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Harnessing CRISPR-Cas9 for Precision Oncological Interventions

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Abstract

CRISPR-Cas9 has brought tremendous changes in genetic engineering world with its unparalleled precision and efficiency. This review is pivoted on the transformative impact of CRISPR CAS9 on precision oncology. This genetic engineering tool has offered oncology new methods of intervention that are beneficial over the traditional method. CRISPR has a role in finding oncogenic mutations, creating tumor models, and also enabling researchers to excel in personalised therapeutic screening. The paper also brings to notice the advancements in CRISPR-enhanced immunotherapy, such as improved CAR-T cell efficacy.

1. Introduction

CRISPR-Cas9, often referred to as CRISPR (Clustered Regularly Inter Spaced Short Palindromic Repeats) is an avant-garde gene editing technology that allows for unequalled precision in modifying genomes. It has a wide range of genome applications, having been used in various cell types and organisms resulting in editing of a single or multiple target genes using sgRNA (Single Guide RNA) for site specific recognition ultimately resulting in correcting genetic defects or modifying plants and crops. It is essentially an adaptive immune system that consists of a programmable RNA molecule and an associated DNA endonuclease Cas9 with the RNA guiding the Cas9 endonuclease to specific DNA sequences to cut double stranded DNA site precisely. In the field of oncology, Crispr-Cas9 is extensively researched to reach novel cancer treatments that aim to correct the genetic mutations that initiate cancerous growths. As cancer stems from numerous genetic/epigenetic aberrations, genetic correction is predicted to stop cancer in its tracks and prevent its regrowth. Current approaches to cancer treatment are quite invasive and have serious side effects. In treating any ailment, diagnosing it at a very early stage is a beneficial and with advancements in diagnostic areas such as imaging, cancer can be detected at a comparatively earlier stage. However, the treatment scheme is often generalised. A double-edged sword in cancer treatment, the chemotherapy is a well-known treatment for cancer but besides causing physiological changes in tumor microenvironment (TME), it targets other fast dividing cells such as hair and gut cells in the body and produces extreme side effects. All though chemotherapy is currently the most effective treatment available for cancer, it unfortunately hasn't been much effective in eradicating cancer. Such "one size fits all" treatments can be quite damaging to the body and are often not specific enough to successfully treat the cancer long term. This is exactly where precision oncological interventions come in. While there are two other mainstream genome editing tools, Zinc Finger Nucleases (ZFNs) and Transcription activator-like effector nucleases (TALENs), CRISPR-cas9 has been showing promising results in this regard, due to the advantages of easy-to-understand design, lesser cost, high efficiency, good-recreatibility and shortercycle. By performing genetic surgery at a molecular level, mutations that cause cancer could be targeted and

corrected. The level of precision CRISPR provides can completely alter the treatment approaches to cancer and its management. This molecular approach requires advanced knowledge of the underlying genetics of cancer - studies in this field have been increasing at a rapid pace and increased focus is on precision editing of genome.

Taxonomic Group	Genes containing cas9	Genes containing other Cas Type Protiens
Bacteria	Cas 9 belongs to Type II system	Cas 10/Cas 7 and Cas 3 /Cas 1
Actinobacteria	15	42
Aquificae	1	16
Bacteroidetes-Chlorobi group	2	36
Chlamydiae-Verrucomicrobia group	1	3
Chloroflexi	2	25
Cyanobacteria	1	21
Firmicutes	17	119
Proteobacteria	20	246
Spirochaetes	1	5

TABLE 1: Prevalence of Cas 9 immune system in BACTERIA groups

1.1. Overview of CRISPR-Cas9 technology

CRISPR (short for clustered regularly interspaced short palindromic repeats) is an adaptive immune system, a family of DNA sequences found in the genomes of prokaryotic organisms such as most of bacteria and archaea. It has been shown that at least 50% of the strains of the bacteria *Streptococcus Pyogenes* have a CRISPR system. When a prokaryote is infected by a foreign genetic element, CAS proteins cut the foreign DNA into short fragments and these fragments are then integrated into the CRISPR array as new spacers. The CRISPR repeated spacer arrays can be transcribed into CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) and CRISPR associated (cas) genes which code for CAS proteins. CAS 9 nuclease produces Double Stranded Breaks (DSBs) at a site upstream to PAM (Protospacer adjacent motif), a short 2-5 Base Pair length DNA sequence which varies in different bacterial species.

Prokaryotes use the cas9 enzyme to chop viral DNA by unwinding it and a complementary base pair is attached to the guide RNA. This immune system of the prokaryotes thus works on memory and quickly recognises foreign DNA during subsequent infections and protects the host.

In 2012, researchers at the University of California, Berkeley led by Jennifer Doudna and Emmanuelle Charpentier proposed transforming CRISPR from a bacterial immune system into a gene-editing tool for eukaryotes. They identified a pivot component of CRISPR system, an RNA molecule which recognises the foreign sequences in bacteria.

