AL-KUFA JOURNAL FOR BIOLOGY
ISSN (Print): 2073-8854 **ISSN (online):** 2311-6544

Evaluation of gene Expression of Interleukin -15 and Interleukin -21of *Human T- cell Leukemia viruses Type-1* of Adult T-cell Leukemia Patients

Zainab majed[1], Fadyia Mahdi Muslim AL Ameedy[2]

[1,2.] *Department of Pathological Analysis, Faculty of Science, Kufa University, Najaf, Iraq.*

Article history

Received: 20/6/2025

Revised: 20/7/2025

Accepted: 27/7/2025

*Corresponding Author: *Zainab Majed , Department of Analysis , Faculty of science*, University of kufa, Kufa, Iraq; Email: Zainabm.aldabagh@uokufa.edu.iq

Abstract: *Human T-lymphotropic viruses (HTLVs)* constitute a family of human Retroviruses classified as oncoviruses due to their ability to induce malignant transformation. To date, four distinct types have been identified: *HTLV-1*, *HTLV-2*, *HTLV-3*, and *HTLV-4* .Among them, *HTLV-1* was the most clinically significant and was the first human retrovirus to be discovered. It was isolated in 1980 from T-cell lines derived from patients with cutaneous T-cell lymphoma and adult T-cell leukemia. Notably, it was also the first Retrovirus conclusively linked to human disease .Human samples from Leukemia patients were collected from 16 December 2024 Until January 2025 .Leukemia was included (18 – 80) years ages patients . The RT-qPCR,Method was used to detect IL-21 and IL-15 of all samples. The population groups studied samples subject groups were distributed into 5 groups including (18-27,28-37,38-47, 48-57 and 58-67) years, changed age to sex. The samples were isolated from Hospital Middle Euphrates Cancer Center in Najaf province,The first study in Iraq to immunity study cytokine in patients with Leukemia by molecular technique RT-qPCR.This technique was performed *Alamin center* for advanced research and biotechnology,in Al-Najaf province. This study employed real-time quantitative PCR (RT-qPCR) to evaluate the gene expression levels of two key immunomodulatory cytokines interleukin-15 (IL-15), a pro-inflammatory marker, and interleukin-21 (IL-21), an anti-inflammatory marker in patients diagnosed with leukemia associated with *Human T-cell Leukemia virus Type-1 (HTLV-1).* The objective was to assess whether *HTLV-1* infection is associated with significant alterations in cytokine expression profiles compared to leukemia patients without *HTLV-1* infection. IL-15 gene expression was significantly upregulated in leukemia patients infected with *HTLV-1* compared to non-infected leukemia controls. IL-21 expression, as depicted in (Figure 3), was significantly downregulated in *HTLV-1*-positive leukemia patients relative to those without *HTLV-1* infection. Interlukin -15 was also significantly elevated in *HTLV-1* positive leukemia patients, and a strong positive correlation was found between IL-15 and miR-146a-5p expression levels, suggesting possible synergistic effects in leukemogenesis. These molecular findings contribute to a better understanding of the pathogenic mechanisms underlying ATL, particularly in the context of *HTLV-1* infection, and may aid in identifying novel therapeutic targets.

Keywords: Leukemia ,*Human T-cell Leukemia virus Type-1* ,RT-qPCR, ATLL, Retrovirus , Interleukin-15, Interleukin -21.

DOI: https://doi.org/10.36320/ajb/v17.i3.20263

1. Introduction

The *Human T-cell Leukemia virus type- 1 (HTLV-1)*, an oncogenic virus belonging to the Deltaretrovirus genus,This virus can infect different types of cells, such as dendritic cells, macrophages, monocytes, and CD8 T cells, still mainly mainly CD4 + lymphocytes act as virus reservoirs. Over 90 % of infected people remain asymptomatic, At the same time the rest may progress to Adult T-cell Leukemia/Lymphoma (ATLL) [1]. This *Human T-cell Leukemia virus Type- 1 (HTLV-1)*,to be mainly transmitted through unprotected sexual intercourse (particularly from men to women), prolonged infant-breastfeeding, and contaminated blood products during transfusion, organs transplantation or intravenous drug administrations [2].

