Advancing Personalized Cancer Care: Integrating CRISPR/Cas9 with Next-Generation Sequencing Technologies

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Abstract

Background: Cancer arises from a complex interplay of genetic and epigenetic abnormalities, presenting significant challenges for conventional therapies. This complexity underscores the urgent need for innovative therapeutic approaches. The integration of the CRISPR/Cas9 system with next-generation sequencing (NGS) presents a promising avenue for rapid identification, validation, and targeting of critical therapeutic targets. Methods: Personalized medicine leverages genetic, phenotypic, and environmental data to tailor healthcare solutions, moving beyond the limitations of standardized treatments. Advances in cancer genome sequencing have facilitated this shift, with NGS offering advantages such as minimal sample requirements and the ability to identify novel biomarkers. Tumor profiling, along with cell-free DNA analysis, proteomics, and RNA studies, enhances our understanding of immunological responses and informs treatment strategies. Results: The CRISPR/Cas9 system enables precise targeting of genetic alterations in tumor cells, providing a mechanism to disrupt genetic pathways responsible for tumorigenesis and metastasis. This targeted approach enhances the potential for more effective and personalized therapies. Combining NGS and CRISPR/Cas9 aims to match treatments to specific tumor profiles and develop bespoke therapeutic strategies tailored to individual tumors. This review highlights the transformative potential of CRISPR/Cas9 and NGS in advancing personalized cancer treatment.

Keywords: CRISPR/Cas9, Next Generation Sequencing (NGS), Personalized Medicine, Cancer Treatment, Liquid Biopsy

Significance | The integration of CRISPR/Cas9 and NGS enhances personalized cancer treatment, enabling precise targeting of genetic alterations and improved therapeutic outcomes.

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Introduction

Cancer remains a leading cause of morbidity and mortality worldwide, characterized by the dysregulation of various signaling pathways associated with critical processes such as cell proliferation, angiogenesis, metastasis, and apoptosis evasion (Huang et al., 2020). Current cancer treatment modalities, including chemotherapy, radiation therapy, and surgical interventions, are often limited in effectiveness due to the heterogeneity of tumors. Each tumor may arise from distinct genetic mutations, and even within the same type of cancer, variability exists among patients, complicating treatment protocols (Almendros et al., 2019). Consequently, there is an increasing need for alternative therapeutic strategies that can address this complexity. In recent years, genome editing has emerged as a promising therapeutic avenue for various diseases, including cancer

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(Zhang et al., 2020). Notably, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system has garnered significant attention for its potential in treating several malignancies, including non-small cell lung carcinoma (NSCLC), breast cancer, multiple myeloma, glioblastoma, and leukemia (Mali et al., 2013). The integration of CRISPR/Cas9 with next-generation sequencing (NGS) technologies further enhances the therapeutic landscape, providing a more rapid and targeted approach to cancer treatment (Jiang et al., 2018).

CRISPR/Cas9 operates by inducing double-strand breaks (DSBs) at specific genomic locations, which are subsequently repaired by cellular mechanisms (Certo et al., 2018). The efficiency and specificity of CRISPR/Cas9 editing rely on guide RNAs that facilitate the precise targeting of desired sequences through Watson-Crick base pairing (Doudna & Charpentier, 2014). However, despite the advantages of this technology, challenges persist in the delivery of CRISPR/Cas9 components to target cells in vivo and in minimizing unintended off-target effects, which are critical considerations for therapeutic applications (Zhou et al., 2021). To overcome these limitations, alternative gene-editing systems, such as prime editors and cytosine base editors, have been developed, offering improved accuracy and reduced off-target effects compared to traditional CRISPR/Cas9 (Anzalone et al., 2019; Liu et al., 2020).

The advancement of precision or personalized medicine further underscores the shift towards more individualized treatment strategies. By leveraging the increased accessibility and affordability of NGS technologies, researchers can obtain comprehensive genomic profiles of cancers, enabling the identification of specific mutations and the development of targeted therapies tailored to a patient's unique cancer characteristics (Gonzalez et al., 2021). NGS profiles facilitate the identification of mutations in signaling pathways, allowing for the design of therapies that directly inhibit these alterations (Davis & Nussbaum, 2019). Consequently, CRISPR/Cas9 gene editing can serve as a powerful tool for correcting mutated genes identified through NGS analysis.

CRISPR/Cas9 technology has demonstrated considerable potential for gene knock-in, knock-out, repair, and transcriptional regulation, positioning it as a vital component of modern cancer treatment strategies (Wang et al., 2020). This review explores the integration of CRISPR/Cas9 and NGS technologies in advancing personalized cancer medicine, emphasizing their role in enhancing therapeutic efficacy and precision.

Personalized Medicine

Personalized medicine, also known as precision medicine, is a revolutionary approach that aims to customize treatment strategies for individual patients, shifting away from the "one-size-fits-all" model of traditional healthcare (Lander, 2016; Wong et al., 2017). The advent of next-generation sequencing (NGS) has been instrumental in advancing this field (figure 1), offering oncologists detailed insights into each patient's unique genetic makeup and mutational landscape (Mardis, 2013; Tewari et al., 2015). By allowing for single-nucleotide resolution analysis, NGS enables the identification of tumor-specific mutations, facilitating the precise targeting of disease drivers (Mardis, 2013). Since the completion of the Human Genome Project, the focus on functional characterization of genetic elements has provided a deeper understanding of both normal biological processes and the mechanisms underlying various diseases (Lander, 2016).

NGS technology empowers clinicians to analyze individual genetic profiles, identifying mutations that can serve as therapeutic targets. Coupled with CRISPR gene-editing, these targets can be precisely modified, enhancing treatment specificity and potential effectiveness (Doudna & Charpentier, 2014). The development of liquid biopsy biomarkers further supports personalized medicine by enabling non-invasive, real-time monitoring of tumor dynamics, which provides crucial insights into disease progression without the need for invasive procedures (He et al., 2015).