In vivo carrier	Examples	
Vector Viruses	1.	AAV
	2.	Baculovirus
	3.	Lentivirus
Biomimetic NPs	1. ROS sensitive PPMMT 2. OA&BCMNs	
Lipid NPs	1.	306-O12B LNP
	2.	Onpattro MC-3 LNP
Polymer NPs	1. pH sensitive MDNP 2. MMP-2- Sensitive PICASSO	
Exosomes	1.	Functionalized Exosomes
	2.	Natural Exosomes
Gold NPs	1. LACPs 2. Hypoxia Responsive APACPs	

TABLE 2: Depicting In Vivo carriers and their examples used in CRISPR technology in three forms of CRIPSR action: pDNA, mRNA, and RNP

This gene editing function in eukaryotes was achieved by replacing the bacterial enzyme with an analogous one that is found in eukaryotic cells, and the DNA of some viruses with the DNA of an organism's choice. By 2013, several groups had demonstrated Crispr-mediated gene editing in a variety of organisms, such as Drosophila, zebrafish, mice, and human cell lines. In the same year, Feng Zhang's group at the Broad Institute reported modification of mammalian genomes using the Crispr-Cas9 system. This technology has not only made gene editing much simpler and more precise, but it has also made it much cheaper. As a result CrisprCas9 gene editing has been hailed as a major breakthrough in genetic engineering.

It offers a significant improvement over other existing gene-editing techniques in terms of efficiency, speed, and most importantly, cost. For these reasons, CrisprCas9 has been adopted by both academic and commercial biomedical researchers around the world for applications in gene therapy, drug discovery, tissue engineering, and in understanding the role individual genes play in health and disease.

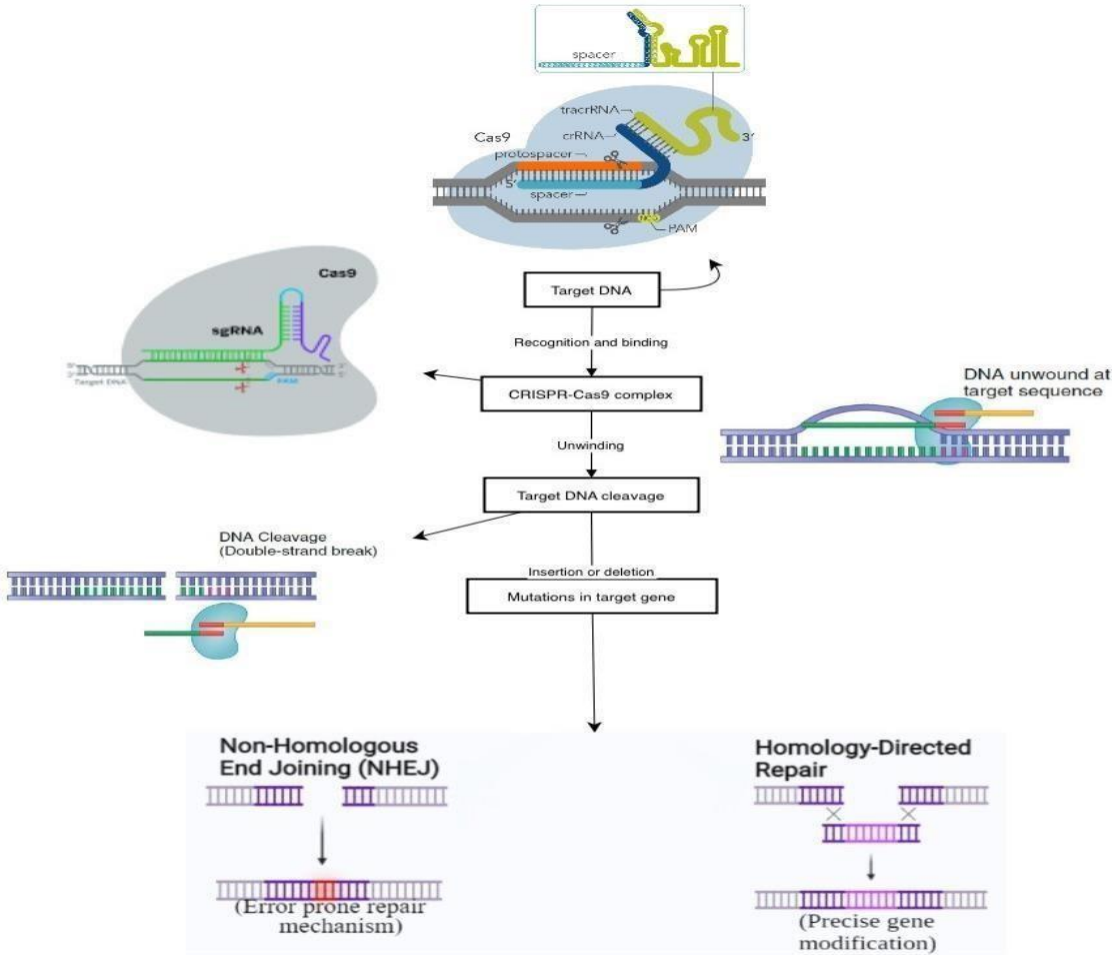


Figure 1. Schematic Representation of CRISPR-Cas9 Gene Editing Mechanism and Subsequent DNA Repair Pathways

The figure illustrates the recognition and binding of the Cas9 protein-guide RNA complex to target DNA, unwinding of DNA, and the introduction of double-stranded DNA breaks. There are two DNA repair pathways: Non-Homologous End Joining (NHEJ), an error-prone mechanism leading to insertions or deletions and potential mutations, and Homology-Directed Repair (HDR), a precise mechanism enabling targeted gene modifications.

