Egyptian Journal of Medical Microbiology Volume 34 / No.3 / July 2025 517-523 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Association between IL-34 Expression and some Clinicopathological Parameter in Colorectal Cancer

[1]Farah T.H. Al Sadawi*, [1]Saad A. Atiyah, [2]Hameed N. Musa
[1]Microbiology Department, Medical College, Thi-Qar University.
[2]Department of Histopathology and Forensic Medicine, Medical College, Thi-Qar University.

Key words:
CRC, Interleukin-34 and IL-34 expression

Corresponding Author:
Farah Thiab Hammed Al Sadawi
Microbiology Department, Medical College, Thi-Qar University
Tel.: 07810718406
farahthiab987@gmail.com

ABSTRACT

Background: *The third most common cause of mortality and morbidity around the globe is colorectal cancer (CRC). Despite its high prevalence, there remains a limited number of reliable biomarkers for the early diagnosis and prognosis of colorectal cancer. Interleukin-34 (IL-34) is a novel cytokine involved in immune regulation and inflammatory processes. Recent studies have demonstrated that IL-34 is significantly overexpressed in colorectal cancer tissues. In this study, we investigated the potential role of IL-34 as a prognostic biomarker for colorectal cancer.* ***Aims of the study:*** *The study was designed to evaluate the IL-34 expression in colorectal cancer tissues and its diagnostic value as a prognostic factor.* ***Methodology:*** *A total of 14 normal colon tissues were gathered as a control group and 74 colorectal cancer tissues were embedded in formalin-fixed paraffin. Immunohistochemistry (IHC) technique was performed to detect IL-34 expression. Following that, IL-34 expression levels were linked to clinicopathological information such as patient sex, age, tumor grade, stage, and lymph node involvement. The statistically analyses were conducted by SPSS version 26, based on the Chi-square test at p. value ≤0.05.* ***Results****: IL-34 expression was detected to be increased significantly in the CRC tissue (٥٨%) in comparison to the control group (0%). Increased IL-34 expression was substantially correlated with advanced tumour stage and lymph node metastases, and grade under (p ≤ 0.05). No significant correlation was found with patient age or sex.* ***Conclusion:*** *the current study indicated that expression in CRC is high and not detected in normal tissue in control group is not. CRC represents a poor prognosis for patients with significant associated with stage, grade and lymph node metastasis with no significant relation of IL 34 with sex and age of patients.*

INTRODUCTION

Colorectal cancer, which ranks third globally in terms of malignancy incidence and second in terms of related mortality for both men and women, is the most common malignant tumour affecting the digestive tract[1]. In males, colorectal cancer (CRC) is the third most prevalent cancer, following lung and prostate cancers; in females, CRC ranks as the second most prevalent cancer after breast cancer[2]. Colorectal cancer is the third most prevalent malignancy in Iraq, with more than 2,210 new cases annually[3]. Approximately 80–85% of colorectal cancer (CRC) cases are classified as sporadic, indicating that they are not attributable to genetic abnormalities. This shows that environmental variables such as nutrition, lifestyle, and microbiome contribute to carcinogenesis[4,5]. Ageing, male gender, excessive intake of fat, alcohol, or red meat, obesity, smoking, and physical inactivity are identified as risk factors for disease development[5]. The likelihood of acquiring colorectal cancer grows with age. The disease predominantly affects those over the age of 50[6], during which over 90% of colorectal cancer cases are identified[7].

CRC diagnosis is predominantly based on histopathological methods, which provide critical information on tissue architecture and cellular abnormalities necessary for prognosis assessment and therapeutic planning[8]. In addition to routine staining procedure, immunohistochemistry (IHC) has become an essential diagnostic tool. It allows for the detection and localization of specific tumor associated antigens, contributing to the classification of tumor subtype and the evaluation of protein expression profile relevant to disease progression and response to treatment[9].

There is increasing evidence that colorectal cancer (CRC) may be prevented from developing, growing, and spreading by the immune system. The balance between tumour invasion and cancer defence depends critically on the immune response at the tumour location. According to recent findings, assessing this immune response may aid in determining the prognosis and, potentially, the course of treatment for both localised and metastatic colorectal cancer[10,11]. Both

immune and non-immune cells in the tumour microenvironment produce different cytokines, which cause fibroblasts to undergo particular signalling and produce a pro-tumorigenic secretum[12].

