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Synergistic CRISPR/Cas9 Gene Editing and CAR T-Cell Therapy for FLT3-Mutated in AML

Derrick Lee

ABSTRACT

Acute Myeloid Leukemia (AML) with FLT3 (FMS-like Tyrosine Kinase 3) mutations poses a significant challenge in oncology. This paper explores the potential of a combined treatment approach involving CRISPR/Cas9 gene editing and CAR T-cell therapy. CRISPR/Cas9 technology can precisely target and correct the FLT3 gene mutation responsible for AML proliferation. Simultaneously, CAR T-cell therapy harnesses the immune system to target FLT3-mutated AML cells using engineered T cells. While promising, these approaches come with challenges, such as off-target effects for CRISPR/Cas9 and complications like cytokine release syndrome for CAR T-cell therapy. Nevertheless, they offer a groundbreaking paradigm shift in AML treatment, potentially providing more effective and personalized therapies for patients with FLT3 mutations. Further research and clinical trials are essential to fully realize their potential and address associated hurdles. This combination therapy offers hope for improving outcomes in AML patients with FLT3 mutations, representing a significant advancement in precision oncology.

Introduction

Leukemia is a type of cancer that affects the blood and bone marrow, leading to the abnormal production of leukocytes (Adithya Chennamadhavuni et al., 2023). Additionally, it is classified as chronic or acute based on the proliferation rate, and lymphocytic and myelocytic based on its origin (Adithya Chennamadhavuni et al., 2023). The combination of these classifications results in four main subtypes: Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL), Chronic Myeloid Leukemia (CML), and Chronic Lymphocytic Leukemia (CLL) (Adithya Chennamadhavuni et al., 2023; Shephard et al., 2016).

Acute Lymphoblastic Leukemia (ALL) is a fast-growing blood cancer that begins in the bone marrow. It primarily causes immature lymphocytes, a type of lymphoblast, to proliferate uncontrollably which crowds out healthy blood cells (Adithya Chennamadhavuni et al., 2023; Puckett & Chan, 2023). This leads to a decrease in red blood cells, white blood cells, and platelets, resulting in anemia and an increased infection risk (Adithya Chennamadhavuni et al., 2023; Puckett & Chan, 2023). Chronic Myeloid Leukemia (CML) is a slow-growing leukemia that typically affects adults (Adithya Chennamadhavuni et al., 2023; Eden & Coviello, 2023). An abnormal mutation leads to the overproduction of immature myeloblasts. These proliferated cells accumulate in the bone marrow in the blood, interfering with healthy cell production (Adithya Chennamadhavuni et al., 2023; Eden & Coviello, 2023). Chronic Lymphocytic Leukemia (CLL) is a slow-growing leukemia that is extremely common in elderly adults (Adithya Chennamadhavuni et al., 2023). CLL is characterized by the gradual accumulation of monoclonal lymphoid cells in the blood, bone marrow, and lymph nodes (Adithya Chennamadhavuni et al., 2023).

Acute Myeloid Leukemia (AML) is a common type of leukemia most commonly found in those 45 years of age and older and has an annual death toll of around 11,310 deaths (*Acute Myeloid Leukemia - Cancer Stat Facts*, 2018; Siegel et al., 2023). AML is characterized by the uncontrolled proliferation and accumulation of immature myeloid cells in the bone marrow and blood (Arber et al., 2016; Cassier et al., 2017). These cells,

known as blasts, fail to mature into healthy blood cells and platelets resulting in the disruption and compromisation of the bone marrow and immune system's ability to fight infections among other issues (*Acute Myeloid Leukemia - Cancer Stat Facts*, 2018; *Acute Myeloid Leukemia | Leukemia Types*, 2022).

