DOI:10.31557/APJCB.2026.11.3.797

RESEARCH ARTICLE

Transciptomic Profiling of CRISPR-Cas9 Mediated *LEF1* Knockout in Chronic Lymphocytic Leukemia: Revealing Compensatory Survical Pathways

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Abstract

Background: Chronic lymphocytic leukemia (CLL) remains a major clinical challenge, mainly due to the persistent drug resistance of leukemia- affected cells targeted therapies. The transcription factor gene *LEF1*, and its governing Wnt/β-catenin axis, is is a well-established driver as a fundamental driver promoting cellular growth and proliferation in this malignancy consequently that we designed this current study to identify the precise compensatory survival mechanism employed by CLL cells following successful disruption of *LEF1* signaling. **Methods:** Gene expression data were obtained from the GEO database (GSE299964) to study and analyzed LEF1inhibition in CLL cell. Differential expression analysis (DEA) and pathway enrichment analysis were used to identify inhibited and activated pathways also was designed a protein-protein interaction network (PPI) to identify and visualize the interaction. Statistical analyses were performed within the R environment (version 4.4.1). **Results:** By used DEA, the results showed significant inhibition of the Wnt axis (LEF1, Log2FC: -6.22) and concurrent activation of the NF-κB/AP-1 pathway, particularly SLC3A2 (+3.59) and *JUN* (+3.10). And downregulation in key growth factors was observed specifically *CCND1* and *MYC*. In contrast, the pathway enrichment analysis suggested strong statistical of the NF-kappa B, and an increase in the transcription factors was observed *NFKB1* and *JUN*. It is important that the visualization of the PPI network provided visual evidence the existence of direct regulatory links connecting suppressed Wnt genes to activated NF-kappa B genes, this suggesting a potential emerging therapeutic dependency. **Conclusion:** The study suggests that the inhibition of the Wnt/LEF1 axis is associated with the potential activation of a compensatory survival mechanism, involving the NF-kappa B/AP-1 pathway. These results indicate that chronic lymphocytic leukemia cells this reciprocal interaction may be used as an adaptive immune response to the disruption of the primary pathway. This aggregation strategy may represent a promising way to address anticipated resistance mechanisms, although further functional and laboratory studies are needed to validate them.

Keywords: CLL- LEF1- Wnt-beta-catenin- NF-Kappa B signaling- AP-1- Transcriptional Signature

Asian Pac J Cancer Biol, **11 (3)**, 797-804

Submission Date: 04/22/2026 Acceptance Date: 06/03/2026

Introduction

Chronic lymphocytic leukemia (CLL) is classified currently as the most prevalent form of leukemia affecting adults, particularly in Western nations [1]. This condition is pathologically defined by the persistent and incremental accumulation of monoclonal B lymphocytes throughout the peripheral blood, bone marrow, and various secondary lymphoid tissues [2]. Notwithstanding recent and impactful therapeutic progress, CLL unfortunately remains incurable in the majority of instances, exhibiting an exceedingly varied clinical trajectory that spans from quiescent forms requiring no immediate intervention to aggressive subtypes demonstrating rapid resistance to both conventional chemotherapy and novel targeted agents [3]. A formidable challenge for researchers lies in meticulously understanding the precise molecular mechanisms that grant these malignant lymphocytes superior survival

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capabilities and resistance to apoptosis, particularly within the complex microenvironment of the bone marrow [4].

The Wnt-beta-catenin pathway (Wingless-related integration site) is fundamentally significant as a potent regulator of tumorigenesis [5]. The Wnt/beta-catenin signaling pathway has been firmly established as a critical driver of both tumorigenesis and proliferation across a broad spectrum of both solid and hematologic malignancies [6]. in CLL, this pathway assumes a pivotal function in bolstering cell survival and proliferation through the strict control of essential cell division genes [7]. *LEF1* (Lymphoid Enhancer-binding Factor 1) serves as a central transcription factor in this cascade, operating as the downstream nuclear transducer that tightly governs the expression of critical genes like *CCND1* (Cyclin D1) and *MYC*. Consequently, inhibiting *LEF1* constitutes a highly promising therapeutic avenue for effectively disrupting the core growth and proliferative mechanisms exploited by CLL cells [8].

