense against cancer [57]. The successful knockout of E6 and E7, showed restored p53, p21, and pRb pathways, which are crucial for tumor suppression. While the knockout of E6 and E7 led to this state, the cells did not exhibit apoptosis markers like cleaved PARP and caspase-3, maintaining a senescent phenotype instead. By checking the levels of these proteins and their phosphorylated states, the researchers could determine the reversing abilities of the CRISPR treatment to cancer [58]. Reintroduction of the E6 protein into E6-knockout senescent cells resulted in decreased p53 levels, but the cells did not resume normal growth and eventually underwent cell death, displaying the critical role of E6 in maintaining the transformed state. The results confirmed that CRISPR/Cas9 can effectively target and disrupt HPV18 oncogenes, leading to the reactivation of tumor suppressor pathways and induction of cellular senescence, rather than apoptosis, in HPV-positive cancer cells [56]. This study shows that CRISPR modifications can also have unintended consequences, such as impairing the long-term functionality of the engineered cells.

2.2. CRISPR in Lung Cancer Research

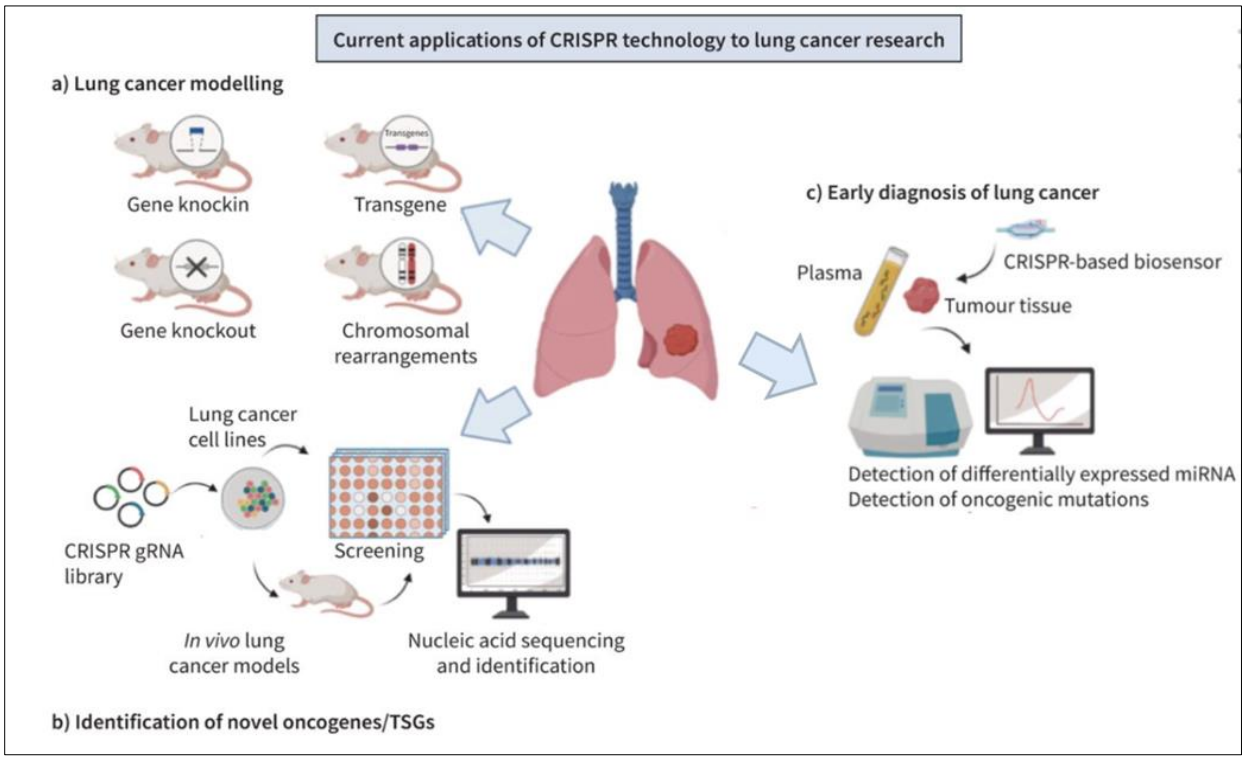

Figure 4 Key applications of CRISPR technology in lung cancer research. In lung cancer modeling, CRISPR/Cas9 allows researchers to induce knockouts to create models that mimic human lung cancer, leading to more precise studies of genetic mutations and their effects. Screening is enhanced by the ability to use CRISPR guide RNA (gRNA) libraries to systematically knock out genes and study their function in lung cancer cell lines. Finally, in early detection and diagnosis, CRISPR enables the detection of lung cancer biomarkers like microRNAs (miRNAs) or specific mutations, significantly improving diagnostic accuracy. Adapted from: Sorrola et al. [59]

2.2.1. CRISPR in Models for Lung Cancer

CRISPR technology has revolutionized lung cancer research by enabling precise gene editing, which helps in modeling the disease more effectively. This has sanctioned researchers to unravel the genetic events that drive research to be closer to a cure in various cancers, but also lung cancer through mice models, offering valuable insights into both tumor initiation and progression, oncogenetic events, that helped in the identification of genes responsible for lung cancer onset, progression, and targeted therapy, and a better understanding of advantages of CRISPR over traditional models.

Researchers Platt et al [60] worked on developing a Cre-dependent Cas9 knock-in mouse model, which they used to induce lung tumors by delivering CRISPR constructs via viral vectors [60]. (Cre-dependent means that Cas9 is only activated in certain tissues or cells when Cre recombinase, an enzyme that recognizes specific DNA sequences (LoxP sites), is present. This allows for targeted gene editing in particular tissues, such as the lungs in this case) [61]. In Platt's model, Cas9, the CRISPR-associated endonuclease that introduces DNA double-strand breaks, was activated in the lungs only when Cre recombinase was present. Then, they injected adenovirus vectors containing sgRNAs targeting the tumor suppressor genes TP53 and LKB1, as well as the oncogene KRAS into the lungs of mice. KRAS is an oncogene that drives cancer development when mutated, and mutations may lead to lung adenocarcinomas [62]. They also used KrasG12D as an HDR mechanism to introduce the most common oncogene present in lung adenocarcinomas [63]. Once the adenovirus vectors were delivered into the lungs the CRISPR/Cas9 system activated and induced mutations in the targeted genes, leading to the formation of lung tumors in less than two months [60].

This was key in furthering the specificity of lung cancer models and demonstrating how CRISPR could be used for somatic genome editing, which refers to altering the non-germline cells of an organism [64]. This contrasts with traditional genetically engineered mouse models (GEMMs), where gene alterations are inherited and affect all cells in the organism. By focusing on somatic mutations, Platt et al's [60] method more accurately reflected the sporadic mutations that occur in human cancer development, in addition to being able to rapidly induce cancerous mutations in specific tissues, using viral vectors. By stimulating stochastic (random) tumor formation, where only a fraction of cells

are genetically edited, similar to the way cancer naturally develops in humans, the production of various tumors, ranging from alveolar carcinomas to invasive adenocarcinomas, was achieved in a short period [60,65].

2.2.2. CRISPR Screening Techniques in Lung Cancer

CRISPR has been utilized in screening procedures, reshaping how researchers identify genetic factors in lung cancer development, progression, and therapy resistance. These screens allow for efficient gene editing, making it possible to knock out, repress, or activate genes across the entire genome or within specific gene subsets. The three main CRISPR-based technologies used in screening are CRISPR knockout (CRISPRko), CRISPR interference (CRISPRi), and CRISPR activation (CRISPRa) [68,69]

CRISPRko use for screening involves the use of Cas9 to create DSBs at target sites in the genome [66,68]. These breaks are typically repaired through the NHEJ repair system, leading to loss-of-function (LOF) mutations.

For example, genes like CDC7, a kinase involved in DNA replication and cell cycle regulation, [69] have been identified as targets to enhance the effectiveness of chemotherapy in small-cell lung cancer (SCLC), by Ling Deng et al. [70] The study found that silencing CDC7 in chemo-resistant SCLC cells enhanced the cells' sensitivity to standard chemotherapy drugs like cisplatin, which form interstrand DNA cross-links and DNA-protein cross-links, which results in apoptosis [71].

