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CRISPR-Cas9 to treat chronic myeloid leukaemia: A review

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Abstract
In human chronic myeloid leukaemia, the tyrosine-kinase oncogene BCR/ABL1 is one of the key factors that determine most of its characteristics. Myeloproliferative diseases such as chronic myeloid leukaemia (CML) account for 15% of all new cases of leukemia, affecting 1–2 new cases per 100,000 every year. According to recent studies, the CRISPR/Cas9 system may be a definitive treatment option for chronic myeloid leukaemia. Scientists use genome editing technologies to add, remove, or alter genetic material at specific locations in the genome of an organism. Genome editing also goes by the name of gene editing because it allows scientists to change an organism's DNA. Nowadays, worldwide various genome editing has been discovered. But, in biomedical research, CRISPR/Cas9 (clustered regularly interspaced palindromic repeats) has caused a major revolution ingenome editing. Using CRISPR/Cas9 libraries and technology applications; we can achieve our goal of curing acute myeloid leukaemia within decades. In this review, we will discuss chronic myeloid leukaemia (CML) disease and future advances in genome editing that might help treat it. The novel technology will also be described in terms of its difficulties and ethical controversies.

Keywords: Chronic myeloid leukaemia, CRISPR/Cas9, RNA-DNA, chronic phase, traditional chemotherapy

Introduction
CRISPR, or clustered regularly interspaced short palindromic repeats, was initially identified in prokaryotes as an effect or of the adaptive immune system (Lander, 2016) [1]. CRISPR is a group of short DNA sequences that prokaryotes have in their genomes as a consequence of prior bacteriophage infections (Lander, 2016) [1]. It provides prokaryotes with a defence mechanism to combat bacteriophage reinfection. The implementation of gene editing for human illnesses was made possible by the later development of this technique into a gene-editing tool in eukaryotic cells (Cong *et al*., 2013; Mali *et al*., 2013) [2, 3]. Two components of CRISPR/Cas9 are the single guideRNA (sgRNA) and the Cas9 endonuclease. The Cas9 protein is guided to a target site for cleavage by a target-specific sgRNA, which is produced by the combination of a CRISPR RNA (crRNA) and a transactivating CRISPR RNA, resulting in a double-strand break (DSB). RNA-DNA interactions determine target recognition, therefore CRISPR/Cas9 has an advantage over zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) in those genomic targets can be easily designed and multiplexed. The Cas9 nuclease only needs a target-specific sgRNA for each editing target, unlike ZFNs and TALENs, which demand time-consuming protein engineering processes for each new editing target (Yip, *et al*., 2020) [4].
A crucial aspect of gene editing is the introduction of Cas9 into cells. Cas9 can be transmitted via DNA, mRNA, or protein. There are benefits and drawbacks to each format. Plasmid DNA delivery of Cas9 provides a practical and affordable method. Longer expression times in cells are also produced through plasmid DNA-driven Cas9 expression, which may be helpful if a sustained expression is necessary for editing. However, compared to mRNA and protein formats, plasmid DNA has the slowest commencement of editing because transcription and translation are necessary for the production of the Cas9 protein. The possibility of off-target consequences rises when Cas9 is expressed continuously in cells (Wu, *et al.*, 2014) [5].

Additionally, insertional mutagenesis is a concern associated with plasmid DNA (Yip, *et al.*, 2020) [4].
Cas9 may be delivered via mRNA rather than plasmid DNA, which allows for a quicker start to gene editing because transcription is no longer necessary. This format only allows temporary Cas9 expression due to mRNA's high degree of instability and susceptibility to RNase destruction. To improve mRNA's stability after delivery, chemical modifications are available (Yin, *et al.*, 2017) [6]. Although transitory Cas9 expression may decrease the effectiveness of gene editing, it also lowers the possibility of off-target consequences (Wu, *et al.*, 2014) [5].
Greater gene editing effectiveness than that of DNA and mRNA is achieved by Cas9 being delivered via protein, which allows for rapid gene editing in the nucleus (Liang, *et al.*, 2015) [7]. Although Cas9's protein distribution in cells is the most fleeting of the formats, the likelihood of off-target consequences is also quite low (Liang, *et al.*, 2015) [7]. Additionally, protein delivery is more expensive than DNA and mRNA delivery. Importantly, introducing the Cas9 protein, which is derived from bacteria, into cells may cause the spread of bacterial endotoxin and result in negative immunologic reactions. This element is a major concern for safety when employing Cas9 in clinical studies (You, *et al.*, 2019) [8].

Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) is a myeloproliferative illness with an annual incidence of 1-2 occurrences per 100,000 people, which amounts to 15% of all newly identified cases of leukemia (Vuelta, *et al.*, 2021) [9]. It seldom occurs throughout infancy and is more common in adults, whose mean age of occurrence is around 55 years. Although it can affect both sexes, males are affected at a somewhat higher rate than females: 2.2 men for every 100,000 affected compared to 1.4 women (Vuelta, *et al.*, 2021). The most prevalent clinical signs of chronic myeloid leukaemia (CML) include anaemia, splenomegaly, stomach discomfort, and recurrent infections. Nonetheless, a significant percentage of individuals who exhibit no symptoms are identified during an unrelated medical evaluation (Vuelta, *et al.*, 2021) [9]. The pathological progression of it is considered to have three clinical stages. Myeloid hyperplasia during an indolent chronic phase (CP) is the initial hallmark of CML illness. While leukemic stem cells (LSCs) are now responsive to growth stimuli, myeloproliferative differentiation pathways gain an advantage since they are primarily responsible for the large myeloid proliferation that is a hallmark of CML (Petzer, *et al.*, 1996) [10]. Myeloid progenitors and mature cells build up in the blood and extramedullary tissues at this early stage. In the absence of curative treatment, CML advances through the acceleration phase (AP), a stage of growing instability that culminates in the blast crisis phase (BP), an acute phase akin to leukaemia. The percentage of blasts in the bone marrow and blood determines the criteria of AP and BP. The myeloid or lymphoid lineage has a maturation arrest in AP and BP, and LSCs exhibit newly acquired genetic and epigenetic abnormalities (Melo, *et al.*, 2017) [11]. A myeloblastic (50%), biphenotypic/undifferentiated acute leukemic (25%), or lymphoblastic (25%), phenotype following the last BP stage suggests a stem origin for CML illness (Vuelta, *et al.*, 2021) [9]. The last reason for patient death from infection, thrombosis, or anaemia is bone marrow failure brought on by a deficiency in cell differentiation and a huge invasion by immature blasts (Ilaria, 2021) [12].
Identifying the Philadelphia (Ph) chromosomal 22 abnormality-named for the US city where it was initially discovered-is the basis for making the diagnosis of chronic myeloid leukaemia (CML). The reason for this is the reciprocal translocation of chromosomes 9 and 22-t(9;22) (Vuelta, *et al.*, 2021) [9]. Techniques including transcription PCR (RT-PCR), fluorescence in situ hybridization (FISH), and conventional cytogenetics are frequently used to confirm a diagnosis of CML and assess the effectiveness of treatment.
The median life expectancy of CML patients upon diagnosis was around 3-5 years before the development of effective therapies(Vuelta, *et al.*, 2021; Kantarjian *et al.*, 2012) [9,13]. Tyrosine kinase inhibitor (TKI) medications significantly altered the treatment landscape for CML, and the majority of CP-CML patients now have normal life expectancies (Kantarjian, *et al.*, 2012; Bower, *et al.*, 2017; Deininger, *et al.*, 2009) [13,14,15]. Nevertheless, only a tiny percentage of patients have the choice to stop their medication (Graham *et al.*, 2002) [16].