Risks HTLV-*1*-associated inflammatory diseases were strongly associated with high proviral load (PVL), the biomarker most consistently associated with clinically relevant outcomes. *HTLV-1* seropositivity is associated with a 60% increased risk of death in all *HTLV-1*a endemic areas where this has been studied. In Central Australia, adverse outcomes, including ATL and *HTLV-1*-associated inflammatory diseases, are associated with an *HTLV-1* PVL exceeding 1% peripheral blood leukocytes. A PVL exceeding this level was found in 36% of adults with *HTLV-1* in a recent community survey, and a high baseline *HTLV-1* PVL prospectively predicted death in two hospital-based Central Australian studies. Reducing the number of *HTLV-1*-infected cells may reduce the future risk of ATL and inflammatory diseases. First-line therapy response rates in ATL patients are high. however, the majority of acute ATL patients relapse within 6–12 months. Novel therapeutic approaches that target the virus before people living with *HTLV-1* develop disease are therefore urgently required [3].

The ‘cytokine’ originates from the Greek words ‘cyto’ (cell) and ‘kinos’ (movement). Recently, cytokines have been referred to as ‘immunomodulatory agents’ due to their ability to adjust immune responses [4].Cytokines were small regulatory proteins with a wide variety of molecular weights, ranging from approximately 6 to 70 kDa . They are produced by both immune and non-immune cells and play a pivotal role in controlling tissue homeostasis and the activation of the body’s immune system [5].

The Interleukin-21 (IL-21) belongs to the class I cytokine family which also comprises IL-2, IL-4, IL-7, IL-9 and IL-15 and is a pleiotropic cytokine with diverse functions in regulating innate and adaptive immune response IL-21is produced mainly by activated CD4+ T cells and promotes the proliferation, survival and differentiation of immune cells [6].

Interleukin-15 (IL-15) was one of the most promising cytokines for cancer immunotherapy.Not only does IL-15 activate natural killer (NK) cells, NKT cells, γδ T cells, and CD8+ T cells, it also stimulates and maintains memory CD8+ T cell responses, does not cause activation-induced cell death,10 and has low effect on regulatory T cell (Treg) expansion [7].

Adult T-cell leukemia/lymphoma (ATLL) was a highly aggressive T-cell malignancy caused by *Human T-cell leukemia virus Type- 1 (HTLV-1)* infection. The disease was characterized by rapid progression, extensive organ infiltration, and profound immunosuppression, rendering patients highly susceptible to opportunistic infections and severe complications [8]. ATL was first discovered in Japan in 1977. According subtypes to (i.e., acute, lymphoma, and unfavourable chronic) [9].

Adult T-cell leukemia (ATLL) was characterized by a combination of clinical manifestations, elevated serum LDH levels, hypercalcemia, and specific morphologic/immunophenotypic characteristics of malignant cells, along with confirmed *HTLV-1* infection. A variety of clinical manifestations in ATLL patients was due to diverse complications of involved organs by ATLL cells, opportunistic infections, and hypercalcemia.These symptoms often contribute to the extremely high mortality of the disease [10] .

The Aim of this study regarding AL-Najaf province, Iraq, was that there had been no previous studies dealing with the molecular detection of *Human T-cell leukemia virus Type-1* in patients with Leukemia. Thus, this work aims to detect *Human T-cell leukemia virus Type-1* in Leukemia patients of different ages and sex. The main objectives of this study included: Detection of *Human T-cell leukemia virus Type-1* from Leukemia patients and Evaluation of IL-15 pro inflammatory and IL-21 anti-inflammatory by RT-qPCR.

DOI: https://doi.org/10.36320/ajb/v17.i3.20263

2. Methodology

Samples collection

A cross-case study design to conduct a molecular investigation of leukemia patients .A 100 total blood include (52 female and 48 male) , were collected from patients aged (18 to 80 years) at the Middle Euphrates Cancer Center in Al-Najaf province. Sample collection took place between November 2024 and January 2025. For each patient, a 5 ml Total blood sample was drawn into an EDTA tube. Samples were immediately labeled, placed into suitable containers, and stored in an icy box for transport. In the laboratory, the collected samples were stored at −80 ∘C until they could be used for the subsequent molecular analysis via Real-Time quantitative Polymerase Chain Reaction (RT-qPCR).

Design and preparation of the primers

The primers essential for this study were prepared strictly following the manufacturer's recommendations. The lyophilized primer sequences were first dissolved in an appropriate volume of nuclease-free water to create a stock solution with a concentration of 100 pmol/µl. Subsequently, a working solution was prepared by diluting this stock to a final concentration of 10 pmol/µl. The specific primers targeting the immunity genes were rationally designed using sequences retrieved from the NCBI database, while the study also employed established primers for the housekeeping gene GAPDH as an internal control.