The human gut produces the new cytokine interleukin-34 (IL-34) naturally, and it is up-regulated in the inflammatory gut of IBD patients and in CRC tissue[13-16]. IL-34 is produced by a range of immune and non-immune cells. In particular, IL-34 is expressed by both CRC cells and non-tumoral cells that infiltrate CRC tissue, and the cytokine promotes the development of CRC cells[15]. In this investigation, we assessed IL-34 in colorectal cancer expression and its possible association with clinicopathological parameters.

METHODOLOGY

Ethics and patient samples

This study is case-control study which include 88 cases of colon and rectum tissue samples divided into two groups: The colorectal cancer group consists of 74 tissue samples and control group consists of ١٤ normal colorectal tissues. The tissue samples and patient data were collected from patients who were attended at AL-Hussein Teaching Hospital and Al-haboubi Hospital (Dhi Qar Specialized Oncology Center) in Thi-qar province during the period from September 2024 to November2024.

The study was approved by the health directorate committee in Thi-Qar province. Under decision number 301/2024 dated 19/12/2024 Permission to conduct this study was also obtained from the committee of publication ethics at the College of Medicine, University of Thi-Qar/ Iraq. Verbal consent was obtained from the patient and his relative at hospitals and clinics to take a biopsy from the collected tissue.

Detection of IL-34 by IHC

Immunohistochemistry technique was used to evaluate the IL-34 expression in CRC and normal tissues. This procedure was performed on 4µm thickness of formalin- fixed paraffin-embedded (FFPE) colorectal cancer tissue (TMA cores) sections in paraffin. Initially, deparaffination was carried out by immersing the slid in xylene followed by rehydration through a graded series of ethanol solution of decreasing concertation. Antigen retrieval was then performed by heating the slides in a retrieval solution, enabling the exposure of antigen sites. To counteract the tissue's natural peroxidase activity, peroxidase was injected after 20 minutes of room temperature cooling and left on for 10 minutes. Subsequently, the slides were washed with phosphate buffered saline for 10 minutes and this step was repeated twice. The sections were then incubated overnight at 4°C with a rabbit polyclonal antibody IL-34 (dilution 1:50). After overnight incubation, the slides were immersed in PBS for 10 minutes and this step was repeated 3 times. Then, the slide was incubated at room temperature for 30 minutes after with anti-mouse labeled polymer-HRP secondary antibody. Following that, the slides were washed in PBS buffer three times, with a 5 minute interval between each wash. Following the Dako technique, the slides were incubated at room temperature for 10 minutes after being stained with drops of DAP chromogen. Then, after 10 minutes, the slides were rinsed in distilled water .The next step was rinsing the slides under running water for two minutes and distilled water for another minute. This process was repeated three times.

The slides were then dehydrated by sequential immersion in 70%, 95%, and 100% ethanol, for one minute each. This was followed by clearing the slides in xylene for two minutes per change. Finally, the slides were mounted using DPX mounting medium, and 22 x 22 mm coverslips were placed over the tissue sections and allowed to dry at room temperature.

Statistical Analysis

The data from this study were statistically analysed using SPSS version 26, employing the Chi-square test at a p-value of ≤0.05.

RESULTS

The study was conducted to evaluate IL34 expression in colorectal tissue. The results showed high significant differences in the expression of IL-34 in CRC tissues when compared with controls, where 43 out of 74 CRC tissue samples revealed appositive results, while all 16 normal value tissues expressed negatively with a p-value ≤0.01, as shown in Table -1.

The present study showed a no significant difference at p. value ≤0.05, between cancer patients according to sex, where a 58.33% of male group have positive IL-34, while 41.67% had negative IL-34. **Also**, in female group the study noted 57.69% give a positive result for IL-34 expression as in table -2.

The current result showed a no significant differences at p. value <0.05, between CRC tissue patient according to age. Although the percentages differed among the groups, the second age group recorded a 32.56% positive IL-34 expression, while the lowest IL-34 positivity rate was detected in the first group, with a p-value ≤ 0.05, as shown in Table 3.