The overarching goal of personalized molecular medicine is to target disease-causing genes while minimizing off-target effects, ultimately aiming for safer and more effective therapies (Ginsburg & Phillips, 2018). As depicted in Figure 1, the integration of NGS with CRISPR/Cas9 is pivotal in refining and advancing personalized treatment approaches.

In assessing therapeutic efficacy, precision medicine leverages biological parameters such as circulating tumor DNA (ctDNA), immune markers, and proteomic data. Analyzing tumor and cell-free DNA, alongside RNA profiling, helps guide individualized treatment options (Meyer et al., 2018). The detailed genetic characterization provided by NGS is central to identifying and acting upon specific therapeutic targets, which is essential for designing effective treatment strategies.

The challenge of drug resistance in cancer has further fueled the need for personalized medicine. Tumors often develop resistance to chemotherapy over time due to genetic mutations or metabolic changes that degrade the efficacy of drugs (Hyman et al., 2015). By analyzing these mutations, clinicians can develop treatment plans tailored to the specific genetic alterations present, improving the likelihood of successful outcomes (Rosenberg et al., 2019).

The effectiveness of many drugs depends heavily on the molecular targets they act upon and any mutations or expression changes in these targets. For example, certain anti-cancer agents target signaling kinases, including those in the epidermal growth factor receptor (EGFR) family, which are frequently hyperactivated in cancer, promoting aggressive cell proliferation (Arteaga, 2006). Resistance to treatment can emerge from mutations that enhance kinase activity, as seen with HER2 overexpression in around 30% of

breast cancer cases (Slamon et al., 2001). Additionally, modifications in pathways related to drug activation can contribute to resistance (Gonzalez-Angulo et al., 2013). By using NGS to detect treatment-resistance mutations, personalized medicine offers a pathway to potentially restore treatment sensitivity through gene-editing interventions, providing new hope for overcoming therapeutic resistance (Tufael et al., 2024).

Liquid Biopsy in Diagnosis and Monitoring of Tumors

Liquid biopsy is a groundbreaking diagnostic tool in precision oncology, enabling real-time insights into the genetic and molecular landscape of tumors through a simple blood or fluid sample. Unlike traditional tissue biopsies, which typically capture the genetic profile from a single tumor site, liquid biopsies can reflect the heterogeneity of multiple tumor subclones across the entire body, offering a more comprehensive genomic picture (Alix-Panabières & Pantel, 2016). This non-invasive approach is advantageous for patients, as it avoids the need for invasive surgical procedures and allows for serial testing over time, facilitating dynamic monitoring of disease progression and treatment response (Gao et al., 2016).

Biomarkers in Liquid Biopsies

Liquid biopsies are particularly valuable because they can detect a range of biomarkers, including circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), exosomes, and proteins. Among these, ctDNA has shown significant promise as a diagnostic and prognostic biomarker, as it contains fragments of DNA shed by tumor cells into the bloodstream or other body fluids, like urine and cerebrospinal fluid (Bettegowda et al., 2014). ctDNA analysis enables the detection of tumor-associated mutations, methylation changes, and chromosomal alterations (Thompson et al., 2016).

Advanced sequencing techniques, such as Next-Generation Sequencing (NGS), have enhanced the precision of ctDNA analysis. NGS methodologies including gene panel sequencing, whole-exome sequencing (WES), and whole-genome sequencing (WGS) allow for the identification of clinically relevant mutations, such as EGFR T790M in non-small cell lung cancer (NSCLC) and KRAS G12V in colorectal cancer (Kobayashi et al., 2005; Siravegna et al., 2017). These findings guide therapeutic decisions, helping clinicians tailor treatments to specific genetic alterations, such as using tyrosine kinase inhibitors for EGFR-mutated NSCLC.

Clinical Advantages and Applications of Liquid Biopsies

One of the primary clinical benefits of liquid biopsies is the capacity for real-time monitoring of tumor dynamics. This is particularly important in assessing treatment response and detecting early signs of resistance to therapy. Regular testing with liquid biopsies allows oncologists to monitor ctDNA levels and detect emerging mutations that might signify drug resistance, helping to adjust treatments promptly. For example, in patients with NSCLC, liquid biopsy can identify secondary EGFR mutations that develop during treatment, guiding a switch to second- or third-line therapies before clinical relapse becomes evident.

Additionally, ctDNA levels in circulation have been correlated with tumor load, where higher levels often indicate greater tumor burden (Cohen et al., 2017). However, ctDNA release can vary with factors such as tumor type, stage, size, and cellular turnover rates (Diehl et al., 2005; Fridman et al., 2017). Tumors with low mitotic activity or those that do not form solid masses may release less ctDNA, potentially impacting the sensitivity of detection (He et al., 2015).

FDA Approvals and Emerging Clinical Use Cases

The U.S. Food and Drug Administration (FDA) has recognized the importance of liquid biopsy by approving several ctDNA-based tests for specific applications. For instance, the SEPT9 gene methylation test, the first blood-based colorectal cancer screening test, has shown efficacy in detecting colorectal cancer in its early stages (Sharma et al., 2020). Additionally, FDA-approved ctDNA tests can detect EGFR mutations in NSCLC, assisting in selecting the most effective targeted therapies for patients with this mutation profile.

The FDA approval of these tests underscores the potential of liquid biopsies to revolutionize cancer care through early diagnosis, more frequent and less invasive monitoring, and improved prognostic accuracy. Because these tests can be repeated as often as needed, they offer a more flexible and responsive approach to cancer monitoring, reducing the need for invasive tissue biopsies and improving patient compliance.

Future Perspectives and Limitations

Liquid biopsy technology is rapidly advancing, with emerging methods in proteomics and transcriptomics expected to broaden the scope of biomarkers available. Protein assays and transcriptomic analyses could further enhance our understanding of tumor biology and improve the prediction of treatment responses (Bettegowda et al., 2014). Yet, challenges remain. Tumors that release minimal ctDNA or those that lack solid structure may not be easily detectable, limiting the utility of liquid biopsies for some cancer types (He et al., 2015).