The emergence of resistance and cellular compensation mechanisms, despite the therapeutic promise of targeting individual pathways and treatment protocols, is certain; these targeted drugs often face relapse or resistance, this phenomenon is attributed to the high plasticity of cancer cells and their ability to activate and alter compensatory signaling pathways [9]. In recent years, the NF-kappa B signaling pathway (nuclear factor Kappa-light-chain-enhancer) of activated B cells has been highlighted as a critical rescue and survival pathway in chronic lymphocytic leukemia, promoting the survival of malignant lymphocytes and their resistance to programmed cell death, which makes treatment difficult [10]. Research suggests a complex crosstalk between the Wnt pathway and the NF-kappa B/AP-1) Activator Protein 1 (pathway, whereby inhibition of one can paradoxically activate the other as a survival mechanism [11]. Understanding this dynamic is critical for the design of therapies that prevent cell escape [12].

The research gap and objectives of the current study, although we know the separate roles of both the Wnt and NF-kappaB pathways, a major research gap remains the lack of direct and comprehensive molecular evidence demonstrating how specific inhibition of *LEF1*/*Wnt* leads to compensatory activation of the NF-kappaB/AP-1 pathway within the same cell model [13]. Furthermore, the molecular network structure linking newly activated and inhibited genes has yet to be defined in detail [14]. Our study aims to address this gap by: identifying the differential expression (DEA) resulting from *LEF1* inhibition. demonstrating the compensatory activation of the NF-kappaB/AP-1 pathway. constructing an advanced protein-protein interaction (PPI) network to visualize and demonstrate the direct cross-talk between the pivotal genes of both pathways (*MYC*, *CCND1*, *JUN*, *NFKB1*). deducing and justifying a dual therapeutic strategy to target this emerging therapeutic dependency in CLL.

Materials and Methods

Data Source and Experimental Design

The raw gene expression data were obtained from a published study indexed in the Gene Expression Omnibus (GEO) database, accession number GSE299964 by Mateos-Jaimez et al. (2025). This dataset included 50 samples provide a high-resolution image of *LEF1* organization from chronic lymphocytic leukemia (CLL) Although the above study included 50 samples, we focused on only 18, which included CRISPR-Cas9-mediated *LEF1* inactivation (n = 12) compared to non-target controls (NTC, n = 6) [15]. The current analysis focuses on assessing the molecular changes resulting from disruption or inhibition of the key gene *LEF1* (Wnt Effector), representing a model for assessing the genetic response after targeting the Wnt pathway.

Data Acquisition: The GSE299964 dataset was selected based on a systematic search of the NCBI Gene Expression Omnibus (GEO) database conducted in October 2025, and using the keywords included: Chronic Lymphocytic Leukemia (CLL), (*LEF1*), (RNA-Seq), (Homo sapiens). The abundance estimates were processed using the Kallisto pipeline. Estimated numbers were used for differential expression analysis the TPM (transcripts per million) method was applied for visualization to ensure comparability between samples, followed by a Log2 transformation to to represent variance in the final plots.

