Interleukin-6 Level among Iraqi Patients with Chronic Lymphocytic Leukemia from Babil Province

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الخلاصة:

مرض إبيضاض الدم اللمفاوي المزمن هو أحد أكثر أنواع إبيضاض الدم إنتشارا بين البالغين. يصيب هذا المرض الخلايا اللمفاوية من النوع (ب) مما يؤثر على انتاج ووظيفة العديد من العوامل المناعية في الجسم . يعتبر عامل الانترلوكين واحد من أهم هذه العوامل ومما يزيد في أهميتة هو غموض دوره في التأثير على هذا المرض.

الهدف من الدراسة:

قياس مستوى الانترلوكين-6 لدى المرضى العراقيين من محافظة بابل والمشخصين حديثا بمرض إبيضاض الدم اللمفاوي المزمن والذين لم تتم معالجتهم لحد لحظة إنضمامهم الى الدراسة.

المرضى وطرق البحث:

لقد شملت هذه الدراسة 106 من مرضى إبيضاض الدم اللمفاوي المزمن المشخصين حديثا وقبل تلقيهم للعلاج المتخصص والذين راجعوا مستشفيات محافظة بابل خلال الفترة الممتدة من الأول من تشرين الثاني 2012 ولغاية الثلاثين من تشرين الأول 2015. وشملت أيضا 106 من الأشخاص الطبيعيين كحالات سيطرة متوافقين بالعمر والجنس مع مجموعة المرضى.

تم اجراء فحص صورة الدم ومستوى الانترلوكين-6 بالدم لكلا المجموعتين مضافا لها فحص نخاع العظم في مجموعة المرضى.

النتائج:

كان عمر مجموعة المرضى يتراوح بين 44-82 سنة بمعدل 61,8 ±6,14 سنة. وكان معدل الذكور الى الاناث هو 1,8 :1 وكان 57,5% منهم ليس لديه أية أعراض مرضيم مهمة.

لم يتم إكتشاف الانترلوكين-6 في دم مجموعة السيطرة بنسبة أكبر من مجموعة المرضى (39,6% ، 13,2% على التوالي). بينما كانت مستويات الانترلوكين-6 أعلى في دم مجموعة المرضى (12,6±5,1 بيكوغرام/مللتر) مقابل 6,2 ±2,3 بيكوغرام/مللتر لدى مجموعة السيطرة (قيمة P<0.05).

الاستنتاج:

ان إرتفاع نسبة الانترلوكين-6 لدى مرضى إبيضاض الدم اللمفاوي المزمن يدل على العلاقة المهمة بينهما. تبقى الحاجة ملحة إلى المزيد من الدراسات لاضاءة الجوانب المظلمة من هذه العلاقة المركبة.

Abstract

Introduction: Interleukin-6 (IL-6) is a pro-inflammatory cytokine and an anti-inflammatory myokine. Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in adults. The exact role of IL-6 in CLL is still contraversial and needs alot of research.

Aim of the study: To measure serum level of interleukin-6 among Iraqi patients from Babil province with a newly diagnosed untreated chronic lymphocytic leukemia and to assess it's association with the stage of the disease and peripheral blood indices.

Materials and methods: This is a case-control study included 106 Iraqi patients with a newly diagnosed untreated chronic lymphocytic leukemia (CLL). They were from Babil province and attended the hospital during the period from 1st of November 2012 to 30th of October

2015, while the control group included 106 age and sex matched healthy individuals. CBC, blood film and serum IL-6 (Human IL-6 ELISA kit) were done for both groups, and bone marrow exam was performed for the patients only.

Results: The age of the patients ranged from 44 to 82 years with mean age of 61.8 ±6.14 years. Male:Female ratio was 1.8:1 and 57.5% of them were asymptomatic. Serum IL-6 was undetected in 39.6% (42/106) of the control cases and in 13.2% (14/106) of the patients; all of them were in CLL stages 0 and 1. Mean serum IL-6 in the patient group was 12.6 ±5.1 pg/ml, while it was 6.2 ±2.3 pg/ml in the control group (P-value was 0.001). Higher levels were documented in stages 3 and 4 (P-value 0.0001).

