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IDO1 and PLXNC1 Genes Expression in Adult Patients with Acute Myeloid Leukemia

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ABSTRACT
Background: IDO1 play a critical part in weakening the body's natural defenses against cancer and promoting tumor resistance., Although PLXNC1 shows aberrant expression in many human malignancies, its role in cancer progression is complex, as it can function as both an enhancer and an inhibitor.
Objectives: To investigate the expression levels of IDO1 and PLXNC1 genes in newly diagnosed adult patients with acute myeloid leukemia (AML) and examined their correlation with various clinical aspects of the disease.
Methods: This case–control study included 44 participants Group I included 32 newly diagnosed adult AML patients and group II included 12 ages and gender- matched apparently healthy controls. The expression of IDO1 and PLXNC1 genes was determined using Quantitative reverse transcriptase PCR (qRT-PCR).
Results: IDO1 gene expression was significantly higher in AML patients compared to healthy controls (P = 0.010) while PLXNC1 expression levels were significantly lower in AML patients compared to healthy controls (P = 0.005). A significant inverse correlation was observed between PLXNC1 expression and initial leukocyte count (P = 0.024) while IDO1 expression showed a significant inverse correlation with hemoglobin (P = 0.007) and WBC count (P = 0.025). other clinic pathological features did not show any significant correlations in our analysis.
Conclusion: IDO1 expression was significantly upregulated while PLXNC1 expression was significantly downregulated in AML patients compared to healthy controls, suggesting IDO1 and PLXNC1 may play a role in AML pathogenesis and could act as potential biomarkers for tracking disease progression and predicting prognosis.
Keywords: AML, IDO1, PLXNC1, Genes Expression, qRT-PCR.

INTRODUCTION

Acute myeloid leukemia (AML) represents an aggressive hematological malignancy characterized by a critical disruption in the normal differentiation of myeloid progenitors, leading to their uncontrolled proliferation within the bone marrow (BM) [1].

Due to the complexity of its molecular and cytogenetic structure, its clinical results are heterogeneous. AML stands out as the most frequently diagnosed blood cancer among adults [2].

Indoleamine 2,3-dioxygenase (IDO) is an immunomodulatory enzyme containing a haem prosthetic group. It functions by catalyzing the oxidative breakdown of the essential amino acid tryptophan's indole ring, resulting in the production of immunoregulatory metabolites known as kynurenines [3]. The PLXNC1 gene encodes a protein belonging to the plexin family, which functions as a receptor for Semaphorin 7A [4]. The Human Protein Atlas indicates elevated expression of PLXNC1 in macrophages, melanocytes, and Kupffer cells. Semaphorins, a diverse family of signaling molecules, play crucial roles in regulating cell motility, migration, and the immune response [5].

Marker genes play a crucial role in refining the prognosis of acute myeloid leukemia (AML). Specific genetic mutations and expression patterns can stratify patients into distinct risk categories, independent of traditional clinical and morphologic factors [6]. The integration of immune gene signature markers has emerged as a significant advancement in refining the prognosis and predicting outcomes in acute myeloid leukemia (AML). Beyond individual gene mutations, the collective expression patterns of immune-related genes provide a comprehensive snapshot of the tumor microenvironment and the host's anti-tumor immune response [7].

Research Gap: While IDO1 and PLXNC1 have each linked individually to immune evasion, no study has examined their concurrent expression in newly diagnosed adult AML or evaluated whether their combined profile refines current risk-scarification models. Addressing this gap could uncover a readily measurable immunogenic signature that predicts outcome beyond standard clinical and cytogenetic factors.

The aim of this work was to investigate the expression levels of IDO1 and PLXNC1 genes in newly diagnosed adult AML patients and to correlate the outcome with different clinical aspects of the disease.

Methods: This study involving case-control comparison was conducted at the Faculty of Medicine, Menoufia University Hospitals' Clinical Pathology Department from August 2023 to March 2024.

The sample size was determined to be at least 38 participants based on calculations from **Ragaini *et al.*** [8] The participants were split into two categories: Group I consisted of 32 adult AML patients, while Group II consisted of 12 healthy donors matched for age and sex, who served as controls.

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All patients underwent a comprehensive full history taking and clinical assessment.

Patients with any other hematological or non-hematological malignancies, patients who already received chemotherapy and pediatric patients are excluded from this study.