FLT3 is a gene that is critical in the normal development and function of hematopoietic stem cells, which are integral to the generation of all blood cells (Anusha Vakiti & Prerna Mewawalla, 2023). In AML, the FLT3 gene can undergo mutations that contribute to the development and progression of leukemia (Anusha Vakiti & Prerna Mewawalla, 2023). FLT3 mutations occur in 20% of patients with the two most common FLT3 mutations being Internal Tandem Duplications (ITD) and Tyrosine Kinase Domain (TKD) and mutations in these genes result in uncontrolled FLT3 signaling which prompts proliferation, survival, and resistance to apoptosis (Anusha Vakiti & Prerna Mewawalla, 2023). FLT3 mutations are often associated with more aggressive conditions and poorer prognosis, ultimately resulting in a higher likelihood of relapse and a decreased rate of survival (Anusha Vakiti & Prerna Mewawalla, 2023).

FLT3 Mutations Beyond AML: Expanding Roles and Therapeutic Implications

Internal tandem duplication (ITD) mutations occurring within the FLT3 gene have emerged as pivotal contributors to the pathogenesis of various malignancies (Daver et al., 2012). These mutations, characterized by the insertion of tandem repeated sequences into the juxtamembrane domain of the FLT3 receptor tyrosine kinase, result in the gain of function by facilitating constitutive activation of downstream signaling pathways (Daver et al., 2012; Hitoshi Kiyoi et al., 2002; Janke et al., 2014). The juxtamembrane domain normally plays a crucial role in regulating FLT3 activity, but with the introduction of ITD mutations, this regulatory mechanism is disrupted (Hitoshi Kiyoi et al., 2002). As a result, uncontrolled and sustained FLT3 signaling occurs, driving aberrant cell proliferation and enhanced cell survival, which are hallmark features of malignancies associated with FLT3-ITD mutations (Hitoshi Kiyoi et al., 2002). This persistent activation, in turn, fosters unregulated cellular proliferation and enhanced cell survival (Daver et al., 2012). Notably, FLT3-ITD mutations have been detected in specific subsets of patients afflicted with other hematologic malignancies such as acute lymphoblastic leukemia (ALL) and myelodysplastic syndromes (MDS), underscoring their broader implications within the hematopoietic system (Daver et al., 2012). Furthermore, recent investigations have unveiled the presence of FLT3-ITD mutations in non-hematologic cancers, including solid tumors like breast, lung, and colorectal cancers (Daver et al., 2012). The expanding spectrum of malignancies harboring FLT3-ITD alterations underscores the imperative to comprehensively comprehend their distinct effects within diverse cellular contexts (Daver et al., 2012).

The FLT3-ITD (Internal Tandem Duplication) mutation is one of the most common FLT3 mutations in AML (Hitoshi Kiyoi et al., 2019). It involves the insertion of multiple duplicated segments of DNA within the FLT3 gene, specifically in the juxtamembrane domain of the receptor tyrosine kinase, resulting in the prediction of an abnormal, constitutively active FLT3 receptor (Ahn & Kim, 2022; Hitoshi Kiyoi et al., 2019). This leads to uncontrolled cell growth and proliferation, contributing to the development and progression of AML (Ahn & Kim, 2022; Hitoshi Kiyoi et al., 2019). The FLT3-ITD mutation is associated with a poorer prognosis in AML patients (Ahn & Kim, 2022; Hitoshi Kiyoi et al., 2019). Additionally, patients with AML with the FLT3-ITD mutations often have a higher white blood cell count at the time of diagnosis, and the presence of this mutation is associated with a poorer prognosis compared to those without (Ahn & Kim, 2022; Hitoshi Kiyoi et al., 2019). However, targeted therapies such as FLT3 inhibitors have shown promise in improving outcomes for AML patients who are FLT3-ITD-positive (Ahn & Kim, 2022; Hitoshi Kiyoi et al., 2019).