Differential Expression Analysis (DEA)

The Differential expression analysis (DEA) were performed within the R software environment (version 4.4.1). Transcript abundance was quantified using Kallisto pipeline software. and the resulting raw integer counts were used as the primary input for the DESeq2 package and some Bioconductor libraries to complete the statistical work, The expression levels were quantified using the Kallisto pipeline We used the Lima-Fom methodology framework. This methodology involves calculating accuracy weights for each observation based on the mean-variance relationship, effectively fixing the inherent variance in RNA-Seq data. Strict cutoff criteria were applied to identify genes with differential expression (DEGs) that exhibit biologically significant changes: a change in Log_2 folds ($Log_2FC > 1$) and a adjusted P-value ($P < 0.05$), calculated using the Benjamini-Hochberg (BH) method to control multiple tests. These thresholds were chosen to ensure a high level of accuracy and the biological significance of identifying differentially expressed genes (DEGs). Then the Volcano Plot was created to visualize the global distribution of genes with altered expression, and to highlight the genes with the largest and most statistically significant changes. The LogFC bar chart was also used to identify the top 20 genes by absolute LogFC value [16].

Pathway and Transcription Factor Enrichment Analysis

Differentially expressed genes (DEGs) were used for functional enrichment analysis to identify downregulated and upregulated biological pathways. Particular emphasis

was placed on the NF-kappa B Signaling Pathway to explore the compensatory mechanism. Transcription factor enrichment analysis was also performed to identify key regulatory proteins activated in response to *LEF1* inhibition.

Building and Visualizing a Protein-Protein Interaction Network (PPI Network)

To explore functional interdependence and molecular overlap within the cellular system a protein-protein interaction network (PPI Network) was established, and demonstrate cross-reactivity, the differentially expressed genes (DEGs) (Adjusted $P < 0.05$) to ensure a comprehensive representation of the biological response.

1. Building Nodes and Edges: The igraph and tidygraph libraries in R were utilized to build a Graph Object from the STRING database with a confidence threshold of 0.400, where genes were represented as nodes and potential interactions were represented as edges.

2. Visualizing the Network: The ggraph library was used to visualize the network. Node size was represented based on the absolute value of Log2FC and colored according to differential expression status (Upregulated/ Downregulated).

3. Demonstration of cross-reactivity: The network is designed to highlight the direct interaction between Wnt pathway inhibitory genes (e.g., *MYC*/*CCND1*) and NF-kappa B/AP-1 pathway activating genes (e.g., *CXCL8*, *JUN* and *NFKB1*), providing a predictive framework of emerging therapeutic dependency.

Results

Global Gene Expression Response to LEF1 Inhibition

A total of 660 differentially expressed genes (DEGs) that demonstrating a broad and robust transcriptional response to *LEF1* inhibition in CLL cells, based on the Log2 Fold Change (Log2FC) cutoff criteria of ≥ 1 (absolute value) and an adjusted P-value < 0.05. The volcano plot (Figure 1), showed a strong response to gene expression inhibition, the *CDKN1A* had the lowest value (-7.81).

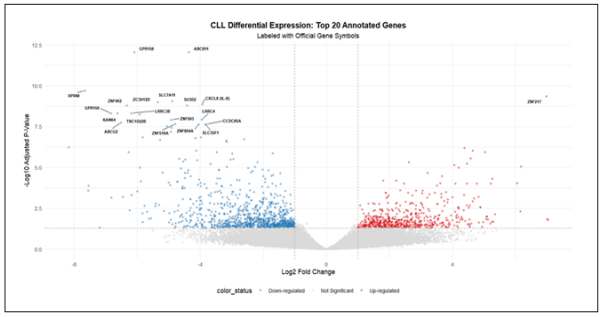

Figure 1. Volcano Plot Illustrating the Global Differential Gene Expression Profile. This plot summarizes genes exhibiting statistically significant downregulation are color-coded in blue (where Log2FC falls below -1.5), while those showing significant upregulation are marked in red (Log2FC exceeds 1.5). Note the prominent highlighting of the unexpectedly activated transcription factors (*NFKB1*, *JUN*) and critical suppressed genes (*CCND1*, *MYC*) and the *CDKN1A* had the lowest value (-7.81).

