

Research Article

Structure-guided CRISPR CAS9 Targeting of ABL1 for Functional Disruption of BCR-ABL1 Fusion in Chronic Myeloid Leukaemia

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Abstract

Chronic myeloid leukaemia (CML) is a clonal myeloproliferative cancer caused by the constant activity of the BCR–ABL 1 fusion protein's tyrosine kinase, resulting from the Philadelphia chromosome translocation, which leads to abnormal cell growth, survival, and disease progression. While tyrosine kinase inhibitors (TKIs) have greatly improved patient outcomes, issues like drug resistance and persistent leukemic stem cells highlight the need for alternative therapies. This study used a structure-guided CRISPR–Cas 9 genome editing approach to identify highly specific single-guide RNAs (sgRNAs) that can disrupt the human ABL 1 gene, a key part of the BCR–ABL 1 fusion. The high-resolution crystal structure of the ABL 1 kinase domain (PDB ID: 8 I 7 S) helped identify essential functional regions, including catalytic and ATP-binding sites, for precise CRISPR targeting. Computational design and filtering of sgRNAs were performed using E-CRISP and CHOPCHOP, focusing on criteria like PAM site accessibility, targeting conserved kinase regions in exons, GC content, predicted efficiency, and low off-target risk. In silico analyses, including specificity scores, mismatch profiles, and sequence alignment across ABL 1 transcript variants, ensured high selectivity and broad coverage. Genomic visualization confirmed accurate targeting within exons encoding vital kinase functions. Protein–protein interaction analysis via STRING showed strong links between ABL 1 and key oncogenic regulators such as BCR, STAT 5, and MAPK pathway components. KEGG pathway analysis further indicated ABL 1's involvement in chronic myeloid leukaemia, PI 3 K–AKT, MAPK signaling, and other cancer-related pathways, emphasising its importance in CML development. This combined computational approach demonstrates that structure-guided CRISPR–Cas 9 targeting of ABL 1 can effectively disrupt BCR–ABL 1 driven cancer signals. The results provide a strong theoretical basis for future experimental validation and genome editing therapies aimed at overcoming TKI resistance and achieving long-lasting molecular remission in CML.

Keywords

CRISPR-Cas9, ABL1, BCR-ABL, Chronic Myeloid Leukaemia, sgRNA Design, E-CRISP, CHOPCHOP, Off-target Analysis

1. Introduction

Chronic myeloid leukaemia (CML) is a stem cell cancer of the blood characterised by the persistent growth of myeloid

cells and driven by the BCR-ABL1 fusion gene. This gene forms from a reciprocal translocation between chromosomes

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9 and 22, creating a continuously active tyrosine kinase that promotes leukemogenesis via disrupted intracellular signaling. [1, 2] Persistent BCR-ABL1 activity causes abnormal cell growth, resistance to cell death, modified interactions with the bone marrow environment, and increased genomic instability. These factors collectively contribute to disease progression from the chronic phase to the advanced blast phase. [3] Although its genetic cause is straightforward, CML shows significant biological complexity due to extensive downstream signaling changes and adaptive survival strategies.

The ABL1 proto-oncogene encodes a widely expressed non-receptor tyrosine kinase that is essential for normal cellular functions, including cytoskeletal remodelling, signal transduction, DNA repair, and apoptosis. Mostly, ABL1 activity is thoroughly controlled by intramolecular autoinhibition and conformational changes. However, fusion with BCR disrupts these controls, leading to sustained kinase activation and oncogenic signalling. [4, 5] The BCR-ABL1 oncoprotein acts as a central signalling hub, triggering multiple pathways like PI3K-AKT, MAPK/ERK, and JAK-STAT, which promote leukemic cell survival, metabolic adaptation, and the self-renewal of leukemic stem cells. [6] These networks are crucial for disease maintenance and also contribute to therapy resistance and ongoing disease persistence. The clinical introduction of tyrosine kinase inhibitors (TKIs) marked a major shift in CML treatment, significantly enhancing patient survival and turning the disease into a more manageable chronic condition. Long-term studies indicate excellent overall survival rates with first- and second-generation TKIs; however, achieving molecular eradication remains challenging. [7] Many patients develop primary or acquired resistance because of kinase domain mutations, activation of alternative signaling pathways, or intolerance to medication. Additionally, leukemic stem cells stay present despite effective kinase suppression, acting as a reservoir for molecular relapse when treatment stops. These challenges highlight the need for therapies that target the oncogenic driver at its genetic source, rather than just inhibiting its enzyme activity. Recent advances in functional genomics reveal that CML cells are highly plastic, allowing them to survive through mechanisms independent of BCR-ABL1. Genome-wide CRISPR-Cas9 screens in CML models have uncovered alternative genetic dependencies, adaptive signaling points, and compensatory pathways that support leukaemia survival under treatment pressure. [8] These results emphasise that achieving long-term disease control likely needs both direct genetic targeting of the oncogenic driver and a systems-level understanding of signaling network dependencies. The CRISPR-Cas9 system for genome editing is a highly effective platform for precise, programmable, and efficient gene disruption. It works by creating site-specific double-strand DNA breaks, which are then repaired in an error-prone manner, allowing for targeted knockout of disease-related genes and functional studies of oncogenic pathways. [9, 10] In blood cancers, CRISPR approaches have shown the potential to disrupt fusion oncogenes, suppress leukaemia