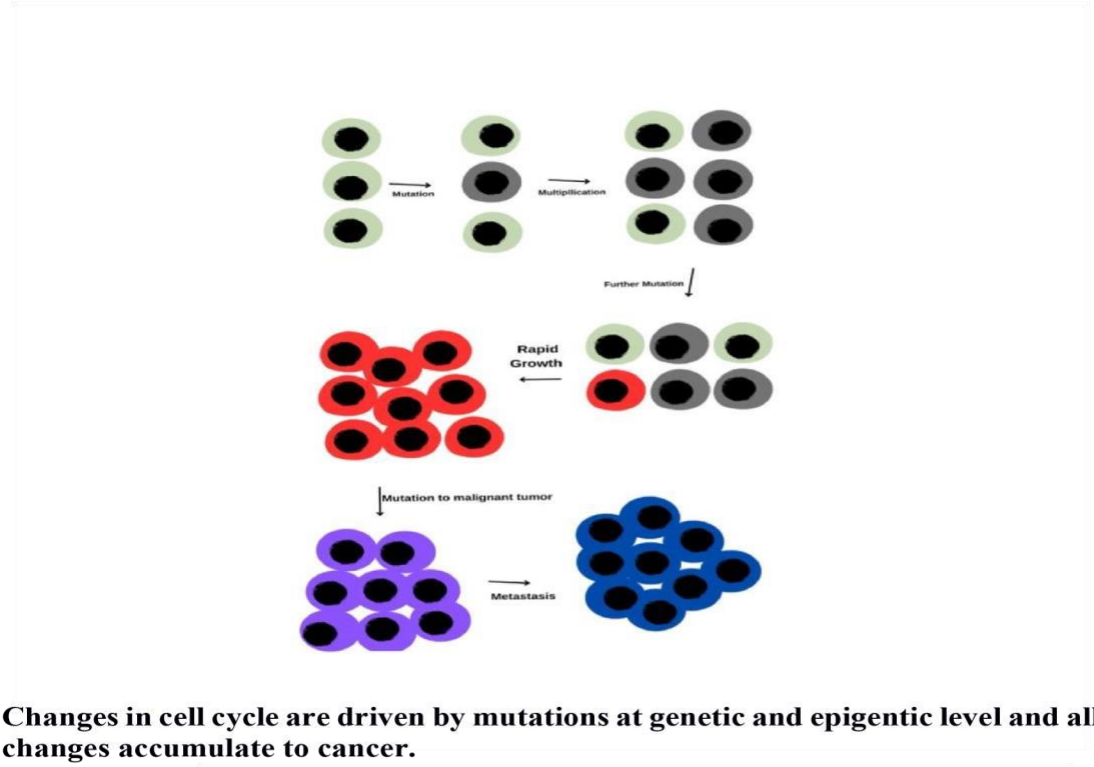
1.2. Importance of precision interventions in oncology

Cancer is a genetic disease caused by uncontrolled cell growth and defined by mass cell mutations, which are a result of progressive accumulation of DNA mutations. There are over 100 types of cancers, and each type is a result of a unique set of mutations in various genes. For example, lung cancer is often associated with mutations in the EGFR (Epidermal Growth Factor Receptor) gene, while skin cancer is often associated with mutations in the BRAF gene of chromosome 7. These changes in the information system of cells that leads to this malformation are genetic and hence require treatments guided accordingly. The knowledge that human genome project (HGP) provided and further with the advent of novel sequencing technologies, oncogenesis and metastasis genetic basis has been clearly defined. and Precision oncology takes information from this genetic study to better understand the prognosis and cancer progression which paves way for a more effective treatment. This effective treatment is essentially guided by the idea that the right drug gets to the right patient and that the generalised treatments for cancer are not as effective as

they are expected. Molecular diagnosis helps a scientist discover different medicine for different people with similar cancer but different stage and this certainly increases the treatment efficacy. Precision treatments are in contrast to traditional chemotherapy which have far more side effects. For targeted therapy to have a clinically positive outcome, it must be preceded by timely and accurate identification of the oncogenic mutations. CRISPR-cas9 is one of the most promising methods that helps in identifying oncogenic genes as drug targets. CRISPR cas9 has been used to identify new drug targets and has shown a powerful impact in the treatment industry. Edwin et al. conducted a genomic wide Loss Of Function (LOF) generic screening in colorectal cancer HTC116 (human colorectal carcinoma cell line) cells that harboured modified KRAS (Kristen Rat Sarcoma) and they found that several genes involved in metabolism were potential therapeutic targets and the inhibition of the enzymes produced by these genes, such as ketohexokinase, weakened the growth of colorectal cancer cells. Konstantinos et al employed CRISPR cas9 in Acute Myeloid Leukemia (AML) study and identified several new therapeutic targets such as DOT1L, BCL 2 and ultimately KAT2A (K lysine acetyltransferase) was prosposed as a new therapeutic target in AML. Nowadays, with the help of machine learning algorithms, scientists are able to develop different computational tools to effectively streamline the data analysis process of cancer genomic studies and this tremendous research has lead to the discovery of numerous genetic biomarkers which help in predicting growth of the cancer or more promising use of them is to identify precision therapies to optimise treatment plan. Such information not only benefits the discovery of new anticancer drugs in the pharmaceutical industry but also sets up the foundation for the development of precision interventions in oncology.

