Applications of CRISPR-Cas9 in treatment of HIV and inherited blood disorders: A Systematic Review

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Introduction

This poster will focus on the use of CRISPR-Cas9 in the treatment of HIV and inherited blood disorders, such as chronic myeloid leukaemia.

Oncogenes play key roles in cancer progression and inactivating or deleting these genes is considered a potential option for oncogene-linked cancers. Human immunodeficiency virus (HIV) is a major global health issue that is treated via anti-retroviral therapy (ART) by reducing the viral load. ART requires lifelong administration, proving to be expensive to treat but CRISPR emerging as an effective tool to reduce the progression of the disease by blocking coreceptors and, therefore, can reduce the progression of the disease by blocking coreceptors and the entry of HIV-1 (Bhowmik and Chaubey, 2022). This systematic review will focus on the use of CRISPR-Cas9 in the treatment of HIV and Inherited blood disorders, such as chronic myeloid leukaemia.

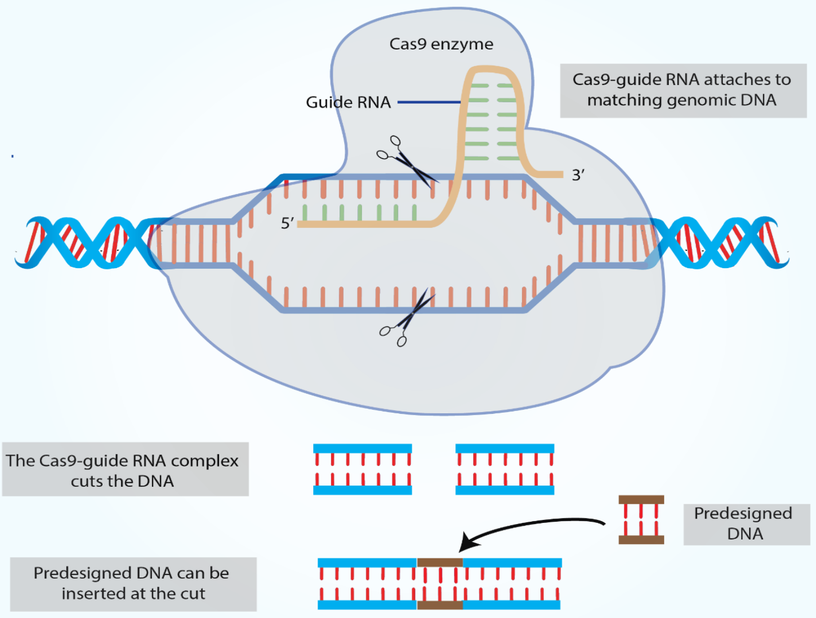
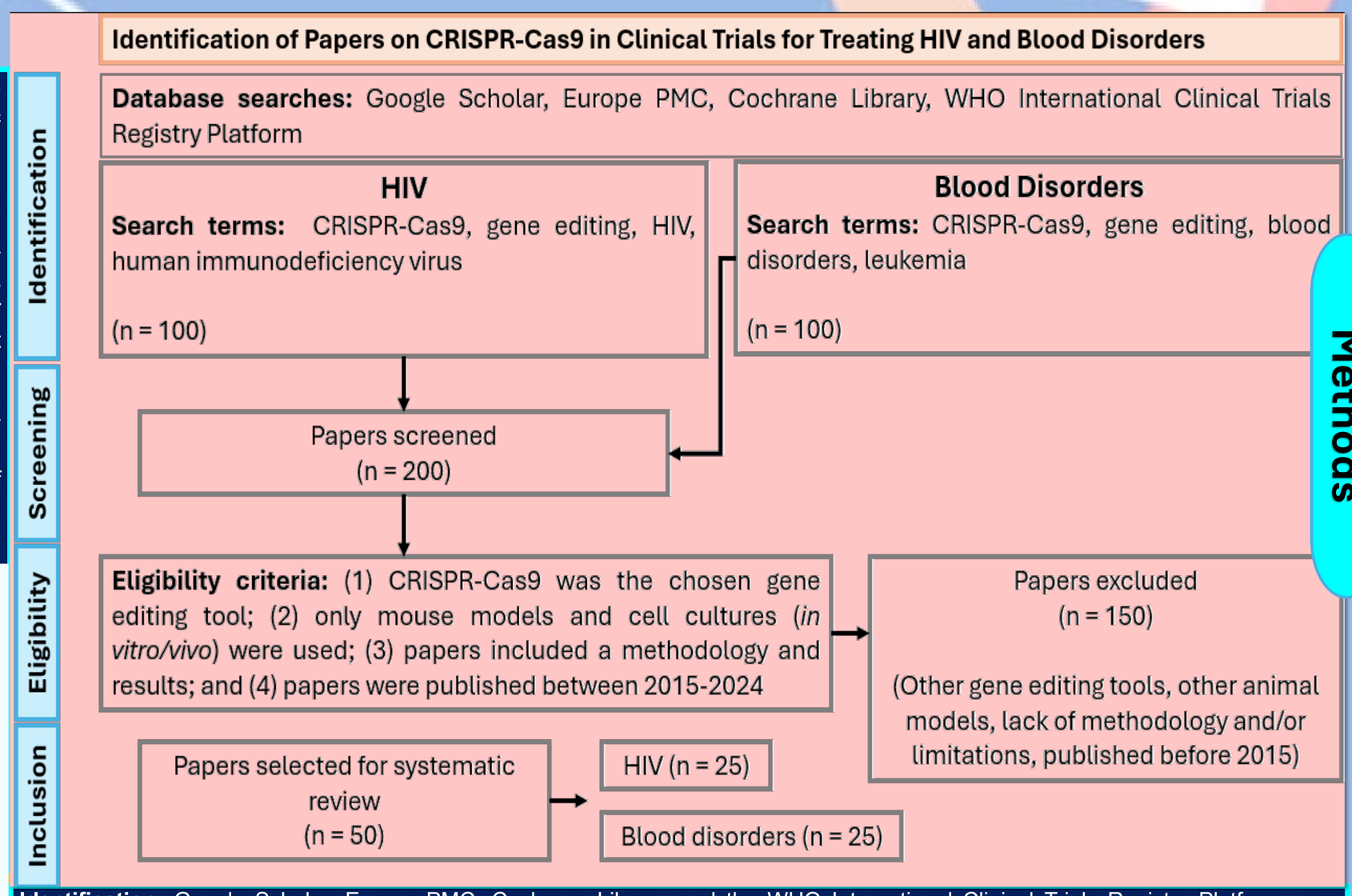