phenotypes, and explore resistance mechanisms with unprecedented detail. [11] Nonetheless, successful clinical translation depends on careful optimization of target site selection, guide RNA (sgRNA) efficiency, and minimising off-target effects. Structure-guided CRISPR design has recently emerged as a strategy to enhance functional specificity by integrating three-dimensional protein structural data with genomic targeting. High-resolution protein structures help identify conserved and crucial residues in catalytic or regulatory domains that cannot tolerate genetic changes, thereby increasing the chance of achieving complete functional loss. [12] For ABL1, having high-resolution structures of the kinase domain, including the recently determined PDB structure 8I7S, offers a solid basis for designing sgRNAs that target regions vital for kinase stability and activity. Computational protein annotation tools like InterProScan enhance structure-guided targeting by identifying functional domains, conserved motifs, and regulatory regions within the ABL1 protein. Combining structural mapping with domain annotation helps prevent the creation of hypomorphic alleles that still have partial oncogenic activity—an important factor in CML, where kinase domain point mutations often lead to TKI resistance while maintaining signaling. This domain-focused approach supports advanced precision genome-editing methods, aiming to enhance therapeutic efficacy and reduce unintended biological effects. Efficient and safe sgRNA design is fundamental to CRISPR-Cas9 applications. Tools such as E-CRISP and CHOPCHOP enable systematic prediction and ranking of sgRNAs based on factors including PAM availability, GC content, exon targeting, predicted cleavage efficiency, and off-target risk. [13, 14] Genome-wide off-target assessment using BLAST against the human genome adds a layer of safety by spotting potential off-target sites with sequence similarity. These multi-parameter design pipelines are regarded as best practices in translational CRISPR research and are increasingly incorporated into therapeutic development. [15] Besides gene disruption, understanding the systems-level effects of ABL1 targeting is key for predicting therapeutic outcomes and resistance. Protein interaction networks via STRING reveal ABL1's central role as a regulatory hub in leukaemia pathways. [16] Mapping BCR-ABL1/ABL1 signaling cascades offers mechanistic insights into how genome editing can modulate multiple oncogenic pathways, possibly overcoming compensatory signaling that hampers single-target therapies. This study introduces an integrated structure-guided CRISPR-Cas9 and cancer bioinformatics framework to identify high-specificity sgRNAs targeting the ABL1 kinase domain, thereby disrupting BCR-ABL1 in chronic myeloid leukaemia. By combining structural analysis of ABL1 (PDB ID: 8I7S), functional domain annotation via InterProScan, sgRNA design using E-CRISP and CHOPCHOP, genome-wide specificity validation via BLAST, and signalling network mapping using STRING, we establish a comprehensive computational pipeline. This approach offers a rational basis for experimental validation and advances next-generation genome editing strategies to overcome resistance

and achieve durable disease control in CML.

2. Materials and Methods

2.1. Experimental Design and Computational Workflow

This study used a structure-guided CRISPR-Cas9 genome editing strategy to specifically disrupt the ABL1 kinase part of the BCR-ABL fusion protein, a major oncogenic factor in chronic myeloid leukemia (CML). The process combined protein structural analysis, rational sgRNA design, off-target risk evaluation, functional annotation, and downstream signaling assessment to achieve precise and biologically relevant targeting of ABL1. All analyses were performed with in silico bioinformatics tools, enabling systematic identification of critical residues and reducing potential off-target effects.

2.2. Identification of Functionally Critical ABL1 Residues

The 3d structure of human ABL1 kinase was obtained from the Protein Data Bank (PDB) and examined to identify key residues vital for kinase activity and oncogenic signaling. The analysis concentrated on critical regions such as the ATP-binding pocket, activation loop, and regulatory motifs that play roles in substrate recognition and signal transduction. Residues were prioritized based on:

- 1) Structural importance within the kinase domain
- 2) Evolutionary conservation
- 3) Functional relevance reported in leukaemia-associated mutations

These residues, essential for structure and function, were the foundation for targeted sgRNA design.

2.3. Structure-informed sgRNA Design

Single guide RNAs (sgRNAs) were carefully designed to target exonic regions that encode essential ABL1 residues, aiming to induce frameshift mutations or premature truncations after CRISPR-Cas9 cleavage. Target selection focused on maximizing disruption of ABL1 kinase activity while reducing the chance of functional protein rescue. sgRNA candidates were created using E-CRISP and CHOPCHOP, with specific parameters.

- 1) Human reference genome (GRCh38)
- 2) SpCas9 nuclease (NGG PAM)
- 3) Preference for coding exons
- 4) High predicted on-target efficiency

Only sgRNAs directly overlapping structurally essential residues were retained for downstream evaluation.

2.4. Off-target Risk Evaluation

Potential off-target effects were carefully examined using

E-CRISP and CHOPCHOP. Each sgRNA was evaluated for:

- 1) Number of predicted off-target sites
- 2) Degree of sequence mismatches
- 3) Genomic context of off-target loci (coding vs non-coding regions)

sgRNAs that may induce off-target effects in critical genes or tumor suppressor regions were eliminated. The selected sgRNAs focused on high specificity scores and minimal off-target risks, leading to safer and more precise genome editing.

2.5. Functional Validation of CRISPR Knockout Targets

To verify the biological significance of the targeted ABL1 regions, BLAST analysis was conducted against the NCBI non-redundant protein database. High sequence conservation across different species was taken as a sign of functional importance. Additionally, InterProScan was employed to annotate protein domains, functional sites, and conserved motifs impacted by CRISPR editing. Disruption of key features such as the protein kinase domain or ATP-binding sites was regarded as proof of a successful functional knockout.

2.6. Simulation of Downstream Signaling Disruption

To assess the effects of ABL1 knockout on downstream oncogenic pathways, protein-protein interaction (PPI) data were collected from the STRING database. ABL1 served as the central node, and interaction networks were built based on high-confidence scores. Key pathways involved in CML progression, such as MAPK, PI3K-AKT, JAK-STAT, and apoptosis-related pathways, were analysed to understand the functional impact of ABL1 disruption. The network's connectivity and interaction dependencies were examined to predict how CRISPR-induced knockout might impair oncogenic signaling cascades.

2.7. Data Visualization and Analysis

Structural data, sgRNA target sites, and interaction networks were visualized with standard molecular visualization and network analysis tools. The results were interpreted descriptively to evaluate structural disruption, functional effects, and signaling consequences related to ABL1 targeting.

3. Results

3.1. Retrieval and Structural Annotation of ABL1 from PDB ID 8I7S

The three-dimensional structure corresponding to PDB ID: 8I7S was retrieved and analyzed to obtain the amino acid sequence and structural features of the ABL1 kinase domain.

The structure represents the human ABL1 tyrosine kinase domain in a resolved conformation suitable for structure-guided functional analysis. Sequence extraction from the PDB file confirmed high integrity of the catalytic domain, including the ATP-binding pocket, activation loop (A-loop), and key regulatory motifs critical for kinase activity. The extracted protein sequence served as the reference for downstream structural validation, mutation mapping, and CRISPR guide RNA (gRNA) design.

InterProScan analysis of the 272-amino-acid ABL1 protein fragment showed that it falls under the TyrPK_CSF1R family, categorising it as a non-receptor tyrosine-protein kinase that plays a role in intracellular signal transduction. The domain architecture analysis detected several conserved kinase-related domains, such as the protein kinase-like (PK-like) domain, PTKc catalytic domain, STYKc domain, and the standard protein kinase domain, collectively affirming its enzymatic kinase activity.



Figure 1. InterProScan-Based Family Classification and Domain Architecture of ABL1.