Conclusion and recommendations: Elevated serum IL-6 was found among Iraqi patients with newly diagnosed untreated CLL, and higher levels were documented in advanced stage disease. Further studies are needed to assess exact role of IL-6 in CLL as it may have prognostic and/or therapeutic implications.

Key words: Interleukin-6 (IL-6), Chronic lymphocytic leukemia (CLL).

Introduction:

Interleukin-6 (IL-6) acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine.[1]

Interleukin-6 is secreted by T cells and macrophages to stimulate immune response, e.g. during infection, inflammation and after trauma. In addition, osteoblasts secrete IL-6 to stimulate osteoclast formation. Smooth muscle cells in blood vessels also produce IL-6 as a pro-inflammatory cytokine. The role of IL-6 as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-alpha and IL-1, and activation of IL-1ra and IL-10.[2] Normally, IL-6 isn't detected in the blood or is present in low quantities.[3]

Interleukin-6 has been shown to interact with interleukin-6 receptors.[4,5,6] and glycoprotein 130 receptors.[7] Chronic IL-6 stimulation was associated with tumorigenesis in many malignancies by increasing tumor cell proliferation and metastasis.[8,9]

Chronic lymphocytic leukemia (CLL) is characterized by sustained lymphocytosis in the peripheral blood of monoclonal $CD5^+$ B-lymphocytes. It is the most common type of leukemia in adults. Most patients are over the age of 50, and the majority are men.[10]

Chronic lymphocytic leukemia (CLL) is staged with either the Rai staging system [11,12] or the Binet classification.[13]

The presence of either cluster of differentiation 38 (CD38) or Z-chain–associated protein kinase-70 (ZAP-70) is associated with more immature cells and aggressive disease course and may be a marker of high risk CLL.[14]

Toll-like receptor 7 (TLR7) has been shown to be present and functional on both normal and malignant B cells.[15] TLR7 stimulation in vitro induces rapid CLL-cells proliferation[16] and increases sensitivity to cytotoxic agents (eg, vincristine) and killing by cytotoxic T lymphocytes in vitro.[15]

Inspite of this fact; results from studies of the TLR7 agonist (852A) in CLL patients showed only modest clinical efficacy after systemic delivery.This suggested the presence of impaired TLR7 signaling in vivo.[17]

Li et al described a novel mechanism by which microenvironment-produced IL-6 acts as a tumor suppressor in CLL by inhibiting TLR7 signaling (Figure 1).[18]

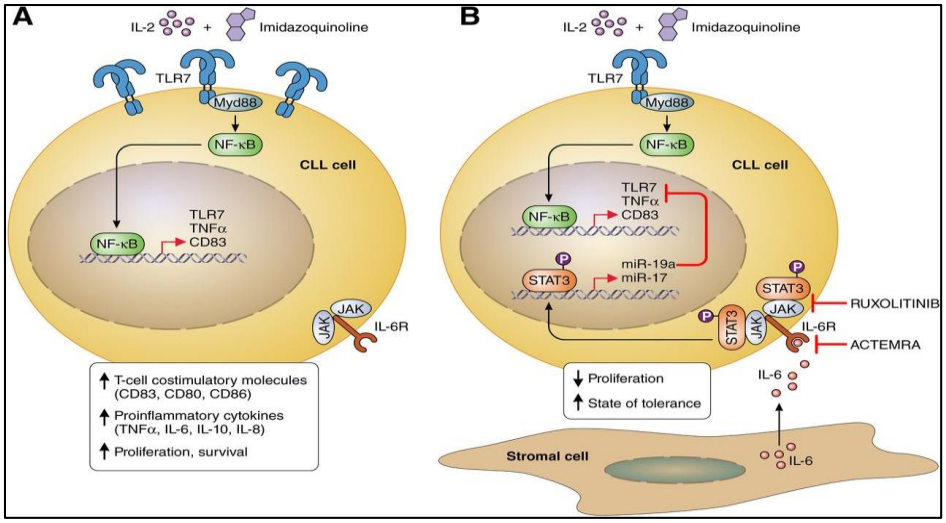

Figure 1: IL-6 inhibits TLR-signaling in CLL cells. (A) Simultaneous treatment of isolated CLL cells with IL-2 and TLR7 agonists (such as the imidazoquinoline resiquimod) increases CD83 and TNF-α, and induces rapid CLL cells proliferation. (B) Contrarily, treatment of stromal-cocultured CLL cells with IL-2 and TLR7 agonists slows down cell proliferation and induces a "state of tolerance" to TLR7 stimuli.