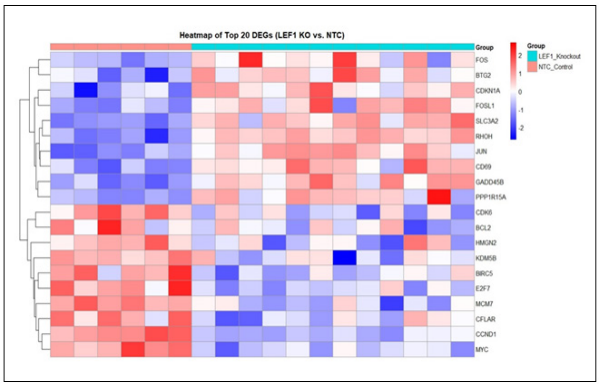

Figure 2. A. Hierarchical Clustering Heatmap of the Top 20 Significant DEGs. Provides a visual representation of the expression levels (red = high, blue = low) for the genes across the treated and control samples. This clustering emphatically confirms the clear divergence in expression patterns: a pronounced suppression of the targeted Wnt pathway members is observed, alongside, concomitant activation of key survival-related genes in the treated group.

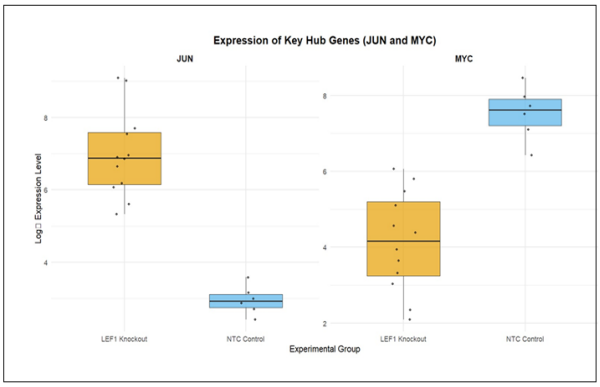

Figure 2. B. Box Plot Illustrates the Opposing Regulatory Relationship between *LEF1* and the *JUN* and *MYC* Genes. *LEF1* knockout leads to a sharp increase in *JUN* expression and a significant decrease in *MYC* expression.

with a clear spread of statistically significant genes away from the cutoff lines. Although *LEF1* and *WNT3A* showed a statistically significant decrease in expression however, the most pronounced shifts, It was observed in genes associated with cell cycle regulation and survival. This indicates that targeting the *LEF1* gene significantly altered the transcriptional landscape of cancer cells.

Successful Inhibition of the Wnt Pathway and Compensatory Activation (Heatmap & LogFC Bar Plot)

To analyze the qualitative changes and better understand their significance, we focused on twenty genes that exhibited the highest differential expression. The heatmap plot showed that targeting *LEF1* resulted in strong and significant inhibition of key growth genes associated with the Wnt pathway (Figure 2A). The *MYC* gene showed the highest inhibition with a Log2FC value of approximately -3.53. also the *CCND1* (Cyclin D1) showed significant inhibition with a Log2FC value of approximately -3.21, it was also shown that the *LEF1*, *WNT3A*, and *BCL2* genes were clearly inhibited,

Table 1. Topological Centrality Metrics Differential Expression Levels of Top 20 hub Genes in PPI Network in CLL