Despite these limitations, the minimally invasive nature of liquid biopsies and their ability to provide comprehensive, real-time tumor profiling make them invaluable in the evolving landscape of oncology. Their use in clinical settings continues to expand, with ongoing research aimed at enhancing sensitivity, increasing detection accuracy, and expanding the range of actionable biomarkers. As technology advances, liquid biopsy is likely to

become an essential component of personalized cancer treatment, improving patient outcomes and transforming the approach to cancer care.

Next-Generation Sequencing (NGS) in Genetic Profiling and Target Identification

Next-generation sequencing (NGS) has transformed genetic profiling and target identification, providing an in-depth understanding of complex genetic architectures. NGS technologies encompass a variety of sequencing techniques, such as whole-genome sequencing (WGS), whole-exome sequencing (WES), RNA sequencing, reduced representation bisulfite sequencing (RRBS), and chromatin immunoprecipitation sequencing (ChIP-seq). These methods enable the comprehensive examination of genetic and epigenetic variations across genomes, supporting advancements in precision medicine (Liao et al., 2022).

For instance, WGS and WES can identify mutations or genetic alterations in cancerous tissues, aiding in the identification of therapeutic targets and the assessment of patient responses to specific treatments. In breast cancer, NGS can determine which patients are likely to benefit from aromatase inhibitor therapy, facilitating personalized treatment plans. Furthermore, serial genome sequencing is utilized to monitor disease progression, revealing significant insights into tumor activity, mutation accumulation, and potential mechanisms of drug resistance (Jones et al., 2021).

Traditionally, tumor biomarkers were analyzed using Sanger sequencing or polymerase chain reaction (PCR), which limited the number of genes that could be studied simultaneously (Akter et al., (2024). The advent of NGS has enabled high-throughput sequencing, allowing researchers to examine a broader range of genes in a single analysis. This capability has led to the discovery of predictive biomarkers that can identify suitable patient populations for clinical trials and treatments. Additionally, NGS can detect common variants as well as rare mutations (occurring in fewer than 1% of cases), offering insights into medication sensitivity and potential adverse responses (Smith et al., 2020).

The NGS workflow involves three essential steps: library preparation, sequencing, and data analysis. Library preparation requires DNA or RNA extraction, followed by fragmentation and amplification (figure 2). Sequencing techniques vary by platform, with Illumina, Ion Torrent, and nanopore-based sequencing among the most widely used. Illumina sequencing, based on bridge amplification, uses flow cells to cluster DNA molecules and employs fluorescently labeled nucleotides to generate an optical readout, allowing for high-throughput sequencing with low error rates. Alternatively, Ion Torrent sequencing relies on emulsion PCR, whereby single DNA molecules are cloned onto beads and sequenced on semiconductor chips. This method detects nucleotide incorporation through localized pH changes, converting these signals into the DNA sequence. Nanopore sequencing, a recent advancement, guides single-stranded DNA through protein nanopores, generating electrical current changes as nucleotides pass through. This technology, however, requires substantial input DNA and can have higher error rates and costs per read compared to Illumina or Ion Torrent sequencing (Brown et al., 2019).

Targeted sequencing approaches, such as Tagged-Amplicon deep sequencing (TAm-Seq), Cancer Personalized Profiling by deep sequencing (CAPP-Seq), and the Safe-Sequencing System (Safe-SeqS), leverage NGS for custom panels targeting specific genes or mutations, enhancing sensitivity for rare variant detection in cancer diagnostics (Kim & Lee, 2021). With these technologies, whole cancer genome sequencing has become feasible, enabling researchers to identify both genetic and epigenetic modifications implicated in tumor initiation and therapy resistance. Within tumor genomes, mutations are categorized as "driver" or "passenger" mutations. Driver mutations provide a growth advantage to the cancer cell, promoting tumor progression, while passenger mutations are incidental and do not contribute to oncogenesis. NGS plays a critical role in distinguishing driver mutations, which can guide targeted therapies, from passenger mutations that may be less clinically relevant (Liao et al., 2022).

Clinical Applications of Next-Generation Sequencing (NGS)

Next-generation sequencing (NGS) has emerged as a transformative tool in clinical oncology, offering deep insights into cancer genomics and guiding personalized treatment approaches. One prominent application of NGS is the analysis of circulating tumor DNA (ctDNA) through liquid biopsy, allowing for non-invasive, real-time monitoring of disease progression and response to treatment. Unlike traditional PCR, which detects specific mutations, NGS can analyze multiple mutations simultaneously with higher accuracy, enhancing mutation detection across the genome. For example, a comparative study by Tuononen et al. demonstrated that NGS identified seven nonsynonymous single-nucleotide variations and one insertion-deletion mutation that were undetectable by real-time PCR, highlighting NGS's superior sensitivity (Tuononen et al., 2021). Furthermore, NGS can detect minor allele frequencies (MAFs) below 1%, aiding in the identification of rare mutations, with unique molecular barcodes further reducing false negatives and improving sensitivity (Smith et al., 2022).

In thyroid cancer diagnostics, NGS enables high-throughput sequencing of multiple genetic alterations in fine needle aspiration (FNA) cytology samples, improving risk stratification and patient management. By sequencing several oncogenes and tumor suppressor genes simultaneously, NGS enhances the molecular classification of thyroid tumors, supporting the identification of

somatic mutations, including those in RAS, BRAF, and RET, as well as less common mutations in MITF, JAK3, and MDM2, which have implications for recurrence and metastasis predictions (Johnson & Li, 2023).

