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Original Research Paper

# Dynamics of IL-35 Expression in Myeloid Leukemia: Implications for Immunosuppression and Chemotherapeutic Efficacy

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**Abstract:** Background: Interleukin-35 (IL-35), an immunosuppressive cytokine, is implicated in tumor immune evasion and myeloid leukemia progression. While prior studies associate elevated IL-35 with acute myeloid leukemia (AML) pathogenesis and poor prognosis, its regulatory dynamics under chemotherapeutic interventions remain underexplored. This study evaluates IL-35 expression in AML, chronic myeloid leukemia (CML), and healthy cohorts, alongside its modulation by Decitabine and cytarabine.
Methods: Serum IL-35 levels were quantified via ELISA in 50 AML patients, 50 CML patients, and 30 healthy controls. Patient cohorts (mean age: AML=65, CML=64; controls=46) were stratified by disease activity and treatment status. Chemotherapeutic impact on IL-35 was assessed in AML patients receiving Decitabine (hypomethylating agent) or cytarabine (antimetabolite). Statistical analysis employed one-way ANOVA.
Results: IL-35 levels were non-significantly reduced in leukemia groups (AML: 98.84±81.03 pg/mL; CML: 73.82±61.09 pg/mL) versus controls (136.14±106.8 pg/mL). Neither Decitabine nor cytarabine significantly altered IL-35 expression in treated AML patients. Intergroup comparisons (AML vs. CML) also showed non-significant differences. High interpatient variability (wide SDs) and paradoxical lower IL-35 in leukemia cohorts contrasted with literature linking IL-35 to immunosuppression.
Conclusion: IL-35 may not serve as a standalone prognostic biomarker in myeloid leukemias due to its context-dependent expression and resistance to chemotherapy-induced modulation. Its role in immune evasion likely involves microenvironmental or receptor-level mechanisms rather than systemic concentration. Future studies should integrate longitudinal IL-35 profiling with multi-omics to refine therapeutic strategies targeting immunosuppression.

**Keywords:** IL-35, AML, CML, Decitabine, Cytarabine.

## 1. Introduction

IL-35, an immunosuppressive cytokine in the IL-12 family, consists of IL-12 p35 α-chain and Epstein-Barr virus gene 3 β-chain, suppressing T cell proliferation and inducing IL-35-induced regulatory T cells (iTR-35) to limit inflammation. It promotes tumorigenesis in hematopoietic malignancies by inhibiting programmed cell death and facilitating cancer progression [1,2]. IL-35 suppresses CD4+ and CD8+ T cells, impairing antitumor immunity [3,4]. It also expands immunosuppressive IL-35-induced B cells (i35-Breg) and iTR-35 cells, reinforcing the tumor microenvironment [5,6]. IL-35 downregulates pro-inflammatory cytokines (IL-6, IL-17, TNF-α), further dampening immune activation [7]. Additionally, it enhances myeloid-derived suppressor cells, promoting immunosuppression and leukemia progression [8]. In chronic myeloid leukemia (CML), IL-35 may aid immune evasion by suppressing anti-tumor responses against leukemic stem cells [9]. Acute

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myeloid leukemia (AML) patients exhibit elevated IL-35 levels, correlating with reduced CD4+/CD8+ T cells and increased regulatory T cells (Tregs) [10]. High IL-35 is linked to disease progression, immune evasion, treatment resistance, and poor prognosis [11,12]. Decitabine, one of the most used chemotherapies in the treatment of AML which is a hypomethylating agent and cytidine analog, functions as a DNA methyltransferase (DNMT) inhibitor by promoting DNA hypomethylation, thereby initiating differentiation and apoptosis in acute myeloid leukemia (AML) blast cells. This mechanism demonstrates enhanced clinical efficacy in older adult populations, as evidenced by improved remission rates, prolonged survival, and superior therapeutic outcomes [13-15]. Nevertheless, resistance to Decitabine often emerges due to genetic mutations, phenotypic plasticity in leukemic cells, or adaptive alterations in pyrimidine metabolism that circumvent DNMT1 degradation, ultimately facilitating disease progression [16,17]. Cytarabine, an antimetabolite chemotherapeutic agent, mediates its cytotoxic effects through high-dose regimens that elevate intracellular AMP/ATP ratios, activate the AMPK-FoxO signaling axis, and deplete dCTP nucleotide reserves, collectively disrupting DNA replication fidelity and inducing cell cycle arrest [18,19]. Clinical evidence demonstrates that cumulative cytarabine dosages exceeding 30g during consolidation phases significantly enhance relapse-free and overall survival in intermediate-risk AML cohorts without exacerbating hematologic toxicity [20]. Despite achieving remission rates of 60% in adults and 80% in pediatric populations, therapeutic resistance frequently arises from hypoxia-inducible factor 1α (HIF-1α) and heme oxygenase-1 (HO-1) overexpression, which shield leukemic stem cells (LSCs) from reactive oxygen species (ROS)-mediated apoptosis [21,22]. Additionally, metabolic reprogramming, characterized by unique Ara-C-associated glycolytic signatures in resistant cellular models, further underpins cytarabine insensitivity [23].