Additionally, a study by Chun Cheng et al. [72] utilized CRISPRko library screening to identify key genes associated with radiation resistance in lung cancer cells. They found that plakophilin 2 (PKP2), a member of the armadillo (ARM) superfamily of proteins, plays a critical role in driving radiation resistance in lung cancer. After radiation therapy, the level of PKP2 was found to be significantly higher, and high PKP2 expression predicted poor overall survival and post-progression survival in lung cancer patients [73]. Further analysis showed that the methylation of PKP2 by PRMT1 stabilized β-catenin which in turn enhanced PKP2's stability and function. This stabilization induced the expression of LIG4, a key DNA ligase in the NHEJ DNA repair pathway, which enhanced the lung cancer cells' resistance to radiation therapy [74]. By promoting LIG4-mediated NHEJ repair, PKP2 increased the cells' ability to repair DNA damage induced by radiation, thus contributing to their radioresistance. Through further testing, Cheng et al [72] identified the PRMT1/PKP2/β-catenin/LIG4 pathway as a potential therapeutic target for overcoming radiation resistance in lung cancer. Additionally, the use of a PRMT1 inhibitor that led to increased sensitivity of lung cancer cells to radiation therapy, demonstrated its potential as a radiosensitizer for lung cancer treatment [72].

Similarly, in Tamar Evron et al's [75] study, a CRISPRko screen was used to identify new regulators of canonical Wnt signaling, which plays a crucial role in cell proliferation, differentiation, and tumorigenesis [76]. This genome-wide screen used the HEK293-TCF-Hygro cell line, which was engineered to express a Wnt pathway reporter. The cells were transduced with a pooled CRISPR library targeting numerous genes, followed by selection with hygromycin. The CRISPR screen identified several potential inhibitors of Wnt signaling, including the DHX29 protein, which was shown to modulate Wnt activity through knockdown and overexpression experiments, revealing DHX29 as a possible tumor suppressor that modulates Wnt signaling in lung cancer [75].

These studies by Deng et al. [70], Cheng et al. [72], and Evron et al. [75] collectively demonstrate the power of CRISPR knockout technology in lung cancer research, emphasizing its effectiveness in identifying genes that drive treatment resistance, cancer progression, and potential therapeutic targets. A key theme is the identification of proteins and kinases, such as CDC7 and PKP2, which contribute to chemotherapy and radiotherapy resistance, alluding to the importance of targeting DNA repair mechanisms and cell cycle regulation. The strength of CRISPRko lies in its precision, enabling researchers to systematically identify and validate key genes driving lung cancer, offering valuable insights into treatment strategies [77]. However, its limitations include potential off-target effects and its inability to capture subtle gene regulatory roles, which approaches like CRISPRa and CRISPRi address more effectively [78]. Despite these challenges, the studies agree on the potential of CRISPRko in advancing targeted cancer therapy, though there remains a need for more *in vivo* research to confirm these findings and explore a more advanced approach to gene modulation.

CRISPRi uses a dCas9 protein, a protein that doesn't cut DNA, fused with transcriptional repressors, such as the Krüppel-associated box (KRAB) domain, which strengthens repression by adding repressive marks to histones, to suppress gene expression without making permanent changes to the DNA sequence [78,79]. This allows researchers to inhibit the transcription of target genes and study their role in lung cancer without permanently altering the cell and makes it perfect for studying gene regulation and non-coding regions [66].

CRISPRi was used in a study by Philipp Raffeiner et al's [81] group to uncover lncRNAs regulated by the MYC protein, essential for human lymphoid cell proliferation, and has been shown to be involved in lung cancer. They used a version dCas9-SID which could effectively repress specific target genes through prediction and theory analysis. The study's procedure involved designing and using a large CRISPR guide RNA library to target the transcription start sites of selected MYC-regulated lncRNAs, then conducting screens to determine which lncRNAs are required for cell growth. The study successfully identified 320 non-coding loci that played a role in cell growth, with transcriptional repression of any lncRNAs reducing the proliferative capacity of the cells, suggesting that lncRNAs have a significant role in MYC-driven cancer cell proliferation [81]. While not specifically involving lung cancer, this study played an important role in lung cancer research since MYC is a driver oncogene in many cancers, including lung cancer, and its regulation of lncRNAs plays a crucial role in cancer cell proliferation. By identifying lncRNAs that are essential for MYC-driven cell growth, the study introduces potential targets for developing new therapeutic techniques [82].

However, research done by Goyal et al [83] on the applications of CRISPRi for long non-coding RNA (lncRNA) genes has proven valuable in the identification of lncRNAs involved in lung cancer progression, yet also in understanding the limitations involved in using CRISRPi. The study used both dCas9, which is a nuclease-deficient Cas9, and dCas9-KRAB, to silence specific lncRNAs in lung cancer cells. They designed sgRNAs to guide dCas9 to the promoter regions of lncRNAs such as NOP14-AS1, LOC389641, MNX1-AS1, HOTAIR, and TP53. CRISPRi effectively knocked down lncRNA expression, but it also affected the expression of nearby or overlapping genes due to the complex genomic structure that surrounds these lncRNA.To overcome these limitations, the researchers used alternative techniques such as using siPOOLs, which are small interfering RNAs that produce more specific gene silencing, and antisense oligonucleotides (ASOs), variations of nucleic acid therapy that target and bind to complementary RNA [83,84,85]. However, unlike CRISPRi, these RNA-based methods didn't affect neighboring gene expression, which shed light on the unintended effects of CRISPRi when targeting lncRNAs. Specifically, 62% of lncRNAs are found to not be "CRISPRable," especially when transcribed from bidirectional or internal promoters, which is the method used in this study [83].

CRISPRi plays a crucial role in lung cancer research by allowing repression of specific genes and helping researchers identify essential genes and lncRNAs that contribute to cancer cell proliferation and survival. Despite its effectiveness, CRISPRi has limitations, such as off-target effects, variability in repression efficiency, and challenges in fully disrupting noncoding RNA function, which has been valuable in understanding the complexity behind gene regulation in cancer cells. These challenges spotlight the need for more precise, targeted approaches, suggesting that therapies may need to combine CRISPRi with other treatment modalities to achieve better efficacy, like Goyal et al's [83] study did. For example, understanding which genes are partially silenced versus fully suppressed can inform potential resistance mechanisms, making it clear that multi-target or combination therapies might be necessary for effective cancer treatment [86]. Additionally, addressing off-target effects could lead to the development of safer, more reliable gene-editing tools, advancing the precision of potential gene therapies for lung cancer [87].

CRISPRa offers a more versatile and efficient gene activation approach compared to other CRISPR-based systems, especially in high-throughput gain-of-function screens and cellular modeling. It is essentially a modification of the CRISPR-Cas9 system to increase the expression of target genes without cutting the DNA and instead of creating double-strand breaks, a dCas9 protein is used, which retains its ability to bind to specific DNA sequences guided by a sgRNA but lacks cutting activity, similar to CRISPRi [79,88]. To activate gene expression, the dCas9 is fused with transcriptional activators, causing the dCas9-sgRNA complex to bind to the promoter region of a target gene, recruiting the cellular transcription machinery and enhancing the transcription of the target gene. This allows researchers to upregulate specific genes, making CRISPRa a powerful tool for studying gene function, disease modeling, and potential therapeutic applications [88,89,90].

In lung cancer, this method has proven imperative in the identification of WWTR1, or TAZ, resistance to EGFR inhibitors in non-small-cell lung cancer (NSCLC) [91], discovered by Müller et al [92]. Resistance to EGFR inhibitors has been a persistent obstacle in NSCLC treatments and regulates the Hippo signaling pathway [93]. The finding that WWTR1 contributes to the resistance EGFR inhibitor, displays its critical role in maintaining tumor cell survival even under targeted therapy [92]. Since EGFR inhibitors are commonly used in NSCLC patients with EGFR mutations, the development of resistance is a significant clinical problem. Targeting WWTR1 alongside EGFR inhibitors could be a possible approach to a combination therapy that prevents or delays resistance in NSCLC [94].

An additional study conducted by Zhang Yang et al [95], utilized CRISPRa screening in identifying PLK1 as a synthetic lethal target in FGFR1-amplified lung cancer. FGFR1 (Fibroblast Growth Factor Receptor 1) amplification is associated with squamous cell carcinoma, a subtype of NSCLC [96]. Despite FGFR1 amplifications being identified as important drivers for tumor growth, FGFR-targeted therapies have not shown successful results regarding responding to FGFR inhibitors, which has allowed cancer cells that developed resistance to continue proliferating even when inhibited

[97,98]. Therefore, by conducting a kinome-wide screen in FGFR1-amplified lung cancer cells treated with FGFR inhibitors, Zhang Yang et al [95] identified PLK1 as a critical factor that mediates resistance to FGFR inhibition. The combination of FGFR inhibitors and PLK1 antagonism, which blocked PLK1 activity and made it harder for cancer cells to divide and repair, enhanced cancer apoptosis in both *in vitro* and *in vivo* models [95,99,100,101]. The discovery of synthetic lethal interactions provides new therapeutic options for patients who do not respond to FGFR-targeted therapies alone. Given that FGFR1 amplifications are present in ~20% of lung cancers, the development of a combination therapy targeting FGFR1 and its synthetic lethal partners could significantly improve outcomes for a large subset of lung cancer patients who currently have limited treatment options [96].