Table 1: Expression of IL-34 in patient with CRC and control group

IL34	Patient		Control		Total	
	No.	%	No.	%	No.	%
Positive	43	58.11	0	0.00	43	47.78
Negative	31	41.89	16	100	47	52.22
Total	74	82.22	16	17.78	90	100
CalX^2= 81.6 TabX^2= 3.84 DF= 1 p. value ≤0.01**						

Table 2: Expression of IL-34 in colorectal cancer patient according to sex

IL34 Sex	Male		Female		Total	
	No.	%	No.	%	No.	%
Positive	28	58.33	15	57.69	43	58.11
Negative	20	41.67	11	42.31	31	41.89
Total	48	64.86	26	35.14	74	100
CalX^2= 0.008 TabX^2= 3.84 DF= 1 p. value 0.930						

Table 3: IL-34 Expression in colorectal cancer patient according to age groups

IL34 Age	Positive		Negative		Total	
	No.	%	No.	%	No.	%
30-40	5	11.63	6	19.35	11	14.87
41-50	14	32.56	9	29.03	23	31.08
51-60	6	13.95	7	22.58	13	17.57
61-70	11	25.58	6	19.35	17	22.97
≥ 71	7	16.28	3	9.68	10	13.51
Total	43	58.11	31	41.89	74	100
CalX^2= 6.49 TabX^2= 9.49 DF= 4 p. value 0.165						

The present results revealed a statistically significant difference ($p \leq 0.05$) in IL-34 expression among colorectal cancer patients according to tumor grade, stage, and lymph node metastasis. IL-34 positive expression was highest in grade II tumors (74.42%) and lowest in grade III (2.33%). IL-34 negativity was most frequent in grade II (83.87%) and least frequent in grade III (16.13%), with no IL-34 negative cases reported in grade I. For tumor stage, the highest IL-34 positivity was observed in stage T3 (72.09%), while the lowest was in stage T4 (9.30%). IL-34 negativity peaked in stage T3 (87.1%) and was lowest in stages T2 and T4 (6.45% each). Patients with lymph node metastasis exhibited a higher IL-34 positivity rate (76.74%) compared to those without lymph node involvement (23.26%). Likewise, IL-34 negativity was more frequent in patients with lymph node metastasis (61.29%) than in those without (38.71%).as in Table-4.

Table4: Expression of IL-34 in cancer patient according to grade, stage and lymph node metastasis of colorectal caner

IL-34 Grades	Positive		Negative		Total		p. value
	No.	%	No.	%	No.	%	<0.05
1st	10	23.26	0	0.00	10	13.51	≤0.01
2nd	32	74.42	26	83.87	58	78.38	
3rd	1	2.33	5	16.13	6	8.11	
Total	43	58.11	31	41.89	74	100	
IL-34 Stages							
T2	8	18.60	2	6.45	10	13.51	≤0.012
T3	31	72.09	27	87.10	58	78.38	
T4	4	9.30	2	6.45	6	8.11	
Total	43	58.11	31	41.89	74	100	
IL-34 Lymph nod							≤0.014
Yes	33	76.74	19	61.29	52	70.27	
No	10	23.26	12	38.71	22	29.73	
Total	43	58.11	31	44.59	74	100	