Sequence motif analysis showed the presence of hallmark conserved kinase motifs such as VAIK, HRD, and DFG, along with a well-defined ATP-binding pocket and Mg^{2+} -binding residues, which are essential for catalytic activity. The activation loop and the key catalytic aspartate residue, which acts as a proton acceptor, were also conserved, indicating a functional phosphorylation mechanism. Functional family classification placed ABL1 within the Protein kinase ATP-binding FunFam (G3DSA:1.10.510.10F), reinforcing its role in ATP-dependent phosphorylation of substrate proteins. Overall, the annotated features confirm that ABL1 functions as an active tyrosine-protein kinase, playing a critical role in cellular signalling, growth regulation, and phosphorylation cascades, and highlighting its relevance as a therapeutic target in cancer and neurodegenerative disorders.

3.2. E-CRISP Analysis of ABL1

E-CRISP analysis was conducted on the ABL1 genomic locus (chr9: 130,713,946–130,887,675; query length: 174,730 bp) to identify optimal CRISPR–Cas9 guide RNAs. An initial total of 4,849 potential sgRNA designs (20 bp + PAM) were identified within the target region. After applying strict filtering criteria, 79 sgRNAs were classified as successful designs, meeting exon-targeting, nucleotide composition, and specificity requirements, and are therefore recommended for efficient and specific targeting of ABL1.

Most candidate guides were excluded because they lacked exon targeting (3,902 guides), while others were filtered out for overlapping CpG islands (129), having suboptimal nucleotide composition (192), containing poly-T sequences (TTTT)

that could cause premature transcription termination (397), or exceeding the maximum number of guides allowed per exon (150). Overall, the E-CRISP analysis produced a high-confidence set of sgRNAs suitable for functional genome editing of the ABL1 gene.

3.2.1. CRISPR–Cas9 gRNA Target Prediction for ABL1

Name	Nucleotide sequence	SAE-Score	Target	Matchstring	Number of Hits
ABL1_33_0	GAAGGAATCATCGAG GCATG NGG		ENSG00000097007:A BL1	Matchstring Info	1
ABL1_31_0	GTACCTATTATTACTTT ATG NGG		ENSG00000097007:A BL1	Matchstring Info	1
ABL1_338_127072	GCGGCTTCACTCAGA CCCTG NGG		ENSG00000097007:A BL1	Matchstring Info	1
ABL1_333_127072	GGCTTCTGGAAGAGA AAGGG NGG		ENSG00000097007:A BL1	Matchstring Info	1
ABL1_339_127072	GTCTGAGTGAAGCCG CTCGT NGG		ENSG00000097007:A BL1	Matchstring Info	1
ABL1_335_127072	GAAGGGCTTCTGGAA GAGAA NGG		ENSG00000097007:A BL1	Matchstring Info	1
ABL1_336_127072	GGCTCAAAGTCAGAT GCTAC NGG		ENSG00000097007:A BL1	Matchstring Info	1
ABL1_27_0	GGGTTCAAGATCTTC CAGAA NGG		ENSG00000097007:A BL1	Matchstring Info	1
ABL1_25_0	GAGGGAATGGCATA TACGT NGG		ENSG00000097007:A BL1	Matchstring Info	1
ABL1_28_0	GAAGATCTTGAACCC TCTTC NGG		ENSG00000097007:A BL1	Matchstring Info	1
ABL1_340_127072	GCTTAGAGTGTATCT CCAC NGG		ENSG00000097007:A BL1	Matchstring Info	1

Figure 2. The Output of CRISPR guide RNA (gRNA) target prediction.

CRISPR guide RNA (gRNA) target prediction identified 11 high-confidence sgRNAs targeting the ABL1 gene (Ensembl ID: ENSG00000097007). Each sgRNA is given a unique identifier and consists of a 20-bp target sequence followed by the canonical NGG PAM, which is essential for SpCas9 recognition and cleavage. All chosen guides showed a single genomic hit, indicating high target specificity and low off-target risk.

3.2.2. Off-target Analysis of CRISPR gRNAs Targeting ABL1

Following the initial CRISPR–Cas9 gRNA target prediction for ABL1, the shortlisted guide RNAs were further evaluated for off-target effects and overall editing safety.

ABL1_238_0	GTGCGGCCACTGTG GCTCTA NGG		ENSG00000224797:R P11-57C19.6	Matchstring Info	2
ABL1_238_0	GTGCGGCCACTGTG GCTCTA NGG		ENSG00000227401:R PL37P1	Matchstring Info	2
ABL1_243_158840	GTGCTCTGCAGCTCT CCTGG NGG		ENSG00000097007:A BL1	Matchstring Info	3
ABL1_243_158840	GTGCTCTGCAGCTCT CCTGG NGG		CHR_HSCHR8_2_CTG 7	Matchstring Info	3
ABL1_243_158840	GTGCTCTGCAGCTCT CCTGG NGG		8	Matchstring Info	3
ABL1_235_0	GGGGTAGCCACATT GCCAC NGG		ENSG00000227950:R P11-312B8.2	Matchstring Info	10
ABL1_235_0	GGGGTAGCCACATT GCCAC NGG		ENSG00000232059:R P11-173A6.2	Matchstring Info	10
ABL1_235_0	GGGGTAGCCACATT GCCAC NGG		ENSG00000224797:R P11-57C19.6	Matchstring Info	10
ABL1_235_0	GGGGTAGCCACATT GCCAC NGG		ENSG00000145592:R PL37	Matchstring Info	10
ABL1_235_0	GGGGTAGCCACATT GCCAC NGG		ENSG00000224627:R PL37P10	Matchstring Info	10
ABL1_235_0	GGGGTAGCCACATT GCCAC NGG		ENSG00000216365:R PL37P15	Matchstring Info	10
ABL1_235_0	GGGGTAGCCACATT GCCAC NGG		13	Matchstring Info	10

Figure 3. Comparative Off-Target and On-Target Efficiency Analysis of ABL1 CRISPR gRNAs.

Each gRNA (e.g., ABL1_238_0) consists of a 20 bp target sequence followed by the NGG PAM, a requirement for SpCas9-mediated cleavage. Guide performance was assessed using three composite scoring parameters representing on-target efficiency, Cas9 activity, and specificity, visualized as colored horizontal

bars. Guides exhibiting higher on-target efficiency scores (dominant orange bars) and lower off-target risk (minimal blue and green bars) were considered optimal candidates. Matchstring analysis confirmed accurate alignment of these gRNAs with

the intended ABL1 genomic locus, while also identifying potential off-target genomic sites. gRNAs with fewer genomic hits demonstrated higher specificity, whereas those with multiple hits were deprioritized due to increased off-target potential. This stepwise evaluation, following initial gRNA identification, enabled the refinement of candidate guides and the selection of highly specific and efficient CRISPR–Cas9 gRNAs, thereby ensuring safer and more precise genome editing of the ABL1 gene.