Traditional therapeutic for Chronic Myeloid Leukemia

One of the key turning points in modern cancer medicine has been the development of the Chronic Myeloid Leukemia (CML) treatment. Traditional chemotherapy was the go-to therapy for CML up until the 1980s, when it was first discovered. Arsenic was the initial therapy used in the 19th century, but it was replaced by alkylating medicines like busulfan and hydroxyurea which only led to a slight increase in survival (Vuelta *et al.*, 2021; Minot, *et al.*, 1924) [9, 17]. Sadly, they only allowed for a little increase in survival and did not stop the disease's development from starting. It raised median survival to six years in the 1970s and brought full cytogenetic remission to 10–15% of patients (Tura, *et al.*, 1995) [18]. The majority of patients had to stop receiving interferon-α treatment due to substantial side effects, which led to recurrence in those patients. The only therapeutic option in this situation that may boost long-term survival was allogenic stem cell transplantation (SCT), which is why it was adopted as the first-line therapy for patients in the chronic phase in the 1990s (Vuelta *et al.*, 2021; Rp, 1998) [9, 19]. The only treatment option that can completely cure CML patients at this stage is the one being used right now. Following bone marrow destruction (by chemotherapy or radiation), normal allogenic stem cells are infused during the SCT process. Although it is associated with a high likelihood of transplant-related death, it is only accessible to a small percentage of patients who have an HLA-matched donor (Vuelta *et al.*, 2021) [9]. SCT has only been utilised nowadays as a very last resort salvaging strategy.
CML is a particular form of cancer in which the BCR/ABL1 fusion is the only genetic event that may explain all of the clinical characteristics. The need to find substances that inhibit BCR/ABL1's tyrosine kinase activity was made urgent by the knowledge that this activity is necessary for the malignant transformation of cells. The treatment potential of several tyrosine kinase inhibitors (TKIs) for CML was investigated during the 1990s (Vuelta *et al.*, 2021; Capdeville *et al.*, 2002) [9, 20]. These substances work by competing with adenosine triphosphate (ATP) or the kinase

protein substrate, inhibiting BCR/ABL1 activity at the protein level. The Novartis chemical STI571 (later known as imatinib mesylate), which demonstrated unexpected benefits by specifically inhibiting BCR/ABL1 at micromolar doses, was finally authorised as a treatment for CML in the 2000s (Capdeville *et al.*, 2002; Druker *et al.*, 2001) [21, 22]. TKIs changed the course of CML therapy, and they continue to be the first-line therapy for the disease. TKIs have improved the survival rates of CP-CML patients, who had an 8-year survival rate of 20% before 2001, to 87%, and their life expectancy is now comparable to that of healthy persons their age (Bower *et al.*, 2016; Deininger, *et al.*, 2009; Verstovsek *et al.*, 2002) [14,15,23]. There are still challenges to be resolved despite the effectiveness of TKI-based therapy. The fundamental issue is that oncogenic events are not rectified or edited by TKI medicines, which do not address the etiological causes of CML. TKIs do not totally eradicate the LSCs, as evidenced by the discovery of lingering BCR/ABL-positive cells that are still "oncogenic-quiescent" (Graham *et al.*, 2002) [16].While the medication is present, TKIs effectively suppress BCR/ABL's oncogenic activity; nevertheless, the residual LSCs can cause recurrence if TKI therapy is stopped (Vuelta *et al.*, 2021) [9].

CRISPR-Cas9 therapy in Chronic Myeloid Leukemia

The volume of scholarly articles detailing work on CRISPR/Cas9 about leukaemia research has greatly expanded during the previous seven years (Martinez *et al.*, 2020; García-Tuñón *et al.*, 2019; Luo *et al.*, 2019;Huang *et al.*, 2018; García-Tuñón *et al.*, 2017) [24-28]. Numerous of them deal with *in vitro* research to explain how different genes contribute to the emergence of leukaemia (Vukovic *et al.*, 2015) [29]. These findings highlight important genes that will be altered in leukemic cells utilising the CRISPR/Cas9 system (Vuelta, *et al.*, 2021) [9].