Stromal cells release high levels of IL-6, which binds to IL-6 receptor on CLL cells stimulating signal transducer and activator of transcription 3 (STAT3) which in turn, activates transcription of microRNA (miR)-17 and miR-19a, that bind to and prevent translation of TNF-α and TLR7 messenger RNAs (mRNAs). This phenomenon can be prevented by the use of IL-6 receptor blocking antibodies (Actemra) or JAK inhibitors (ie, ruxolitinib).[19]

Recently, an anti-cancer role for IL-6 has been proposed where IL-6 stimulates proliferation of leukocyte populations and mobilizes antitumor T-lymphocytes.[8] This dual effect of IL-6 was explained by some authors who showed that in the absence of exogenous TLR7 stimulation, IL-6 enhances replication of CLL cells in vivo, whereas in the presence of TLR7 agonists, IL-6 acts as a tumor suppressor and slows down CLL progression.[15,18]

Aim of the study:

The objective of this study is to measure serum level of interleukin-6 among Iraqi patients from Babil province with a newly diagnosed untreated chronic lymphocytic leukemia and to assess the association of serum IL-6 level with the stage of the disease and peripheral blood indices.

Patients and methods:

This is a case-control study included 106 Iraqi patients with a newly diagnosed untreated chronic lymphocytic leukemia (CLL). They were from Babil province 100 km south of Baghdad. They attended hematology center in Merjan Teaching Hospital during the period from 1st of November 2012 to 30th of October 2015. They were either complained from one of variable symptoms, referred cases or attended hospital for routine check up.

Detailed medical history was taken, full physical examination was performed and

relevant investigations (radiological or ultrasound) were sent for the patients in addition to the following laboratory tests; complete blood count (CBC), blood film, bone marrow aspiration with or without biopsy and serum IL-6. Then, CLL was staged according to Rai staging system.

Interleukin-6 (IL-6) was assessed with human IL-6 ELISA kit from Aviscera Bioscience Inc.(CA-USA). The kit pack contains detection antibody concentrate, positive control, avidin-HRP conjugate, dilution buffer, wash buffer, TMB substrate solution, plate sealer and stop solution. Other supplies used during the work included microplate reader, microplate shaker (250-300 rpm), pipettes with their tips, distilled water, 100 ml and 500 ml graduated cylinders and automated microplate washer. Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) was used for ALL samples collected to prevent sample degradation (0.5 TIU per ml of sample solution). The standards were assayed in duplicate. The minimum detectable dose (MDD) of IL-6 in this kit was 1.56 pg/mL.

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for human IL-6 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, Avidin link HRP is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of IL-6 bound in the initial step. The color development is stopped and the intensity of the color is measured.

After getting the results, I average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Then a standard curve was constructed by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph (Figure 2).

The control group included 106 age and sex matched healthy individuals who attended the hospital for routine check up and had found normal or they were relatives with other patients. For all control cases, medical history was taken and physical examination was done to exclude any significant acute or chronic disease, inflammation or infections, and blood samples were taken for CBC, blood film and IL-6.

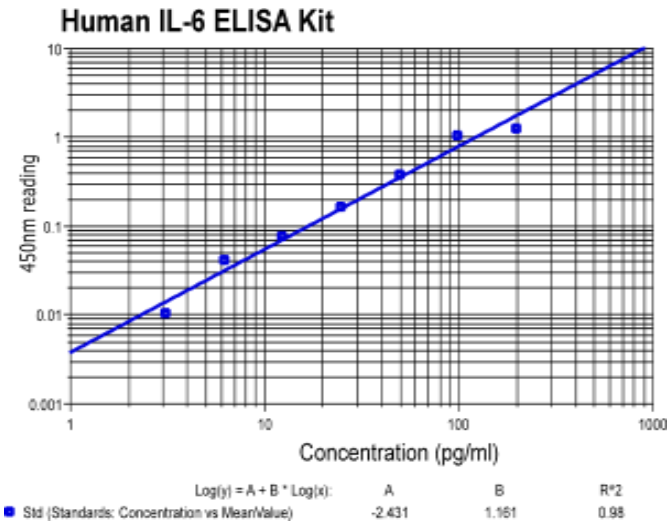

Figure 2: The standard curve that was obtained in IL-6 assessment by ELISA.