The CRISPR screening methods, including CRISPR activation (CRISPRa), CRISPR interference (CRISPRi), and CRISPR knockout (CRISPRko), have become effective instruments for understanding the intricacies of lung cancer biology and pinpointing possible targets for treatment. However, CRISPRko and CRISPRi have the potential to cause off-target effects, which can limit their efficacy in some situations [66]. CRISPRi offers a more refined technique by permitting gene repression without total deletion, which makes it useful for researching critical genes, but it frequently necessitates careful adjustment [102].

CRISPRa, in comparison, offers a more advanced and successful technique to examine gene activation and identify oncogenes or prospective therapeutic targets by upregulating gene expression both *in vivo* and *in vitro* [103,104]. Its ability to precisely upregulate gene expression without introducing a DSB minimizes the risks of unwanted mutations, reducing cell toxicity and preserving genomic integrity, which is particularly important for therapeutic research [89]. Furthermore, CRISPRa allows for the activation of gene networks at endogenous levels, providing a more significant understanding of gene functions in their natural context, making it more effective for gain-of-function studies [90]. CRISPRa's capacity to precisely regulate gene expression makes it an indispensable instrument for furthering lung cancer research and treatment.

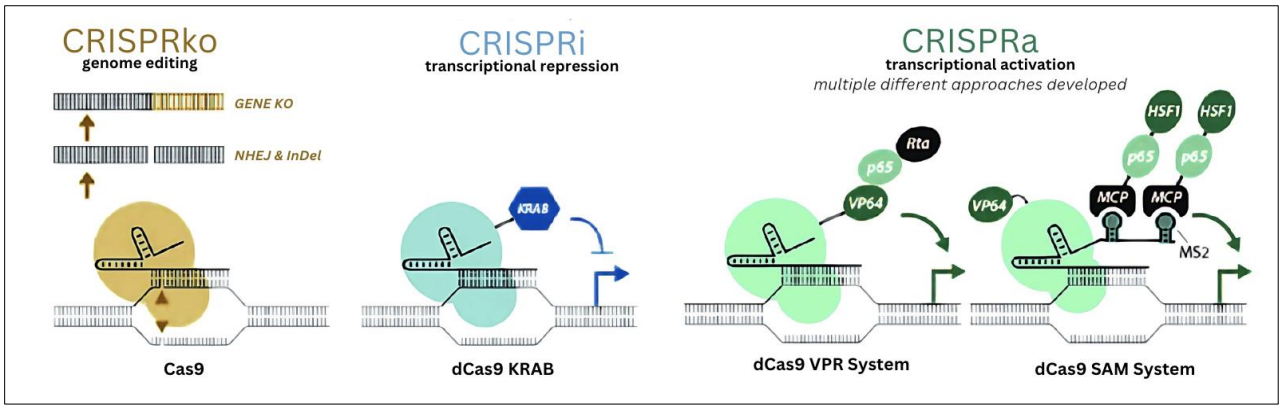

Figure 5 Three different CRISPR approaches: CRISPRko (knockout), CRISPRi (interference), and CRISPRa (activation). CRISPRko uses Cas9 to create double-strand breaks, causing gene knockouts. CRISPRi utilizes dCas9 fused with KRAB to repress gene transcription, while CRISPRa employs activators to upregulate gene expression without cutting DNA. Taken from Sage et al [105]

Table 2 Use of genome-wide CRISPR screens across several cancer research areas, focusing on chemotherapy resistance, radiation resistance, and identifying novel cancer targets. CRISPR screens have been crucial in identifying vulnerabilities in lung cancer cells, such as genes involved in resistance to chemotherapy, providing insights into potential new therapeutic targets. Taken from Shen et al [66]

	Library	Stress	Model	Readout.	Refs.
Overcoming chemotherapy resistance	Genome-wide CRISPR activation	EGFR inhibitor osimertinib	Human lung cancer PC9 cells	NA	Müller et al. (2023)
	Genome-wide knockout	cisplatin (DDP) or etoposide (VP16)	Human chemo-resistant SCLC cell line H69-AR	*In vitro* growth for 7 days	*Deng et al. (2023)*
	Brunello human genome-wide	CHK1 inhibitor prexasertib	Human NSCLC cell lines A549-Cas9 and NCI-H460-	*In vitro* growth for	*Braniga n et al.*

	lentiviral gRNA pooled library		Cas9	19 days	*(2021)*
	Genome-wide CRISPR interference	CDK9 inhibitor and MCL1 inhibitor	Human lung cancer LK2 cells c-KRAB	NA	Kabir et al. (2019)
Overcoming radiation resistance	Genome-wide knockout	Radiation	Human NSCLC cell line A549	*In vitro* growth for 4 days	*Cheng et al. (2021)*
Identifying novel targets	4,915 mouse genes corresponding to 5,347 human orthologs	NA	Cell lines derived from genetically engineered mouse models (GEMMs) of three cancers: SCLC ($Trp53^{-/-}$; $Rb1^{-/-}$), LUAD ($Kras^{G12D/+}$; $Trp53^{-/-}$), and PDAC ($Kras^{G12D/+}$; $Trp53^{R172H/-}$)	*In vivo* growth for 26 days	*Li et al. (2019)*
	Genome-scale knockout	NA	Lung cancer cell line, EKVX, for stable expression of Cas9	*In vivo* growth for 2 weeks	*Liu et al. (2022)*
	Genome-wide knockout	NA	Five cell lines derived from a genetically engineered autochthonous Rb1/Trp53-deleted SCLC mouse model	NA	Norton et al. (2021)
	Druggable-genome library targeting 4,915 genes	NA	KP tumor-derived cell lines	After 8 population doublings	Romero et al. (2020)

2.2.3. CRISPR in Early Detection and Diagnosis for Lung Cancers

Another method of utilizing CRISPR in lung cancer is through early detection and diagnosis using microRNAs (miRNAs) and crRNAs designed to target specific mutations.

Qiu et al [106] developed an innovative, sensitive, and cost-effective CRISPR-Cas9-based method for detecting miRNAs, which holds promise for lung cancer detection, specifically for NSCLC. Using rolling circle amplification (RCA), the target miRNA, let-7a, a known biomarker for NSCLC and has been meta-analyzed by Zhang et al [107] in its viability in cancer research as a biomarker, is captured using probes that initiate amplification by the phi-29 enzyme [106,107]. It then becomes a target for a dCas9 protein and fused with fragments of split-horseradish peroxidase (split-HRP). This fusion enables further signal amplification, where the dCas9 precisely binds to the amplified miRNA, resulting in a detectable color change in the presence of the TMB substrate, which is often used in simulation with split-HRP to produce a blue-colored reaction in response to enzyme activity [108]. The system showed exceptional sensitivity, detecting miRNA concentrations as low as 1 femtomolar (fM) and distinguishing even single-base mismatches, demonstrating its accuracy as specific concentrations of miRNA serve as biomarkers of certain lung cancers. Additionally, this method effectively identified the miRNA let-7a in serum samples, differentiating NSCLC patients from healthy individuals, making it a highly promising non-invasive diagnostic tool. Its high sensitivity and low cost make it a valuable tool in lung cancer treatment, as early detection is crucial in improving the chances of successful treatment and survival [106].

Additionally, a study by Zhou et al [109] focused on using the CRISPR–Cas12a system to detect KRAS mutations with greater sensitivity compared to traditional PCR methods. The procedure involved amplifying DNA samples from lung cancer tissues using PCR, followed by the use of CRISPR–Cas12a with crRNAs designed to target specific KRAS mutations at G12C, G12S, and G12D sites. The fluorescent readout indicated whether mutations were present. This method demonstrated a limit of detection at 0.01%, compared to PCR's 0.1%, and was able to detect mutations in five out of twenty tumor samples, whereas PCR only detected them in three. The CRISPR-Cas12a-based detection method proved to be more advantageous due to its high sensitivity, specificity, and rapid detection capability for KRAS mutations, which are responsible for about 30% of NSCLC [110]. Compared to traditional methods, CRISPR-Cas12a offers faster results

without the need for complex equipment, making it more accessible for clinical settings. Its ability to differentiate between mutant and wild-type sequences with high specificity makes it particularly effective for detecting mutations in a heterogeneous sample [109].