Before therapy initiation, 2 ml of venous blood were withdrawn under complete aseptic condition in EDTA tube for performing CBC analysis by (XN1000, Sysmex, Kobe, Japan) and preparation of Leishman stained peripheral blood (PB) smears. BM aspiration was performed for all patients: the first few drops were used for spreading smears to be examined by Leishman and additionally about 4ml was divided into 2 EDTA tubes first tube to be divided as the following: 1st 2ml for routine immunophenotyping (IPT) performed by (Cytoflex, Bekman Coulter Corporation, Hialeah, FL, USA) to confirm the diagnosis, second 2ml for analysis of IDO1 and PLXNC1 Genes expression. Mononuclear cells isolated from BM samples by Ficoll density gradient centrifugation washed twice with phosphate buffered saline (PBS) and preserved in 300 µL PBS in Eppendorf tubes at −20 °C till analysis.

RNA was extracted from mononuclear cells obtained from BM samples using the (RNeasy Mini Kit, Qiagen, Hilden, Germany using the (RNeasy Mini Kit, Qiagen, Hilden, Germany) according to manufacture protocol. Reverse transcription was performed and cDNA was generated using the (Revert Aid First Strand cDNA Synthesis Kit, Thermo Fisher Scientific, Massachusetts, USA).

The PCR amplification mix was done by (Maxima SYBR Green qPCR Master Mix, Thermo Fisher Scientific, Applied Biosystems, CA, USA) (K0251). The specific Primers sequence for IDO1 Forward primer: (5`-AGCAGACTGCTGGTGGAGGACATG-3`) and Reverse primer: (5`-TTCACACAGGCGTCATAAGCTTCC-3`) and The Specific Primers sequence for PLXNC1 Forward primer: (5`-TGATGTTCTCCTTCGGTTCTTG-3`) and reverse primer: (5`-TTCCTCCTCCTGATGTCTTCTC-3) and The Specific Primers sequence for GAPDH Forward primer (5`-ACCCACTCCTCCACCTTTGAC-3`) and Reverse primer (5`- TGTTGCTGTAGCCAAATTCGTT-3`).

Reagents and primers were provided by (Thermo Fisher Scientific, Massachusetts, USA). PCR reaction with 25µl final volume was prepared by adding 12.5µl Master mix, 1µl Forward primer, 1µl Reverse primer, 1 µL cDNA1 and 9.5 µl sterile deionized water into PCR wells. Cycling conditions of RT-qPCR were started at 95°C for 10 minutes, followed by 40 cycles at 95 °C for 15 secs and 60°C for 1 min

IDO1 and PLXNC1 expression levels were calculated relative to that of GAPDH (refrence gene) using the equation 2-ΔΔCT, where CT is the threshold cycle of each sample and ΔΔCT = Δ CT (tested sample) –ΔCT (control sample).

Ethical approval: The Research Ethics Committee of Medical Research, Faculty of Medicine, Menoufia University" issued the ethical approval with IRP number and date of 06/2023 CPATH 7, which was followed in conducting the study. All participants provided informed written consent. The study adhered to the Helsinki Declaration throughout its execution.

Statistical analysis

The results were gathered, organized, and subjected to statistical analysis were performed using SPSS software (version 20.0, IBM Corp, Armonk, NY, USA) on a personal computer compatible with IBM.

The analysis included two types of statistical methods: Descriptive statistics were expressed in: Number (n), percentage (%), average ($\overline{x}$), and standard deviation (SD). Analytical statistics involved Student's t-test, Mann Whitney's test, Kruskal Wallis test, Chi-square test ($\chi 2$), Shapiro-Wilk test, and Spearman coefficient (rs).

RESULTS

Our study included 32 adult patients with acute myeloid leukemia 18 males (56.3%) and 14 females (43.8%) and 12 age and gender matched healthy controls, the age of studied patients ranged from 19-75 years with a median of 53.0 years while median age of controls was 42.5 years and ranged from 20-60 years. The two studied groups did not show a statistically significant difference in terms of age and gender.

The clinic pathological characteristics of AML patients at time of their diagnosis were outlined in (**Table 1)**.