## 2. Methodology

*Samples collection*

Blood samples were collected from May 1st, 2024, to March 2nd, 2025. Seventy samples were analyzed, comprising 50 patients with acute myeloid leukemia (AML), 50 patients with chronic myeloid leukemia (CML), and 30 healthy controls. Disease activity was considered in patient categorization. Diagnosis of AML and CML was established by a hematologist at the Cancer Oncology Department of Al-Forat Al-Awsat Hospital (Iraq) following established leukemia classification criteria. For each participant, one samples that serum samples. Serum samples was frozen immediately until cytokine evaluation using ELISA technology.

IL-35 protein levels were quantified using ELISA. The detection of serum levels of (IL-35). The serum levels were measured using (ELISA), according to the manufacturer's instructions for the ELISA kit (Solar-bio, China). A double-antibody sandwich ELISA method was used to assess the concentration of the target proteins in the samples. The serum was added to wells that were pre-coated with monoclonal antibodies specific to the target proteins, and the mixture was incubated. After washing was performed to remove unbound enzyme, Chromogen Solution (antibody conjugate and streptavidin-horseradish peroxidase (HRP)) were added. This caused the liquid to change color from blue to yellow due to the acidic reaction. The intensity of the (yellow color) was directly correlated with the concentration of the target protein in the samples changed from (blue to yellow) under acidic conditions. The color intensity was directly proportional to the concentration of the target protein in the samples. The concentration of IL-35 was measured dependently on the stander curve as demonstrated below in the figure 1.

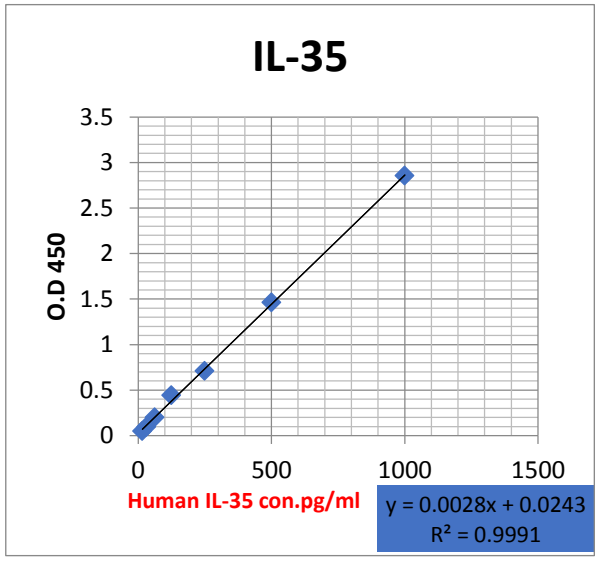


Fig 1: The stander curve of the IL-35 that have been used in the calculation of the IL-35 concentration.

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## 3. Results and discussion

### *Age distribution*

The age distribution of the study cohorts is summarized in table 1. The AML (Acute Myeloid Leukemia) and CML (Chronic Myeloid Leukemia) groups each comprised 50 participants, while the control group included 30 individuals. Age distributions differed notably across the groups: the AML cohort had a mean age of 65 years (Standard Deviation [SD] = 13.59), and the CML cohort exhibited a comparable mean age of 64 years (SD = 11.65). In contrast, the control group was significantly younger, with a mean age of 46 years (SD = 11.54).