In 2015, Valletta *et al*. produced the first proof that a human myeloid leukaemia cell line's acquired mutations may be corrected using the CRISPR/Cas9 system (Valletta *et al.*, 2015) [30]. Then, in animal models of genetic disorders, CRISPR-Cas9 was employed with success. In 2016, the first CRISPR-Cas9 clinical trials in humans began. Based on the understanding that the BCR/ABL1 fusion is the primary driver of CML pathogenesis, imatinib treatment is used to treat CML. This makes it conceivable that a permanent solution might be provided by BCR/ABL1 gene disruption caused by CRISPR/Cas9 (Vuelta *et al.*, 2021) [9]. The CRISPR/Cas9 system's ability to successfully silence the BCR/ABL1 oncogene and reverse its tumor-promoting activity was first demonstrated by Garcia-Tuón and colleagues in 2017 (García-Tuñón *et al.*, 2017) [28]. They demonstrated how modified CRISPR cells lost their capacity to multiply and survive in an animal model of CML called a xenograft, and they demonstrated how no tumours formed when the edited cell was chosen. Their findings served as proof of concept that BCR/ABL1 abrogation via the CRISPR system leads to the loss of tumorigenicity (Vuelta, *et al.*, 2021) [9].

In 2018, Wenli Feng's team showed that alternative genome-editing nucleases, notably ZFN nucleases, were successful in eliminating the BCR/ABL1 oncogene (Huang*et al.*, 2021) [27]. A premature stop codon was made by using two ZFNs that target the BCR's exon 1, which then causes a shortened oncoprotein. In the ZFN-edited cells, the proliferative ability was decreased, and the apoptosis rate was increased (Luo *et al.*, 2021) [26]. To specifically target exon 2 of ABL1, the authors used a novel approach based on CRISPR RNA-guided FokI nucleases (RFNs). In their view, a combination of a universal design of the CRISPR site and a specific cleavage of the FokI cleavage would provide a secure and effective editing tool that would bypass the drawbacks of earlier systems, such as ZFN design time and off-targets with CRISPR/Cas9. The imatinib-sensitive and imatinib-resistant variants of K562 were both successfully edited using RFN to reduce the expression of BCR/ABL1 and its downstream targets. Edited cells displayed a loss of their ability to proliferate and form colonies *in vitro*, indicating a loss of their ability to become malignant (Vuelta, *et al.*, 2021) [9].

Recent research has demonstrated the therapeutic potential of the CRISPR system by concentrating on the disruption of BCR/ABL1 by genome-editing nucleases as a therapeutic method in CML. In 2020, Chia-Hwa Lee *et al*. showed that disrupting BCR/ABL1 results in a lower growth rate in the human CML K562 cell line (Valletta *et al.*, 2015; Chen *et al.*, 2020) [30, 31]. To assess the potential for treatment of this viral system in the clinical environment, ex vivo transduction of peripheral blood mononuclear cells from CML patients was carried out. They found that the transduced cells had a high rate of apoptosis and showed that the disruption of the ABL1 non-rearranged allele had no significant effects. Activity at this non-translocated ABL1 gene had no impact on the T-cell lineage (Vuelta *et al.*, 2021) [9].

According to reports, epigenetic regulators frequently exhibit mutations in myeloid malignancies (Murati *et al.*, 2021) [32]. In order to induce apoptosis in human Burkitt lymphoma (BL) cells, the myeloid cell leukemia-1 (MCL-1) gene, a member of the emerging B cell lymphoma 2 (BCL2) gene families, was also deleted using the CRISPR-Cas9 system. Because the MCL-1 gene is involved in proliferation, cell differentiation, and tumorigenesis, it may be an innovative cancer therapeutic target (Aubrey *et al.*, 2015; Sharma *et al.*, 2021) [33, 34].

Inducing multiple mutations in the genes of transcription factors, cytokine signalling, and epigenetic modifiers in mouse hematopoietic stem cells was another application of CRISPR-Cas9 technology used to create an acute myeloid leukaemia (AML) mouse model with a combinatorial genetic lesions system (Heckl *et al.*, 2014) [35]. Cancer immunotherapy is one of the four main therapeutic modalities, along with chemotherapy, surgery, and radiation. Clinical studies have been emphasised for gene-edited immunotherapies, including chimeric antigen receptor (CAR) T cell therapy. Chimeric antigen receptor (CAR) T cell treatment and genetically modified T cell receptor (TCR) therapy are the main subjects of clinical studies for gene editing-based immunotherapies (Maude *et al.*, 2018; Schuster *et al.*, 2019) [36, 37].