Table (1): The clinicopathological features of AML patients

Variable	No.	%
Performance status		
2	11	34.4
3	11	34.4
4	10	31.3
Splenomegaly		
No	13	40.6
Yes	19	59.4
Hepatomegaly		
No	17	53.1
Yes	15	46.9
Lymphadenopathy		
No	28	87.5
Yes	4	12.5
FAB classification		
M1	3	9.4
M2	14	43.8
M3	4	12.5
M4	1	3.1
M5	10	31.3
CD34		
Negative	7	21.9
Positive	25	78.1
Fate		
Died	27	84.37
Alive	5	15.63
Hb		
Mean ± SD.	7.92 ± 1.56	
WBCs (10^9/L)		
Mean ± SD.	68.42 ± 8.24	
Platelets (10^{11}/unit)		
Mean ± SD.	38.91 ± 3.31	
PB blasts (%)		
Mean ± SD.	46.81 ± 8.80	
ESR		
Mean ± SD.	90.22 ± 7.49	
LDH((U/L)		
Mean ± SD.	726.47 ± 624.52	
Uric acid((mg/dL))		
Mean ± SD.	6.68 ± 1.79	
B.M blasts (%)		
Mean ± SD.	76.5± 19.83	

FAB: French American-British; WBC: White Blood Cell; IQR: Inter quartile range; SD: Standard deviation. IDO1 was significantly higher in AML patients in comparison with control group (P=0.010) **(Figure 1).**

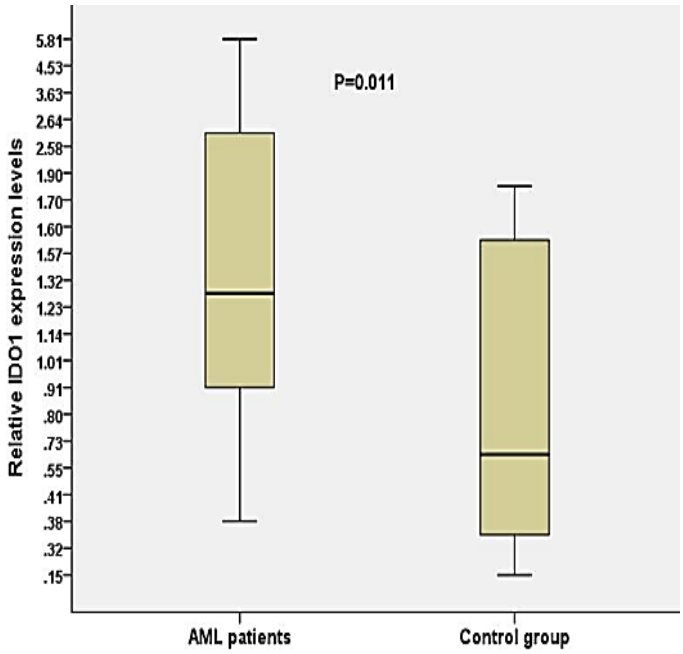

Figure 1: Comparison between AML patients and controls according to IDO1 expression

PLXNC1 expression levels were significantly lower in AML patients as compared to controls (P=0.005). (**Figure 2).**

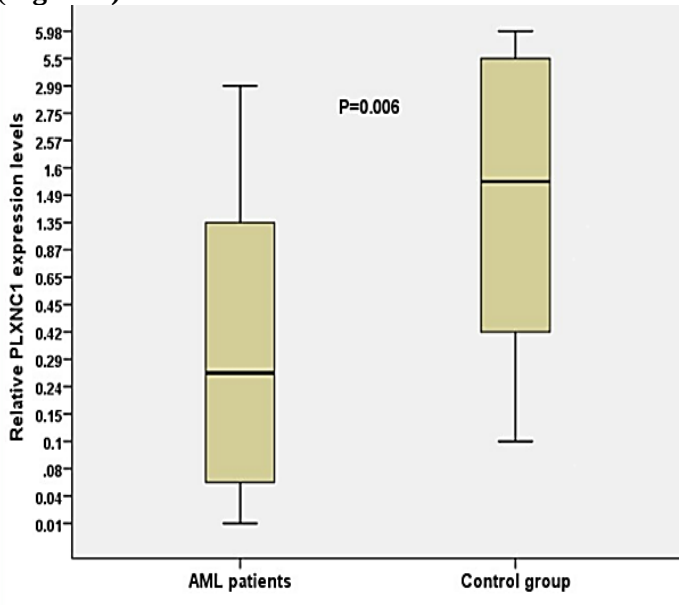

Figure 2: Comparison between AML patients and controls according to PLXNC1 expression.