IL-34 expression in colorectal cancer

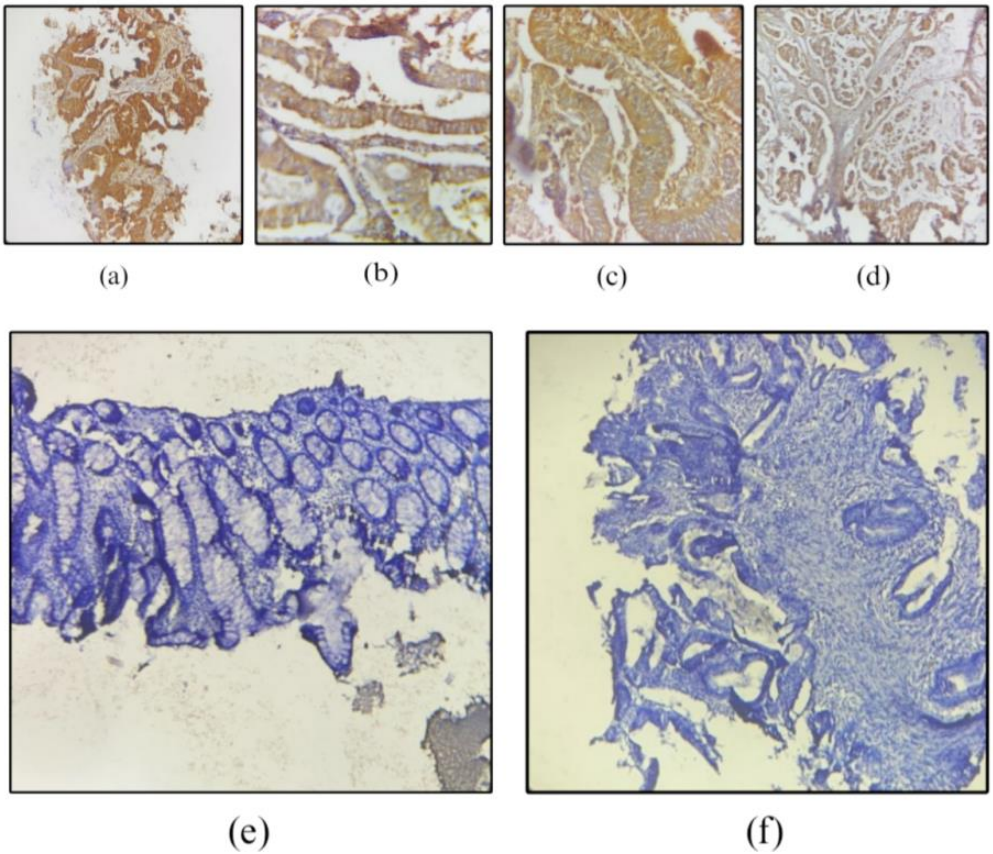

Fig. 1: Immunohistochemical Evaluation of IL34 in Malignant and Benign Colorectal Tissue. **Sections (a–d)** show colonic adenocarcinoma with positive cytoplasmic immunohistochemical staining for IL34, indicating intracellular expression of the protein within malignant epithelial cells. In contrast, **Section (e)** shows colonic adenocarcinoma with negative IL34 staining, while **Section (f)** shows colonic tubular adenoma also lacking IL34 expression.

DISCUSSION

In recent years, IL-34, a recently identified cytokine, has been demonstrated to affect cancer by promoting cell proliferation and invasion. Our primary objective in doing this work was to see if IL-34 might be expressed by colorectal cancer cell lines and clinical samples taken from individuals diagnosed with this disease.

Based on the immunohistochemistry result, IL-34 expression is noticeably high in CRC tissues (58.11%) compared to controls (0%), with a highly significant p-value (≤0.01). The results demonstrate a significant correlation between IL-34 expression and colorectal cancer. Given that IL-34 is absent from normal tissues, it could have a role in tumorigenesis, immune system modulation, or even as a sign of disease. The present result aligns with the previous finding of Eleonora Franzè et.al[17], which confirmed IL-34 expression is increased in tumoral samples of CRC patients.

However, Eleonora study disagree when show that IL34 is also expressed in surrounded normal tissue as well as agree with earlier studies on other cancers such as lung ovarian, and gastric tumors[18-20].

IL-34 expression across different age groups also examined among CRC patients which showed the highest proportion of IL-34-positive cases was noted in the 41–50 age group, followed by the 61–70 group, the lowest proportion was seen in the 30–40 age group. No statistically significant correlation was identified between IL-34 expression and patient age. This may be attributed to several factors, including relatively small sample size in certain age groups (particularly the 30-40 group) may have limited the statistical power to detect significant differences. It is also possible that IL-34 expression is more strongly influenced by other clinical or pathological factors, such as tumor stage, grade, or local immune response, rather than patient age, this result is in line with previous literature, which has also reported no consistent correlation between IL-34 levels and age in cancer patients[21].

Similarly, IL-34 expression showed no significant association with patient sex ($p = 0.930$), with nearly equal proportions of IL-34 positivity among male (58.33%) and female (57.69%) patients. Closely linked, to tumor biology and immune modulation, rather than demographic variables. IL-34 expression is mainly influenced by tumor-intrinsic factors such as the tumor microenvironment and oncogenic signaling pathways, rather than patient sex. Its regulation by tumor-derived signals likely overrides hormonal or demographic influences, reinforcing its potential as a sex-independent biomarker[22,23]. In contrast, it disagreed with other previous findings[24].