Table 1: The primer design of Human T-cell Leukemia virus Type-1

Type	sequences	length	Product size
IL-15	F TTTGGGCTGTTTCAGTGCAG	20bp	150bp
	R ACTTTGCAACTGGGGTGAAC	20bp	
IL-21	F AAGGTCAAGATCGCCACATG	20bp	147bp
	R AGCAGGAAAAAGCTGACCAC	20bp	
GAPDH	F AATTCCATGGCACCGTCAAG	20bp	207bp
	R ATCGCCCCACTTGATTTTGG	20bp	

Real Time -quantitative polymerase chain reaction (RT- qPCR) Technique

This technique, utilizing Real-Time quantitative Polymerase Chain Reaction (RT-qPCR), was employed to amplify and detect both viral genes and the expression levels of key immunity genes (specifically IL-15 and IL-21) in all whole blood samples. The study utilized commercial real-time kits, including the Script RT Master (2x Conc.) Script RT Master ® 2-Step RT-qPCR System kit (Script, Korea) and SyBR Master (Add Bio, Korea). The final reaction mixture for the assay was prepared with a total volume of 20 µl by combining all components as detailed in the accompanying tables (Tables 2, 3, 4, and 5). This molecular analysis was conducted at the Al-Amin Center for Advanced Research and Biotechnology in Al-Najaf province.

Table 2: The Components of RT-qPCR Mixture for ,IL-15 and IL-21

Item	**Volume**
SYBR Master	10µl
Forward Primer	1 µl
Reverse Primer	1 µl
cDNA template	5 µl
Nuclease-Free Water	3µl
Total reaction volume	20µl

DOI: https://doi.org/10.36320/ajb/v17.i3.20263

Table (3) The Thermo cycler conditions for the amplification Two step

Step	Condition	No. of cycle
Pre –Denaturation	25 °C/10 min	1
Denaturation	80 °C/5 min	45
Annealing	58°C/60 min	
Extension		
Detection (Scan)		
Melt cerve	12°C- ∞ °C	

Table (4) The cDNA master mix of RT-qPCR gene expression (IL-15 and IL-21)

Item	Volume
Script RT Master	10 µl
RNA Template	5 µl
DEPC Water	3 µl
oligo dT20 (random hexamer)	2 µl
Total	20 µl

Table (5) The thermo cycler conditions for the amplification of *HTLV-1* , Immunity parameters (IL-15 and IL-21)

Step	Condition
cDNA Synthesis	95□, 10 min
RT inactivation/ hot start activation	95□, 15-30 sec
PCR Cycling (45Cycles)	55-65□, 15-30 sec 72□, 30 - 60sec
Dissociation	60-90□

Gene Expression

This study aims to evaluate the gene expression of microRNAs and cytokines, specifically the pro-inflammatory marker IL-15 and the anti-inflammatory marker IL-21, in one hundred leukemia patients who tested positive for Human T-cell Leukemia Virus type-1 (HTLV-1). After the extraction of RNA and microRNA and subsequent cDNA synthesis following manufacturer protocols , expression levels were quantified using RT-qPCR. The relative expression ($2^{-\Delta\Delta Ct}$) was calculated by comparing the results to one hundred matched infected control samples, using the housekeeping gene GAPDH for normalization across both patient and control groups.

Equation Delta Ct equations to analyse RT-qPCR results

The threshold cycle (Ct) value was calculated as below: ΔCt Value (Control) (ΔCtC) = Average control Ct Value (test gene) - Average experimental Ct value (housekeeping gene) ΔCt value experimental (ΔCtE) = Average experimental Ct value (test gene) - Average experimental Ct value (housekeeping gene)

Delta Ct value (ΔΔCt) = ΔCtE – ΔCtC The fold change (ΔΔCq) = 2- ΔΔCt

Statistic alanalysis

The statistical analysis involved is the un paired T test was used to compare the two groups.The reasults are shown as mean ± SD. The correlation test was done by pearson correlation test.