3.2.3. Genomic Browser Analysis of ABL1

After confirming minimal off-target effects of the designed CRISPR gRNAs, the genomic browser view was used to map these gRNAs onto the ABL1 locus on chromosome 9 (~170 kb).

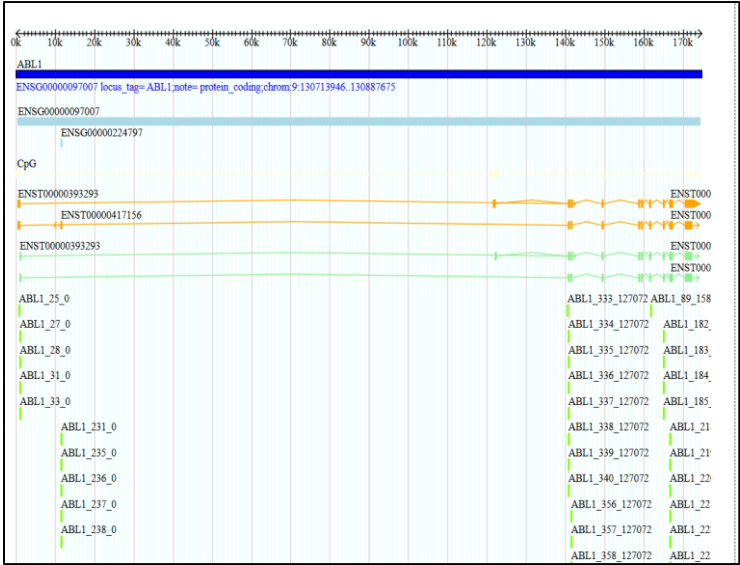


Figure 4. Display of genomic browser, illustrating the ABL1 gene structure.

The blue track depicts the full ABL1 genomic region, with the light-blue layer marking gene boundaries. Orange tracks display multiple protein-coding ABL1 transcript isoforms, while light-green tracks represent non-coding splice variants. CpG islands highlight potential regulatory regions. The vertical green bars indicate CRISPR gRNA target sites, spread

across key regions of the gene, and grey grid lines show genomic coordinates. Overall, this step integrates transcript structure with CRISPR targeting, confirming that the gRNAs—already validated by off-target analysis—are correctly positioned within the ABL1 gene for effective CRISPR-based experiments.

3.2.4. Genomic Annotation Map of ABL1

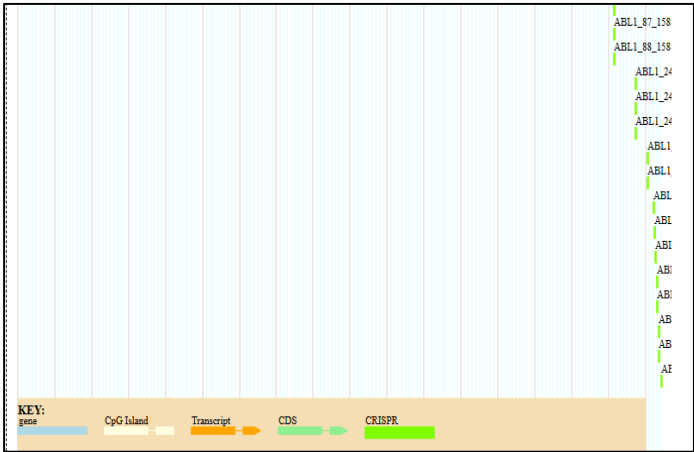


Figure 5. Genomic Organization of the ABL1 Locus Showing Transcript Variants, CpG Islands, and CRISPR Sites.

The Genomic Annotation map offered a detailed view of the ABL1 gene structure. CpG islands highlighted regulatory regions involved in gene expression control. Transcript tracks displayed multiple ABL1 transcript variants, while the CDS regions marked the protein-coding parts of each transcript. CRISPR sites pointed out suitable target locations for CRISPR–Cas9 genome editing within the gene.

The map revealed extensive alternative splicing, the presence of regulatory CpG islands, and well-positioned CRISPR target sites, supporting the functional complexity of ABL1 and

its importance for experimental and therapeutic genome-editing applications.

3.3. CHOPCHOP Genome Browser Result

After E-CRISP identified candidate CRISPR gRNAs for ABL1, CHOPCHOP was employed as a supplementary validation and visualization tool. It confirmed gRNA locations within the genomic context, verified targeting across multiple transcript isoforms, and offered an intuitive genome browser view of exon–intron structure.

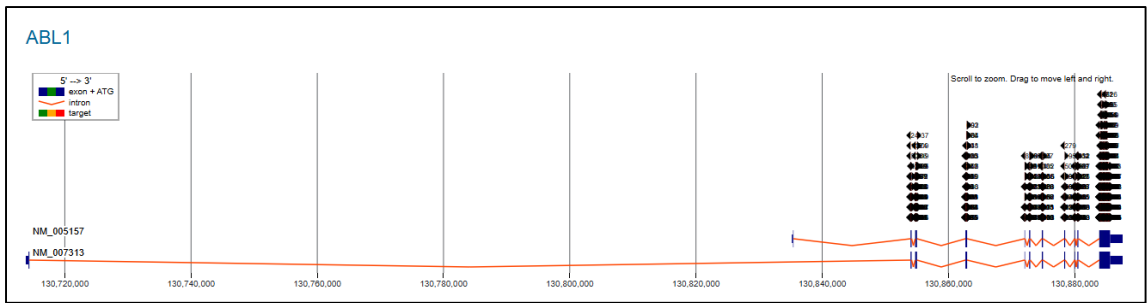


Figure 6. CHOPCHOP-Based Genomic Mapping of CRISPR Targetable Regions within the ABL1 Gene.

The figure displayed the extensive genomic span of ABL1, with black boxes indicating exons and connecting lines showing introns, confirming the gene's structural complexity. Multiple layers of exon structures emphasised the presence of several transcript variants, aligning with the extensive alternative splicing reported earlier. Reference mRNA sequences (e.g., NM_007313) validated the annotated transcript models.

This visualisation confirmed that the gRNAs selected by E-CRISP were located within well-annotated exonic regions shared across major ABL1 isoforms, thereby optimising on-target efficiency. Overall, the result supported the robustness of the E-CRISP predictions and reinforced the suitability of

ABL1 as a multi-isoform oncogenic target for CRISPR-based genome editing in leukaemia studies.