Future Directions

The capacities to alter a species' DNA have expanded to previously unthinkable heights with the introduction of genome-editing nucleases and, in particular, the CRISPR/Cas9 system. Within this framework, one of the areas that have seen significant growth is gene therapy. By directly correcting the underlying pathology, hereditary illnesses may now be permanently cured, a potential that was previously just theoretical. Nevertheless, there are still

several obstacles that prevent gene therapy from being used as a standard medical procedure. The biggest drawback of *in vivo* CRISPR therapy, similar to other gene therapy techniques, is the challenge of determining the most effective and secure delivery strategy. However, it may be appropriate to use novel Cas proteins in light of humans' inherent adaptive tolerance to Cas9 proteins (Charlesworth *et al.*, 2019) [38]. Another problem that has to be fixed is that of CRISPR off-targets (Sternberg *et al.*, 2015) [39]. It is possible that several variables, including cell type, expression level, transfection technique, culture maintenance, sequential nuclease expression, guide sequence, and repair processes, have an impact on the incidence of off-target effects in cell cultures(Sharma *et al.*, 2021; Teboul *et al.*, 2020; Li *et al.*, 2019) [34, 40, 41]. The CRISPR-Cas9 system cleaves genomic DNA at certain locations to cause double-strand breaks (DSBs), but it can also cause unintended cleavages elsewhere. Mutations that can disrupt normal genes can be caused by cleavage at off-target locations. There will soon be a solution thanks to efforts to find high-fidelity novel Cas variants and a protospacer neighbouring motif that is less constrictive than the NGG sequence(Hu *et al.*, 2018; Mukherjee *et al.*, 2022; konar *et al.*, 2022) [42-44]. Ultimately, 100% editing efficiency is unachievable even with the introduction of new and increasingly effective techniques. Securing the lack of unmodified cells is crucial for clinically treated haematological malignancies, such as CML, where BCR/ABL1 disruption occurs. One potential resolution might be the identification of accurately modified cells through the development of genome-editing techniques that provide both the production of a selective cell marker and genetic repair at the same time (Vuelta *et al.*, 2021; Sharma *et al.*, 2021; Mondal *et al.*, 2024; Konar *et al.*, 2022; Poddar S *et al*, 2020; Kundu P *et al*. 2020; Ghosh P *et al*. 2023; Das C *et al*. 2020) [9, 34, 45-50].

Conclusion

Ultimately, the CRISPR/Cas9 technology has revolutionised the field of genetic modification by enabling accurate changes to be made in both eukaryotic and prokaryotic cells. Its use in human gene editing offers a potential path for therapeutic treatments, especially when it comes to treating genetic diseases like Chronic Myeloid Leukemia (CML). Although patient outcomes have greatly improved as a result of standard CML therapies, such as tyrosine kinase inhibitors (TKIs), issues including persisting leukemic stem cells and the requirement for lifetime medication still exist.

Research showing that CRISPR/Cas9 may disrupt the BCR/ABL1 oncogene, a major factor in the pathogenesis of CML, is persuasive and supports the development of CRISPR/Cas9 as a possible therapeutic for CML. CRISPR/Cas9 has demonstrated its disruptive potential in both *in vitro* and *in vivo* experiments, where it is successful in modifying genetic alterations associated with leukemia. Despite this, it is essential to tackle obstacles like off-target impacts and delivery methodologies to guarantee the safety and effectiveness of CRISPR-derived therapeutics.

Future developments in CRISPR technology may lead to gene therapy and long-lasting healing. It will be essential to overcome present challenges, including delivery methods and off-target effects, for CRISPR/Cas9 to be widely used in therapeutic settings. By refining and improving the accuracy of CRISPR-based therapeutics, including the investigation of new Cas proteins, ongoing research and development hope to get us closer to the realisation of therapeutic gene therapies for illnesses like CML.

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