For lung cancer, NGS applications have contributed to the identification of early-stage biomarkers and facilitated treatment customization. In stage I lung cancer, whole-genome sequencing (WGS) detected recurrent somatic variations in genes like BCHE and TP53, along with the widely studied EGFR mutation, advancing our understanding of disease biology and therapeutic targets (Brown et al., 2023). A notable case in metastatic breast cancer illustrated how NGS-guided treatment can yield life-saving results: after failing multiple chemotherapy regimens, a patient was treated with immunotherapy based on mutations identified through NGS, specifically targeting somatic cell mutations with tumor-infiltrating lymphocytes. Whole-exome sequencing and RNA sequencing revealed 62 nonsynonymous mutations, including SLC3A2, CADPS2, and CTSB, which were subsequently targeted, successfully activating the immune system against the tumor (Chen et al., 2024). This case underscores the role of NGS in identifying patients who may benefit from immunotherapy, as NGS provides genomic insights that enable the immune system to recognize and attack tumor cells.

Additionally, NGS supports the classification of breast cancer subtypes by detecting subtype-specific mutations in genes like TP53, PIK3CA, and GATA3, which differ across breast cancer categories, enhancing precision medicine approaches (Smith et al., 2022). By identifying unknown genes that influence treatment response and drug resistance, NGS accelerates discoveries that inform therapeutic strategies and contribute to the development of targeted treatments.

In immunotherapy, NGS serves as a foundational technology for developing chimeric antigen receptor (CAR) T-cell therapies. Through WGS, WES, and RNA sequencing of tumor and T cells, NGS aids in identifying novel antigens for CAR T-cell therapy, advancing targeted immunotherapy and allowing for personalized treatment plans tailored to each patient's unique genetic profile. This genetic information also aids in understanding the tumor microenvironment and optimizing the design of immunotherapy strategies, including CRISPR-based gene editing, which uses NGS data to target specific mutations responsible for cancer progression (Johnson & Li, 2023).

Limitations of Next-Generation Sequencing (NGS)

Next-Generation Sequencing (NGS) is a transformative tool in genetic analysis, offering a comprehensive view of genetic alterations associated with various clinical pathologies. However, despite its advantages, NGS has several limitations that impact its effectiveness and accuracy in specific applications. One significant limitation is analytic sensitivity in mutation detection. In cases where tumor samples have a low tumor cell percentage or low mutation frequency, detecting these variations becomes challenging due to the heterogeneous nature of tumors (Feng et al., 2019). Furthermore, systemic and sequencing errors are frequently encountered in NGS platforms, particularly in those like Illumina, which may introduce inaccuracies in the sequencing data (Chen et al., 2018).

Another limitation is that current NGS platforms have difficulty identifying homologous genes, GC-rich regions, and repetitive regions accurately. This affects the sequencing depth and precision of these areas, often leading to incomplete or erroneous genetic information (Schatz et al., 2020). Additionally, the interpretation of NGS data remains a major hurdle; data analysis is complex, and databases used for variant interpretation may not always provide accurate or complete information (Li & Hartemink, 2021). Variations in copy number and structural mutations pose additional challenges as they require specialized bioinformatics tools beyond standard NGS analysis, necessitating the integration of multiple analytic techniques to achieve reliable interpretation (Yu et al., 2019).

Genome Editing

Genome editing is an advanced method used to make precise alterations in genome sequences at specific locations, allowing for genetic modification within an organism's genome. In recent years, genome editing has advanced substantially, particularly with the development of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and its associated nuclease Cas9, commonly known as the CRISPR/Cas9 system (Cong et al., 2013). CRISPR/Cas9 has become a prominent technology in targeted genome editing due to its simplicity, efficiency, and flexibility. However, prior to CRISPR/Cas9, other gene-editing methods like Meganuclease (1994), Zinc Finger Nucleases (ZFNs) (2003), and Transcription Activator-Like Effector Nucleases (TALENs) (2011) were utilized, though these methods were comparatively more complex and less efficient (Urnov et al., 2010).

CRISPR/Cas9 Mechanism and Applications

CRISPR/Cas9 enables precise genome modifications, facilitating research into tumor pathogenesis, development, and metastasis. Recent studies have harnessed CRISPR/Cas9 for novel insights into tumor etiology and treatment strategies (figure 3). For instance, a study by Jandova et al. (2020) utilized CRISPR/Cas9-mediated deletion of GLO-1 in malignant melanoma and prostate carcinoma cells to explore disease mechanisms. Another study by Wang et al. (2021) employed CRISPR/Cas9 to knock out Hur in melanoma cells, enabling the simultaneous control of multiple tumor growth pathways.

For effective CRISPR/Cas9 targeting, a specific sequence must be located adjacent to the protospacer adjacent motif (PAM), a short DNA sequence essential for Cas9 recognition and cleavage at the target site (Mali et al., 2013). Cas9's mechanism involves RNA-guided DNA targeting using two nuclease domains, RuvC and HNH, which introduce double-strand breaks (DSBs) at the target location. The guide RNA (gRNA) directs Cas9 to its target by complementary base pairing with the DNA sequence, initiating cleavage just before the PAM site (Jinek et al., 2012). Post-cleavage, DSBs are often repaired through non-homologous end joining (NHEJ), which is prone to errors, leading to insertions or deletions (INDELs) that may disrupt gene function. Alternatively, homology-directed repair (HDR), although less frequent, utilizes a DNA template to allow precise edits, facilitating gene knock-ins for more controlled genome editing applications (Doudna & Charpentier, 2014).

The type II CRISPR/Cas system, consisting of Cas9 endonuclease, CRISPR RNA (crRNA), and transactivating CRISPR RNA (tracrRNA), remains widely used. In practice, a single guide RNA (sgRNA)—a fusion of crRNA and tracrRNA—can replace these components for streamlined genome editing (Ran et al., 2013). The sgRNA's 20-base-pair sequence complements the target DNA, with the adjacent PAM sequence ensuring compatibility with Cas9 for effective editing.