There was no statistically significant difference between IDO1 gene expression and the clinical variables including age, sex, performance state, hepatomegaly, splenomegaly, lymphadenopathy, response to therapy and fate of patients (P> 0.05). regarding FAB classification there is no statistically significant difference except for M4 patients who show lower IOD1 expression levels with significant p value (P=0.022). No associations were found between PLXNC1 and different clinical features of AML patients (P > 0.05). **(Table 2).**

Table (2): Relation between IDO1, PLXNC1 and clinical features of AML patients

	IDO1	PLXNC1
	Mean ± SD.	Mean ± SD.
Sex		
Male	1.68 ± 1.31	0.56 ± 0.77
Female	2.06 ± 1.63	0.92 ± 1.09
(p)	0.372	0.128
Age (years)		
<60	1.65 ± 1.29	0.57 ± 0.82
≥60	2.16 ± 1.67	0.96 ± 1.08
(p)	0.284	0.205
PS		
2	1.67 ± 1.13	0.55 ± 0.80
3	2.4 ± 1.96	1.14 ± 1.21
4	1.42 ± 0.92	0.43 ± 0.52
(p)	0.762	0.336
Splenomegaly		
No	2.20 ± 1.63	0.78 ± 0.94
Yes	1.60 ± 1.29	0.67 ± 0.94
(p)	0.328	0.565
Hepatomegaly		
No	2.0 ± 1.64	0.80 ± 1.0
Yes	1.66 ± 1.21	0.61 ± 0.86
(p)	0.777	0.395
Lymphadenopathy		
No	1.8 ± 1.31	0.70 ± 0.86
Yes	2.15 ± 2.46	0.82 ± 1.45
(p)	0.864	0.458
FAB classification		
M1	4.52 ± 1.66	1.96 ± 1.57
M2	1.67 ± 1.29	0.79 ± 0.90
M3	2.46 ± 1.21	0.44 ± 0.27
M4	0.55#	0.01
M5	1.17 ± 0.63	0.41 ± 0.69
(p)	0.022*	0.074
CR		
No	1.88 ± 1.57	0.85 ± 1.03
Yes	1.75 ± 1.07	0.32 ± 0.31
(p)	0.777	0.446
Fate		
Died	1.84 ± 1.51	0.76 ± 1.0
Alive	1.87 ± 1.16	0.47 ± 0.30
(p)	0.640	0.856

SD: Standard deviation; PS: Performance status; CR: Complete Remission; U: Mann Whitney test; H: H for Kruskal Wallis test; *: Statistically significant at P ≤ 0.05; #: Excluded from the Relation due to small number of case (n = 1)

No association was found between IDO1 and PLXNC1 expression levels and different laboratory data including hemoglobin, platelets count, P.B &BM blast %, ESR, LDH and CD34 (P> 0.05). Statistically significant association was found between initial leucocyte count and IOD1 and PLXNC1 expression (lower expression levels in patients at presentation) (P=0.023) and (P=0.024) respectively **(Table 3).**

DISCUSSION

The progression of various types of tumors involves the participation of the IDO1 and PLXNC1 genes. Identifying the characteristics of the AML tumor microenvironment and its methods of immune evasion is essential for designing new treatments. (IDO) is particularly relevant, as it has been found in AML blast cells in both bone marrow and peripheral blood. Nevertheless, its exact role in the advancement of the disease is still not fully understood. **[9]**. this research examined the expression of IDO1 and PLXNC1 genes and their potential correlation with clinical features and outcome in newly diagnosed AML patients.

In this case control study, 44 participants were divided into two categories: the first group consisted of 32 adult AML patients, and the second group consisted of 12 healthy donors of similar age and sex were selected as the control group. We observed a significant upregulation of IDO1 expression in AML patients relative to these healthy controls. this aligns with previous study by **Mougiakakos *et al.*** **[10]** which reported elevated IDO1 levels in various cancers, suggesting a common mechanism of immune evasion utilized by tumors. IDO1 upregulation has also been reported by **Wang *et al.*** **[11]** in other diseases such as melanoma and colorectal cancer, suggesting a broader role for IDO1 beyond AML and this open the door to considering IDO1 inhibitors as therapeutic approach holds potential not only for AML but also for other cancers where the regulation of immune checkpoints is disrupted.