3. Results and Discussion

This study employed real-time quantitative PCR (RT-qPCR) to evaluate the gene expression levels of two key immunomodulatory cytokines interleukin-15 (IL-15), a pro-inflammatory marker, and interleukin-21 (IL-21), an anti-inflammatory marker in patients diagnosed with leukemia associated with Human T-cell Leukemia virus Type-1 (HTLV-1). The objective was to assess whether HTLV-1 infection is associated with significant alterations in cytokine expression profiles compared to leukemia patients without HTLV-1 infection.

The total RNA is extracted from peripheral blood samples, and cDNA is synthesized using a two-step reverse transcription kit. Quantitative PCR is performed

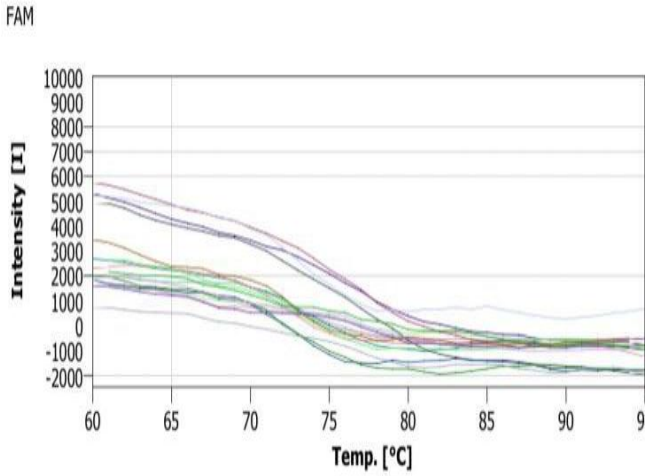
DOI: https://doi.org/10.36320/ajb/v17.i3.20263

with GAPDH as the housekeeping reference gene. Gene expression fold changes were calculated using the 2^(-ΔΔCt) method. comparative analysis between HTLV-1-positive and HTLV-1-negative leukemia cohorts was performed using unpaired T-Tests, with statistical significance set at $P < 0.05$.

As shown in (Figure 1,Figure 2) IL-15 gene expression was significantly upregulated in leukemia patients infected with HTLV-1 compared to non-infected leukemia controls. The mean fold increase in IL-15 expression is statistically significant, indicating a strong pro-inflammatory response in the HTLV-1-positive cohort. These findings underscore the role of IL-15 in HTLV-1-mediated leukemogenesis and immune activation.

The conversely, IL-21 expression, as depicted in (Figure 3), was significantly downregulated in HTLV-1-positive leukemia patients relative to those without HTLV-1 infection. This result suggests that IL-21, typically recognized for its anti-inflammatory and immunomodulatory properties, may be suppressed in the context of HTLV-1 infection. The difference in IL-21 expression was also highly statistically significant ($P < 0.001$), as determined by unpaired T-test.

To date, no previous studies have comprehensively examined the differential expression of IL-21 in HTLV-1–associated leukemia, making these findings (Table 6). The observed downregulation of IL-21 may reflect an immune evasion strategy utilized by HTLV-1, or it may be indicative of a disrupted cytokine milieu associated with chronic viral infection.



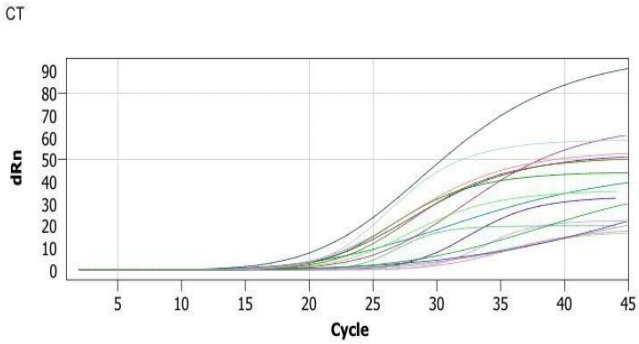


Fig 1: Detection of IL-15, IL-21 gene expression for Leukemia with HTLV-1 and Leukemia without HTLV-1 with RT-qPCR

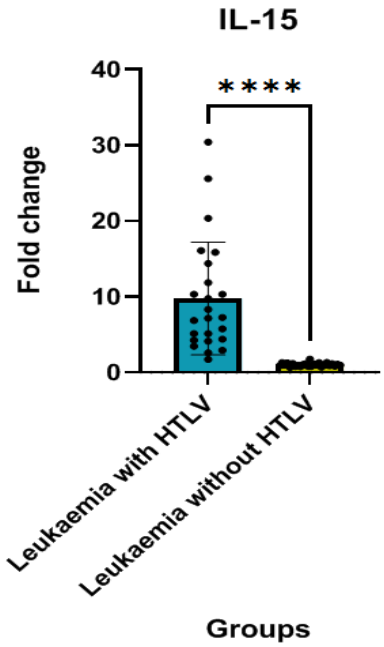


Fig.2. Comparing level of IL-15 of Gene Expression leukemia Positive infected HTLV-1 patients and Leukemia Negative infected HTLV-1 with RT-qPCR