Figure 1: Diagram representing CRISPR-Cas9 complex targeting DNA and creating a double-strand break.



Identification: Google Scholar, Europe PMC, Cochrane Library, and the WHO International Clinical Trials Registry Platform were searched to identify peer-reviewed articles on CRISPR-Cas9 in clinical trials for HIV and blood disorders. Specific search terms (CRISPR-Cas9, gene editing; HIV, human immunodeficiency virus; blood disorders, leukemia) were used to narrow down available articles for screening.
Screening: A total of 200 articles were selected for eligibility assessment (HIV = 100; blood disorders = 100).
Eligibility: Articles were assessed using four inclusion-exclusion criteria: (1) CRISPR-Cas9 was the chosen gene editing tool; (2) only mouse models and cell cultures (*in vivo/in vitro*) were used; (3) research papers included methodology and limitations; and (4) articles that were published between 2015-2024. A total of 50 articles (HIV = 25; blood disorders = 25) were selected for systematic review.
Analysis: Articles were analysed to determine the impact of CRISPR-Cas9 in clinical trials for treating HIV and blood disorders. Results were categorised based on the following criteria: (1) whether the trials were conducted *in vivo* or *ex vivo*; (2) safety profiles, such as off-target edits; and (3) accessibility (for example, costs). Key observations were also documented, including the common trends and the overall limitations of the research area.