2. Understanding Oncological Mutations

Even though many genetic alterations occur throughout the life a cancer cell, but the progression of cancer is mainly driven by what are termed as driver mutations. These driver mutations result in imperfect cell cycle control such as no response to growth inhibitory messages or escape from necessary checkpoints which are necessary for the correctness of major events of cell development in cell cycle including replication, chromosome integrity and segregation at mitosis. A normal cell cycle starts with a resting phase or what is known as Quiescent or G0 phase which is followed by a phase of both the physical growth and increase in the volume of organelles and proteins. This is termed as G1 phase where the organelles copies are created. The S phase, which follows, synthesizes a complete copy of the DNA. Mitosis in the cell begins when the next phase, the G2 phase, ends. This Gap phase result into more cell growth, more protein production and organisation of cell contents for a proper division. Ultimately the cell division proper occurs with the mitotic phase. The daughter cells formed can re-enter the G0 phase. Movement of a cell through cell cycle from one phase to another is strictly regulated and a cell with any slight imperfection either exits the cell cycle or gets repaired before re-entering the cell cycle. This regulation is carried out by checkpoints and the first checkpoint is at the end of the G1 phase called the G1 checkpoint which checks for cell size, nutrients within the cell, correct molecular signals, necessary growth factors, DNA integrity and decides whether the cell should divide or not. The G2 checkpoint checks for DNA damage, DNA replication exactitude. A cell passing this checkpoint meets the spindle checkpoint at M phase which checks for “straggler” chromosomes or the chromosome attachment at metaphase plate.



Changes in cell cycle are driven by mutations at genetic and epigentic level and all mutational changes accumulate to cancer.

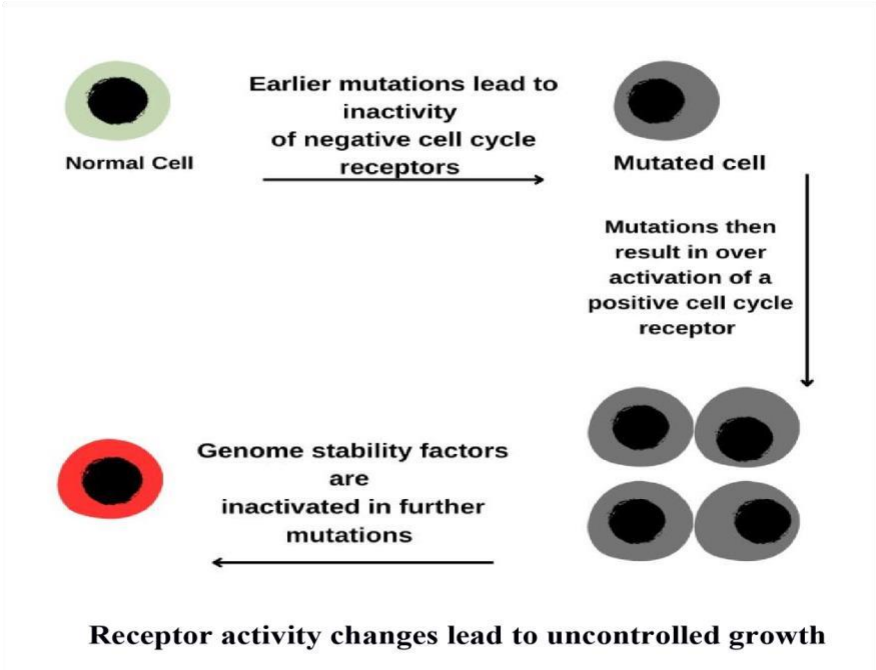


Figure 2. Diagrammatic Overview of Cancer Progression Driven by Genetic and Epigenetic Mutations in Cell Cycle Regulation

Accumulation of genetic and epigenetic changes lead to altered cell cycle regulation and cancer development. In activation of negative cell cycle receptors, leads to a mutated cell. More mutations in genome stability factors result in rapid cell growth, formation of malignant tumours, and eventual metastasis. In the bottom part, the sequential mutation-driven progression, that enhance changes in receptor activity and genome stability that contribute to oncogenesis is depicted.

The central machines that drive cell cycle progression are actually the cyclins and cyclin-dependent kinases (CDKs). When a cell decides to enter the cell cycle, in early G1phase cyclin D and CDK 4/6 are produced, this cyclin D binds with CDK 4/6 and creates an active complex with unique substrate specificity. This births a reaction inside the cell which detaches E2F from Retinoblastoma (Tumor supressor protein) and when released, E2F contains one or more conserved DNA binding domains (DBDs) that bind target promoters and regulate their expression and act as a transcription factor allowing the cell to progress through cell cycle. Towards the end of G1 phase, CDK 2 and Cyclin E are produced, CDK 1/2 and Cyclin A at S phase and CDK 1 and Cyclin B at G2 phase are also produced that allow further progress in cell cycle. Simply, if the amount of of CDKs is too low,the cell cycle won't progress and if the amount of CDKs is too much,the cells divide continuously which is essentially the mechanism of cancer. The elevation in CDK levels has been ascribed to a mechanistic basis which could be gene amplification of protein overexpression of either CDK or Cyclin subunit.

Mutations or Epigenetics, which have emerged as fundamental pathway in the pathogenesis of malignancies, can induce transcriptional alterations, encompassing both gene silencing and activation phenomena. In a cancer cell activation of oncogenes such as RAS gene, MYC gene involves mutations to activate protooncogenes through structural alterations in their encoded proteins which lead to continuous activity of mutated protein or these mutations may cause inactivation of tumour suppressor genes such as P53, APC, BRCA 1/2.