The FLT3-TKD (Tyrosine Kinase Domain) mutation involves alterations within the tyrosine kinase domain of the FLT3 protein (Ahn & Kim, 2022; Hitoshi Kiyoi et al., 2019). This mutation leads to increased

kinase activity and signaling which is similar to that of FLT3-ITD but occurs through a different mechanism and contributes to uncontrolled cell growth, proliferation, and survival of leukemic cells along with similar clinical features such as a higher white blood cell count at diagnosis (Ahn & Kim, 2022; Li et al., 2023). Unlike FLT3-ITD, FLT3-TKD mutations occur within the tyrosine kinase domain of the FLT3 receptor tyrosine kinase (Ahn & Kim, 2022; Hitoshi Kiyoi et al., 2019). This tyrosine kinase domain is critical for the proper regulation of FLT3 signaling (Hitoshi Kiyoi et al., 2019). The presence of FLT3-TKD mutations in AML patients can influence the response to specific therapies as FLT3-TKD mutations are associated with a less aggressive disease compared to that of FLT3-ITD (Ahn & Kim, 2022; Hitoshi Kiyoi et al., 2019). However, FLT3-TKD may still impact the effectiveness of targeted therapies that specifically target FLT3 receptors, such as FLT3 inhibitors (Ahn & Kim, 2022; Hitoshi Kiyoi et al., 2019).

Beyond its firmly established role in acute myeloid leukemia (AML), the tyrosine kinase domain (TKD) of the FLT3 gene has gained significant attention as a mutation hotspot driving tumorigenesis across a wider spectrum of cancers (Ahn & Kim, 2022; Li et al., 2023). These TKD mutations, often characterized by point mutations occurring within the FLT3 receptor's kinase domain, bestow a gain-of-function trait upon it (Ahn & Kim, 2022; Li et al., 2023). This leads to the initiation of downstream signaling pathways independently of ligand binding, thereby promoting uncontrolled cell growth and enhanced survival (Li et al., 2023). While extensively explored within hematologic malignancies, particularly in the context of AML relapse, emerging evidence underscores their presence in diverse cancer types (Ahn & Kim, 2022; Li et al., 2023). Notably, FLT3-TKD mutations have been identified in subsets of patients with solid tumors, such as glioblastoma multiforme and prostate cancer (Ahn & Kim, 2022; Li et al., 2023). This expanding landscape of malignancies harboring FLT3-TKD aberrations underscores the imperative need for a comprehensive understanding of their distinct functional implications within various cellular contexts (Li et al., 2023). Such insight opens exciting avenues for the development of targeted therapeutic strategies tailored to the unique demands of these diverse settings (Li et al., 2023). As we progress to the subsequent paragraph, we delve into the intricate mechanisms governed by FLT3-TKD mutations, shedding light on their potential as promising druggable targets within the realm of precision oncology (Ahn & Kim, 2022; Li et al., 2023).

Therapeutic Strategies and Combinations in the Management of FLT3-Mutated AML

In the therapeutic landscape of acute myeloid leukemia (AML) with FLT3 mutations, innovative approaches are being explored, including leveraging the unique properties of stem cells and radiotherapy. Stem cell transplantation, particularly allogeneic hematopoietic stem cell transplantation (HSCT), has emerged as a curative option for FLT3-mutated AML (He et al., 2022). The graft-versus-leukemia effect associated with allogeneic HSCT can target and eliminate residual leukemic cells harboring FLT3 mutations, offering a potential cure for this high-risk subset of AML patients (He et al., 2022). Additionally, radiotherapy, traditionally employed to target solid tumors, is now being investigated for its potential in treating FLT3-mutated AML. Preclinical studies suggest that localized irradiation can disrupt leukemic bone marrow niches and sensitize leukemic cells, including those with FLT3 mutations, to subsequent chemotherapy or targeted therapies (Allodji et al., 2020). These novel approaches not only underscore the dynamic nature of AML treatment strategies but also highlight the promising potential of combining conventional therapies with innovative techniques to combat FLT3-mutated AML effectively (Allodji et al., 2020).