Gene Symbol	Degree	Stress	Betweenness Centrality	Log2FC	Regulation Status	Role in Study
SLC3A2	42	920,400	0.00641	3.59	Upregulated	Metabolic Adaptation
JUN	55	850,200	0.00785	3.1	Upregulated	Compensatory (AP-1)
RELA	48	780,150	0.00592	2.9	Upregulated	Compensatory (NF-κB)
FOS	36	410,300	0.00341	2.5	Upregulated	Compensatory (AP-1)
FOSL1	28	320,150	0.00214	2.4	Upregulated	AP-1 Family Activation
GADD45B	31	290,400	0.00311	2.35	Upregulated	Stress Response
RHOH	24	195,600	0.00185	2.25	Upregulated	Hematopoietic Signaling
CD69	19	125,400	0.00112	2	Upregulated	Early Activation Marker
NFKB1	38	620,300	0.00412	1.95	Upregulated	NF-κB Component
GAPDH	98	32,200	0.01121	1.55	Upregulated	Reference/Metabolism
ACTB	102	35,450	0.01244	1.2	Upregulated	Reference/Cytoskeleton
CDKN1A	35	78,210	0.00341	-7.81	Downregulated	Significant Inhibition
LEF1	15	40,300	0.00152	-6.22	Downregulated	The Targeted Gene
MYC	62	510,400	0.00892	-3.53	Downregulated	Suppressed Growth
CCND1	58	480,200	0.00751	-3.21	Downregulated	Suppressed Cell Cycle
CDK6	32	195,400	0.00315	-2.8	Downregulated	Cell Cycle progression
BIRC5	25	165,300	0.00284	-2.45	Downregulated	Suppressed Survival
WNT3A	20	110,400	0.00195	-2.15	Downregulated	Wnt Ligand inhibition
BCL2	41	390,500	0.00482	-1.8	Downregulated	Suppressed Anti-apoptosis
CTNNB1	34	210,150	0.00395	-1.65	Downregulated	Wnt Signaling (Beta-Catenin)

confirming the effectiveness of the Wnt targeting strategy in these pathways.

Conversely, a significant increase in genes associated with the NF-kappa B/AP-1 pathway was observed, most notably, the gene SLC3A2 showed the highest significant increase in Log2FC values, reaching approximately +3.59. Additionally, the *NFKB1*, *JUN*, and *RELA* genes showed significant upregulation and gene expression, with Log2FC values exceeding approximately +2.90, indicating activation of a compensatory rescue pathway. This clear contrast could provide direct evidence of the cell's shift towards dependence on the NF-kappa B/AP-1 axis for survival, thus increasing the risk and persistence of cancer.

Pathway Enrichment Analysis

Analysis of pathway enrichment confirmed that the genetic changes were not randomly. The suppressed pathways showed a clear dominance of the KEGG:Wnt signaling pathway (Figure 3), while the KEGG:NF-kappa B signaling pathway dominated the activated pathways with high statistical power. This statistical evidence confirms that NF-kappa B is the main compensatory pathway.

The figure illustrates the most statistically significant pathways. The horizontal axis represents the negative logarithm of the adjusted p-value (statistical significance of the enrichment). Inhibited pathways (blue bars): Clearly show the dominance of the KEGG: Wnt signaling pathway and cell cycle regulation pathways, confirming the successful targeting of *Wnt/LEF1* functions. Compensatory activated pathways (orange bars): Clearly show that the strongest and most enriched pathway is the KEGG:NF-kappa B signaling pathway, followed by cellular response to stress pathways. This strong statistical enrichment demonstrates that NF-kappa B has become the primary pathway upon which the cell depends for survival after Wnt inhibition.

Demonstrating Cross-Interaction via the PPI Network

To visualize the molecular relationship between inhibitory and activating pathways, a protein-protein interaction network (PPI Network) was constructed using the 20 most differentially expressed genes (Figure 4). The network exhibited a high density of overlap edges between nodes. The key genes (*RELA*, *WIN3A*, *MYC*, *CCND1*, *LEF1*, *BCL2*, *JUN*, and *NFKB1*) exhibit the highest levels of connectivity, confirming their central role in the newly established network. The orange color means

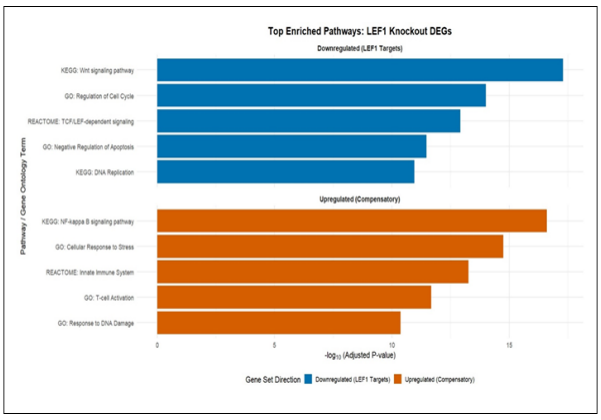

Figure 3. Pathway Enrichment Analysis of Differentially Expressed Genes (DEGs).