These two processes are critical steps in tumour initiation and progression which involve increased proliferation, invasiveness, and metastatic tendencies. Normal cells have a RAS gene which produce intracellular RAS proteins which are binary molecular switches that cycle between active Guanosine Triphosphate (GTP)- bound and inactive Guanosine diphosphate (GDP) bound states. These

RAS proteins are activated by Growth factors such as Epidermal Growth Factor (EGF) and a cascade of intracellular phosphorylation occurs which in turn activate a transcription factor to make proteins for cell cycle, the CDKs already discussed. Essentially, if the RAS genes are mutated, the RAS proteins are frozen in the GTP bound state and thus there is a continuous production of proteins for cell growth.

Most cancers require also the sustained MYC activation along with other genetic events. Genetic events that abrogate cell-cycle checkpoints necessary for regulating proliferative arrest, apoptosis etc often cooperate with MYC to induce malignant transformation for example the over-expression of BCL-2, loss of P53 or loss of P19 ARF.

The activation of oncogenes, such as the RAS and MYC genes, facilitates the evasion of cell cycle checkpoints, promoting uncontrolled cellular proliferation.

Ordinarily, cells possess mechanisms to impede the progression of aberrant cells through the cell cycle. Tumor suppressor genes play a pivotal role in this regulatory process. In the context of cellular regulation, the presence of damaged DNA can lead to cell cycle arrest, particularly at the G2 phase, mediated by key regulatory proteins such as p53. Upon detection of DNA damage, p53, which is one of the Tumor suppressor genes, functions as a transcription factor and reads the DNA to produce protiens for cell arrest (e.g., p21), DNA repair, and apoptosis. Notably, p21 inhibits cyclin-dependent kinases (CDKs) or bind to proliferating cell nuclear antigen (PCNA), thereby preventing progression through the cell cycle, inhibiting DNA replication and modulating various PCNA dependent DNA repair processes. Concurrently, p53 promotes the expression of factors crucial for DNA repair and, if repair is unsuccessful, initiates apoptosis to eliminate aberrant cells. Mutation in DNA resulting in inactivation of tumor suppressor genes, such as the inactivation of p53, disrupts the regulatory cell cycle network. In most cases TP53 gene suffers

one nucleotide mutation leading to a single amino acid substitution in P53 protien. It has been reported that most frequent mutations are represented by G:C> A:T or G:C>T:A

Thus, the cells with inactivated tumor suppressor genes can evade cell cycle checkpoints, leading to uncontrolled proliferation. Thus, multiple genetic and epigenetic alterations observed in cancer, characterised by the inactivation of tumor suppressor genes and the activation of oncogenes, contribute to an abnormal cell cycle progression and sustained proliferation.

Category	Component	Normal Function	Cancer-Related changes	Consequences
Cell Cycle Regulators	Cyclin D + CDK 4/6	\Early G1 phase progression	Over-activation	Uncontrlled G1
	Cyclin E + CDK 2	Late G1 phase progression	Over-activation	Accelerated S phase entry
	Cyclin A + CDK 1/2	S phase progression	Over-activation	Unregulated DNA replication
	Cyclin B + CDK 1	G2 phase progression	Over-activation	Premature mitosis entry
Oncogenes	RAS	Growth Signaling	GTP bound state mutation	Continuous Growth signaling
	MYC	Cell Proliferation	Sustained activation	Abnormal Cell Proliferation
Transcription Factors	E2F	Regulates Gene Expression	Hyperactivation	Increased Cell Cycle Progression
Checkpoint Protiens	p21	Inhibits CDKs	Downregulation	Loss of cell cycle arrest
Tumor Supressors	p53	DNA damage	Mutation	Loss of cell cycle check points

		response/cell cycle arrest		
	Rb	Regulates G1/S transition	Inactivation	Unregulated transition
DNA repair	Various	Genome Stability	Impaired Genes	Mutation Accumulation
Apoptosis Regulators	BCL-2	Regulates Cell Survival	Over-expression	Apoptosis Resistance
Epigenetic Factors	Various	Gene Expressionn Regulation	Altered Methylation Patterns	Abberant Gene Expression

TABLE 3: Key components of cell cycle and their alterations in cancer

2.1 Gene Editing Concepts and Strategies

In recent years, advancements in gene editing technology have revolutionised the field of cancer research, offering novel approaches to treating this complex and heterogeneous disease. Genome editing has extended the ability to explain the contribution of genetics in cancer progression. Targeted DNA alterations commence with the generation of Nuclease induced Double Stranded breaks (DSBs). DNA breaks accidentally or can be broken purposefully by nucleases. These DSBs can be repaired by cells themselves but the repair isn't always correct and can generate alterations in DNA sequence. DSBs stimulate DNA repair by non homologous end joining (NHEJ) which results in deletion or insertion of DNA sequence and disrupt the gene function. However, a homologous stretch of DNA can be introduced into the cell as donor template and the homology directed repair (HDR) can thus result in a more accurate repair. If this Homologous Stretch contains a preset alteration, the result can be manifested in the repaired DNA. These cellular mechanisms form the fundamentals of gene editing.