Both studies have proven prevalent in the discussion of CRISPR technology in lung cancer, however, the key difference lies in the target molecules—Qiu et al [106] targeted miRNA levels as an indirect marker of cancer, while Zhou et al [109] targeted specific genetic mutations, KRAS, in tumor DNA. This makes Qiu et al's [106] approach broader for early detection through biomarkers, whereas Zhou et al's [109] approach offers high specificity for known genetic mutations in lung cancer diagnostics.

2.3. Future Perspectives: Potential for Utilizing CRISPR to Eliminate Tumorigenic Effects of EGFR Mutations in Lung Cancers

A few of the most common types of persistent proto-oncogenes in lung cancers include KRAS, EGFR, and ALK driving tumor growth, metastasis, and resistance to therapies [111]. Current treatments often face challenges due to tumor heterogeneity and the adaptive nature of these proto-oncogenes, leading to limited long-term efficacy, which can also be seen in previous studies mentioned above [112]. The interest in applying CRISPR to proto-oncogenes comes from their linked resistance to traditional therapies, making them ideal for CRISPR-mediated intervention – specifically, EGFR which is a proto-oncogene that plays a critical role in lung cancer and NSCLC. Mutations in the EGFR gene lead to its persistent activation, driving tumorigenesis, uncontrolled cell growth, and resistance to standard therapies [113]. Targeting the EGFR pathway has been a cornerstone of lung cancer treatment, but resistance to EGFR inhibitors often develops, necessitating novel therapeutic strategies. While tyrosine kinase inhibitors (TKIs) like osimertinib have shown efficacy in targeting EGFR, the emergence of drug-tolerant persister cells (DTPs) poses a major challenge, leading to treatment resistance [114]. This is why, the potential of using CRISPR technology to mitigate the tumorigenic effects of mutant EGFR in lung cancer cells by activating alternative tumor suppressor pathways that counteract EGFR signaling is imperative to further lung cancer studies.

The EGFR proto-oncogene is a well-established driver of NSCLC, with activating mutations such as exon 19 deletions and L858R substitutions leading to constitutive signaling that promotes tumor growth, survival, and metastasis [115]. However, it has been identified that DTPs develop resistance to TKIs like osimertinib, possessing vulnerabilities in pathways such as MEK, AURKB, BRD4, and TEAD, and through secondary mutations, like the T790M mutation, rendering the inhibitors less effective over time [116].

In agreement with Tang et al's [117] proposal to overcome EGFR mutations via CRISPR using gRNAs to induce DSBs at these mutation sites to interrupt EGFR protein activity, we propose a similar theory with some alterations.

We agree with their use of HDR to induce a stop codon or an indel using NHEJ to alter the EGFR protein to a non-functional state, leaving it without oncogenic characteristics. However, instead of broadening which TK domains to be affected by CRISPR, narrowing it to 3 classes of EGFR mutations: an L858R missense mutation, deletions in exon 19, and insertions in exon 20, which are known to represent the most common alteration in EGFR mutant NSCLC, will be more beneficial [118]. They are known to influence the tumor's sensitivity to treatments [119].

Additionally, while the proposed delivery method has the potential to work, using lipid nanoparticles (LNPs) in collaboration with inhalation delivery will prove to be more advantageous than packaging CRISPR systems into viruses. Intratracheal delivery ensures that the CRISPR components reach the lung tissue efficiently, making it an effective treatment for lung cancers, whereas intravascular delivery can treat both primary lung tumors and metastases, offering a broader treatment approach [120,121]. Yet, both intratracheal and intravascular delivery might face challenges in fully penetrating tumor tissues, especially in dense or poorly vascularized areas of the lung [122] Additionally, inhalation proves to be more successful in distributing content particles more evenly in comparison to intratracheal delivery, which will be more beneficial in the context of LNP delivery [123].

LNPs are nonviral delivery vehicles capable of encapsulating large nucleic acid payloads, such as the Cas9 mRNA and sgRNA needed for CRISPR applications [124]. In contrast to viral vectors, which are often restricted by carrying capacity, immunogenicity, and cellular targeting limitations, LNPs have shown the ability to bypass these issues effectively [125,126,127]. Using LNPs for delivering CRISPR components enhances the therapy's precision and reduces the likelihood of off-target effects [127]. Additionally, LNPs can be chemically modified to improve stability and minimize immune responses, making them more favorable for inhalation-based delivery. The inhalation method allows for localized and uniform targeting directly to the lungs, which improves drug concentration in the target tissue while minimizing systemic exposure, potential toxicity, and bypassing first-pass metabolism, thereby minimizing side effects

[128]. In comparison to the intratracheal or intravascular methods proposed by Tang et al [117], incorporating CRISPR technology with LNP-based delivery through inhalation provides enhanced therapeutic efficacy, better targeting capabilities, and reduced side effects, making it a superior method for addressing lung cancer compared to traditional viral vector-based delivery systems. While the inhalation method does have its shortcomings from lack of control over the dosage, greater expense, and impotent in handling highly toxins, in this context, it works as a far better delivery method with LNPs [128].

Furthermore, the combination of CRISPR therapy with existing lung cancer treatments holds great potential due to the complexities posed by tumor heterogeneity. In lung cancer, subpopulations of cancer cells often carry different genetic mutations, making it difficult for a single treatment to be effective against all cells [129] By integrating CRISPR with established treatments like chemotherapy or tyrosine kinase inhibitors, a more comprehensive attack on diverse cancer cell populations becomes possible. For instance, chemotherapy works by targeting rapidly dividing cells but may miss slower-growing cancer cells with specific mutations [130]. Using CRISPR alongside chemotherapy could ensure that these resistant cells, often driven by persistent oncogenes, are also targeted, reducing the chances of relapse. Additionally, TKIs, which target specific signaling pathways in cancer cells, can be paired with CRISPR to knock out genes that contribute to resistance mechanisms [131]. This dual approach enhances the likelihood of attacking cancer cells from multiple angles, preventing them from adapting to the CRISPR treatment.

An aspect to consider when approaching the use of CRISPR in EGFR mutations is minimizing off-targets. Various studies such as Goyal et al [83] and others in the context of CRISPRi have seen off-target effects negatively affecting CRISPR's efficacy. One way to tackle this is by using high-fidelity Cas9 variants, such as eSpCas9 and SpCas9-HF1, which are engineered to enhance specificity by altering Cas9 structure to reduce interactions with unintended DNA sequences [132]. This reduces the risk of off-target mutations, which is particularly important in lung tissue, where unintended edits could disrupt crucial genes and exacerbate the disease [133]. Another key approach is multiple gRNA screening. By designing and pre-screening several gRNAs, researchers can select those with the highest specificity and minimal off-target effects for EGFR mutations. This ensures that the CRISPR system accurately targets cancerous cells without affecting healthy lung cells, addressing the genetic diversity within lung tumors [134].

Lastly, regarding the testing of utilizing CRISPR in EGFR, a series of *in vitro* and *in vivo* studies could be conducted. EGFR-mutant lung cancer cell lines such as PC9, H1975, and HCC827 can be employed to evaluate the efficacy and specificity of CRISPR-mediated knockout or repression of EGFR mutations. These cell lines possess different EGFR mutations, such as exon 19 deletions and the T790M resistance mutation, which makes them ideal for testing CRISPR systems targeting EGFR [135]. For in vitro experiments, cells can be treated with CRISPR reagents to disrupt or suppress EGFR expression. Additionally, to assess CRISPR's therapeutic potential in a complex biological environment, patient-derived xenografts (PDX) can be used, which involve implanting human lung cancer tissue with EGFR mutations into immunocompromised mice. These models maintain the tumor's genetic heterogeneity and microenvironment, providing a realistic setting for testing CRISPR therapy [136]. Additionally, genetically engineered mouse models (GEMMs) harboring EGFR mutations, such as L858R or T790M, enable the evaluation of CRISPR-mediated editing directly in the lung tissue [137]. Administering CRISPR via LNPs in these models allows for tracking efficiency, tumor regression, and off-target effects, thereby determining the potential of CRISPR as a targeted lung cancer therapy.

3. Conclusion

Precision medicine has made significant strides with the introduction of CRISPR technology into lung cancer treatment. CRISPR provides a way to directly modify genes that cause cancer by focusing on persistent oncogenes like EGFR, offering a path to overcome the inherent challenges of tumor heterogeneity and resistance to conventional therapies. This review has emphasized brilliant approaches such as integrating CRISPR with existing therapeutic strategies to tackle multiple tumor cell types efficiently. The potential of CRISPR to offer long-lasting and efficient therapeutic choices appears to be increasingly captivating and effective as research advances. Further studies and clinical trials will be crucial in validating these approaches and ensuring they can be safely and effectively translated into clinical practice, potentially revolutionizing the treatment paradigm for lung cancer.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Siegel, R. L., Giaquinto, A. N., Jemal, A. Cancer statistics, 2024. CA: A Cancer Journal for Clinicians 2024 Jan 17, 74(1), 12.