Our data showed that IL-34 positivity is highest in Grade 2 tumors_suggesting that IL_34 expression may be associated with moderately differentiated tumors and decrease in poorly differentiated ones. This expression may be linked to moderately differentiated tumors and could reflect specific stages of tumor progression rather than simply indicate aggressiveness. This agreed with previous findings of Takuto Kobayashi [22] which indicated expression of IL-34 increased in Moderate Differentiation.

One possible explanation is that IL-34 contributes to shaping the tumor microenvironment by supporting macrophage survival and promoting immune cell recruitment, which are more prominent in intermediate-grade tumors. In contrast, the highly disorganized structure and immune-suppressive nature of poorly differentiated tumors may result in reduced IL-34 expression. This aligns with findings Franzè [14].

The findings indicated a substantial correlation between IL-34 expression and lymph node metastasis (LNM) in patients with colorectal cancer. IL-34 expression was detected in 76.74% of patients with positive lymph nodes, in contrast to 61.29% in patients without lymph node involvement. This may result from IL-34's function in fostering a tumour-promoting immunological milieu, facilitating angiogenesis, and activating pathways such as ERK and STAT3 that encourage cancer cell invasion and migration. IL-34 additionally activates CSF-1R signalling and exacerbates chronic inflammation, both of which facilitate tumour development and metastasis. The current findings align with the research by Kobayashi[22], which identified a strong association between IL-34 expression and lymph node metastasis, hence reinforcing the function of IL-34 as a prognostic marker in cancer development.

Ultimately, we assessed the expression levels of IL-34 at each stage in individuals with colorectal cancer. IL-34 exhibited the highest expression in stage T3, suggesting a correlation between IL-34 expression and stages of colorectal cancer, perhaps serving as a criterion for progression. The present data corroborates the prior CRC study[22]. These findings correspond with prior studies demonstrating that IL-34 facilitates tumor proliferation and immunological modulation in colorectal cancer. Franzè[17] indicated that IL-34 augments the activity of tumor-associated macrophages, facilitating cancer cell invasion. Monteleone et al.[25] similarly identified IL-34 as a prospective biomarker for unfavorable prognosis and treatment resistance.

CONCLUSION

The present study noted that IL-34 plays a substantial role in the pathogenesis and prognosis of colorectal cancer cells, exhibiting a strong correlation with clinicopathologic tumour parameters. It can serve as an immunological marker for assessing the prognosis of colorectal cancer (CRC).

Acknowledgment
The authors express gratitude to the staff members of AL-Hussein teaching hospital and Al-Haboubi hospital (cancer unit) in Nasiriyah city, for collecting the samples.

Author contribution
Farah and Saad conceptualized the project, conducted the experimental procedures, drafted the initial articles, and conducted the statistical analysis. Farah and Saad managed the data collection. All authors collaborated on writing, reviewing, and editing the material. The authors have reviewed and approved the final manuscript.