One of the primary techniques of gene editing in cancer cells, CRISPR-Cas9,allows for precise modification of the DNA sequence, enabling researchers to target and disable genes associated with cancer development. By guiding the Cas9 nuclease to a specific region of the genome using a short RNA sequence, scientists can induce double-strand breaks in the DNA, leading to gene knockout or insertion of desired sequences. Moreover, CRISPR-Cas9 can be used in combination with other techniques like homology-directed repair to correct mutations in cancer-related genes. Researchers have proposed various CRISPR based strategies for cancer therapy including inactivating cancer growth driver genes, enhancing immune response to cancer cells, repairing mutations that cause cancer ,or even delivering cancer killing molecules directly to tumor cells. The treatment proposals range from the inactivation of MYC (the effects of activation have been discussed in section 2) gene to halt tumour growth to repair of mutations in case of inherited cancers caused by BRCA 1 and BRCA 2.

3. CRISPR Applications in precision oncology

The application of CRISPR-Cas9 in precision oncology presents with an immense potential for advancing cancer research and therapy. CRISPR technology facilitates precise manipulation of genes associated with oncogenesis, offering a transformative instrument for investigating tumor biology and discovering novel therapeutic targets. Recent advancements in CRISPR-Cas9 platforms are widely facilitating precise genome editing for investing roles of genes in tumorigenesis, Moreover, CRISPR/Cas9's potential in correcting genetic mutations associated with cancer has lead researches to strive for targeted gene therapy and personalised medicine, it holds immense potential for transforming the landscape of cancer treatment. CRISPR technology is currently used in genomic silencing, knock out-knock in experiments as well as in the transcriptional activation and repression. CRISPR enables for a precise and efficient gene manipulation. Thus this ability of CRISPR has made correction of cancer associated alterations an important objective for advancements in cancer treatment, therapy, diagnosis and related applications.

Scientists strive to understand complex mechanisms that drive cancer progression at molecular level. Advancements in this understanding is a necessity to develop cancer treatments. One of the applications of CRISPR to better understand cancer mechanisms was the development of tumor models both in vitro and in vivo. CRISPR has been used in mammalian cell lines with single or multiple genes deletion to generate in vitro cancer models. CRISPR has also been used to knock in or knock out functional alleles to enhance or develop drug resistance in vitro. Drug targets such as NAMPT have been identified for anti cancer agents such as KPT-9247. It is evident that the genetics and epigenetics of cancer patients differ and they exhibit different transcriptome, proteome, and metabolome even for the same cancer type. This describes why cancer therapeutical regimen needs to be researched and diversified and hence why CRISPR plays a pivotal role in cancer treatment. Cancer has been found to evolve to resist therapy which is often attributed to the difference in mutation landscape and copy number variation (CNV) of driver oncogenes. With the advent of CRISPR-Cas based techniques, personalised screening of genetic elements has become easier and formation of therapeutics is properly guided. This CRISPR based Genome-wide or sub-pool. CRISPR screens are implemented with the help of RNA libraries. RNA library is a pool of multiple sets of guide RNAs. Each guide RNA set targets an annotated genetic element in the genome. Cas9 expression is achieved either by incorporation of its expression cassette in the sgRNA backbone or a separate plasmid is used. These CRISPR-based screenings have lead to the expose of several genetic vulnerabilities and the genes that mandate drug response in wide variety of cancer cell lines.

An example to highlight the impact of CRISPR comes from the deletion screen performed in the presence of inhibitors targeting the RTK(Receptor Tyrosine Kinases)/MAPK(Mitogen activated protein kinases) pathway in lung cancer cells that had identified the loss of KEAP1 gene as a decisive factor in providing resistance and promoting cell survival. Another example comes from the cancer dependency map (DepMap). It's a viability-based CRISPR knockout screen performed in about 800 cancer cell lines. DepMap provides invaluable information for identifying gene essentiality and dependency in specific cancer case types.

One of the most recent advancements in cancer treatment with CRISPR has been the technology to augment the amount of MHC class 1 in cancer cells. These Major Histocompatibility Complex (MHC) class 1 molecules are the immune complexes present on surface of human cells. These are required by the immune system to recognise and eliminate cancer. Cancer impairs the CD8+ cell mediated immune response and suppresses the expression of MHC-1 molecules and thus flees from immune surveillance. This molecule is regulated by a gene NLRC5 which is turned off by switches on DNA in cancer cells

through methylation. kobayashi et all with their CRISPR based technology known as TRED-1 (Targeted Reactivation and Demethylation for MHC-1) were able to restore DNA methylation of NLRC5 gene and activate it to increase levels of MHC class 1 without any severe side effects. TRED-1, tested on cancer models, also increases activity of cytotoxic CD8+T cells

The TP53 gene that encodes the transcription factor and oncosuppressor p53 whose role in cancer has been discussed is the most frequently mutated gene in cancer cells. This is one of the reasons why it's often considered for gene editing tools. Scientists have proposed a highly tumor specific delivery system for TP53 which will restore the normal p53 phenotype in tumor cells by replacing the mutant type with a fully functional wild-type copy through homologous recombination leading to normal expression of P53 protein. This theory is also based on CRISPR technology because its system allows for making large insertions. CRISPR has also been used to create a genetic tool that specifically eliminates p53 deficient cells using a genetic sensor to detect expression of wild type p53.

These finding elucidate the immense potential that CRISPR based genome editing and technologies posses to be used to edit TP53 gene and regulate the mechanism of cell cycle growth thereby preventing tumor progression and development.