Results

Progress in treatment of Leukemia

CD58-mediated CRISPR-Cas9 knockout in all cells halted t-cell activation and proliferation in acute lymphoblastic leukaemia (Vuelta et al., 2021). This was achieved by exploring the role of PAX5, a transcription factor vital for B cell development (Vuelta *et al*., 2021). Further testing for the efficacy of CRISPR-Cas 9 was investigated in patient-derived xenograft of pre-B acute lymphoblastic leukaemia revealing delayed progression and prolonged survival in mice treated with lentivirus that have been modified. This indicates the potential of reverting the leukaemia phenotype to improve outcomes (Yu-tin *et al*., 2020).

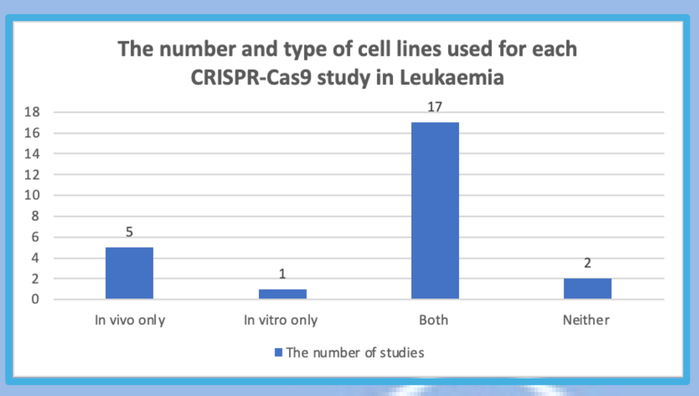


Figure 2: Bar chart represents a comparison of: "In vivo only," "In vitro only," "both," and "neither." Data from articles exploring CRISPR-Cas9 for the manipulation of somatic mutations and fusion genes in cell lines and mice models.

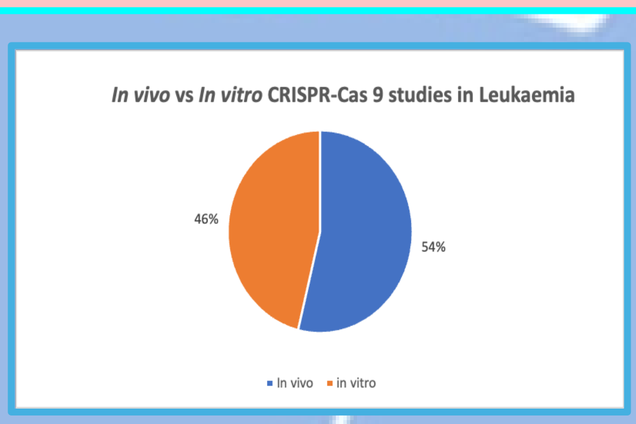


Figure 3: A bar graph illustrating the percentage of In vivo compared to In vitro experiments in the context of the application of CRISPR-Cas9 technology in the treatment of leukaemia.

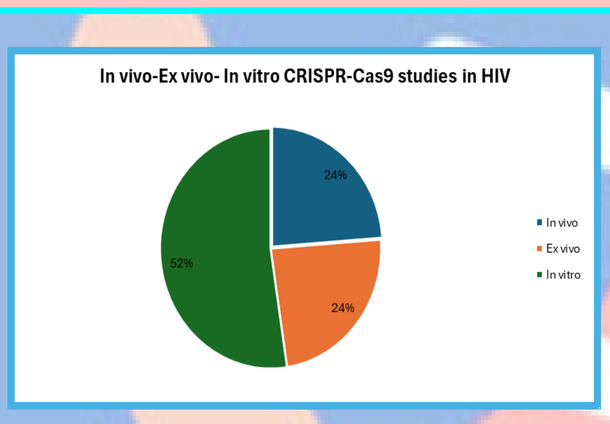


Figure 4: A pie chart displaying the use of 'In vivo', 'Ex vivo' and 'In vitro' techniques from a selection of 25 research articles focused on CRISPR-based approaches treatment of HIV.