3.3.1. CHOPCHOP-based Selection and Evaluation of Optimal CRISPR–Cas9 sgRNA Candidates

CHOPCHOP was used to identify and rank potential CRISPR–Cas9 sgRNA target sites within a specific gene region on chromosome 9. The tool evaluated guides based on predicted cutting efficiency, GC content, self-complementarity, and potential off-target effects (MM0–MM3 mismatches).

Rank	Target sequence	Genomic location	Strand	GC content (%)	Self-complementarity	MM0	MM1	MM2	MM3	Efficiency
1	CACGGTGACCTGCCCGGAGGG	chr9:130884740	-	75	0	0	0	0	0	48.28
2	CCTCCAGCGGATGCCAGCG	chr9:130885342	+	70	1	0	0	0	0	48.47
3	ATCTCAGCGAGATGACCTCTGG	chr9:130855024	-	55	0	0	0	0	0	47.30
4	ACCCAGACGTCCGACTTGAAG	chr9:130875052	-	60	0	0	0	0	0	46.85
5	CTCGTCAGCCATGGAGTACCTGG	chr9:130872993	+	60	1	0	0	0	0	43.51
6	ATCTCCCACTTGTCTGATTTGG	chr9:130862901	-	45	1	0	0	0	0	27.38
7	GGGCCGAGACATCAGCAAGGG	chr9:130884201	+	65	0	0	0	0	1	71.12
8	ACGGTAAAGTACCCATCCCGGG	chr9:130880155	+	55	2	0	0	0	1	72.41
9	TCTCCTGAGATACGAGGAGG	chr9:130855035	+	55	1	0	0	0	1	70.78
10	CAGTACGGGAGGTGTACGAGG	chr9:130862967	+	60	2	0	0	0	1	70.00
11	ACGGTGACCTGCCCGGAGGG	chr9:130884739	-	75	0	0	0	0	1	56.35
12	CACAGTCCGCCAAGCCGTCGGG	chr9:130885187	+	70	1	0	0	0	1	54.84
13	AGACGGTAAAGTACCCATCCCGG	chr9:130880153	+	50	2	0	0	0	1	55.63
14	TCAGCGGCAATTCGGGACACGG	chr9:130854938	-	65	0	0	0	0	1	51.35
15	CTCCATCAAGTCCGAGCTCTGG	chr9:130875030	+	55	0	0	0	0	1	48.29
16	CCACAGTCCGCCAAGCCGTCGGG	chr9:130885186	+	70	1	0	0	0	1	40.23
17	AAAGAGCGAGGTCCCCGGAGGG	chr9:130884013	+	65	0	0	0	1	0	62.55
18	GGGGACCTCGCTTTTCGAGGG	chr9:130884006	-	60	2	0	0	1	0	52.49

Figure 7. CHOPCHOP Ranking and Characterization of High-Confidence CRISPR–Cas9 sgRNA Targets on Chromosome 9.

The figure showed 18 candidate sgRNAs identified by CHOPCHOP ranked according to their predicted cutting efficiency. Each target sequence ended with an NGG PAM motif (highlighted in red: GGG/AGG/CGG), which was required for Cas9 binding. All sgRNAs mapped to the same genomic region on chromosome 9 and originated from both DNA strands; strand orientation did not affect editing efficiency, provided the target region was accessible.

The analysis indicated that sgRNAs with GC content within the optimal range (40–60%), low self-complementarity, and minimal off-target mismatches (MM0–MM3) were predicted to perform better. Based on these combined parameters and higher efficiency scores, sgRNAs 7, 8, 9, 10, and 11 were identified as the most suitable candidates for CRISPR–Cas9–mediated genome editing.

3.3.2. Evaluation of Off-target Effects of the Selected CRISPR–Cas9 sgRNA for ABL1

The selected ABL1-targeting sgRNA identified by CHOPCHOP (Rank 7) was evaluated for potential off-target effects across the genome. Analysis showed that predicted off-target sites contained multiple mismatches relative to the guide sequence, especially within the PAM-proximal seed region, which is critical for Cas9 specificity. Most predicted off-targets were located in intronic or intergenic regions rather than

coding exons. The limited number and low-risk nature of these sites indicate a low probability of unintended genome editing, supporting the specificity and suitability of this sgRNA for targeted ABL1 gene disruption. Now clarify that although red bars indicate predicted off-target sites with mismatches, these sites are considered low risk based on the following criteria:

Mismatch Distribution:

The majority of predicted off-target sites contain ≥ 3 mismatches, particularly within the PAM-proximal seed region, which is critical for Cas9 cleavage specificity. Such mismatch patterns are known to significantly reduce Cas9 cutting efficiency.

Genomic Context:

Nearly all predicted off-target sites are located in intronic or intergenic regions, with no enrichment in coding exons, tumour suppressor genes, or regulatory promoter regions.

Absence of Functional Overlap:

No predicted off-target loci overlap with genes involved in hematopoiesis, cell-cycle control, or known oncogenic pathways, minimizing the likelihood of biologically adverse effects.

Comparative Ranking:

The selected sgRNA (Rank 7, CHOPCHOP) showed a better balance between predicted on-target efficiency and off-target safety compared to other candidate guides.

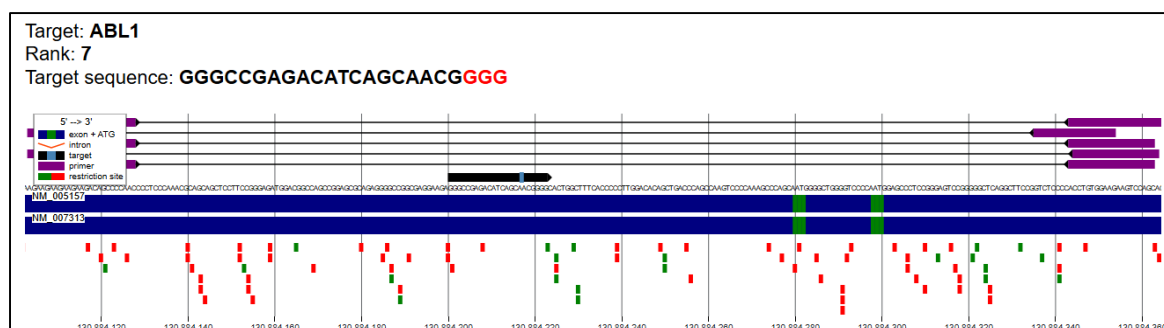


Figure 8. Genome-Wide Off-Target Profiling of the Selected CRISPR–Cas9 sgRNA Targeting ABL1.