CRISPR technology has been used to target mutated versions of EGFR gene which helps cells grow and thus mutated versions would cause the cells to grow too much causing cancer. Using CRISPR cas9 mediated genome editing, scientists targeted EGFR gene to either repair or destroy it in Non Small Cell Lung Cancers. In vivo, it resulted in cancer cell death and tumor size reduction.

In breast cancer, CRISPR mediated knockout of the FASN (Fatty Acid Synthase) gene, which is over-expressed and hyper-activated in biologically aggressive sex steroid related tumours such as breast cancer, can reduce the proliferation and metastasis of breast cancer . On Similar lines Estrogen receptor beta (ERβ) which is the most prevalent Estrogen receptor in the human prostate was identified and eliminated using CRISPR Technology.

Chimeric Antigen Receptor (CAR) T-cell therapy, which uses T lymphocytes to eliminate cancer cells, can also be improved by combining it with CRISPR technology. It's a form of precision medicine therapy where T cells from the patient are collected and re-engineered to produce CAR proteins on their surface which recognise and bind to antigens on cancer cells. The persistent expression of inhibitory receptors including PD-1,LAG3 and more leads to the exhaustion of T-Cells. These receptors do so by binding to the ligands and inhibit T-cell activation and decreases the release of cytotoxic cytokines like IFN-γ, IL-2, and TNF-α. The exhausted CAR-T cells lose their cytotoxic and long-term survival abilities. CRISPR technology can potentially overcome this setback by disrupting inhibitory receptors, thereby enhancing CAR-T cell resistance to the immunosuppressive tumor microenvironment. Various clinical trails have demonstrated that CRISPR/Cas9-mediated PD-1 disruption could effectively suppress CAR-T cell exhaustion and prolong CAR-T cell persistence in vivo. Adenosine also mediates immunosuppression in Tumor micro environment and the CRISPR mediated knockout of Adenosine A2A receptor gives CAR-T cells resistance to adenosine mediated immunosuppression and enhances the ability of the Janus kinase/signal transducers and activators of transcription pathway (JAK/STAT). The JAK-STAT pathway is a critical signaling cascade that mediates the effects of a wide range of cytokines, growth factors, and other extracellular signaling molecules. JAK activation stimulates cell proliferation, differentiation, cell migration and apoptosis.

Therapies generally lead to decrease in circulating T cells in patients and as T cells are to be taken from the patients to be reengineered, this become as major hindrance in this therapy. Off-the-shelf allogeneic

CAR-T cells are engineered to overcome this hindrance but it still faces major set backs such as the Graft Versus Host disease (GVHD), a serious complication that can arise when donor T cells recognise and attack the recipient's healthy tissues due to the recognition of recipient alloantigens by donor T-cell receptors (TCRs). Moreover, the recognition of donor human leukocyte antigen (HLA) molecules by the recipient's immune cells may to graft rejection. Yet again research has shown that the disruption of TCR and HLA-I expression is necessary to generate offthe-shelf universal CAR-T cells and CRISPR can certainly be used to achieve this disruption.

By utilising CRISPR/Cas9 technology, researchers and clinicians have been able to generate off-the-shelf universal CAR-T cells that are less likely to induce GVHD or be rejected by the recipient's immune system. These universal CAR-T cells can be manufactured in advance and made readily available for treatment, potentially improving the accessibility and efficiency of CAR-T cell therapy for cancer patients.

TABLE 4: Summary of CRISPR Application in Tumor Research and Therapy

Application	Description
Investigating tumor biology and discovering therapeutic targets	Manipulation of genes associated with oncogenesis.
Genomic silencing, knockout-knock-in experiments, transcriptional activation/repression	
Developing in vitro and in vivo tumor models	Deleting single or multiple genes in mammalian cell lines, as well as to knock in or knock out functional alleles to enhance or develop drug resistance in vitro.
Identifying drug targets	CRISPR has helped identify drug targets such as NAMPT for anti-cancer agents like KPT-9247 and many other potential targets such as CD38, CXCR2,MASTL and RBX2.
Personalised screening of genetic elements	genome-wide or sub-pool CRISPR screens, implemented with RNA libraries, have been enhanced, enabling personalised screening of genetic elements and guiding the formation of therapeutics.
Identifying genetic vulnerabilities and drug response	CRISPR based screening has exposed several genetic vulnerabilities and genes that mandate drug response in various cancer cell lines.
Cancer dependency mapping	(DepMap), a viability-based CRISPR knockout screen performed in about 800 cancer cell lines, provides valuable information for identifying gene essentiality and dependency in specific cancer types.
Enhancing immune recognition of cancer cells	TRED-1 (Targeted Reactivation and Demethylation for MHC-1) have been used to restore DNA methylation and activate the NLRC5 gene, increasing MHC class I

	expression and enhancing cytotoxic CD8+ T-cell activity against cancer cells.
Editing TP53 gene	CRISPR could target tumors by restoring p53 or eliminating p53-deficient cells using genetic tools.
Targeting mutated EGFR gene	CRISPR has been used to target mutated EGFR gene, which can cause uncontrolled cell growth, leading to cancer cell death and tumor size reduction in non-small cell lung cancers.
Targeting FASN and ERβ genes	CRISPR-mediated knockout of the FASN (Fatty Acid Synthase) gene, overexpressed in breast cancer, can reduce proliferation and metastasis, while CRISPR has been used to eliminate the Estrogen receptor beta (ERβ) in prostate cancer.
Enhancing CAR-T cell therapy	CRISPR technology can potentially improve CAR-T cell therapy by disrupting inhibitory receptors like PD-1 and LAG3, enhancing resistance to the immunosuppressive tumor microenvironment, and generating off-the-shelf universal CAR-T cells by disrupting TCR and HLA-I expression to prevent graft-versus-host disease and graft rejection.