FLT3 inhibitors are targeted therapies designed to block the activity of FLT3 kinase, which is often overactive in the presence of FLT3 mutations. Some examples of FLT3 inhibitors used in the treatment of FLT3-mutated AML include multiple drugs: Midostaurin, an oral multi-kinase inhibitor that received FDA approval in 2017 as a combination therapy with standard chemotherapy for newly diagnosed FLT3-positive

AML patients; Gilteritinib, a medication approved by the FDA in 2108 for relapsed or refractory FLT3-positive AML; Quizartinib, an oral FLT3 inhibitor that has shown efficacy in clinical trials for the treatment of relapsed or refractory FLT3-positive AML (Fedorov et al., 2023; Pratz & Levis, 2008; Zhao et al., 2022).

Chemotherapy is a standard treatment approach for AML, including AML cases with FLT3 mutations, and it is often used in combination with FLT3 inhibitors for the treatment of FLT3-mutated AML (Fedorov et al., 2023; Pratz & Levis, 2008). One of the most common chemotherapy regimens used for FLT3 AML is called "7+3" which consists of two main drugs: cytarabine and anthracyclines (Fedorov et al., 2023; Pratz & Levis, 2008).

Cytarabine (also known as Ara-C) is an antimetabolite drug that interferes with DNA synthesis in rapidly dividing cells, including leukemia cells (Fedorov et al., 2023; Neubauer et al., 2008; Pratz & Levis, 2008). It is typically administered through continuous intravenous infusion over several days (Fedorov et al., 2023; Neubauer et al., 2008; Pratz & Levis, 2008).

Anthracyclines (Daunorubicin or Idarubicin) are potent chemotherapy drugs that work by damaging the DNA in cancer cells, leading to their death (Fedorov et al., 2023; Pratz & Levis, 2008). They are usually administered as intravenous infusions for a few days during the chemotherapy cycle (Fedorov et al., 2023; Pratz & Levis, 2008).

The "7+3" regimen involves seven days of continuous cytarabine infusion and three days of anthracycline treatment (Pratz & Levis, 2008). The cycle is typically repeated a few times over several weeks, depending on the patient's response and tolerance to the treatment (Pratz & Levis, 2008). This intensive chemotherapy aims to induce remission by reducing the number of leukemic cells in bone marrow and peripheral blood (Pratz & Levis, 2008). Additionally, Hematopoietic Stem Cell Transplant (HSCT) may be considered a curative treatment option for those in complete remission after initial therapy.

Challenges and Limitations of the Current Treatment of AML

Despite the promising potential of stem cell transplantation and radiotherapy in addressing FLT3-mutated AML, several challenges and downsides merit consideration. Stem cell transplantation, while curative for some patients, poses significant risks such as graft-versus-host disease and transplant-related complications, limiting its applicability to older or medically frail individuals (He et al., 2022). Moreover, the scarcity of suitable donors and the intricacies of matching processes can further impede the widespread use of this approach (He et al., 2022). In the case of radiotherapy, its systemic toxicities and potential damage to healthy tissues surrounding the bone marrow underscore the need for precise dosing strategies to minimize adverse effects (Allodji et al., 2020). Additionally, the impact of radiotherapy on leukemia-initiating cells and the potential for therapy-induced secondary malignancies necessitate careful evaluation (Allodji et al., 2020). Furthermore, the potential emergence of radioresistant leukemic clones and the challenges associated with targeting FLT3 mutations within the bone marrow microenvironment call for innovative strategies to enhance treatment efficacy (Allodji et al., 2020). While these challenges are substantial, they prompt researchers and clinicians alike to work towards refining these therapeutic modalities to maximize their benefits while mitigating their limitations, ultimately improving outcomes for patients with FLT3-mutated AML.

Chemotherapy for AML has been effective in reducing remission and improving survival rates for many patients (Fedorov et al., 2023; Pratz & Levis, 2008; Zhao et al., 2022). However, it comes with several challenges and drawbacks that can impact patients' quality of life and overall treatment outcomes.