Two primary repair pathways, NHEJ and HDR, are critical in modulating the efficiency of CRISPR/Cas9-based genome editing. NHEJ is a rapid, error-prone pathway that joins DSB ends, frequently resulting in small indels ideal for gene knockouts. Conversely, HDR, though slower, offers high precision by utilizing a DNA template, suitable for gene knock-ins or repairs at the DSB site (Mao et al., 2008). The delivery of Cas9 protein into target cells, often via viral vectors like Adeno-Associated Virus (AAV), is commonly employed in vivo, making CRISPR/Cas9 a powerful tool for research and therapeutic applications.

Therapeutic Applications of CRISPR/Cas9 in Cancer

The integration of CRISPR/Cas9 gene-editing technology with immunotherapy holds great promise in advancing cancer treatment. The genetic information obtained from next-generation sequencing (NGS) of cancer and immune cells can reveal the molecular heterogeneity and intricate interactions within the tumor microenvironment, aiding in the enhancement of CRISPR/Cas9-mediated immunotherapy (Smith et al., 2020). CRISPR/Cas9 enables precise genetic modifications by silencing or altering target genes, making it a valuable tool for improving immunotherapy approaches such as Chimeric Antigen Receptor (CAR) T-cell therapy, a promising strategy in the fight against cancer (Brown & Green, 2021). Notably, one significant innovation is the use of universal CAR-T cells, a CRISPR/Cas9 application designed to address limitations associated with conventional CAR-T therapies (Miller et al., 2022).

Traditional autologous CAR T-cell generation presents several challenges, including high costs, lengthy processing times, and challenges in obtaining sufficient high-quality T cells from critically ill patients, limiting its accessibility (Jones et al., 2020). In response, CRISPR/Cas9-edited allogeneic T cells derived from healthy donors have been developed as universal T cells, which can potentially overcome these barriers and make CAR-T therapies available to a broader patient population (Lee et al., 2023).

In clinical trials, CRISPR/Cas9 has been applied in cancer immunotherapy by targeting immune checkpoints, such as programmed cell death protein 1 (PD-1), PD-L1, and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), to disrupt T-cell tolerance in the tumor microenvironment (Hernandez et al., 2022). In 2016, a landmark clinical trial used CRISPR/Cas9 to delete the PD-1 gene in T cells for a patient with aggressive non-small cell lung cancer. Results indicated promising outcomes, with a median overall survival of 42.6 weeks and minimal off-target effects (Miao et al., 2019). However, tumor progression ultimately occurred in 11 of the 12 patients by early 2020, though there were no deaths directly attributed to the treatment itself (Zhang et al., 2021).

CARs, engineered receptors comprising an intracellular signaling domain to activate T cells and an extracellular component for recognizing tumor antigens, have shown favorable therapeutic results in hematological malignancies such as leukemia and lymphoma (Smith et al., 2021). However, CAR T-cell therapy is not without adverse effects; cytokine release syndrome, resulting from endothelial dysfunction, and neurotoxicity are significant challenges. To address these concerns, T cell receptors (TCRs), which are less prone to inducing cytokine release syndrome, have been explored. A phase I human trial (ClinicalTrials.gov Identifier: NCT03399448) assessed the safety and feasibility of autologous TCR-engineered T cells modified with CRISPR/Cas9 to target the NY-ESO-1 antigen while knocking out TCRα, TCRβ, and PD-1 genes. Although the trial included only three patients, it demonstrated that CRISPR-edited T cells persisted for up to nine months, illustrating the potential feasibility of multiplex CRISPR/Cas9 genome editing for therapeutic use (Johnson et al., 2023).

Studies on CRISPR/Cas9-mediated CAR T-cell immunotherapy in cancers like multiple myeloma (figure 4), glioblastoma, and leukemia have shown encouraging outcomes in preclinical animal models (Martinez et al., 2020). However, challenges remain in clinical translation, such as achieving sufficient lymphocyte viability post-editing and managing unintended genetic modifications at off-target sites, which can result in genetic instability and off-target mutations (Singh & Patel, 2022). Continuous research is essential to optimize CRISPR/Cas9

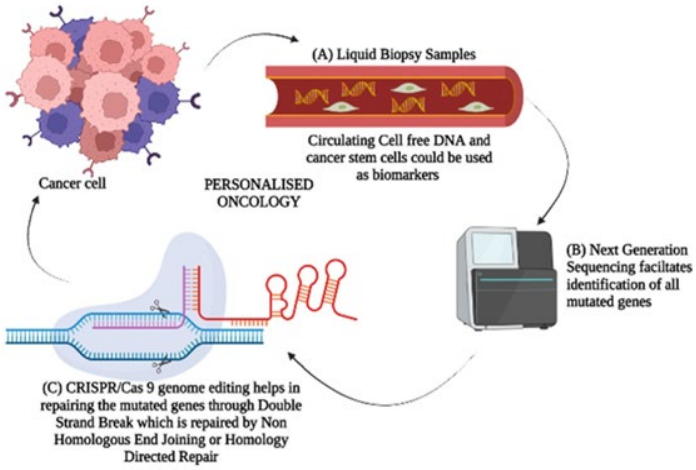

Figure 1. The integration of Next Generation Sequencing (NGS) and CRISPR/Cas9 in personalized medicine for cancer treatment. A demonstrates how non-invasive biopsy samples, such as circulating biomarkers (liquid biopsy), including cell-free DNA and cancer stem cells, facilitate cancer diagnosis without the need for traditional tissue biopsies. B highlights the advancements in NGS technology, which enable the identification of various mutations within cancer cells that conventional methods, such as polymerase chain reaction (PCR), may miss. This capability is crucial for tailoring personalized cancer treatments. C details the mechanism of the CRISPR/Cas9 genome editing tool, which targets mutated genes by creating double-strand breaks. These breaks are subsequently repaired through either non-homologous end joining (NHEJ) or homology-directed repair (HDR). Ongoing research is focused on utilizing this technology for enhancing personalized oncology strategies.