3.1 Target Identification and validation

Target identification is essential for developing effective cancer therapeutic interventions, diagnostic tools. This target can be applied in a broader sense to a range of biological units such as the proteins, genes, and the RNA. The target must be affected by a drug and must elicit a biological response. One of the most common approaches employed in target identification and validation is the highthroughput screening technique, such as genomics, proteomics, and metabolomics, to identify potential targets and one of the most recent successful tools used in the drug identification is CRISPR. Generally , CRISPR screening is dependent on a pooled CRISPR guide RNA library, which is introduced into the target cell by lentiviral transduction, opening new avenue of possibilities in gRNA sequencing. In this transduction, the viral vector (virion) infects the cells and transports DNA into nucleus, not dependent on other actions. CRISPR based target identification was used to identify that BDW568 targets stimulator of interferon genes (STING) to activate interferon signaling. These IFNs increase the cytotoxic activity of both innate and adaptive immunity both must for tumour growth to be inhibited, and cancer cells are eliminated by innate and adaptive immunity orchestrated by IFN.

Once potential targets are identified, they ought to undergo meticulous validation processses to confirm the target's involvement in cancer or the biological process of cancer.

CRISPR has been employed in techniques such as gene knockdown, knockout, or overexpression studies. These methods are utilized to evaluate functional consequences of genetic manipulation. The PTEN (Phosphatase and TENsin) in prostrate cancer cells was knocked out conforming the role of this gene in tumor supression and identifying the targets in the PI3K/AKT pathway Genome-wide CRISPR knockout (GeCKO) and CRISPR activation (CRISPRa) screens have been frequently used for identification of

genes that play essential role in cancer cell survival, proliferation and drug resistance. SP11 transcription factor was identified as a drug target using a CRISPR screen in Acute Myeloid Leukemia (AML). This target inhibition impaired growth of AML cell selectively and spared normal hematopoietic cells. CRISPR-Cas9 based screens have identified genes involved in resistance to BRAF inhibitors in melanoma. This research validated MITF (Microphthalmia associated transcription factor) as the key component in drug resistance and a target for combination therapies.

Successful in vivo target validation was used to develop MCAP (Massively Parallel CRISPR-Cpf1/cas12a crRNA array profiling). This array targeted 325 pairwise combinations of genes implicated in metastasis. This approach allowed for a rapid in vivo validation of therapeutic targets and cancer drivers in mouse models.

Target discovery and validation are essential elements in the development of successful cancer therapies. CRISPR/Cas9 has changed this technique by enabling high-throughput functional genomic screening and precise gene editing in cancer cell lines and model organisms.

3.3 Synthetic Lethality and Combination Therapy

CRISPR/Cas9 technology has been instrumental in uncovering synthetic lethal interactions and guiding the development of rational combination therapies in cancer treatment. Synthetic lethality occurs when the simultaneous perturbation of two genes or pathways leads to cell death, while the individual perturbation is tolerated. This concept holds immense potential for exploiting cancer-specific vulnerabilities and identifying effective combination therapies.

In this context, one effective use of CRISPR/Cas9 is to generate isogenic cell line models with precise gene knockouts or mutations. Researchers can find synthetic lethal interactions and possible treatment targets by making comprehensive genetic changes to cancer-related genes. CRISPR Cas9 has been used to create a panel of isogenic cell lines with single and combined knockouts of tumor suppressor genes in ovarian cancer. A synthetic lethal interaction was found between PTEN and NF1 loss. Combined inhibition of PTEN and NF1 thus was found to hold a significant therapeutic potential.

On a genome wide scale a CRISPR/cas9 knockout screen in BRCA1/2 deficient ovarian cancer cells was performed to identify essential genes for survival of cancerous cells but where inessential for non-cancerous cells thus identifying PARP1 as synthetic lethal target for these ovarian cancers.

Conclusion:

Throughout the review, the transformative potential of **CRISPR-Cas9** technology in precision oncology is well evidenced. Owing to its exceptional ability to change genetic information with high specificity and efficiency, CRISPR aided genetic engineering has been tremendously used in oncology research. CRISPR facilitates high-throughput genetic screens that accelerate the identification of critical oncogenes, tumor suppressor pathways, and mechanisms of drug resistance. These capabilities support the development of targeted therapeutic strategies tailored to individual patients, aligning with the principles of personalised medicine.

Moreover, CRISPR has been used it enhancing cancer immunotherapies, such as improving the efficacy of **CAR-T cell** therapies by altering inhibitory pathways and tame immunosuppressive tumor environments. The strategic knockout and modification of pivotal genes like **TP53** and **EGFR** have illustrated CRISPR's role in directly targeting mutations that drive malignancies. These minimise off-target effects typically associated with conventional cancer therapies. However, challenges related to off-target effects, delivery mechanisms, and ethical considerations remain. Addressing these issues is crucial

for the successful clinical application of CRISPR technology. Nevertheless, its potential to revolutionise cancer treatment is significant, aiming for more precise, effective, and patient-specific therapeutic interventions. As research progresses, CRISPR-Cas9 stands poised to play a pivotal role in advancements in oncological research.

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