[2] Debela, D. T., Muzazu, S. G., Heraro, K. D., Ndalama, M. T., Mesele, B. W., Haile, D. C., Kitui, S. K., Manyazewal, T. New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Medicine 2021 Aug 12, 9

[3] Krzyszczyk P, Acevedo A, Davidoff EJ, Timmins LM, Marrero-Berrios I, Patel M, White C, Lowe C, Sherba JJ, Hartmanshenn C, O'Neill KM, Balter ML, Fritz ZR, Androulakis IP, Schloss RS, Yarmush ML. The growing role of precision and personalized medicine for cancer treatment. Technology (Singap World Sci). 2018 Sep-Dec, 6(3-4):79-100

[4] Brown JS, Amend SR, Austin RH, Gatenby RA, Hammarlund EU, Pienta KJ. Updating the Definition of Cancer. Mol Cancer Res. 2023 Nov 1;21(11)

[5] Cooper GM. The Cell: A Molecular Approach. 2nd edition. Sunderland (MA): Sinauer Associates; 2000. The Development and Causes of Cancer.

[6] Siddiqui F, Vaqar S, Siddiqui AH. Lung Cancer. [Updated 2023 May 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan.

[7] Bagheri R, Haghi SZ, Dalouee MN, Nasiri Z, Rajabnejad A. Evaluation of the results of surgery treatment in patients with benign lung tumors. Lung India. 2015 Jan-Feb;32(1):29-33

[8] Mercadante AA, Kasi A. Genetics, Cancer Cell Cycle Phases. [Updated 2023 Aug 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan

[9] Baba AI, Câtoi C. Comparative Oncology. Bucharest (RO): The Publishing House of the Romanian Academy; 2007. Chapter 2, CARCINOGENESIS

[10] Hill W, Weeden CE, Swanton C. Tumor Promoters and Opportunities for Molecular Cancer Prevention. Cancer Discov. 2024 Jul 1;14(7)

[11] Celià-Terrassa T, Kang Y. Distinctive properties of metastasis-initiating cells. Genes Dev. 2016 Apr 15;30(8)

[12] Burger GA, van de Water B, Le Dévédec SE, Beltman JB. Density-Dependent Migration Characteristics of Cancer Cells Driven by Pseudopod Interaction. Front Cell Dev Biol. 2022 Apr 25;10

[13] National Institutes of Health (US); Biological Sciences Curriculum Study. NIH Curriculum Supplement Series [Internet]. Bethesda (MD): National Institutes of Health (US); 2007. Understanding Cancer.

[14] Martin TA, Ye L, Sanders AJ, et al. Cancer Invasion and Metastasis: Molecular and Cellular Perspective. In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience; 2000-2013

[15] Yayan J, Franke KJ, Berger M, Windisch W, Rasche K. Adhesion, metastasis, and inhibition of cancer cells: a comprehensive review. Mol Biol Rep. 2024 Jan 22;51(1):165

[16] Stages of Non-Small Cell Lung Cancer [Internet]. CancerHelp®; © 2017 [cited 2024 Oct. 5]. Available from https://cancerhelpessentiahealth.org/

[17] Haschek WM, Rousseaux CG, Wallig MA, Fundamentals of Toxicologic Pathology (Second Edition), Academic Press, 2010, 9-42,

[18] Chial, H., Proto-oncogenes to oncogenes to cancer. Nature Education, 2008 1(1):33

[19] Song X, Liu C, Wang N, Huang H, He S, Gong C, Wei Y, Delivery of CRISPR/Cas systems for cancer gene therapy and immunotherapy, Advanced Drug Delivery Reviews, 2021 Jan, 168, 158-180.

[20] Tchakarska G, Sola B. The double dealing of cyclin D1. Cell Cycle. 2020 Jan;19(2):163-178.

[21] Kaufman NEM, Dhingra S, Jois SD, Vicente MDGH. Molecular Targeting of Epidermal Growth Factor Receptor (EGFR) and Vascular Endothelial Growth Factor Receptor (VEGFR). Molecules. 2021 Feb 18;26(4)

[22] Purba ER, Saita EI, Maruyama IN. Activation of the EGF Receptor by Ligand Binding and Oncogenic Mutations: The "Rotation Model". Cells. 2017 Jun 2;6(2):13.

[23] Wee P, Wang Z. Epidermal Growth Factor Receptor Cell Proliferation Signaling Pathways. Cancers (Basel). 2017 May 17;9(5):52

[24] Xu Y, Li Z. CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. Comput Struct Biotechnol J. 2020 Sep 8;18:2401-2415.

[25] Tamura R, Toda M., Historic Overview of Genetic Engineering Technologies for Human Gene Therapy. Neurol Med Chir (Tokyo). 2020 Oct 15;60(10):483-491.

[26] Ishino Y, Krupovic M, Forterre P. History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology. J Bacteriol. 2018 Mar 12;200(7)

[27] Rath D,Amlinger L, Rath A, Lundgren M, The CRISPR-Cas immune system: Biology, mechanisms and applications, Biochimie, Oct 2015, 17,119-128

[28] Tao S, Chen H, Li N, Liang W. The Application of the CRISPR-Cas System in Antibiotic Resistance. Infect Drug Resist. Aug 2022 ;15

[29] Hillary VE, Ceasar SA. A Review on the Mechanism and Applications of CRISPR/Cas9/Cas12/Cas13/Cas14 Proteins Utilized for Genome Engineering. Mol Biotechnol. Mar 2023; 65(3):311-325

[30] Le Rhun A, Escalera-Maurer A, Bratovič M, Charpentier E. CRISPR-Cas in Streptococcus pyogenes. RNA Biol. 2019 Apr;16(4):380-389.

[31] Katti, A., Diaz, B.J., Caragine, C.M. et al. CRISPR in cancer biology and therapy. Nat Rev Cancer. Feb 2022. 22, 259–279

[32] Xue C, Greene EC. DNA Repair Pathway Choices in CRISPR-Cas9-Mediated Genome Editing. Trends Genet. 2021 Jul;37(7):639-656.

[33] Mao Z, Bozzella M, Seluanov A, Gorbunova V. DNA repair by nonhomologous end joining and homologous recombination during cell cycle in human cells. Cell Cycle. 2008 Sep 15;7(18):2902-6

[34] Allemailem KS, Almatroodi SA, Almatroudi A, Alrumaihi F, Al Abdulmonem W, Al-Megrin WAI, Aljamaan AN, Rahmani AH, Khan AA. Recent Advances in Genome-Editing Technology with CRISPR/Cas9 Variants and Stimuli-Responsive Targeting Approaches within Tumor Cells: A Future Perspective of Cancer Management. Int J Mol Sci. 2023 Apr 11;24(8):7052.

[35] Gostimskaya I. CRISPR-Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing. Biochemistry (Mosc). 2022 Aug;87(8):777-788

[36] Liu H, Zhu Y, Li M, Gu Z. Precise genome editing with base editors. Med Rev (2021). 2023 Feb 22;3(1):75-84

[37] Zhang D, Zhang Z, Unver T, Zhang B. CRISPR/Cas: A powerful tool for gene function study and crop improvement. J Adv Res. 2020 Oct 21;29:207-221.

[38] Lino CA, Harper JC, Carney JP, Timlin JA. Delivering CRISPR: a review of the challenges and approaches. Drug Deliv. 2018 Nov;25(1):1234-1257

[39] Baghini S, Gardanova ZR, Abadi SAH, Zaman BA, İlhan A, Shomali N, Adili A, Moghaddar R, Yaseri AF. CRISPR/Cas9 application in cancer therapy: a pioneering genome editing tool. Cell Mol Biol Lett. 2022 May 4;27(1):35.

[40] Nussinov R, Jang H, Tsai CJ, Cheng F. Review: Precision medicine and driver mutations: Computational methods, functional assays and conformational principles for interpreting cancer drivers. PLoS Comput Biol. 2019 Mar 28;15(3)

[41] Porta-Pardo E, Valencia A, Godzik A. Understanding oncogenicity of cancer driver genes and mutations in the cancer genomics era. FEBS Lett. 2020 Dec;594(24):4233-4246.