DOI: https://doi.org/10.36320/ajb/v17.i3.20263

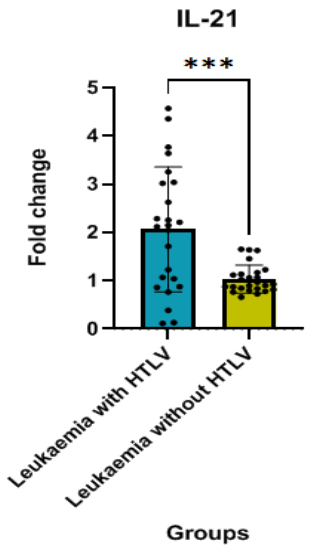


Fig.3. Comparing level of IL-21 of Gene Expression leukemia Positive infected *HTLV-1* patients and Leukemia Negative infected *HTLV-1* with RT-qPCR

Cytokine	**Leukemia (Positive for *HTLV-1*) mean (SD)**	**Leukemia(Negative for *HTLV-1*) mean (SD)**	**P value**
IL-15	9.7(4)	1(0.2)	P <0.0001
IL-21	2(1.3)	1(0.28)	P < 0.001

Table 6: Comparing the level of IL-15 and IL-21 Gene Expression of leukemia with infected *HTLV-1* patients and Leukemia Without infected *HTLV-1* with RT-qPCR

In vivo and ex vivo studies had consistently demonstrated the critical role of the HTLV-1-encoded viral protein Tax in sustaining the survival of HTLV-1-transformed adult T-cell leukemia/lymphoma (ATLL) cells. Tax not only promotes cellular transformation but also drived the expression and secretion of a wide array of cytokines and growth factors that exert anti-apoptotic effects. Among these, interleukin-15 (IL-15) has emerged as a key cytokine, with it was transcription been directly activated by Tax through the NF-κB signaling pathway. In addition, Tax enhances the expression of IL-15 receptor subunits, such as IL-15Rα, thereby amplifying downstream signaling cascades [11] .

The Interleukin-15 agonists and engineered cytokine-receptor complexes have been explored for their potential to augment anti-tumor immune responses. These agents were under investigation both as standalone therapies and in combination with immunotherapies or adoptive cell transfer approaches. However, data from recent clinical trials indicate that IL-15 monotherapy was insufficient to achieved significant tumor regression. Consequently, its utility was now being considered primarily in combination regimens. Notably, IL-15 also appears to had a pathogenic role in the initiation and progression of several hematological malignancies, including both B- cell and T- cell neoplasms. Therapeutic strategies aimed at inhibiting IL-15 signaling particularly targeting the JAK/STAT pathway were being developed to mitigate this effected .The a unique challenge in hematologic cancers was the dual identity of malignant cells as components of the immune system. This overlap may explain IL-15's paradoxical role in both promoting tumor survival and enhancing immune function. Furthermore, the effects of exogenous IL-15 administration remain complex and incompletely understood. While tumor cells and their surrounded microenvironment may produce IL-15 in an autocrine or paracrine manner to support malignancy, exogenous IL-15 may disrupt this feedback loop, potentially led to therapeutic benefit by downregulating endogenous IL-15 expression [12] .

The Given high prevalence of HTLV-1 infection in mature double-positive (DP) T cells, recent investigations have characterized their phenotype in asymptomatic carriers using high-dimensional flow cytometry. Both Tax-positive and Tax-negative DP T cells exhibited a highly pro-inflammatory profile upon stimulation, with elevated expression of IL-21 [13].

The potential of IL-21 as an anti-cancer cytokine was first highlighted by G. Wang et al. in 2003, who demonstrated it was significant anti-tumor activity in murine models. This was further supported by a Phase I clinical trial led by J.A. Thompson in 2008, which evaluated recombinant human IL-21 in patients with advanced malignancies. Mechanistically, IL-21 enhanced the cytotoxic functions of $CD8^+$ T cells and NK cells, suppresses regulatory T cell (Treg) activity, promotes apoptosis in tumor cells, and inhibits metastasis. These immunomodulatory properties had positioned IL-21 as a promising agent in cancer immunotherapy, prompting continued research into its molecular mechanisms of action [14].

