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ORIGINAL RESEARCH

Circulating MicroRNAs in Patients with Psoriasis Treated with Anti-IL-23: A Cohort Study

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ABSTRACT

Introduction: Psoriasis is characterized by aberrant keratinocyte activity and immune cell infiltration, driven by immune-mediated pathways. MicroRNAs (miRNAs) play crucial roles in regulating these processes, offering insights into disease mechanisms and therapeutic targets.
Objectives: This study aimed to investigate changes in circulating miRNAs in psoriasis patients undergoing risankizumab therapy, an anti-IL-23 monoclonal antibody, to understand its impact on disease pathogenesis and treatment response.
Methods: Plasma samples from 12 psoriasis patients were collected before (T0) and after 1 year (T1) of risankizumab treatment and analyzed using small RNA sequencing. Findings were validated in a separate cohort of 23 patients using quantitative real-time PCR (qRT-PCR). T-regulatory cell (Treg) numbers and pro-inflammatory cytokine levels were also assessed.
Results: Significant clinical improvement was observed in all patients after 1 year of treatment, accompanied by increased Treg counts and

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reduced levels of pro-inflammatory cytokines. Twenty-four miRNAs exhibited differential expression post-treatment; 9 were downregulated and 15 upregulated. Notably, miR-200a-3p showed a significant correlation with baseline Psoriasis Area Severity Index (PASI), indicating its potential as a severity marker. Risankizumab therapy also decreased peripheral blood levels of IL-23, IL-1β, and IL-8.

Conclusions: This study identifies specific circulating miRNAs, including miR-200a-3p, as potential biomarkers for monitoring treatment responses in psoriasis patients. The findings underscore the therapeutic efficacy of risankizumab in modulating miRNA profiles and immune pathways associated with psoriasis pathogenesis. Overall, these results provide new insights into the mechanisms of risankizumab action and highlight miRNAs as promising candidates for personalized medicine approaches in psoriasis management.

Keywords: Psoriasis; Immunomodulatory therapies; Immunodermatology; miRNAs

Key Summary Points

Why carry out this study?
Psoriasis is characterized by abnormal immune activity, and microRNAs (miRNAs) play a critical role in regulating these processes, providing potential therapeutic insights.
This study aimed to explore changes in circulating miRNAs in psoriasis patients undergoing risankizumab therapy to understand its effects on disease pathogenesis and treatment response.
What did the study ask?
The study hypothesized that specific miRNAs would change following risankizumab treatment, correlating with clinical improvement and immune modulation.
What was learned from the study?
Twenty-four miRNAs showed altered expression post-treatment, with miR-200a-3p significantly correlating with disease severity.
What has been learned from the study?
The study highlights the potential of miRNAs, like miR-200a-3p, as biomarkers for monitoring psoriasis treatment and suggests that personalized medicine approaches could optimize therapeutic outcomes.
Risankizumab therapy modulates both miRNAs and immune markers, including T-regulatory cells and pro-inflammatory cytokines, offering new insights into immunomodulatory therapy mechanism.

INTRODUCTION

Psoriasis, a chronic inflammatory skin disorder, involves immune dysregulation, abnormal keratinocyte proliferation, and immune cell infiltration into skin tissue. Key cytokines in its pathogenesis include TNF-α, IL-17, IL-22, IL-23, and GM-CSF, which trigger inflammatory cascades [1, 2]. In the realm of immune function, T-regulatory cells (Tregs) maintain immune homeostasis by suppressing immune responses, but in psoriasis, an imbalance between T-helper 17 cells and Tregs compromises their regulatory function [3].

The role of miRNAs in disease pathogenesis has been widely discussed in recent years, suggesting that miRNAs modulating the inflammatory response might be involved in the heterogeneity of psoriatic features and clinical responses to treatments [4].

MicroRNAs (miRNAs) are small, noncoding RNA molecules, 19–25 nucleotides long, involved in post-transcriptional gene regulation in nearly all human genes [5–7]. The primary action of mature miRNAs within RISC is to repress gene expression, often by binding to the 3′ UTR of target mRNAs through partial sequence complementarity [8]. This enables

miRNAs to regulate multiple pathways simultaneously, including those involved in immune cell behavior and keratinocyte functions in psoriasis [5].

Recently, 11 immune-related circulating miRNAs were evaluated in a cohort of 100 patients with psoriasis. The results of the study revealed that all miRNAs examined could serve as effective biomarkers for autoimmune skin disease, with miR-145 exhibiting the strongest potential [9].

Additionally, miR-203 was found to be involved in the pathogenesis of psoriasis not only on a local level in skin lesions, but also in the circulation, possibly contributing to the systemic symptoms of the disease [10].

In a recent study, the analysis of plasma miRNAs revealed a significant difference in expression patterns between psoriasis patients and healthy volunteers, with 15 miRNAs upregulated and 15 miRNAs downregulated. Particularly, the most highly upregulated miRNAs, including miR-214-3p, miR-7-5p, miR-761, and miR-665, were found to target crucial pathways in psoriasis pathogenesis [4].