Interestingly research by **Memarian & Abdolmaleki** **[12]** has demonstrated a significant association between IDO1 overexpression and reduced survival rates in AML patients, reinforcing its role as an unfavorable prognostic factor. Research by **Folgiero *et al.*** **[13]** demonstrated that observation is consistent with findings in other hematologic malignancies, where elevated IDO1 activity has been linked to worse clinical outcomes. In lymphomas and multiple myeloma, for example, elevated IDO1 expression has been correlated with increased regulatory T-cell (Treg) activity and impaired cytotoxic T-cell responses, leading to unchecked tumor growth. Further supporting the role of IDO1 as a prognostic biomarker, **Ragaini *et al.*** **[8]** identified an IDO1-related immune gene signature that was predictive of overall survival in AML patients. Their findings emphasize the potential of IDO1-based profiling for risk stratification and treatment decision-making.

The negative correlation observed between IDO1 expression and initial leukocyte count in our study and hemoglobin levels aligns with findings by

Mangaonkar *et al.* [14], who demonstrated an association between IDO1 expression and early mortality risk in AML. This suggests that patients with lower leukocyte counts and higher IDO1 expression may experience more aggressive disease progression due to an impaired immune response. Our findings are also consistent with **Iachininoto *et al.* [15],** who suggested that IDO1 expression in AML blasts constrains interferon-gamma (IFN-γ) production, thereby impairing anti-tumor immunity. IFN-γ plays a critical role in orchestrating immune responses against malignant cells by enhancing cytotoxic T-cell activity and antigen presentation. By reducing IFN-γ levels, IDO1 further limits the immune system's ability to recognize and attack AML cells. This finding aligns with **Kierdorf *et al.* [16],** who reported that elevated kynurenine levels, a downstream metabolite of IDO1, were associated with poor prognosis in AML patients.

PLXNC1, is a transmembrane receptor belong to the plexin family, which primarily serves as receptor for semaphorins-proteins involved in axon guidance, immune responses, and cell migration. Beyond neural development, PLXNC1 plays a role in immune regulation and inflammation. It is regulated by the transcription factor IRF5, implicating it in immune response pathway [17]. PLXNC1 identified as a part of an IDO1 –related immune gene signature that predicts overall survival in AML patients, furthermore PLXNC1 expression levels have been found to correlate with immune cell infiltration and the expression of immune check points in AML, suggesting a role in modulating the tumor microenvironment [8]. This study revealed a statistically significant reduction in PLXNC1 expression in AML patients that aligns with study done by **Qin *et al.* [18]** that highlighted PLXNC1's role in immune cell interactions, reinforcing the idea that its loss may weaken immune surveillance against leukemic cells, and further underscores its potential as a biomarker for AML progression and immune dysregulation. **Corradi *et al.* [19]** also reported similar findings, that reduced PLXNC1 expression disrupts key immune microenvironment interactions, further enhancing AML progression Also, study made by **Toledano & Neufeld [20]** demonstrated that PLEXNC1 expression was down regulated in primary malignant melanoma as compared with begin melanocytic nevi and reached its lowest levels in metastatic carcinoma, the downregulation correlated with increased tumor progression and metastasis, suggesting that loss of plexin-C1 expression may contribute to the aggressive behavior of melanoma cells.

Overall, the current findings contribute to the growing body of evidence suggesting that IDO1 upregulation and PLXNC1 downregulation play significant roles in AML pathogenesis.

This study has some limitations that should be acknowledged. First, the relatively small sample size may limit the generalizability of the findings. Second, the study was conducted at a single center, which may not represent the broader AML population. Lastly, although we assessed gene expression levels, functional studies were not performed to explore the mechanistic roles of IDO1 and PLXNC1 in AML pathogenesis. Future studies with larger multicenter cohorts and in vitro/in vivo experiments are warranted to validate and expand upon our findings.

CONCLUSION

In AML patients, our research revealed IDO1 expression was significantly upregulated in AML patients, while PLXNC1 expression was significantly downregulated compared to healthy controls, suggesting IDO1 and PLXNC1 may play a role in AML pathogenesis have the potential to serve as biomarkers for tracking disease progression and predicting prognosis.