[42] Dana H, Chalbatani GM, Mahmoodzadeh H, Karimloo R, Rezaiean O, Moradzadeh A, Mehmandoost N, Moazzen F, Mazraeh A, Marmari V, Ebrahimi M, Rashno MM, Abadi SJ, Gharagouzlo E. Molecular Mechanisms and Biological Functions of siRNA. Int J Biomed Sci. 2017 Jun;13(2):48-57.

[43] Austin CP, Battey JF, Bradley A, Bucan M, Capecchi M, Collins FS, Dove WF, Duyk G, Dymecki S, Eppig JT, Grieder FB, Heintz N, Hicks G, Insel TR, Joyner A, Koller BH, Lloyd KC, Magnuson T, Moore MW, Nagy A, Pollock JD, Roses AD, Sands AT, Seed B, Skarnes WC, Snoddy J, Soriano P, Stewart DJ, Stewart F, Stillman B, Varmus H, Varticovski L, Verma IM, Vogt TF, von Melchner H, Witkowski J, Woychik RP, Wurst W, Yancopoulos GD, Young SG, Zambrowicz B. The knockout mouse project. Nat Genet. 2004 Sep;36(9):921-4.

[44] Ishibashi, A., Saga, K., Hisatomi, Y. et al. A simple method using CRISPR-Cas9 to knock-out genes in murine cancerous cell lines. Sci Rep. December 2020; 10

[45] Limaye A, Hall B, Kulkarni AB. Manipulation of mouse embryonic stem cells for knockout mouse production. Curr Protoc Cell Biol. 2009 Sep; 19 (19).

[46] Gurumurthy CB, Lloyd KCK. Generating mouse models for biomedical research: technological advances. Dis Model Mech. 2019 Jan;12(1).

[47] Jung CJ, Zhang J, Trenchard E, Lloyd KC, West DB, Rosen B, de Jong PJ. Efficient gene targeting in mouse zygotes mediated by CRISPR/Cas9-protein. Transgenic Res. 2017 Apr;26(2):263-277.

[48] Wei W, Chen ZN, Wang K. CRISPR/Cas9: A Powerful Strategy to Improve CAR-T Cell Persistence. Int J Mol Sci. 2023 Aug 1;24(15)

[49] Tasian SK, Gardner RA. CD19-redirected chimeric antigen receptor-modified T cells: a promising immunotherapy for children and adults with B-cell acute lymphoblastic leukemia (ALL). Ther Adv Hematol. 2015 Oct;6(5):228-41.

[50] Stenger, D.; Stief, T. A.; Kaeuferle, T.; Willier, S.; Rataj, F.; Schober, K.; Vick, B.; Lotfi, R.; Wagner, B.; Grünewald, T. G.; Kobold, S.; Busch, D. H.; Jeremias, I.; Blaeschke, F.; Feuchtinger, T. Endogenous TCR promotes in vivo persistence of CD19-car-T cells compared to a CRISPR/Cas9-mediated TCR knockout CAR. Blood. September 2020,136(12), 1407–1418.

[51] Justiz Vaillant AA, Modi P, Mohammadi O. Graft-Versus-Host Disease. StatPearls. Treasure Island (FL): StatPearls Publishing; 2024 Jan.

[52] Szeto C, Lobos CA, Nguyen AT, Gras S. TCR Recognition of Peptide-MHC-I: Rule Makers and Breakers. Int J Mol Sci. 2020 Dec 23;22(1):68.

[53] Cappell, K.M., Kochenderfer, J.N. A comparison of chimeric antigen receptors containing CD28 versus 4-1BB costimulatory domains. Nat Rev Clin Oncol, May 2021, 18, 715–727.

[54] Bouza E, Martín Jiménez M, Alemany L, Arribas J, Bañares R, Barragán MB, Eiros Bouza JM, Felip E, Fernández-Capetillo O, Gracia D, López-Vélez R, Bautista Mollar J, Muñoz P, Paz-Ares L, Torné A, Tovar J, Valencia E, Palomo E. Overview of virus and cancer relationships. Position paper. Rev Esp Quimioter. 2021 Dec;34(6):525-555.

[55] Moore PS, Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. Nat Rev Cancer. 2010 Dec;10(12):878-89.

[56] Inturi R, Jemth P. CRISPR/Cas9-based inactivation of human papillomavirus oncogenes E6 or E7 induces senescence in cervical cancer cells. Virology. Oct 2021. 562, 92-102,

[57] Purcell M, Kruger A, Tainsky MA. Gene expression profiling of replicative and induced senescence. Cell Cycle. Dec 2014;13(24):3927-37.

[58] Boulares H, Yakovlev A, Ivanova V, Stoica B, Wang G, Iyer S, Smulson M. Role of Poly(ADP-ribose) Polymerase (PARP) Cleavage in Apoptosis: CASPASE 3-RESISTANT PARP MUTANT INCREASES RATES OF APOPTOSIS IN TRANSFECTED CELLS. Journal of Biological Chemistry. Aug 1999. 274(33). 22932-22940

[59] Sorolla A, Parisi E, Sorolla MA, Marques M, Parcel JM. Applications of CRISPR technology to lung cancer research. European Respiratory Journal. 2022 Jan; 59(1).

[60] Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR, Dahlman JE, Parnas O, Eisenhaure TM, Jovanovic M, Graham DB, Jhunjhunwala S, Heidenreich M, Xavier RJ, Langer R, Anderson DG, Hacohen N, Regev A, Feng G, Sharp PA, Zhang F. CRISPR-Cas9 knockin mice for genome editing and cancer modeling. Cell. 2014 Oct 9;159(2):440-55.

[61] Carullo NVN, Hinds JE, Revanna JS, Tuscher JJ, Bauman AJ, Day JJ. A Cre-Dependent CRISPR/dCas9 System for Gene Expression Regulation in Neurons. eNeuro. 2021 Aug 18;8(4)

[62] Román M, Baraibar I, López I, Nadal E, Rolfo C, Vicent S, Gil-Bazo I. KRAS oncogene in non-small cell lung cancer: clinical perspectives on the treatment of an old target. Molecular Cancer. Feb 2018. 17(33).

[63] Hashimoto T, Owada Y, Katagiri H, Yakuwa K, Tyo K, Sugai M, Fuzimura I, Utsumi Y, Akiyama M, Nagashima H, Terasaki H, Yanagawa N, Saito H, Sugai T, Maemondo M. Characteristics and prognostic analysis of patients with detected KRAS mutations in resected lung adenocarcinomas by peptide nucleic acid-locked nucleic acid polymerase chain reaction (PNA-LNA PCR) clamp method. Transl Lung Cancer Res. 2023 Sep 28;12(9):1862-1875.

[64] Polcz S, Lewis A. CRISPR-Cas9 and the non-germline non-controversy. Journal of Law and the Biosciences. 2016 Aug. 3(2). 413

[65] Foo J, Leder K, Michor F. Stochastic dynamics of cancer initiation. Phys Biol. 2011 Feb;8(1).

[66] Shen W, Hu F, Pan L, Tang Y. Applications of CRISPR screening to lung cancer treatment. Frontier Cell Development Biology. Dec 2023. 11.

[67] Zhao Y, Zhang M, Yang D. Bioinformatics approaches to analyzing CRISPR screen data: from dropout screens to single-cell CRISPR screens. Quant Biol. 2022 Dec;10(4):307-320.

[68] Wang, SW., Gao, C., Zheng, YM, Lu JC, Huang XY, Cai JB, Zhang PF, Cui YH, Ke AW. Current applications and future perspective of CRISPR/Cas9 gene editing in cancer. Molecular Cancer, Feb 2022, 21(57)

[69] Martin JC, Sims JR, Gupta A, Hagoel TJ, Gao L, Lynch M, Woloszynska A, Melendy T, Kane J, Kuechle J, Ohm J. CDC7 kinase (DDK) inhibition disrupts DNA replication leading to mitotic catastrophe in Ewing sarcoma. Cell Death Discovery. Feb 2022. 8(85)

[70] Deng L, Yang L, Zhu S, Li M, Wang Y, Cao X, Wang Q, Guo L. Identifying CDC7 as a synergistic target of chemotherapy in resistant small-cell lung cancer via CRISPR/Cas9 screening. Cell Death Discov. 2023 Feb 2;9(1).40.

[71] Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol. 2014 Oct 5; 740. 364-78

[72] Cheng C, Pei X, Li SW, Yang J, Li C, Tang J, Hu K, Huang G, Min WP, Sang Y. CRISPR/Cas9 library screening uncovered methylated PKP2 as a critical driver of lung cancer radioresistance by stabilizing β-catenin. Oncogene. Mar 2021. 40

[73] Wu Y, Liu L, Shen X, Liu W, Ma R. Plakophilin-2 Promotes Lung Adenocarcinoma Development via Enhancing Focal Adhesion and Epithelial-Mesenchymal Transition. Cancer Manag Res. 2021 Jan 22;13. 559-570.