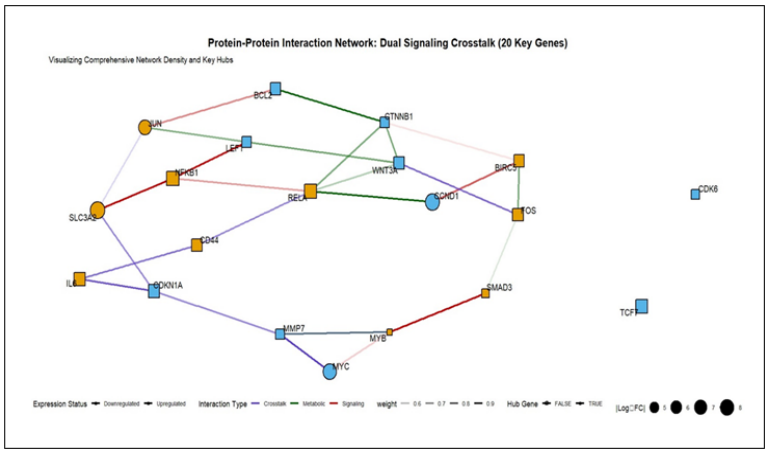

Figure 4. Protein- protein Interaction Network Showing the Dual Crosstalk between 20 Genes Key. The network indicates that the pivotal genes *LEF1*, *JUN* and *RELA* is up-regulated, while the *MYC* gene is down-regulated. The interactions (lines) reveal strong signaling overlap linking cell growth pathways (*WNT*), inflammation (NF-κB), and cell death/survival axes (*JUN*/*BCL2*). Orang as Upregulated , blue as Downregulated

Upregulated, while the blue color means Downregulated. For example, the pivotal genes *LEF1*, *JUN*, and *RELA* are Up-regulated, while *LEF1* and *MYC* genes acted as a significantly downregulated central node.

To identify the most influential proteins within the PPI network interactions associated with *CLL*, we conducted a topological analysis of complex and interwoven networks Using specialized software such as Cytoscape 3.8 and StringApp, the top 20 pivotal genes were ranked based on their stress centrality scores (Table 1), the genes *UBQLN2*, *AP2A2*, and *CDKN1A* have emerged, It is worth noting that the *CDKN1A* gene, which showed the most significant decrease in our genomic data (Log2FC: -7.81) it also showed high stress centrality and intercentrality this underscores its strategic role in the network architecture and it's optional as a major organizer in CLL

We can analysis the PPI network according the number of connections with others genes for example *RELA* gene has five connections that means this gene is expressed at high levels in the state being analyzed in this network and is associated with five other genes, (with *CTNNB1*, *WNT3A*, *CD44*, *NFKB1* and *CCND1*), the most important of which is the link between the three key genes via green lines with *CTNNB1*, *WNT3A* and *CCND1* as A interactions, and the *CD44* factor. *RELA* appears in orange, indicating that it is upregulated. Also *WNT3A* appears in blue, indicating that it is downregulated, it interacts directly with two key genes via green lines with *LEF1*, *CTNNB1* and *RELA* as A interactions *LEF1/WNT3A* pathway and the inflammatory pathway (NF-κB, of which *RELA* is a part) and the *CD44* factor.