The figure illustrated the off-target analysis of the selected ABL1 guide RNA (Rank 7), which indicated moderate predicted editing efficiency. The target sequence (GGGCCGAGACATCAGCAACGGGG) contained a 3' NGG PAM motif, confirming compatibility with Cas9. The gene annotation track showed the genomic context of ABL1, including exon–intron organization, and indicated that the gRNA target site was located within a gene-associated region, suggesting functional relevance. The off-target mismatch distribution displayed predicted off-target sites across the genomic region, where red bars represented higher mismatch frequencies and green bars represented fewer mismatches, indicating potential off-target concerns. The genomic coordinate

axis demonstrated that these predicted off-targets were distributed across the region rather than being highly clustered. Overall, the figure demonstrated that the selected ABL1-targeting gRNA exhibited good specificity with minimal high-confidence off-target effects. Despite being ranked seventh, the guide maintained a favourable balance between targeting efficiency and genomic safety, supporting its suitability for functional genomics studies or therapeutic modeling, pending experimental validation.

3.3.3. In Silico Design and Specificity Assessment of PCR Primers Targeting the ABL1 Locus

Multiple primer pairs were designed to amplify the selected ABL1 genomic region on chromosome 9 flanking the

CRISPR–Cas9 target site. All primer pairs showed optimal melting temperatures (~59–62°C), balanced GC content, and

generated PCR products ranging from ~250–261 bp, suitable for downstream validation assays.

Pair	Left primer coordinates	Left primer	Left primer Tm	Left primer off-targets	Right primer coordinates	Right primer	Right primer Tm	Right primer off-targets	Pair off-targets	Product size
1	chr9:130884107-130884129	AGAAGAAGAAGACAGCCCAAC	61.1	0	chr9:130884344-130884364	GCTGGACTTCTCCACAGGT	59.3	0	0	257
2	chr9:130884104-130884126	AGAAGAAGAAGAAGACAGCCCC	60.4	0	chr9:130884345-130884365	TGCTGGACTTCTCCACAGG	61.4	0	0	261
3	chr9:130884108-130884129	GAAGAAGAAGACAGCCCAAC	60.2	0	chr9:130884344-130884364	GCTGGACTTCTCCACAGGT	59.3	0	0	256
4	chr9:130884104-130884126	AGAAGAAGAAGAAGACAGCCCC	60.4	0	chr9:130884336-130884355	CTTCCACAGGTGGGAGAC	60.5	0	0	251
5	chr9:130884107-130884129	AGAAGAAGAAGACAGCCCAAC	61.1	0	chr9:130884344-130884366	GTGCTGGACTTCTCCACAGGT	62.9	0	0	259
Off-targets										
Location		Number of mismatches			Sequence (including mismatches)					
chr5:178265527		3			CCAC TT TCTGTGATGCTCGG CT C					

Figure 9. In Silico Design and Specificity Analysis of PCR Primer Pairs Targeting the ABL1 Genomic Region.

This figure summarises the in silico design and evaluation of five primer pairs (Pairs 1–5) targeting a genomic region on chromosome 9 within the ABL1 locus. All primers were 18–22 bp in length, which was optimal for efficient and specific PCR amplification. The melting temperatures (Tm) of the forward and reverse primers within each pair differed by less than 3°C, indicating good thermodynamic compatibility.

The lowest Tm observed was approximately 59.3°C, which supported a recommended PCR annealing temperature of about 56–57°C (acceptable range 55–58°C). In silico off-target analysis showed no predicted off-targets for all primer pairs, confirming high specificity towards the target region.

Based on balanced Tm values and suitable amplicon sizes (~250–260 bp), Primer Pair 1 and Primer Pair 3 were identified as the most appropriate candidates for experimental validation. Overall, the designed primers were suitable for routine PCR and qPCR applications, as the annealing temperature

range was both safe and effective, according to experimental results.

3.4. BLAST-based Validation of CRISPR-Cas9 Target Specificity for ABL1

To confirm the specificity and effectiveness of the designed CRISPR-Cas9 guide RNAs targeting the ABL1 gene, the edited sequences were subjected to BLAST analysis against the human protein database. The top hits consistently matched Chain A, Tyrosine-protein kinase ABL1 (Homo sapiens) with 100% query coverage and high sequence identity (99.63–100%), indicating that the CRISPR-induced modifications were highly specific to the intended target. No significant off-target alignments were observed, supporting the precision of the CRISPR knockout strategy for functional studies.

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒	Chain A, Tyrosine-protein kinase ABL1 [Homo sapiens]	Homo sapiens	573	573	100%	0.0	99.63%	408	4XEY_A
☒	Chain A, Tyrosine-protein kinase ABL1 [Homo sapiens]	Homo sapiens	572	572	100%	0.0	99.63%	448	8SSN_A
☒	Chain A, Tyrosine-protein kinase ABL1 [Homo sapiens]	Homo sapiens	570	570	100%	0.0	99.63%	310	7CC2_A
☒	Chain A, Tyrosine-protein kinase ABL1 [Homo sapiens]	Homo sapiens	570	570	100%	0.0	99.63%	287	6XR6_A
☒	Chain A, Tyrosine-protein kinase ABL1 [Homo sapiens]	Homo sapiens	570	570	100%	0.0	100.00%	276	ZWY_A

Figure 10. Sequence Similarity search in BLAST.

All rows represented ABL1 (chain A) from *Homo sapiens*. The maximum/total score was near the upper limit (570–573), indicating very high alignment similarity. The query covered 100% of the sequence, and the E-value was 0.0, showing statistically significant matches. Percent identity ranged from 99.63% to 100%, demonstrating almost complete sequence identity with reference proteins. The query sequence corresponded to ABL1 kinase, closely matching multiple high-quality crystal structures.

3.5. STRING-based Protein Interaction Analysis of Downstream Effects Induced by ABL1 Knockout

After high-efficiency sgRNAs were designed and validated using E-CRISP and CHOPCHOP, ABL1 was targeted for CRISPR-Cas9-mediated knockout to investigate its role in

downstream signaling regulation. The objective of this approach was to assess how loss of ABL1 altered interconnected signaling pathways by integrating protein–protein interaction (PPI) data from the STRING database.