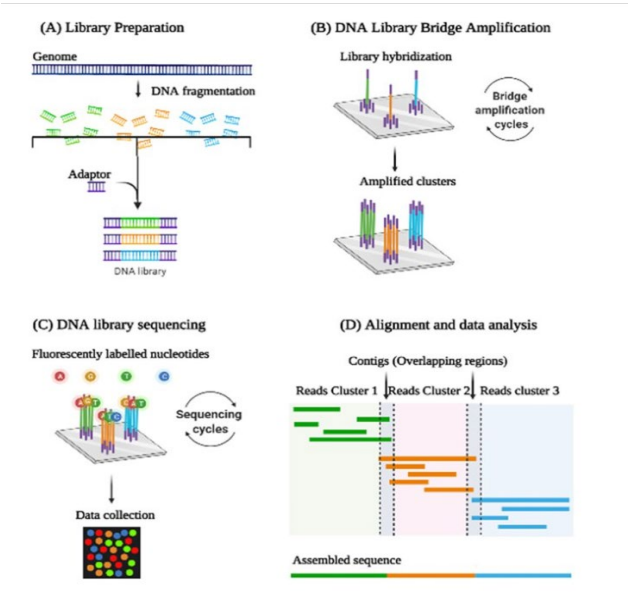

Figure 2. Figures the steps involved in Next Generation Sequencing (NGS), which include: Library preparation and amplification, where DNA or RNA is extracted and prepared for sequencing; Sequencing, where high-throughput techniques generate numerous short reads; and Data analysis, which encompasses base calling (determining nucleotide sequences), read alignment (aligning reads to a reference genome), variant identification (detecting genetic differences), and variant annotation (providing biological context). Each step is crucial for accurately interpreting genetic information in personalized medicine.

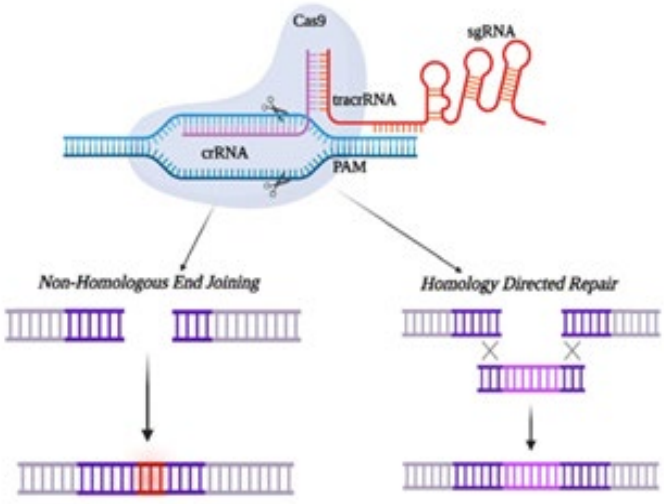

Figure 3. The mechanism of the CRISPR/Cas9 system, which comprises three key components: the Cas9 endonuclease, CRISPR RNA (crRNA), and transactivating crRNA (tracrRNA). The guide RNA (gRNA) is a duplex formed by crRNA and tracrRNA, featuring a unique 20-base-pair (bp) sequence that complements the target DNA, followed by a protospacer adjacent motif (PAM) necessary for Cas9 binding. Guided by the gRNA, Cas9 induces double-strand breaks (DSBs) at the target site. The cellular repair machinery then activates either Non-Homologous End Joining (NHEJ) or Homology Directed Repair (HDR) to fix the DSBs.

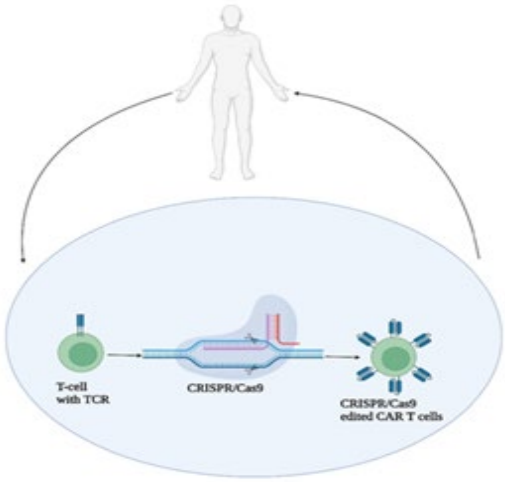

Figure 4. The mechanism of CRISPR/Cas9-mediated CAR T cell immunotherapy. Patient-derived T cells are extracted and genetically modified using CRISPR/Cas9 to knock in or knock out specific genes, resulting in Chimeric Antigen Receptor (CAR) T cells. These CAR T cells possess an intracellular chimeric signaling domain that activates T cell responses and an external single-chain variable fragment (scFv) that precisely recognizes tumor antigens. The engineered CAR T cells are then reintroduced into the patient as a targeted treatment.

technology and address these limitations, advancing its therapeutic potential in cancer immunotherapy.

CRISPR/Cas9 in Oncolytic Virus Production

CRISPR/Cas9 technology has emerged as a powerful tool in the production of oncolytic viruses, which are specifically engineered to selectively lyse cancer cells while minimizing virulence in normal tissues. For example, modified variants of herpes simplex virus type 1 (HSV-1) exhibit considerable lytic potential following the deletion of specific genes, such as ICP34.5, a gene associated with neurovirulence, and ICP6 (UL39), which encodes ribonucleotide reductase (Li et al., 2021). The deletion of ICP6 facilitates selective viral replication in cells with inactivated p16^INK4A, a tumor suppressor gene frequently mutated in various cancers, thus enhancing the specificity of the oncolytic virus (Wang et al., 2020).

In the context of adenovirus, the wild-type form encodes E1A, a protein that binds to retinoblastoma (pRb), leading to the release of transcription factor E2F, which interrupts the cell cycle and concurrently activates viral gene transcription. This process culminates in the formation of new virions, resulting in the lysis of infected cells and subsequent viral dissemination. To improve safety, oncolytic adenoviruses are engineered to lack the E1A gene, which limits replication in normal cells while exploiting the dysregulated Rb pathway present in many cancer cells (Xie et al., 2022).