The data were analyzed by using computerized SPSS (Statistical Package of Social Science) program version 20.0 (SPSS Inc., Chicago, Illinois, USA); Independent t-test was used to estimate differences between two groups in continuous variables while the analysis of variance (ANOVA) were used to determine the differences between the CLL stages 0, 1, and 2. A p-value < 0.05 was considered to be statistically significant (Daniel, 1999).

Results:

This study included 106 patients with a newly diagnosed untreated CLL (depending on morphological features of blood film and bone marrow) and 106 healthy normal controls.

The age of patients ranged from 44 to 82 years with mean age of 61.8 years ±6.14. Majority of them 92.45% (98/106) were between 50 and 70 years. Most patients 64.15% (68/106) were males while 35.85% (38/106) were females. The patient and control groups were age and sex matched(P-value >0.05). The charecteristics of the patients and controls were summerized in tables 1 and 2.

Table 1: The age and gender of the patient and control groups.

The group	Number	Male % (number)	Mean age ±2SD (year)
Patients	106	64.15 (68)	61.8 ±6.14
Control	106	64.15 (68)	62.7 ±5.62
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Table 2: The charecteristics of the patients accordiong to the stage of CLL.

The stage of CLL	Total number	Male % (number)	Mean age ±2SD (years)
0	25	68 (17)	60.56 ±5.3
1	33	63.6 (21)	61.64 ±6.5
2	33	57.6 (19)	62.42 ±6.3
3	9	66.7 (6)	61.89 ±5.9
4	6	66.7 (4)	64.33 ±5.2

The presenting features in the patient group were summerized in table 3; however, majority of them were asymptomatic discovered accidentally during investigations done for routine check up or for other unrelated complaints.

Table 3: The presenting features of the patients included in the study.

The compliant	Number of patients	%
Asymptomatic	61	57.5
Lymphadenopathy	32	30.2
Splenomegally	28	26.4
Features related to blood elements deficiency (e.g. pallor, bleeding, infections)	21	19.8
Hepatomegally	14	13.2
Non-specific symptoms (Fatigue, cramps, malaise, and night sweating)	14	13.2
Fever	10	9.4
Weight loss (> 10 Kg over last 6 months)	5	4.7
Abdominal distension	5	4.7
Others	4	3.8

Note: Some patients may be presented with more than one feature e.g. fever, lymphadenopathy and hepatosplenomegally.

Within the patient group, no statistically significant effect for the patient's age (r = 0.03 , P value > 0.05) on mean serum IL-6 level and the age had no effect within the control group (r = 0.018 , P-value >0.05) (Figure 3).

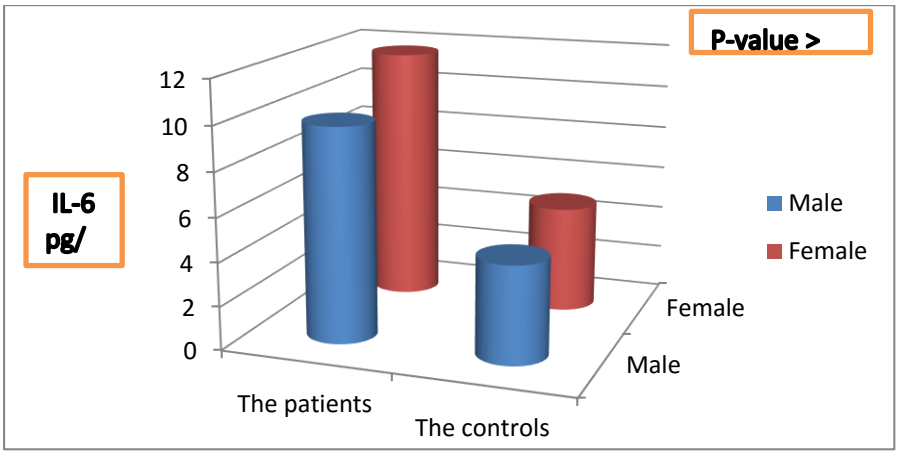

Figure 3: The association between gender and IL-6 concentration in the patient and control groups.