DOI: https://doi.org/10.36320/ajb/v17.i3.20263

Conclusion

Interlukin -15 was also significantly elevated in HTLV-1 positive leukemia patients, and a strong positive correlation was found between IL-15 and miR-146a-5p expression levels, suggesting possible synergistic effects in leukemogenesis.

These molecular findings contribute to a better understanding of the pathogenic mechanisms underlying ATL, particularly in the context of HTLV-1 infection, and may aid in identifying novel therapeutic targets.

Ethical Approval

The study protocol has been approved by ethical committee from university of kufa/faculty of science and permission were taken from Al-Najaf health precedence.

References

1. Ali, S., Sangwan, S., Srivastava, P., et al. (2024). From Bronchial Asthma to Tuberculosis: Unravelling the Cytokine-Macrophage Axis in Diverse Diseases. *SunText Review of Pharmaceutical Sciences*, *5*(1), 128.
2. Amiri, A., Mardi, S., Bahavar, A., et al. (2025). Investigating the circadian rhythm signaling pathway in HTLV-1 pathogenesis. *Virus Research*. doi:10.1016/j.virusres.2025.199539
3. An, J., Hu, E., Shi, Y., et al. (2025). Single-cell analysis of oncolytic virotherapy in adult T-cell leukemia/lymphoma. *Journal for Immunotherapy of Cancer*, *13*(3), 011265.
4. Bellon, M., & Nicot, C. (2024). HTLV-1 Tax Tug-of-War: Cellular Senescence and Death or Cellular Transformation. *Pathogens*, *13*(1), 87.
5. Champiat, S., Garralda, E., Galvao, V., et al. (2025). Nanrilkefusp alfa (SOT101), an IL-15 receptor $\beta\gamma$ superagonist, as a single agent or with anti-PD-1 in patients with advanced cancers. *Cell Reports Medicine*, *6*(2), 101967.
6. Cooney, J., Hirons, A., Jansz, P., Doerflinger, M., & Pellegrini, M. (2025). Combination antiretroviral therapy and MCL-1 inhibition mitigate HTLV-1 infection in vivo. *Cell*.
7. Li, X., Wu, M., Lu, J., Yu, J., & Chen, D. (2024). Interleukin-21 as an adjuvant in cancer immunotherapy: Current advances and future directions. *Cancer Letters*, *1879*(2), 189084.
8. Maher, A., Aristodemou, A., Giang, N., Tanaka, Y., Bangham, C., Taylor, G., & Villar, M. (2024). HTLV-1 induces an inflammatory $\text{CD}4^{+}\text{CD}8^{+}$ T cell population in HTLV-1-associated myelopathy. *JCI Insight*, *9*(1), 173738.
9. Nakashima, M., Tanaka, Y., Okamura, H., et al. (2024). Development of

Innate-Immune-Cell-Based Immunotherapy for Adult T-cell Leukemia–Lymphoma. *Cells*, *13*(128).

10. Neurath, N., & Kesting, M. (2024). Cytokines in gingivitis and periodontitis: from pathogenesis to therapeutic targets. *Immunology*, *15*.
11. Oloumbou, F., Lentombo, L., Nkoghe, J., et al. (2025). Clinical and molecular evidences of HTLV-1 infection in inpatients diagnosed with diseases previously described as associated to this infection. *PLOS Neglected Tropical Diseases*. doi:10.1371/journal.pntd.0013075.
12. Shafiee, A., Seighali, N., Ghahfarokhi, N., et al. (2023). Zidovudine and Interferon Alfa based regimens for the treatment of adult T-cell leukemia/lymphoma (ATLL). *Virology*, *20*(118).
13. Sindaco, P., Pandey, H., Isabelle, C., Chakravarti, N., Brammer, J., Porcu, P., & Mishra, A. (2023). The role of interleukin-15 in the development and treatment of hematological malignancies. *Immunology*, *14*, 1141208.
14. Li, X., Wu, M., Lu, J., Yu, J., & Chen, D. (2024). Interleukin-21 as an adjuvant in cancer immunotherapy: Current advances and future directions. *Cancer Letters*, *1879*(2), 189084.