[74] Kumari N, Antil H, Kumari S, Raghavan SC. Deficiency of ligase IV leads to reduced NHEJ, accumulation of DNA damage, and can sensitize cells to cancer therapeutics. Genomics. 2023 Nov. 115(6).

[75] Evron T, Caspi M, Kazelnik M, Shor-Nareznoy Y, Armoza-Eilat S, Kariv R, Manber Z, Elkon R, Sklan EH, Rosin-Arbesfeld R. A CRISPR knockout screen reveals new regulators of canonical Wnt signaling. Oncogenesis. 2021 Sep 22;10(9).63.

[76] Liu J, Xiao Q, Xiao J, Niu C, Li Y, Zhang X, Zhou Z, Shu G, Yin G. Wnt/β-catenin signaling: function, biological mechanisms, and therapeutic opportunities. Signal Transduct Target Ther. 2022 Jan 3;7(1):3.

[77] Sanson KR, Hanna RE, Hegde M, Donovan KF, Strand C, Sullender ME, Vaimberg EW, Goodale A, Root DE, Piccioni F, Doench JG. Optimized libraries for CRISPR-Cas9 genetic screens with multiple modalities. Nat Commun. 2018 Dec 21;9(1)

[78] Yang C, Lei Y, Ren T, Yao M. The Current Situation and Development Prospect of Whole-Genome Screening. Int J Mol Sci. 2024 Jan 4;25(1)

[79] Cai R, Lv R, Shi X, Yang G, Jin J. CRISPR/dCas9 Tools: Epigenetic Mechanism and Application in Gene Transcriptional Regulation. Int J Mol Sci. 2023 Oct 3;24(19)

[80] Ying Y, Yang X, Zhao K, Mao J, Kuang Y, Wang Z, Sun R, Fei J. The Krüppel-associated box repressor domain induces reversible and irreversible regulation of endogenous mouse genes by mediating different chromatin states. Nucleic Acids Res. 2015 Feb 18;43(3):1549-61.

[81] Raffeiner P, Hart J, García-Caballero D, Bar-Peled L, Weinberg MS, Vogt PK. An MXD1-derived repressor peptide identifies noncoding mediators of MYC-driven cell proliferation. PNAS. 2020 Mar. 117(12). 6571-6579.

[82] Fatma H, Siddique HR. Role of long non-coding RNAs and MYC interaction in cancer metastasis: A possible target for therapeutic intervention. Toxicology and Applied Pharmacology. 2020 July. 399

[83] Goyal A, Myacheva K, Groß M, Klingenberg M, Duran Arqué B, Diederichs S. Challenges of CRISPR/Cas9 applications for long non-coding RNA genes. Nucleic Acids Res. 2017 Feb 17;45(3)

[84] Hannus M, Beitzinger M, Engelmann JC, Weickert MT, Spang R, Hannus S, Meister G. siPools: highly complex but accurately defined siRNA pools eliminate off-target effects. Nucleic Acids Res. 2014 Jul;42(12):8049-61.

[85] Dhuri K, Bechtold C, Quijano E, Pham H, Gupta A, Vikram A, Bahal R. Antisense Oligonucleotides: An Emerging Area in Drug Discovery and Development. J Clin Med. 2020 Jun 26;9(6)

[86] Chehelgerdi, M., Chehelgerdi, M., Khorramian-Ghahfarokhi, M. et al. Comprehensive review of CRISPR-based gene editing: mechanisms, challenges, and applications in cancer therapy. Mol Cancer 23, 9 (2024)

[87] Liu W, Li L, Jiang J, Wu M, Lin P. Applications and challenges of CRISPR-Cas gene-editing to disease treatment in clinics. Precis Clin Med. 2021 Jul 10;4(3):179-191.

[88] Ding X, Yu L, Chen L, Li Y, Zhang J, Sheng H, Ren Z, Li Y, Yu X, Jin S, Cao J. Recent Progress and Future Prospect of CRISPR/Cas-Derived Transcription Activation (CRISPRa) System in Plants. Cells. 2022 Sep 28;11(19).

[89] Zhang X, Lv S, Zhenhuan L, Hu Y, Peng X, Lv J, Zhao S, Feng J, Huang G, Wan GL, Lui J, Huang H, Luan B, Wang D, Zhao X, LinY, Zhou Q, Zhang ZN, Rong Z. MiniCAFE, a CRISPR/Cas9-based compact and potent transcriptional activator, elicits gene expression in vivo. Nucleic Acids Research. 2021 Jun. 49(7). 6002.

[90] Heidersbach AJ, Dorighi KM, Gomez JA, Jacobi AM, Haley B. A versatile, high-efficiency platform for CRISPR-based gene activation. Nature Communications. 2023 Feb. 14(902).

[91] Kim MH, Kim J. Role of YAP/TAZ transcriptional regulators in resistance to anti-cancer therapies. Cell Mol Life Sci. 2017 Apr;74(8):1457-1474.

[92] Müller N, Lorenz C, Ostendorp J, Heisel FS, Friese UP, Cartolano M, Plenker D, Tumbrink H, Heimsoeth A, Baedeker P, Weiss J, Ortiz-Cuaran S, Büttner R, Peifer M, Thomas RK, Sos ML, Berg J, Brägelmann J. Characterizing Evolutionary Dynamics Reveals Strategies to Exhaust the Spectrum of Subclonal Resistance in EGFR-Mutant Lung Cancer. Cancer Res. 2023 Aug 1;83(15):2471-2479.

[93] Zeng R, Dong J. The Hippo Signaling Pathway in Drug Resistance in Cancer. Cancers (Basel). 2021 Jan 16;13(2):318.

[94] Sattler M, Mambetsariev I, Fricke J, Tan T, Liu S, Vaidehi N, Pisick E, Mirzapoiazova T, Rock AG, Merla A, Sharma S, Salgia R. A Closer Look at EGFR Inhibitor Resistance in Non-Small Cell Lung Cancer through the Lens of Precision Medicine. J Clin Med. 2023 Mar 1;12(5)

[95] Yang Z, Liang SQ, Saliakoura M, Yang H, Vassella E, Konstantinidou G, Tschan M, Hegedüs B, Zhao L, Gao Y, Xu D, Deng H, Marti TM, Kocher GJ, Wang W, Schmid RA, Peng RW. Synergistic effects of FGFR1 and PLK1 inhibitors target a metabolic liability in KRAS-mutant cancer. EMBO Mol Med. 2021 Sep 7;13(9)

[96] Heist RS, Mino-Kenudson M, Sequist LV, Tammireddy S, Morrissey L, Christiani DC, Engelman JA, Iafrate AJ. FGFR1 amplification in squamous cell carcinoma of the lung. J Thorac Oncol. 2012 Dec;7(12):1775-1780.

[97] Zhang P, Yue L, Leng Q, Chang C, Gan C, Ye T, Cao D. Targeting FGFR for cancer therapy. Journal of Hematology and Oncology. 2024 Jun. 17(39)

[98] Du S, Zhang Y, Xu J. Current progress in cancer treatment by targeting FGFR signaling. Cancer Biol Med. 2023 Jul 24;20(7):490–9.

[99] Mah, LJ., El-Osta, A. & Karagiannis, T. γH2AX: a sensitive molecular marker of DNA damage and repair. Leukemia. 201 Deb. 24 679–686

[100] Patil M, Pabla N, Dong Z. Checkpoint kinase 1 in DNA damage response and cell cycle regulation. Cell Mol Life Sci. 2013 Nov;70(21):4009-21

[101] Lin WC, Lin FT, Nevins JR. Selective induction of E2F1 in response to DNA damage, mediated by ATM-dependent phosphorylation. Genes Dev. 2001 Jul 15;15(14):1833-44.

[102] Larson MH, Gilbert LA, Wang X, Lim WA, Weissman JS, Qi LS. CRISPR interference (CRISPRi) for sequence-specific control of gene expression. Nat Protoc. 2013 Nov;8(11):2180-96.

[103] Clark T, Waller MA, Loo L, Moreno CL, Denes CE, Neely GG.CRISPR activation screens: navigating technologies and applications. Trends in Biotechnology. 2024 Aug. 42(8). 1017-1034.

[104] Ravichandran M, Maddalo D. Applications of CRISPR-Cas9 for advancing precision medicine in oncology: from target discovery to disease modeling. Front Genet. 2023 Oct 16;14.