No significant effect for the gender on serum IL-6 within the patient group (P-value > 0.05) or within the control group (P-value > 0.05)

Serum IL-6 was undetected in 39.6% (42/106) of the control cases and in 13.2% (14/106) of the patients; all of them were in stages 0 and 1 (Table 4). Mean serum IL-6 in the patient group was 12.6 ±5.1 pg/ml, while it was 6.2 ±2.3 pg/ml in the control group. This difference was statistically significant (P-value was 0.001).

It had been found that mean serum IL-6 was significantly higher in the patients presented with CLL stages 3 and 4 compared to those with stages 0, 1 and 2 (P-value was 0.004) (Table 4).

Table 4: Mean serum IL-6 concentration in patients according to the stage of CLL.

The stage of CLL	Number of cases with undetected IL-6	Mean IL-6 Pg/ml	P-value
0	7	5.3	0.004
1	7	7.68	
2	0	9.37	
3	0	31.15	
4	0	45.59	

Table 5 shows the association of peripheral blood elements (PCV, WBC count and platelets count) with mean serum IL-6 in the patients group.

Table 5: The association between blood profile and mean serum IL-6 concentration.

		PCV%	WBC count	Platelets count	IL-6
PCV%	Pearson correlation Sig. (2-tailed) N	1 91	.000 .997 91	-.099- .352 91	.027 .799 91
WBC count	Pearson correlation Sig. (2-tailed) N	.000 .997 91	1 91	-.051- .629 91	.102 .336 91
Platelets count	Pearson correlation Sig. (2-tailed) N	-.099- .352 91	-.051- .629 91	1 91	.052 .627 91
IL-6	Pearson correlation Sig. (2-tailed) N	.027 .799 91	.102 .336 91	.052 .627 91	1 91

Correlation was significant at 0.05 level (2-tailed).

The association of peripheral blood elements with mean serum IL-6 was only tested among patients in CLL stages 0, 1, and 2 (91 patients) and no statistically significant correlation was found.The patients in stages 3 and 4 were excluded because they already had either anemia or thrombocytopenia. Correlation coefficient (r) was considered significant at a level of 0.05 (2-tailed).

Discussion:

Chronic lymphocytic leukemia (CLL) is treated by chemotherapy, radiation therapy, biological therapy, or bone marrow transplantation. While generally considered incurable, CLL progresses slowly in most cases. Many people with CLL lead normal and active lives for many years—in some cases for decades. Because of its slow onset, early-stage CLL is, in general, not treated since it is believed that early CLL intervention does not improve survival time or quality of life. Instead, the condition is monitored over time to detect any change in the disease pattern.[20] The decision to start CLL treatment is often difficult and taken when the patient's clinical symptoms or blood counts indicate that the disease has progressed to a point where it may affect the patient's quality of life. Clinical "staging systems" such as the Rai 4-stage system and the Binet classification can help to determine when and how to treat the patient;[21] however, efforts are ongoing to find out new markers that help this decision and serum IL-6 might be a reasonable choice.

In the current study, IL-6 was detected in a significantely higher number 86.8% (92/106) of the patients compared to 39.6% (64/106) of the healthy controls. Similar results was found by Luis et al where IL-6

was detected in 59% (89/151) of the patients with CLL compared to 29% (16/55) of the normal volunteers.[22]

It is well known that the patients with CLL are mostly (60-70%) asymptomatic discovered on routine complete blood count with absolute lymphocytosis.[23] In this study, a higher ratio of patients were presented at a later stages of the disease (stage 2) and this may be because of high rates of poverty, low social class, and ignorance with bad primary health care in Iraq that enforce the patients to visit doctors less frequently and only on need without regular check up.