REFERENCES

1. Ueno M, Muto T, Oya M, Ota H, Azekura K, Yamaguchi T. Multiple primary cancer: an experience at the Cancer Institute Hospital with special reference to colorectal cancer. Int J Clin Oncol. 2003 Jun;8:162–7.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021 May;71(3):209–49.
3. Al Alwan NA. General oncology care in Iraq. In: Cancer in the Arab world. Singapore: Springer Singapore; 2022. p. 63–82. [https://doi.org/10.1007/978-981-16-7945-2]
4. Lee JE, Wei EK, Fuchs CS, Hunter DJ, Lee IM, Selhub J, et al. Plasma folate, methylenetetrahydrofolate reductase (MTHFR), and colorectal cancer risk in three large nested case–control studies. Cancer Causes Control. 2012 Apr;23:537–45.
5. Watson AJ, Collins PD. Colon cancer: a civilization disorder. Dig Dis. 2011 Jul 5;29(2):222–8.
6. Molanaie N, Rahimi E, Aiobi S. Epidemiology of Colorectal Cancer in Kurdistan province during 1995-1999. Sci J Kurdistan Univ Med Sci. 2000 Sep 10;5(1):22–5.
7. Edwards EJ, Osborne CP, Strömberg CAE, Smith SA, C4 Grasses Consortium, Bond WJ, et al. The origins of C4 grasslands: Integrating evolutionary and ecosystem science. Science. 2010;328(5978):587–91.
8. Hagen CE, Farooq A. Histologic evaluation of malignant polyps and low-stage colorectal carcinoma. Arch Pathol Lab Med. 2019;143:1450–4.
9. Sun H, Ding Q, Sahin AA. Immunohistochemistry in the diagnosis and classification of breast tumors. Arch Pathol Lab Med. 2023;147(10):1119–32. [https://doi.org/10.5858/arpa.2022-0464-RA].
10. Mlecnik B, Tosolini M, Charoentong P, Kirilovsky A, Bindea G, Berger A, et al. Biomolecular network reconstruction identifies T-cell homing factors associated with survival in colorectal cancer. Gastroenterology. 2010;138(4):1429–40.
11. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, et al. International validation of the consensus Immunoscore for the classification of colon cancer: A prognostic and accuracy study. Lancet. 2018;391(10135):2128–39.
12. da Cunha BR, Domingos C, Stefanini ACB, Henrique T, Polachini GM, Castelo-Branco P, et al. Cellular interactions in the tumor microenvironment: The role of secretome. J Cancer. 2019;10(19):4574–87.
13. Franzè E, Monteleone I, Cupi ML, Mancia P, Caprioli F, Marafini I, et al. Interleukin-34 sustains inflammatory pathways in the gut. Clin Sci (Lond). 2015;129(3):271–80. [https://doi.org/10.1042/CS20140762]
14. Franzè E, Dinallo V, Rizzo A, Di Giovangiulio M, Bevivino G, Stolfi C, et al. Interleukin-34 sustains pro-tumorigenic signals in colon cancer tissue. Oncotarget. 2017;9(3):3432–45. [https://doi.org/10.18632/oncotarget.23102]
15. Zwicker S, Martinez GL, Bosma M, Gerling M, Clark R, Majster M, et al. Interleukin 34: A new modulator of human and experimental inflammatory bowel disease. Clin Sci (Lond). 2015;129(3):281–90. [https://doi.org/10.1042/CS20140791]
16. Meszaros M, Pageaux GP, Altwegg R. Management of ulcerative colitis using vedolizumab after liver transplantation for primary sclerosing cholangitis. J Crohns Colitis. 2016 Feb 1;10(2):236.
17. Franzè E, Stolfi C, Troncone E, Scarozza P, Monteleone G. Role of interleukin-34 in cancer. Cancers (Basel). 2020 Jan 20;12(1):252.
18. Baghdadi M, Endo H, Takano A, Ishikawa K, Kameda Y, Wada H, et al. High co-expression of IL-34 and M-CSF correlates with tumor progression and poor survival in lung cancers. Sci Rep. 2018 Jan 11;8(1):418.
19. Endo H, Hama N, Baghdadi M, Ishikawa K, Otsuka R, Wada H, et al. Interleukin-34 expression in ovarian cancer: A possible correlation with disease progression. Int Immunol. 2020 Mar;32(3):175–86. [https://doi.org/10.1093/intimm/dxz060]
20. Li CH, Chen ZM, Chen PF, Meng L, Sui WN, Ying SC, et al. Interleukin-34 promotes the proliferation and epithelial-mesenchymal transition of gastric

cancer cells. World J Gastrointest Oncol. 2022 Oct 15;14(10):1968–78.

21. Franzè E, Monteleone I, Cupi ML, Mancia P, Caprioli F, Marafini I, et al. Interleukin-34 sustains inflammatory pathways in the gut. Clin Sci (Lond). 2015 Aug 1;129(3):271–80.
22. Kobayashi T, Baghdadi M, Han N, Murata T, Hama N, Otsuka R, et al. Prognostic value of IL-34 in colorectal cancer patients. Immunol Med. 2019 Oct 2;42(4):169–75.
23. Franzè E, Dinallo V, Rizzo A, Di Giovangiulio M, Bevivino G, Stolfi C, et al. Interleukin-34 sustains pro-tumorigenic signals in colon cancer tissue. Oncotarget. 2017 Dec 15;9(3):3432–45.
24. Baghdadi M, Umeyama Y, Hama N, Kobayashi T, Han N, Wada H, et al. Interleukin-34, a comprehensive review. J Leukoc Biol. 2018 Nov;104(5):931–51.
25. Monteleone G, Franzè E, Maresca C, Colella M, Pacifico T, Stolfi C. Targeted therapy of interleukin-34 as a promising approach to overcome cancer therapy resistance. Cancers. 2023 Feb 3;15(3):971.