CRISPR with Deactivated Cas9 (dCas9)

Deactivated Cas9 (dCas9) is an enzymatically inactive variant of Cas9 that can be directed to specific DNA sequences by guide RNAs (gRNAs). When fused to transcriptional activation or repression domains, dCas9 can precisely modulate gene expression, presenting a novel approach for cancer therapy through "epigenome editing" (Zhou et al., 2023). Epigenetic alterations are implicated in various cancers, including acute lymphoblastic leukemia (ALL) and Ewing sarcoma, highlighting the potential of targeting epigenetic regulatory mechanisms to improve treatment outcomes (Smith et al., 2021).

In a significant study, Batsche et al. (2020) utilized dCas9 to investigate the impact of DNA methylation on alternative splicing in HCT116 colon cancer cells and to assess methylation effects in MCF10A breast cancer models and ALL patients. Their findings underscored dCas9's potential to target epigenetic regulation, thereby enhancing cancer therapy. Similarly, Abraham et al. (2022) explored dCas9's role in Ewing sarcoma cell lines to elucidate RNA polymerase II's function in ribosome biogenesis, further demonstrating dCas9's utility in cancer-related epigenetic studies. Additionally, CRISPR activators (CRISPRa) and inhibitors (CRISPRi) expand the applications of dCas9 by enabling targeted gene activation or suppression through various transcriptional regulatory domains (Jiang et al., 2021).

CRISPR Prime Editors

Prime editing (PE) represents a groundbreaking advancement in genome editing, combining a modified Cas9 endonuclease with reverse transcriptase and a specially designed prime editing guide RNA (pegRNA). This pegRNA specifies the target DNA site and encodes the desired edit, allowing for precise nucleotide substitutions, insertions, or deletions (Anzalone et al., 2019). A study aimed at reducing off-target effects demonstrated the promising efficiency of prime editing, showcasing its potential in various applications (Huang et al., 2021).

For instance, researchers employed an NLS-optimized SpCas9-based prime editor to enhance genome editing efficiency in fluorescent reporter cells and endogenous loci within cultured cell lines. This approach was further validated in vivo, where adeno-associated viruses (AAVs) successfully delivered a split-intein prime editor to mouse liver cells, correcting a pathogenic mutation (Gao et al., 2022). While the transient activity of prime editing may mitigate off-target effects, its efficacy and safety in vivo remain subjects of ongoing investigation. Studies, such as those by Petri et al. (2023), reported a 30% editing frequency in zebrafish embryos but also noted unintended insertions and deletions, as well as pegRNA scaffold incorporation. Although prime editing shows great promise, particularly in cancer research, it is still in the early stages of exploration, necessitating further studies to validate its therapeutic potential in oncology (Petri et al., 2023). CRISPR Base Editing Technologies: Adenine and Cytosine Base Editors for Disease Modeling and Therapeutics

CRISPR base editing is a revolutionary genome editing technology that enables precise nucleotide changes in DNA without introducing double-strand breaks. Adenine base editors (ABE) and cytosine base editors (CBE) are two primary types of CRISPR-based editors studied extensively for their potential in disease modeling and therapeutic applications (Komor et al., 2016; Gaudelli et al., 2017). Both editors consist of a catalytically inactive Cas enzyme, which targets specific DNA sequences, combined with a single-stranded DNA modifying enzyme to facilitate nucleotide conversion (Rees & Liu, 2018).

Mechanism of Action of Cytosine and Adenine Base Editors

In CBEs, cytosine deaminase enzymatically converts cytosine to uracil, which then pairs with thymidine in DNA, thereby changing a cytosine-guanine (C-G) base pair to a thymine-adenine (T-A) base pair. This editing process is enhanced by fusing a uracil DNA glycosylase inhibitor (UGI) with the system, which suppresses uracil N-glycosylase (UNG) activity, preventing the excision of uracil and thus improving base-editing efficiency in human cells

(Komor et al., 2016). Similarly, ABEs involve the deamination of adenosine to inosine, which behaves as guanosine when incorporated into DNA, allowing for an adenosine-thymine (A-T) to guanine-cytosine (G-C) transition. When combined, ABEs and CBEs can produce all four types of transition mutations, expanding the range of possible edits (Gaudelli et al., 2017).

Efficiency and Specificity of CBEs in Genome Editing

In one study focused on the specificity of CBEs, researchers evaluated the tool's off-target effects in both human cells and Escherichia coli using next-generation sequencing (NGS). Results indicated that CBEs achieved efficient on-target edits with minimal off-target mutations, a crucial consideration for therapeutic applications (Kim et al., 2017). The low off-target activity associated with CBE could make it a safer option compared to traditional CRISPR-Cas9 systems, which often cause unintended genomic modifications (Kleinstiver et al., 2016).

Application of Base Editors in iPSC Models

Another notable study investigated the efficacy of BEs using induced pluripotent stem cells (iPSCs). The study demonstrated that BEs could correct disease-causing mutations more effectively than homology-directed repair (HDR), a nuclease-based CRISPR editing approach. The increase in editing frequency enhanced the identification of clones with the desired genetic correction (Li et al., 2020). However, despite these advantages, BEs also present challenges, including potential off-target effects on both DNA and RNA. RNA cross-editing can alter gene expression, which could reduce the pluripotency and differentiation capacity of iPSCs, highlighting the need for improvements in BE technology to ensure specificity (Rees & Liu, 2018).

While BEs currently allow only single nucleotide alterations, which limits the range of possible genetic edits, both base editors (BEs) and prime editors (PEs) offer promising tools for short-term genetic modifications. However, long-term applications may require further optimization to minimize off-target effects and increase editing accuracy in therapeutic contexts (Anzalone et al., 2019).