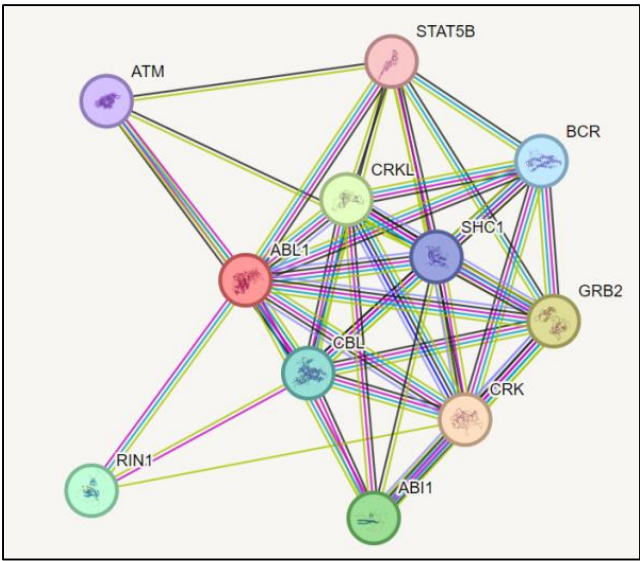


Figure 11. Protein–Protein Interaction Network of ABL1 and Associated Downstream Signaling Proteins.

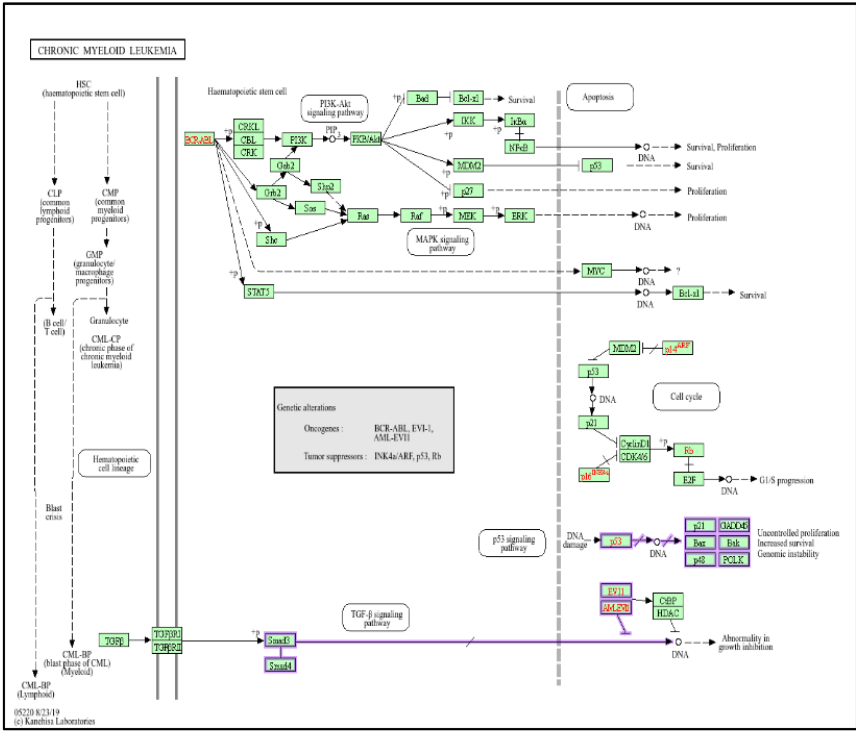


Figure 12. Mapping of BCR-ABL/ABL1-Associated Signaling Cascades in Chronic Myeloid Leukemia.

ABL1 is considered a central signaling hub, and its knock-out was expected to disrupt interactions with key partners such as CRKL, STAT5B, GRB2, SHC1, CBL, BCR, ATM, and CRK, as represented in the STRING interaction network. STRING-based analysis was used to identify direct and indirect interacting proteins whose signaling behavior was likely affected following ABL1 disruption.

By combining CRISPR-mediated gene knockout with STRING network analysis, the study predicted cascade effects on pathways involved in cell proliferation, survival, DNA damage response, and leukemogenic signaling. This integrative strategy facilitated the identification of critical downstream effectors and provided mechanistic insight into how

targeted ABL1 knockout rewired oncogenic signaling networks in leukemia.

4. Discussion

Chronic myeloid leukaemia is a clonal myeloproliferative disorder caused by the BCR-ABL1 fusion gene. This gene encodes a constitutively active tyrosine kinase that disrupts many signaling pathways, affecting cell growth, survival, and genome stability. The development of tyrosine kinase inhibitors (TKIs) has revolutionized CML and changed it from a lethal disease into a manageable chronic illness. Still, challenges like treatment resistance, intolerance, and the persistence of leukemic stem cells impede the complete eradication of the disease. Recent genome-wide CRISPR screening studies revealed that CML cells can develop BCR-ABL1-independent survival mechanisms, highlighting the need for therapies that target the oncogenic driver at the genomic level, rather than solely inhibiting its activity. [8] This research applied a structure-guided CRISPR-Cas9 strategy targeting the ABL1 kinase domain to disable the BCR-ABL1 fusion protein's function. Using the detailed crystal structure of human ABL1 (PDB ID: 8I7S), the researchers precisely identified conserved catalytic and regulatory regions essential for kinase activity. In this study, specific targeting is justified by focusing on structurally and functionally indispensable regions of the ABL1 kinase domain that are critical for the oncogenic activity of the BCR-ABL1 fusion protein. Although the ABL1 kinase domain is shared between the fusion and wild-type ABL1, leukemic cells exhibit a strong dependency on constitutively active BCR-ABL1 signaling for survival and proliferation, a phenomenon known as oncogene addiction. By designing CRISPR-Cas9 sgRNAs against highly conserved catalytic motifs such as the ATP-binding pocket, activation loop, and HRD/DFG motifs, the strategy aims to induce frameshift mutations that abolish kinase activity and selectively impair leukemic signaling networks. Normal hematopoietic cells, which rely on tightly regulated ABL1 activity and possess greater signaling redundancy, are expected to be less vulnerable to partial ABL1 disruption. Nevertheless, we acknowledge that this domain-based approach does not fully distinguish between wild-type ABL1 and BCR-ABL1 at the genomic level. Therefore, the present work is positioned as a predictive, structure-guided framework for functional disruption, while highlighting fusion-junction-specific sgRNA design, base editing, or prime editing as future strategies to achieve absolute therapeutic specificity and minimize systemic toxicity. Structural mapping guided the design of guide RNAs (gRNAs) targeting critical motifs, thereby increasing the likelihood of complete oncogenic inactivation rather than partial disruption. This approach is particularly pertinent in CML, where kinase-domain mutations often drive TKI resistance but may remain susceptible to gene editing. [17, 18] To enhance accuracy and safety, gRNA design involved prediction tools like E-CRISP