[105] Le Sage C, Lawo S, Cross BCS. CRISPR: A Screener's Guide. SLAS DISCOVERY Advancing the Science of Drug Discovery. 2019 Oct. 25(3)

[106] Qui XY, Zhu LY, ZhuCS, Ma JX, Hou T, Wu XM, Xe SS, Min L, Tan DA, Zhang DY, Zhue L. Highly Effective and Low-Cost MicroRNA Detection with CRISPR-Cas9. 2018 Feb. 7(3). 807–813

[107] Zhang WT, Zhang GX, Gao SS. The Potential Diagnostic Accuracy of Let-7 Family for Cancer: A Meta-Analysis. Technol Cancer Res Treat. 2021 Jul;20

[108] Josephy PD, Eling T, Mason RP. The horseradish peroxidase-catalyzed oxidation of 3,5,3',5'-tetramethylbenzidine. Free radical and charge-transfer complex intermediates. Journal of Biological Chemistry. 1982 Apr. 257(7). 3669-3675

[109] Zhou H, Tsou JH, Leng Q, Jiang F. Sensitive Detection of KRAS Mutations by Clustered Regularly Interspaced Short Palindromic Repeats. Pathology and Molecular Diagnostics. 2021 Jan. 11(1). 125

[110] Reita D, Pabst L, Pencreach E, Guérin E, Dano L, Rimelen V, Voegeli AC, Vallat L, Mascaux C, Beau-Faller M. Direct Targeting KRAS Mutation in Non-Small Cell Lung Cancer: Focus on Resistance. Cancers (Basel). 2022 Mar 4;14(5).

[111] Chevallier M, Borgeaud M, Addeo A, Friedlaender A. Oncogenic driver mutations in non-small cell lung cancer: Past, present and future. World J Clin Oncol. 2021 Apr 24;12(4):217-237.

[112] Maleki EH, Bahrami AR, Matin MM. Cancer cell cycle heterogeneity as a critical determinant of therapeutic resistance. Genes Dis. 2023 Jan 14;11(1):189-204.

[113] Iyer RS, Needham SR, Galdadas I, Davis B, Roberts SK, Man RC, Zanetti-Domingues LC, Clarke DT, Fruhwirth GO, Parker PJ, Rolfe DJ, Gervasio FL, Martin-Fernandez ML. Drug-resistant EGFR mutations promote lung cancer by stabilizing interfaces in ligand-free kinase-active EGFR oligomers. Nature Communications. 2024 Mar. 15(2130).

[114] Suda K, Mitsudomi T. Drug Tolerance to EGFR Tyrosine Kinase Inhibitors in Lung Cancers with EGFR Mutations. Cells. 2021 Jun 24;10(7):1590.

[115] Harrison PT, Vyse S, Huang PH. Rare epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer. Semin Cancer Biol. 2020 Apr;61:167-179.

[116] Criscione SW, Martin MJ, Oien DB, Gorthi A, Miragaia RJ, Zhang J, Chen H, Karl DL, Mendler K, Markovets A, Gagrica S, Delpuech O, Dry JR, Grondine M, Hattersley MM, Urosevic J, Floc'h N, Drew L, Yao Y, Smith PD. The landscape of therapeutic vulnerabilities in EGFR inhibitor osimertinib drug-tolerant persister cells. NPJ Precis Oncol. 2022 Dec 27;6(1):95.

[117] Tang H, Shrager JB. CRISPR/Cas-mediated genome editing to treat EGFR-mutant lung cancer: a personalized molecular surgical therapy. EMBO Mol Med. 2016 Feb 1;8(2):83-5.

[118] Das AK, Sato M, Story MD, Peyton M, Graves R, Redpath S, Girard L, Gazdar AF, Shay JW, Minna JD, Nirodi CS. Non–Small Cell Lung Cancers with Kinase Domain Mutations in the Epidermal Growth Factor Receptor Are Sensitive to Ionizing Radiation. Cancer Research. 2006 Oct. 66(19). 9601–9608.

[119] Wang F, Li C, Wu Q, Lu H. EGFR exon 20 insertion mutations in non-small cell lung cancer. Transl Cancer Res. 2020 Apr;9(4):2982-2991.

[120] DuPage M, Dooley AL, Jacks T. Conditional mouse lung cancer models using adenoviral or lentiviral delivery of Cre recombinase. Nat Protoc. 2009;4(7):1064-72.

[121] Vogl TJ, Shafinaderi M, Zangos S, Lindemayr S, Vatankhah K. Regional chemotherapy of the lung: transpulmonary chemoembolization in malignant lung tumors. Semin Intervent Radiol. 2013 Jun;30(2):176-84.

[122] Huang X, Chisholm J, Zhuang J, Xiao Y, Duncan G, Chen X, Suk JS, Hanes J. Protein nanocages that penetrate airway mucus and tumor tissue. PNAS. 2017 Jul. 114(32). 6595-6602.

[123] Osier M and Oberdorster G. Intratracheal Inhalation vs Intratracheal Instillation:Differences in Particle Effects. FUNDAMENTAL AND APPLIED TOXICOLOGY. 1997 Oct. 40, 220-227.

[124] Kazemian P, Yu SY, Thomson SB, Birkenshaw A, Leavitt BR, Ross CJD. Lipid-Nanoparticle-Based Delivery of CRISPR/Cas9 Genome-Editing Components. Mol Pharm. 2022 Jun 6;19(6):1669-1686.

[125] Wang H, Qin L, Zhang X, Guan J, Mao S. Mechanisms and challenges of nanocarriers as non-viral vectors of therapeutic genes for enhanced pulmonary delivery. J Control Release. 2022 Dec;352:970-993.

[126] Ghosh S, Brown AM, Jenkins C, Campbell K. Viral Vector Systems for Gene Therapy: A Comprehensive Literature Review of Progress and Biosafety Challenges. Appl Biosaf. 2020 Mar 1;25(1):7-18.

[127] Farsani MA, Mokhtari N, Nooraei S, Bahrulolum H, Akbari A, Farsani ZM, Khatami S, Ebadi MS, Ahmadian G. Lipid nanoparticles: The game-changer in CRISPR-Cas9 genome editing. Heliyon. 2024 Jan 11;10(2)

[128] Leong EWX, Ge R. Lipid Nanoparticles as Delivery Vehicles for Inhaled Therapeutics. Biomedicines. 2022 Sep 2;10(9):2179.

[129] Maleki EH, Bahrami AR, Matin MM. Cancer cell cycle heterogeneity as a critical determinant of therapeutic resistance. Genes Dis. 2023 Jan 14;11(1):189-204.

[130] Anand U, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, De Falco V, Upadhyay A, Kandimalla R, Chaudhary A, Dhanjal JK, Dewanjee S, Vallamkondu J, Pérez de la Lastra JM. Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. Genes Dis. 2022 Mar 18;10(4):1367-1401.

[131] Vuelta E, García-Tuñón I, Hernández-Carabias P, Méndez L, Sánchez-Martín M. Future Approaches for Treating Chronic Myeloid Leukemia: CRISPR Therapy. Biology (Basel). 2021 Feb 4;10(2)

[132] Kleinstiver BP, Pattanayak V, Prew MS, Tsai SQ, Nguyen NT, Zheng Z, Joung JK. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. Nature. 2016 Jan 28;529(7587):490-5.

[133] Alapati D, Morrisey EE. Gene Editing and Genetic Lung Disease. Basic Research Meets Therapeutic Application. Am J Respir Cell Mol Biol. 2017 Mar;56(3):283-290.

[134] Herring-Nicholas A, Dimig H, Roesing M, Josephs EA. Selection of Extended CRISPR RNAs with Enhanced Targeting and Specificity. bioRxiv [Preprint]. 2023 Jan.

[135] Jung S, Kim DH, Choi YJ, Kim SY, Park H, Lee H, Choi CM, Sung YH, Lee JC, Rho JK. Contribution of p53 in sensitivity to EGFR tyrosine kinase inhibitors in non-small cell lung cancer. Sci Rep. 2021 Oct 4;11(1).

[136] Liu Y, Wu W, Cai C, Zhang H, Shen H, Han Y. Patient-derived xenograft models in cancer therapy: technologies and applications. Signal Transduct Target Ther. 2023 Apr 12;8(1).

[137] McFadden DG, Politi K, Bhutkar A, Chen FK, Song X, Pirun M, Santiago PM, Kim-Kiselak C, Platt JT, Lee E, Hodges E, Rosebrock AP, Bronson RT, Socci ND, Hannon GJ, Jacks T, Varmus H. Mutational landscape of EGFR-, MYC-, and Kras-driven genetically engineered mouse models of lung adenocarcinoma. Proc Natl Acad Sci U S A. 2016 Oct 18;113(42).