Chemotherapy drugs target rapidly dividing cells, which include cancer cells but also affect normal cells in the body (Fedorov et al., 2023; Pratz & Levis, 2008). This can lead to a range of side effects, such as nausea, vomiting, hair loss, fatigue, anemia, and susceptibility to infections (Fedorov et al., 2023; Pratz & Levis, 2008). The side effects can be physically challenging for patients and may require medical interventions and support (Fedorov et al., 2023; Pratz & Levis, 2008).

Chemotherapy used for AML can suppress the bone marrow's ability to produce healthy blood cells, leading to myelosuppression (Fedorov et al., 2023; Pratz & Levis, 2008). Myelosuppression can result in low levels of red blood cells (anemia), white blood cells (neutropenia), and platelets (thrombocytopenia), increasing the risk of infections, bleeding, and fatigue (Fedorov et al., 2023; Pratz & Levis, 2008).

Some AML patients may develop resistance to chemotherapy drugs over time, leading to a reduced response to treatment and increased difficulty in achieving remission (Fedorov et al., 2023; Pratz & Levis, 2008). This can complicate further treatment decisions and may necessitate alternative therapies, such as targeted therapies or stem cell transplantation (Fedorov et al., 2023; Pratz & Levis, 2008).

While chemotherapy can effectively reduce the bulk of leukemia cells, it may not eliminate all leukemia stem cells responsible for disease relapse (Joshi et al., 2019). These stem cells can remain dormant and become active again, leading to leukemia recurrence in some cases (Joshi et al., 2019).

Innovative Synergy: CRISPR/Cas9-Mediated Gene Editing and CAR T-Cell Therapy for AML with FLT3 Mutations

CRISPR-Cas9 is a revolutionary gene editing technology that allows scientists to precisely modify DNA within living organisms (Gostinska, 2022; Redman et al., 2016). CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) are segments of DNA that can be programmed to target specific genes, and Cas9 is an enzyme that acts as molecular scissors to cut the DNA at the targeted location (Gostinska, 2022; Redman et al., 2016). This technology has immense potential for various applications, including genetic research and therapeutic development (Gostinska, 2022; Redman et al., 2016). When it comes to AML (Acute Myeloid Leukemia) with FLT3 mutations, CRISPR/Cas9 can be utilized in several ways:

Researchers can design sgRNAs (single guide RNA) that specifically target the mutated region of the FLT3 gene; Cas9 then introduces a double-stranded break at that location (Gostimskaya, 2022; Kasidet Hiranniramol et al., 2020; Redman et al., 2016). When the cell's repair machinery fixes the break, errors can occur, resulting in gene disruption or modifications that may render the mutated FLT3 gene nonfunctional (Gostimskaya, 2022; Kasidet Hiranniramol et al., 2020; Redman et al., 2016).

In some cases, researchers may aim to correct the FLT3 mutation back to its normal state using CRISPR/Cas9 (Gostimskaya, 2022; Ho et al., 2021; Kasidet Hiranniramol et al., 2020; Redman et al., 2016). This approach requires providing a corrected DNA template along with the CRISPR/Cas9 components, allowing the cell to use the template to repair the gene accurately (Gostimskaya, 2022; Kasidet Hiranniramol et al., 2020; Ho et al., 2021; Redman et al., 2016).

CRISPR/Cas9 can be used alongside other treatments. For example, editing the FLT3 gene to increase the sensitivity of leukemia cells to standard chemotherapies might enhance the effectiveness of these treatments (Gostimskaya, 2022; Ho et al., 2021; Redman et al., 2016).

CRISPR/Cas9-edited cells can serve as models to test potential drugs targeting FLT3 mutations (Gostimskaya, 2022; Ho et al., 2021; Redman et al., 2016). These models help researchers identify compounds that selectively kill AML cells with FLT3 mutations while sparing healthy cells (Gostimskaya, 2022; Ho et al., 2021; Redman et al., 2016).