Table 1: The age distribution of the patient used in the experiment.

| Age | Patient (n=x) | Mean ± S.D |
|---|---|---|
| AML | 50 | 65 ± 13.59 |
| CML | 50 | 64 ± 11.65 |
| control | 30 | 46 ± 11.54 |

### *Gender classification*

The age distribution of the study cohorts is summarized in table 2. Gender distribution varied across the cohorts. In the AML group, males constituted 60.00% (n = 30) of participants, with females representing 40.00% (n = 20). The CML cohort demonstrated a near-balanced gender distribution, with males accounting for 52.00% (n = 26) and females 48.00% (n = 24). Among controls, males comprised 63.33% (n = 19) of the sample, while females represented 36.67% (n = 11). These findings highlight age and gender disparities between the disease groups and the control population, with AML and CML cohorts reflecting older age profiles and differing gender ratios compared to controls.

Table 2: The gender classification of the patient and their percentage.

| Disease | Gender | Patient (n =x) | percentage (%) |
|---|---|---|---|
| AML | Male | 30 | 60.00% |
| | Female | 20 | 40.00% |
| CML | Male | 26 | 52.00% |
| | Female | 24 | 48.00% |
| Control | Male | 19 | 63.33% |
| | Female | 11 | 36.67% |

The regulation of IL-35 protein expression in acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) cases following treatment with various chemotherapeutic agents. The results demonstrated that IL-35 protein expression was non-significantly in AML patients, where the treated groups with Decitabine, cytarabine untreated group was non-significant. In contrast, IL-35 expression in untreated CML cases was non-significant. Furthermore, the difference in IL-35 expression between the AML and CML groups was non-significant. As demonstrated in figure 2. Furthermore, the lowest mean of the IL-35 was observed in the CML patient (73.82) with a S.D of (61.09), followed by the AML patient with a mean of (98.84) with a S.D of (81.03) when compared to the control group that had the highest mean of (136.14) with a S.D of (106.8) as shown in the table 3.

Table 3: The mean and the S.D comparison between the AML, CML and the control.

| Disease | Mean ± S.D |
|---|---|
| AML | 98.46 ± 81.03 |
| CML | 73.82 ± 61.09 |
| Control | 136.14 ± 106.8 |

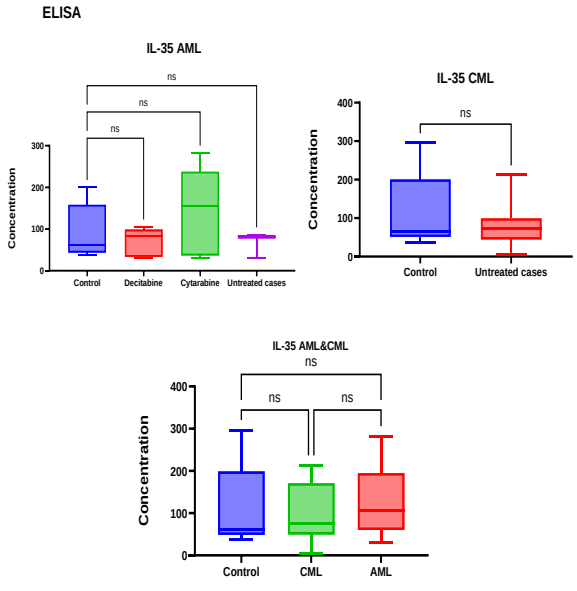


Fig 2: IL-35 Protein Expression in Acute and Chronic Myeloid Leukemia: Association with Chemotherapeutic Protocols and Clinical Outcomes. This figure illustrates the changes in IL-35 protein concentration measured by ELISA in patients diagnosed with AML and CML

compared to healthy individuals. IL-35 levels were quantified using a standard curve derived from known concentrations of recombinant protein, and optical density values were normalized to background measurements. The results demonstrate significant alterations in IL-35 protein levels between healthy and disease groups. Statistical significance was evaluated using one-way ANOVA, where * $p<0.1$, ** $p<0.01$, and **** $p<0.0001$ denote significant differences, and "ns" indicates non-significant results.