JUN, has three connections (with *BCL2*, *LEF1*, *SLC3A2*), one of the strongest axes, connects *LEF1* to the cellular survival pathway (*NFKB1*, *BCL2*),

Discussion

Successful Targeting of LEF1 and Confirmation of its Molecular Role in CLL

Chronic Lymphocytic Leukemia (CLL) is the most common type of leukemia in adults in the Western world, and is characterized by the progressive accumulation of mature, inactive B lymphocytes in the blood, bone marrow, and lymphoid tissues, genetic studies have revealed that the cause of this disease depends largely on the activation of certain signaling pathways that receive their signals from the environment to the tumor [17]. In this context, the *Wnt*/*LEF1* pathway is increasingly active in CLL cells, the transcription factor *LEF1* is important in this pathway, as it is overexpressed in patients and acts as a prosurvival factor that inhibits apoptosis [18].

Current study aimed to evaluate the genetic changes resulting from the specific inhibition of the axial transcription factor *LEF1* (the master driver of the Wnt/beta-catenin pathway) in chronic lymphocytic leukemia (CLL) cells. Differential expression analysis (DEA) demonstrated clear success in disrupting the cell growth axis. This was manifested in the dramatic decrease in the expression of key growth-axis genes (as shown in Figures 2A and 2B), most notably *CCND1* and *MYC*, as well as cell death regulatory genes such as *BCL2*.

These results are strongly consistent with previous research confirming that *LEF1* is overexpressed in the majority of CLL cases, and that this overexpression is associated with a worse disease prognosis [19]. Furthermore, other studies in different laboratories have supported the finding that molecular intervention targeting *Wnt*/*LEF1* reduces cell viability, confirming that our findings provide a solid foundation for the rest of our analysis concerning the resistance mechanism [20]. The originality of our results lies in providing a full transcriptional signature demonstrating not only the inhibition of known Wnt genes but also the differential expression pattern that immediately follows this inhibition, thus paving the way for the discovery of a fully undiscovered compensatory mechanism [21].

Unveiling the Compensatory Mechanism: Activation of the NF-kappa B/AP-1 Pathway

The most significant finding of this study is the clear compensatory activation of the NF-kappa B signaling pathway as an immediate response to *LEF1* inhibition. Pathway enrichment analysis (Figure 3) confirmed that NF-kappa B was the most strongly and statistically enriched pathway among the activated genes (orange bars). This may explain how a cancer cell attempts to escape programmed cell death and maintain "cell survival" for a longer period. This interpretation is likely supported by the simultaneous activation of pathways such as the cellular stress response, suggesting that the cell entered a state of acute genomic stress, which in turn activated its rescue pathway[7].

The pathway enrichment at the individual gene level was further supported by LogFC and Heatmap plots (Figure 2A and 2B). Key transcription factors in this pathway, such as *JUN* and *NFKB1*, showed significantly increased expression. The explicit activation of *JUN*/*FOS* (AP-1 components) and *NFKB1*/*RELA* (NF-kappa B components) demonstrates that the cell does not rely on a single pathway, but rather on the NF-kappa B/AP-1 biosynthesis as a functional substitute. In current study also, we found the significant increase in the level of SLC3A2 protein (CD98hc) as particular importance in the context of metabolic adaptation to chronic lymphocytic leukemia as a key component of amino acid carrier, This protein also supports the increased nutritional needs of leukemia cells, it plays a crucial role in antioxidant defense by regulating the absorption of cysteine its presence in our results indicates that a CLL cells it may undergo metabolic reprogramming as a survival strategy to counteract the stress caused by inhibition of the *LEF1* protein.

The NF-kappa B pathway is known to be highly active in CLL and is associated with chemotherapy resistance. However, previous studies have focused on its activity as a baseline pathway in the disease [22]. The originality of our work may lie in demonstrating that NF-kappa B/AP-1 activation here is an acquired/induced response, not a baseline activity. That is, *Wnt*/*LEF1* inhibition "provokes" the cell to activate NF-kappa B as a rescue axis, creating an "emergent therapeutic dependency" that has not been clearly identified in previous studies examining both pathways together in the context of targeted therapy, Our study offers a unique perspective by focusing on its adaptive role specifically, the results highlight a dynamic shift where the NF-κB/AP-1 axis is further regulated as a compensatory survival mechanism after targeting the *Wnt*/*LEF1* path to be disabled this distinction is important to determines the transcription factor NF-κB not only as a key factor but also as a major mediator of acquired resistances suggests that ealsthe inhibition may be mor effectives when used as a concomitantly with LIF1 inhibitor. This interpretation shifts the focus from the basic resistance pathway to the cellular adaptation mechanism against treatment [23, 24].