and CHOPCHOP, followed by BLAST analysis to assess potential off-target effects in the human genome. Combining multiple algorithms reduced prediction bias and increased confidence in the selected gRNAs, addressing a key challenge in therapeutic CRISPR—achieving genome-wide specificity. Advances in CRISPR technology, including improved gRNA design guidelines and high-fidelity Cas9 variants, bolster the translational potential of these computational strategies. Supporting this approach, experimental studies have demonstrated that CRISPR-Cas9-mediated disruption of BCR-ABL1 with one or two gRNAs significantly decreases fusion gene expression, slows cell proliferation, and induces cell death in CML models. [19, 20] This confirms the feasibility of targeting this oncogenic fusion at the genetic level. In addition, the impact of ABL1 targeting was examined through protein interaction and signaling network analyses. Using STRING mapping, ABL1 was identified as a key signalling hub that integrates multiple oncogenic pathways, including PI3K-AKT, MAPK, JAK-STAT, and DNA damage responses. Consequently, disrupting ABL1 is expected to exert broad antileukemic effects by simultaneously downregulating multiple survival and growth signalling pathways. This systemic influence contrasts with TKIs, which mainly inhibit kinase activity without removing the fusion gene, potentially allowing ongoing signaling or pathway reactivation. Moreover, genome-wide CRISPR screens in CML have highlighted compensatory signaling networks, emphasizing the importance of targeting central regulatory nodes rather than individual downstream effectors. This study presents a comprehensive computational framework that integrates structural biology, CRISPR gRNA design, genome-specificity analysis, and signalling network mapping. It supports targeting ABL1 as a rational strategy for genome editing in CML. Recent reviews indicate that CRISPR-based methods are promising for overcoming TKI resistance and achieving long-term disease control, particularly when paired with improved delivery systems and advanced editing tools, like base editors and prime editors. [21] While further experimental validation and optimization are needed, these findings contribute to the growing evidence that genome editing could become a potential curative approach for chronic myeloid leukaemia.

5. Limitations of the Study and Future Scope

While promising *in silico* results are encouraging, several limitations need to be acknowledged. This study relies solely on computational predictions. Even though structural guidance and multiple gRNA design tools enhance theoretical specificity and efficacy, experimental validation, such as CML cell lines, primary patient cells, and animal models is essential to confirm editing outcomes, cellular impacts, and safety. Techniques like targeted deep sequencing, Western blotting, and viability or clonogenic assays are important to evaluate

on-target effects and phenotypic changes. A key limitation of CRISPR-Cas9 is the risk of off-target mutations, which may lead to unintended, potentially harmful effects. Although computational predictions can reduce, though not eliminate, this risk, future work should incorporate empirical detection methods such as GUIDE-seq, CIRCLE-seq, or comprehensive genome-wide sequencing to assess specificity. Another challenge is the non-specific targeting of wild-type ABL1 and BCR-ABL1 fusion proteins. Since ABL1 is crucial for normal blood cell production, its complete knockout could impair cellular function. Future approaches might include using fusion-junction-specific gRNAs, base editing, or prime editing to selectively target and disrupt the oncogenic fusion without affecting wild-type ABL1. The structural model (PDB 8 I 7 S) provides a static view of the ABL1 kinase domain. In living organisms, factors like conformational shifts, post-translational modifications, and interactions with regulatory proteins can influence CRISPR's access and effectiveness. Incorporating molecular dynamics simulations and structures of key mutant kinases, like T315I, could enhance design precision. [22] Delivering CRISPR components efficiently to target cells remains challenging. Although viral vectors such as lentiviruses and AAV are effective, they pose safety risks, including insertional mutagenesis and immune responses. Therefore, exploring non-viral delivery methods- including lipid nanoparticles, ribonucleoprotein complexes, and ex vivo editing with autologous transplantation- is important. Future research should validate predicted effects on downstream signaling pathways through proteomic and phosphoproteomic analyses, clarifying how ABL 1 disruption affects oncogenic networks and identifying compensatory mechanisms. Combining CRISPR editing with TKIs, immunotherapy, or small- molecule inhibitors may enhance therapeutic efficacy and help overcome resistance. ABL1 kinase undergoes conformational transitions, including DFG-in and DFG-out states, which regulate catalytic activity and inhibitor binding. Although CRISPR-Cas9 targeting is performed at the genomic DNA level, structural conformational plasticity remains relevant for identifying functionally intolerant residues, ensuring that sgRNA-induced mutations disrupt essential catalytic motifs regardless of kinase state. Dynamic conformational states could influence chromatin accessibility and local DNA-protein interactions, potentially affecting editing efficiency in vivo. In summary, while structure-guided CRISPR targeting of ABL 1 shows promise for CML treatment, comprehensive experimental validation, improved specificity, safe delivery methods, and translational studies are crucial to transition from computational predictions to clinical application.

6. Conclusions

This study presents a robust structure-guided CRISPR-Cas9 platform for specifically disrupting the ABL1 oncogene involved in chronic myeloid leukaemia. By combining insights from protein structure, cancer genomics, and strict

sgRNA design and filtering methods, a small set of highly specific CRISPR targets was identified within crucial regions of the ABL1 kinase domain. In silico evaluations confirmed these targets offer optimal on-target efficiency with minimal off-target risks, and genomic visualization validated accurate sgRNA placement across different ABL1 transcript variants. Overall, this work provides a reliable computational framework for detailed functional analysis of ABL1 and supports future genome-editing therapies for BCR-ABL1-driven leukaemia.

Abbreviations

ABL1	Abelson Murine leukemia Viral Oncogene Homolog 1
BCR	Breakpoint Cluster Region
BCR-ABL1	Breakpoint Cluster Region-Abelson 1 Fusion Gene
BLAST	Basic Local Alignment Search Tool
Cas9	CRISPR-Associated Protein 9
CML	Chronic Myeloid Leukemia
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DNA	Deoxyribonucleic Acid
dsDNA	Double-Stranded Deoxyribonucleic Acid
ERK	Extracellular Signal-Regulated Kinase
GC	Guanine-Cytosine
InterProScan	Integrated Protein Signature Recognition Tool
JAK-STAT	Janus Kinase-Signal Transducer and Activator of Transcription
MAPK	Mitogen-Activated Protein Kinase
PAM	Protospacer Adjacent Motif
PI3K-AKT	Phosphoinositide 3-kinase-Protein Kinase B Signaling Pathway
PPI	Protein-Protein Interaction
sgRNA	Single-Guide RNA
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
TCGA	The Cancer Genome Atlas
TKI	Tyrosine Kinase Inhibitor

Author Contributions

Sumita Katal: Data Curation, Investigation, Methodology, Resources, Formal Analysis, Writing – original draft

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Uma Kumari: Conceptualization, Formal Analysis, Supervision, Writing – review & editing

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Data Availability Statement

The data is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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