### *Chemotherapy-Modulated IL-35 Expression in Myeloid Leukemia: Linking Protein Dynamics to Clinical Outcomes and Therapy Efficacy via ELIS*

The present study evaluated IL-35 expression in AML and CML patients, revealing non-significant differences between treated/untreated cohorts and healthy controls, despite numerical trends suggesting lower IL-35 levels in leukemia groups. These findings contrast with prior literature reporting elevated IL-35 in AML patients [5] and its association with immune suppression, disease progression, and poor prognosis [6,7]. The discrepancy may stem from methodological or cohort-specific variables, including disease stage, genetic heterogeneity, or sample size limitations. For instance, the non-significant reduction in IL-35 levels in AML (mean: 98.84 ± 81.03) and CML (73.82 ± 61.09) compared to controls (136.14 ± 106.8) could reflect dynamic fluctuations in IL-35 during leukemia evolution. While IL-35 is implicated in suppressing anti-tumor immunity [19,20] and expanding immunosuppressive subsets [1,2], its role as a biomarker may depend on temporal or spatial factors not captured in this cross-sectional analysis.

Notably, neither Decitabine nor cytarabine significantly modulated IL-35 expression in treated AML patients. This observation aligns with reports of epigenetic and metabolic resistance mechanisms [11,12,16,18], which may insulate cytokine networks from chemotherapeutic effects. Decitabine, a DNMT inhibitor, primarily targets DNA hypermethylation to reactivate tumor suppressor genes [8–10], but its failure to alter IL-35 levels suggests that IL-35 regulation may operate independently of promoter methylation in this context. Similarly, cytarabine's cytotoxicity hinges on nucleotide depletion and AMPK-FoxO activation [13,14], pathways not directly linked to IL-35 production. The persistence of IL-35 in treated AML patients may reflect residual immunosuppressive niches maintained by leukemic stem cells (LSCs) or stromal interactions, which are less responsive to conventional therapies.

The paradoxically higher IL-35 levels in controls compared to leukemia groups warrants further exploration. While IL-35 is classically viewed as immunosuppressive, its physiological role in immune homeostasis remains poorly defined. Healthy individuals may require baseline IL-35 to temper excessive inflammation, whereas leukemic cells could exploit this pathway to evade immune surveillance. Alternatively, technical factors such as assay specificity (e.g., cross-reactivity with other IL-12 family members) or pre-analytical variables (e.g., sample collection timing) may confound these results. The broad standard deviations across groups further underscore significant interpatient variability, potentially masking subgroup-specific IL-35 dynamics.

In CML, the non-significant IL-35 reduction aligns with its proposed role in immune evasion [4] but challenges the hypothesis that IL-35 overexpression is a hallmark of myeloid malignancies. This may imply that IL-35's immunosuppressive effects are mediated through receptor sensitivity rather than circulating levels, or that localized production within the bone marrow microenvironment is more critical than systemic concentrations. Similarly, the lack of intergroup significance between AML and CML cohorts suggests overlapping immunosuppressive mechanisms across myeloid leukemias, though disease-specific pathways (e.g., BCR-ABL1 in CML) may modulate IL-35 activity at a tissue level.

## Conclusion

In summary, this study challenges the prevailing narrative of IL-35 as a uniformly elevated immunosuppressive driver in AML and CML, instead highlighting the complexity of its role in leukemia pathogenesis. The non-significant reduction in IL-35 levels across leukemia groups, coupled with resistance to Decitabine/cytarabine-mediated modulation, suggests that IL-35 may not serve as a standalone prognostic biomarker or therapeutic target in myeloid malignancies. Instead, its function may be context-dependent, influenced by disease stage, genetic landscape, and microenvironmental crosstalk. Future investigations should prioritize longitudinal IL-35 profiling, paired with functional assays to delineate its cellular sources

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and mechanisms of action, particularly in therapy-resistant LSCs. Integrating IL-35 with multi-omic biomarkers and targeting it in combination with immune checkpoint inhibitors may offer a more nuanced therapeutic strategy to counteract immune evasion in leukemia.

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## Author's Contributions

Mortada Hamed Mahmood conceived and designed the experiments, performed the experimental work, analyzed the data, and wrote the manuscript. Affiliation: Department of Laboratory Investigations, Faculty of Science, University of Kufa.
Second Author: Professor Dr. Hawraa Abd Alameer Ali contributed to the experimental work. Affiliation: Department of Laboratory Investigations, Faculty of Science, University of Kufa.

## Ethics

This study was conducted under approval by the medical ethics committee at the University of Kufa (2017). Verbal and written consent was provided by parents and agreement for publication was obtained from both participants and researchers.

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