Network Evidence of Cross-Relationship and Recommendation for Dual Therapy

The protein-protein interaction network (PPI) (Figure 4) provided Preliminary physical and molecular findings of a cross-relationship between the suppressed (*Wnt*/*LEF1*) and compensatoryly activated (NF-kappa B/AP-1) axis. The network demonstrated direct regulatory links between nodes, specifically between *JUN* (activated) and *CCND1* (suppressed), as well as between *NFKB1* and *WNT3A*. This network interconnectedness confirms that the pathways do not operate in isolation; rather, inhibition of one pathway directly releases or regulates the activation of another [25, 26]. Previous studies have demonstrated a functional relationship between growth and survival pathways [27]. Citation A indicated that Wnt inhibition can increase the activity of other pathways. Citation B focused on identifying hub genes in CLL in general [28].

The strength of our work lies in the fact that, unlike research that focused solely on functional relationships, we provided direct, visual network evidence demonstrating that cellular plasticity manifests as an emerging therapeutic dependency. The four key genes (*MYC*, *CCND1*, *JUN*, and *NFKB1*) were identified as the highest-connected hubs in the network (Figure 4), confirming that they represent the most vital and central targets of the two axes involved in the acquired resistance mechanism [29]. Dual targeting strategy, Based on the strong genetic, statistical, and network evidence from our study, monotherapies targeting the Wnt pathway will potentially lead to treatment failure and early relapse due to the rapid activation of the compensatory NF-kappa B pathway [30].

Recommendation: We recommend a simultaneous dual-targeting strategy, including:

LEF1/*Wnt* inhibitor: to disrupt the axis of cell growth and proliferation.

NF-kappa B/AP-1 inhibitor: To block the survival axis and resist cell death.

This dual-study approach, supported by interconnected molecular network analysis, is an optimal pathway to deliver a better and more effective clinical response to overcome the challenge of cell resilience in chronic lymphocytic leukemia and its progression, opening the door to clinical trials investigating this therapeutic combination [31].

In conclusion, this study was concerned with finding direct scientific evidence showing that this compensatory pathway NF-κB/AP-1 is an active alternative pathway after inhibition of the major *Wnt*/*LEF1* axis in patients with chronic lymphocytic leukemia (CLL). The results confirmed three pivotal discoveries: First, the successful radical inhibition of genes driven by the Wnt pathway, most notably the *MYC* and *CCND1* genes Secondly, the detection of clear statistical and genetic activation of the NF-κB/AP-1 cellular survival pathway (represented by the *JUN* and *NFKB1* genes) as a compensatory resistance mechanism; Third, demonstrating the existence of reciprocal activation and direct functional overlap between the two axes, which has been fully proven through protein-protein interaction network analysis. Based on this molecular interrelationship, we can conclude that

relying solely on *Wnt*/*LEF1*-targeted therapy is insufficient to achieve a sustained response due to the immediate emergence of new therapeutic dependencies. Therefore, we recommend a dual, simultaneous therapeutic strategy targeting both the *Wnt*/*LEF1* (growth) and NF-κB/AP-1 (survival) axis as the optimal approach to overcoming resistance mechanisms and achieving a comprehensive therapeutic response in CLL.

Acknowledgments

Statement of Transparency and Principles

• The authors declare no conflict of interest.

• The study was approved by the Research Ethics Committee of the authors' affiliated institution.

• The study data are available upon reasonable request.

• All authors contributed to the implementation of this research.

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