Chimeric Antigen Receptor (CAR) T-cell therapy is a type of immunotherapy used to treat certain cancers, including blood cancers like leukemia. It involves modifying a patient's T cells (a type of immune cell) to express a CAR on their surface using processes such as CRISPR-Cas9, viral vectors, electroporation, transposon systems, and RNA transfection (Jogalekar et al., 2022; Magnani et al., 2020; Pohl-Guimarães et al., 2020; Singh et al., 2017; Zhang et al., 2022). This CAR is designed to recognize a specific protein (antigen) present in cancer cells. When infused back into the patient's body, these engineered CAR T cells target and destroy cancer cells bearing the antigen (Jogalekar et al., 2022; Zhang et al., 2022).

CAR T-cell therapy can be engineered to target the specific FLT3 mutation or an associated antigen present in leukemia cells (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022). This approach aims to harness the patient's immune system to directly attack and eliminate AML cells carrying the FLT3 mutation. (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022)

In the context of AML with FLT3 mutations, researchers identify an antigen that is uniquely or significantly expressed on the surface of AML cells carrying FLT3 mutations (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022). This antigen serves as the target for CAR T-cell therapy (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022).

T cells are collected from the patient's bloodstream through a process called leukapheresis. These T cells are then genetically modified in the laboratory using viral vectors to express the CAR (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022 Singh et al., 2017; Zhang et al., 2017; Zhang et al., 2022). These vectors, derived from engineered viruses, are designed to carry specific genetic material, such as chimeric antigen receptors (CARs), into T cells (Singh et al., 2017; Zhang et al., 2017). Once introduced to T cells in the lab, the viral vectors deliver the genetic payload, often integrating it into the T cell's DNA for relative permanence (Singh et al., 2017; Zhang et al., 2017). The modified T cells are then expanded in culture and infused back into the patient, where they can target and eliminate specific cells, such as cancer cells (Singh et al., 2017; Zhang et al., 2017). This genetic modification provides a long-lasting therapeutic effect, making viral vector-based T-cell therapies a promising approach to treating various diseases, including cancer and genetic disorders (Singh et al., 2017; Zhang et al., 2017). The CAR is a synthetic receptor that consists of an antigen-recognition domain (derived from an antibody), a signaling domain (typically from T-cell receptors or co-stimulatory molecules), and other necessary components for activation (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022). Once the T cells are successfully engineered to express the CAR, they are cultured and expanded in the laboratory to generate a sufficient number of CAR T cells (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022). These engineered cells are activated and primed for their anti-cancer function (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022). The expanded and activated CAR T cells are infused back into the patient's body (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022). The CAR T cells circulate and target AML cells with the specific antigen associated with FLT3 mutations (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022). Upon encountering AML cells, the CAR T cells bind to the antigen and initiate an immune response, leading to the destruction of cancer cells (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022).

Potential New Treatments for AML

The treatment landscape for AML (Acute Myeloid Leukemia) with FLT3 mutations is rapidly advancing, and a groundbreaking approach involves the synergistic use of CRISPR/Cas9 gene editing and CAR T-cell therapy. In this innovative strategy, CRISPR/Cas9 technology is employed to precisely target and correct the FLT3 genetic mutation responsible for driving leukemia growth. By editing the mutated gene, researchers aim to restore normal cellular functions and hinder cancer cell proliferation. Simultaneously, CAR T-cell therapy is harnessed to engage the immune system in eradicating remaining FLT3-mutated AML cells. Engineered CAR T cells, equipped with chimeric antigen receptors designed to recognize the mutated FLT3 antigen, are infused into the patient, providing a targeted immune response against leukemia cells. This combined approach leverages the power of gene editing to rectify the underlying mutation, while CAR T-cell therapy capitalizes on the immune system's ability to seek out and destroy cancerous cells. While still in the experimental stages, the

potential of this tandem therapy offers a hopeful glimpse into the future of personalized and precision medicine for AML FLT3 patients.