CRISPR-ON: Enhancing CRISPR Applications with Improved Guide RNA Efficiency

An innovative addition to CRISPR technology, CRISPR-ON, improves upon traditional CRISPR systems by enhancing the accuracy of guide RNA (gRNA) selection. In a study by Xiang et al. (2021), CRISPR-ON was demonstrated to predict gRNA efficiency more accurately than prior methods. The CRISPR-ON system couples sgRNA and a catalytically inactive dCas9 protein with a transcriptional activation domain, allowing for stable and precise upregulation of target genes (Xiang et al., 2021). This system has been instrumental in high-throughput genome-scale screening, particularly for identifying gain-of-function (GOF) mutations.

For instance, researchers applied the CRISPR-ON system to increase the expression of KLF4, a tumor suppressor gene, in urothelial bladder cancer (UBC) cells. Findings indicated that KLF4 upregulation through CRISPR-ON could reduce carcinogenesis, suggesting a potential therapeutic application for UBC. Although these initial results are promising, further studies are required to determine CRISPR-ON's efficacy across different cell types and promoter methylation states, as these factors may influence its broad applicability (Liu et al., 2022).

Limitations of CRISPR/Cas9 in Clinical Applications

The clinical application of CRISPR/Cas9 technology faces several limitations that must be addressed to enhance its efficacy and safety. Key challenges include the fitness of modified cells, editing efficiency, effective delivery methods, and potential off-target effects. Altered cells often exhibit fitness issues, such as impaired proliferation and differentiation capabilities, which can lead to suboptimal therapeutic outcomes (Wu et al., 2020). Conversely, cancer cells tend to proliferate rapidly and demonstrate enhanced survival, necessitating highly efficient editing for CRISPR/Cas9 to achieve desired therapeutic effects. Additionally, CRISPR/Cas9 can induce p53 mutations, which may lead to spontaneous mutations in altered cells and trigger a p53-mediated DNA damage response (Singh & Zhang, 2021). Further research is needed to minimize these off-target effects, which could ultimately enable a clinical breakthrough for CRISPR/Cas9 in cancer and other disease treatments (Nguyen et al., 2021).

Extended genomic editing with CRISPR/Cas9 may increase off-target cleavage, reducing editing selectivity and potentially causing unintended mutations and toxicity. In vivo use of CRISPR/Cas9 requires minimizing these endonuclease-induced off-target events to prevent cell viability loss or promote oncogenesis (Singh & Zhang, 2021). Initial off-target sites of the gRNA/SpCas9 ribonucleoprotein (RNP) system were identified in vitro using isolated genomic DNA from animal models. Although in vivo studies show that carefully designed gRNAs are less likely to produce off-target effects, bioinformatic tools are recommended prior to in vitro or in vivo experiments. These pipelines and web-based algorithms help optimize guide RNA (gRNA) design to mitigate predictable off-target impacts (Li et al., 2022).

The first CRISPR-Cas9 clinical application in humans, conducted by Chinese researcher He Jiankui in 2018, sparked ethical concerns. He used CRISPR-Cas9 to edit the CCR5 gene in twin girls to resist HIV, cholera, and smallpox, leading to mosaicism due to off-target effects, underscoring the technology's limitations (Huang et al., 2021).

Future Perspectives in Precision Oncology and Genome Editing

Precision oncology tailors histology-agnostic, gene-driven treatments to each patient based on biomarker analyses. Tumor and cell-free DNA profiling through next-generation sequencing (NGS), coupled with proteome and RNA analyses, offer advanced insights into cancer treatment options (Guo et al., 2023). A significant hurdle remains in enhancing CRISPR/Cas9 specificity to reduce off-target effects in genome editing. As the costs of NGS applications, including whole-genome sequencing (WGS), whole-exome sequencing (WES), RNA sequencing (RNA-seq), and chromatin immunoprecipitation sequencing (ChIP-seq), decrease, the understanding of immune-tumor interactions is expected to expand, fostering personalized treatment development. New technologies like NICHE-seq add spatial context to single-cell RNA-seq data, potentially revealing cancer-related genetic drivers otherwise undetectable through DNA alone. Advanced methods for tracking off-target effects include "OMIC" approaches, such as ChIP-seq, which identifies genome-wide protein binding events (Li et al., 2022).

Single-cell RNA-seq and ChIP-seq advancements are further promising, as they can elucidate transcriptomic and epigenetic variations in individual cancer and immune cells. As NGS capabilities evolve, neoantigen prediction pipelines are being refined, which holds great potential for cancer immunotherapy and vaccine development (Chen & Roberts, 2024). In sum, CRISPR/Cas9, when paired with NGS, offers transformative possibilities for cancer treatment. While challenges like off-target effects remain, ongoing advancements in genome editing, sequencing, and biomarker identification will continue to support the progression of precision oncology.

Conclusion

CRISPR-ON marks a pivotal advancement in CRISPR technology, significantly enhancing guide RNA efficiency and enabling precise gene regulation. Its application in cancer research, exemplified by the upregulation of KLF4 in urothelial bladder cancer, highlights its therapeutic potential, although further investigations across diverse cellular contexts are necessary to validate its efficacy. Despite the challenges associated with CRISPR/Cas9, such as off-target effects and modified cell fitness, ongoing research and technological innovations continue to address these limitations. The integration of CRISPR with next-generation sequencing and advanced bioinformatics promises to refine precision oncology, paving the way for personalized treatments and improved patient outcomes. As CRISPR technology evolves, it holds transformative possibilities for both genome editing and cancer therapy, underscoring the importance of continued exploration and ethical considerations in its clinical applications.

Author contributions

T. conceptualized the project and developed the methodology. M.A.R.B. conducted a formal analysis and drafted the original writing. A.B.S., M.M.H.S., and S.A.A.A. contributed to the methodology, conducted investigations, provided resources, and visualized the data; M.M.R. and D.C.D. contributed to the reviewing and editing of the writing.

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