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REFERENCES

1. **Short N, Rytting M, Cortes J (2018):** Acute myeloid leukemia. Lancet, 392(10147):593-606.
2. **Pourrajab F, Zare-Khormizi M, Hashemi A *et al.* (2020):** Genetic characterization and risk stratification of acute myeloid leukemia. Cancer Manag. Res., 12:2231-2253.
3. **Fallarino F, Grohmann U, Puccetti P (2012):** Indoleamine 2,3-dioxygenase: from catalyst to signaling function. Eur. J. Immunol., 42(8):1932-1937.
4. **Liu H, Juo Z, Shim A *et al.* (2010):** Structural basis of semaphorin-plexin recognition and viral mimicry from Sema7A and A39R complexes with PlexinC1. Cell, 142(5):749-761.
5. **Zhou Y, Gunput R, Pasterkamp R (2008):** Semaphorin signaling: progress made and promises ahead. Trends Biochem. Sci., 33(4):161-170.
6. **Smith A, Hill J, Fisher M *et al.* (2022):** The evolving role of molecular marker in the diagnosis and risk stratification of acute myeloid leukemia. Ann. Hematol., 101(7):1401-1415.
7. **Chen X, Wang Y, Zhang Z *et al.* (2023):** Landscape of immune gene signature in acute myeloid leukemia: Prognostic implications and therapeutic opportunities. Nat. Commun., 14(1):1234.
8. **Ragaini S, Wagner S, Marconi G *et al.* (2022):** An IDO1-related immune gene signature predict overall survival in acute myeloid leukemia. Blood Adv., 6(1):78-99.
9. **Arandi N, Ramzi M, Safaei F *et al.* (2020):** Overexpression of indoleamine 2,3-dioxygenase correlates with regulatory T cell phenotype in acute myeloid leukemia patients with normal karyotype. Blood Res., 55(4):294-298.
10. **Mougiakakos D, Kock A, Jager G (2010):** IDO1 a key player in cancer immunotherapy. Cancer Immunol. Immunother., 59(8):1155-1163.
11. **Wang Y, Zhang Z, Li H *et al.* (2024):** IDO1 upregulation is associated with increased immune

infiltration in colorectal cancer. Cancer Immunol. Immunother., 73(4):789-802.

12. **Memarian A, Abdolmaleki S (2015):** Preferential expression of CD200 and Indoleamine 2, 3-dioxygenase-1 (IDO-1) in Acute Myeloid Leukemia Patients with Relapse. Jorjani Biomed. J., 3(2):14-25.
13. **Folgiero V, Cifaldi L, Pira G *et al.* (2015):** TIM-3/Gal-9 interaction induces IFNγ-dependent IDO1 expression in acute myeloid leukemia blast cells. J. Hematol. Oncol., 8:36.
14. **Mangaonkar A, Mondal A, Fulzule S *et al.* (2017):** A novel immunohistochemical score to predict early mortality in acute myeloid leukemia patients based on indoleamine 2, 3 dioxygenase expression. Sci. Rep., 7:1292.
15. **Iachininoto M, Nuzzolo E, Bonanno G *et al.* (2013):** Cyclooxygenase-2 (COX-2) inhibition constrains indoleamine 2, 3-dioxygenase 1 (IDO1) activity in acute myeloid leukemia cells. Molecules, 18(9):10132-10145.
16. **Kierdorf F, Zimmer P, Joisten N *et al.* (2022):** L-kynurenine as a prognostic marker for early mortality in patients with acute myeloid leukemia. Leuk. Lymphoma, 63(8):2001-2004.
17. **Zhang Y, Wang H, Li X *et al.* (2023):** A novel transmembrane receptor involved in semaphorin signaling and immune regulation. J. Cell. Immunol., 5(3):245-256.
18. **Qin L, Li B, Wang S *et al.* (2024):** Construction of an Immune-Related Prognostic Signature and lncRNA-miRNA-mRNA ceRNA Network in Acute Myeloid Leukemia. J. Leukoc. Biol., 115(3):1-41.
19. **Corradi G, Bassani B, Simonetti G *et al.* (2022):** Release of IFNγ by acute myeloid leukemia cells remodels bone marrow immune microenvironment by inducing regulatory T cells. Clin. Cancer Res., 28(14):3141-3155.
20. **Toledano S, Neufeld G (2023):** Plexins as regulators of cancer cell proliferation, migration, and invasivity. Cancers, 15(16):4046.