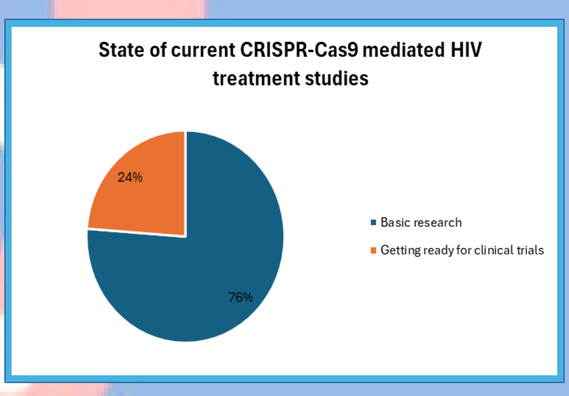


Figure 5: A pie chart illustrating the percentage of 25 studies progressing to advanced stages, such as human clinical trials, compared to those under basic research.

Key Observations

Leukemia:
A common trend observed is improving the specificity and accuracy of CRISPR-Cas9 by detecting off-target effects through amplification and prediction-based strategies (Barghout et al., 2021; Mihaila and Topircean, 2021).
Studies have demonstrated significant improvement in survival rates following hematopoietic stem cell transplantation (HSCT) for a range of illnesses, including multiple myeloma, acute myeloid leukaemia, acute lymphoblastic leukaemia, and Hodgkin's disease.
(Bortesi et al., 2016; Wang et al., 2022).

HIV:
CRISPR-Cas9-based gene editing is being tested widely for HIV/AIDS treatment and many studies have highlighted developments in this area (Bhowmik and Chaubey, 2022).

Limitations

Leukemia:
Low correction efficiency, possible off-target effects, and the requirement for additional validation in increasingly complicated models are among the publications' limitations. Additionally, the publications demonstrated improved survival post CRISPR editing using cell line models and mouse xenografts; this suggests that to fully evaluate the impact on leukaemia and the response to treatment, validation in more clinically relevant models is required (Tirado-Gonzalez et al., 2021; Valletta et al., 2015).

HIV:
The application of CRISPR-Cas9 as an intracellular defence against HIV-1 infection in human cells is limited by issues with the viral rebound, mutations at the target areas' ignition site, and the errors of CRISPR-Cas9 editing. Studies demonstrate that while CRISPR-Cas9 may inhibit the virus, viral rebound is visible in treated cells despite its potential to become the dominant genome editing technique for eradicating HIV-1 infection. Furthermore, alterations in the target areas have been found, proving a requirement for improved accuracy in editing and indicating ongoing research required to maximise the application of CRISPR-Cas9 in fighting HIV-1 (Bhowmik and Chaubey, 2022; Herskovitz et al., 2021; Hussein et al., 2023).

A CRISPR cure for HIV in the horizon?

- Single gRNA CRISPR therapy halts HIV replication (Wang et al., 2016; Yin et al., 2017)
- Combinational double gRNA CRISPR therapy at two different sites inhibits HIV replication and prevent HIV viral escape both *in vivo* and *ex vivo* (Wang et al., 2016; Kaminski et al., 2016)
- Continuous use of CRISPR/Cas9 can lead to hypermutations (large deletions) at the gRNA target sites possibly accelerating viral escape and resistance (possible mechanisms include the NHEJ repair system) (this could also go to limitations) (Wang et al., 2016)
- CRISPR helps identify new factors and target sites necessary for HIV replication like *CCNT1* gene (Hafer at al., 2023)
- CRISPR/Cas9 targets HIV co-receptors encoding genes *CCR5, CXR4, tat* and *rev* offers HIV protection (Xu et al., 2017; Liu et al., 2017).
- No significant off-target effects detected across most studies (Chung et al., 2020; Olson et al., 2020)

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Conclusion

CRISPR-Cas9 is a revolutionary technology that has the potential to change the era of genetic engineering and to be used as a treatment for a variety of diseases, not limited to HIV and blood disorders like Leukemia as discussed in this review. Several limitations surrounding the technology include; the specificity and accuracy of detecting off-target effects, the use of more complicated models, and the ability to use this research for future clinical trials. There is still a wide range of ethical issues concerned with CRISPR-Cas9 contributing to a lack of ongoing clinical trials as well as the inability to use human models. By continuing research on animal models with a variety of *in vivo, ex vivo,* and *in vitro* techniques the CRISPR-Cas9 technology holds potential for creating a positive impact on global health and economic benefits for healthcare systems.