CRISPR/Cas9 Gene Editing

CRISPR/Cas9 gene editing offers remarkable potential for addressing AML (Acute Myeloid Leukemia); however, it comes with notable challenges. A key concern is the risk of off-target effects, where unintended genetic alterations could occur (Gostimskaya, 2022; Ho et al., 2021; Redman et al., 2016). Delivering the gene-editing machinery accurately to bone marrow cells housing leukemia cells proves complex (Gostimskaya, 2022; Ho et al., 2021; Redman et al., 2016). Moreover, introducing CRISPR/Cas9 components might trigger immune reactions or cellular toxicity, affecting treatment viability (Gostimskaya, 2022; Ho et al., 2021; Redman et al., 2016). AML's genetic complexity, often involving multiple mutations, presents a challenge in targeting a single mutation like FLT3 (Gostimskaya, 2022; Ho et al., 2021; Redman et al., 2016). Long-term consequences and safety are uncertain, and ethical and regulatory aspects require careful consideration (Gostimskaya, 2022; Ho et al., 2021; Redman et al., 2016). Clinical experience with CRISPR/Cas9 for AML remains limited, necessitating extensive research and trials (Gostimskaya, 2022; Ho et al., 2021; Redman et al., 2016). Each patient's variability further complicates treatment personalization (Gostimskaya, 2022; Ho et al., 2021; Redman et al., 2016). While offering transformative potential, the deployment of CRISPR/Cas9 in AML necessitates addressing these hurdles rigorously before widespread clinical implementation (Gostimskaya, 2022; Ho et al., 2021; Redman et al., 2016).

CAR T-Cell Therapy

CAR T-cell therapy is an innovative cancer treatment, but it is not without its limitations. One major concern is cytokine release syndrome (CRS), an immune response that can lead to severe complications (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022). Neurological toxicity, manifested as confusion and seizures, is also observed in some cases (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022). While CAR T-cell therapy can produce dramatic initial responses, its durability varies, and cancer cells can develop resistance mechanisms, reducing its long-term effectiveness (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022). The choice of target antigen is crucial, as unintended targeting of healthy tissues can lead to adverse effects (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022). The complex manufacturing process, patient eligibility criteria, and high costs can limit accessibility (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022). Moreover, CAR T-cell therapy's delayed action and potential for immune suppression warrant careful consideration (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022). Despite these challenges, ongoing research seeks to enhance the therapy's outcomes and broaden its utility in treating various cancers (Jogalekar et al., 2022; Mardiana & Gill, 2020; Paresh Vishwasrao et al., 2022; Zhang et al., 2022).

Conclusion

In conclusion, the emergence of AML with FLT3 mutations presents a formidable challenge in the field of oncology. The integration of advanced gene-editing techniques and immunotherapies holds significant promise for revolutionizing the treatment landscape. CRISPR/Cas9 technology offers the potential to rectify the underlying genetic aberration responsible for the aggressive growth of FLT3-mutated leukemic cells, offering a fundamental shift from conventional therapeutic approaches. Concurrently, the advent of CAR T-cell therapy capitalizes on the immune system's inherent power to target and eliminate cancer cells, driven by the precise recognition of specific antigens on the cell surface. By harnessing the synergistic potential of both strategies, a novel therapeutic paradigm emerges that aims to combine genetic correction with immunomodulation, tailored to the

individual patient's molecular profile. Although challenges such as off-target effects, immune responses, and complexities in delivery persist, ongoing research and clinical trials pave the way for refining these therapies. As the convergence of genetic engineering and immunotherapy progresses, the envisioned treatment strategy offers a glimpse into the potential of personalized precision medicine to reshape the future of AML FLT3 treatment, potentially altering the trajectory of this devastating disease and improving patient outcomes.

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