The effect of age and sex on the results of serum IL-6 was excluded in this study for both groups (patient and control) by statistical analysis.

In the recent study, mean serum IL-6 was significantly higher in patients with CLL compared to normal healthy controls, and it was even higher in patients at advanced stages of the disease (3 and 4) compared to the earlier stages (0 - 2) and this might be related to a higher tumor cell mass in advanced stages of the disease.

These results agreed with that stated by Luis et al who also detected high levels of IL-6 in the patients with CLL and higher levels in Rai stages 3 and 4.[22] Similar findings were documented by earlier reports in 1996 when Reittie et al found increased serum IL-6 level in 54% of the patients with CLL and even higher levels in Rai stages 3 and 4. They suggested an autocrine manner of IL-6 effect as it may inhibit DNA synthesis but prolong survival in CLL cells.[24] These results were consistent with that documented by Parfienczyk et al in Polish.[25]

In this study, the higher levels of serum IL-6 in CLL stages 3 and 4 may indicate that high IL-6 levels are associated with more aggressive or advanced stage of CLL. This fact may help finding out new therapeutic interventions that act on this line (role of IL-6 in pathogenesis of CLL) as treatment of CLL focuses on controlling the disease and its symptoms rather than on an outright cure. On the other hand; higher serum levels of IL-6 in advanced stage CLL make IL-6 a reliable prognostic factor and may help taking decision when to start treatment.

Few studies from 80s and 90s had found a reduction in serum IL-6 level in patients with CLL,[26,27] while others found a reduction in advanced stages (Binet III) of the disease.[28] These results might be related to low sensitivity of tests used for detection of IL-6 at that period.

In lymphoma, Fayad et al had found a correlation between high serum levels of IL-6 and B-symptoms and poor outcome.[29] However; no similar association was found in my study between IL-6 level and symptoms among the patients in stages 0, 1 and 2. This may be due to a different source of production and/or effect of IL-6 in CLL and lymphoma.

Herman et al found that treating CD40 or B-cell receptor (BCR) activated CLL with PCI-32765 resulted in inhibition of Bruton tyrosine kinase phosphorylation and effectively downstream tumor cell survival by blocking survival signals provided externally to CLL cells from microenvironment including soluble factors like IL-6 and TNF-α.[30]

Other studies found high levels of serum soluble IL-6 receptors (sIL-6R) in the

patients with CLL and even higher levels in those presented with stage B according to Binnet's classification.[31,32]

Brown et al thought that the possible cause for the lack of a curative potential of cytostatic chemotherapy in CLL is the very low proliferation rate of tumor cells. They suggested that recombinant human (rh) IL-6 might increase the in vivo proliferation rate leading to a higher sensitivity for chemotherapy or might induce CD20 expression prior to anti-CD20 anyibody treatment.[33] Van Kooten et al found an inhibitory effect for IL-6 in high doses on TNF-α induced cell proliferation and differentiation in CLL.[34] Further studies are indicated to assess the significance of these results and the possible therapeutic implications.

Another new idea was raised by Ennas et al suggesting a causative role for IL-6 gene polymorphism in CLL. They found a 4.5-fold increased risk of CLL in association with genotype homozygous for IL6-174C allele and 11-fold increased risk with genotype homozygous for IL6-174C allele and IL 1B-511C allele.[35] Further studies are needed to confirm these results as they can help early recognition of at risk individuals, interfer with disease progression or even find out new therapies.

In the recent study, no positive or negative correlation was found between serum IL-6 and peripheral blood elements (PCV%, WBC count and platelets count) in the patients with CLL stages 0, 1, and 2. Similar results were found by Robak and Luis.[22,32] This might be because of low burden of tumor cells in early stages of the disease on both bone marrow production and peripheral consumption of blood elements.

Conclusion:

In this study, elevated serum levels of IL-6 were found among Iraqi patients with newly diagnosed untreated CLL compared to normal control individuals, and higher levels were documented in advanced stage disease. IL-6 has an important role in CLL progression through interaction with TRL7, hence a better understanding of IL-6 role and TLR7 signaling in the CLL microenvironment will clarify the mechanisms by which IL-6 promote tumor progression and allow the development of novel therapeutic strategies.

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