Moreover, the sequencing of circulating miRNAs in plasma samples of 12 patients with psoriasis vulgaris was conducted before and after acitretin treatment. Three miRNAs (miR-146a-5p, miR-122-5p, and miR-21-5p) were identified as having significantly reduced levels following acitretin treatment [11, 12].

Accordingly, miRNAs may serve as an innovative approach to uncovering the unknown aspects of disease development and understanding the biologic mechanisms that impact the effectiveness of advanced therapies in individuals with psoriasis.

Advances in understanding psoriasis pathophysiology have led to biologic therapies targeting specific cytokines like TNF-α, IL-12/23, IL-17, and IL-23, demonstrating efficacy in managing moderate to severe plaque psoriasis [13, 14]. Risankizumab, an anti-IL-23 monoclonal antibody, has shown effectiveness in inhibiting the proinflammatory effects of IL-23 in psoriasis treatment [15].

However, the modulation of circulating miRNAs in response to anti-IL-23 therapy remains unclear. Therefore, our study aims to investigate circulating miRNA profiles using small RNA-seq in psoriatic patients treated with risankizumab. We will also assess changes in Treg populations and pro-inflammatory cytokine levels before and after anti-IL-23 therapy to elucidate the underlying mechanisms of treatment response in psoriasis.

METHODS

First Cohort of Patients

From January 2021 to July 2022, 12 subjects with moderate-severe plaque psoriasis were selected from the Clinic of Dermatology at the Università Politecnica delle Marche (UNIVPM)—Azienda Ospedali Riuniti Hospital in Ancona and included in this cohort study. Baseline characteristics (T0) of the study patients are summarized in Table 1.

Patients were screened for HBV, HCV, HIV, and TB, as recommended by guidelines [16, 17]. Disease severity was assessed using PASI (Psoriasis Area Severity Index), BSA (Body Surface Area), PGA (Physician Global Assessment), and DLQI (Dermatology Life Quality Index). Selection criteria were:

- Patients older than 18 years with moderate to severe plaque psoriasis according to guidelines [17].
- Patients who had not received local or systemic corticosteroid treatment or immunosuppressive therapy 1 month prior to enrollment.
- Patients eligible for biologic therapy with anti-IL-23 agents.

Exclusion criteria included:

- Severe cardiovascular, cerebrovascular, hepatic, renal, and hematopoietic diseases.
- Pregnant or lactating patients.
- Patients noncompliant with other clinical evaluations.

Patients who received biologic therapy with risankizumab, administered as two 75-mg

Table 1 Baseline demographic and clinical characteristics of the patients of the first cohort

Variables	*N* = 12
Age (years)—*m* (DS)	44.9 ± 12.5
Gender (M)—*n* (%)	9 (75)
Weight (kg)—*m* (DS)	83.3 ± 16.6
BMI (kg/m^2)—*m* (DS)	27.3 (5.1)
Smokers—*n* (%)	6 (50)
Comorbidities—*n* (%)	
Dyslipidemia	2 (16.7)
Hypertension	1 (8.3)
Diabetes mellitus type II	1 (8.3)
Atopic dermatitis	1 (8.3)
Anxiety and depressive disorders	1 (8.3)
Thyroiditis	2 (16.7)
Age of onset of psoriasis (years)—*m* (SD)	29.3 ± 15.1 (10–64)
Duration of psoriasis (years) *m* (SD)	15.6 ± 14 (1–46)
Familiarity *n* (%)	3 (25)
Patients with special sites involvement (%)	10 (83.3)
Two or more sites	3 (25)
Facial lesions	2 (16.7)
Palmoplantar lesions	3 (25)
Scalp lesions	7 (58.3)
Genital lesions	2 (25)
Previous biologic treatment *n* (%)	
No	10 (83.3)
Yes	2 (16.7)
1	1 (8.3)
2	0
3 or more	1 (8.3)
PASI *m* (DS)	19.9 ± 14.8 (3–33.2)
BSA *m* (%) (DS)	27.8 ± 0.2 (3–75)
PGA *m* (DS)	3.3 ± 0.5 (3–4)
DLQI *m* (DS)	7.1 ± 3.6 (2–15)

Data are presented as the mean ± standard deviation (SD), with or without the range in parentheses, or as the count (number of patients) with the percentage in parentheses

BMI body mass index, *PASI* psoriasis area severity index, *BSA* body surface area, *PGA* physician global assessment, *DLQI* dermatology life quality index

injections subcutaneously at week 0, after 4 weeks, and every 12 weeks thereafter. Monthly follow-up was conducted for clinical evaluation and assessment of adverse events, with a reassessment at 1 year (T1) after the first administration. Blood samples were collected at T0 and T1 using tubes containing anticoagulants (5 ml serum in citrate and 5 ml in EDTA for plasma and whole blood). This study was performed in accordance with the Helsinki Declaration of 1964 and its later amendments. The ethics committee of UNIVPM approved the study protocol, and all enrolled participants provided written informed consent. The patients in this study gave written informed consent to publication of their case details.

Second Cohort of Patients

A second group of 11 new patients with plaque psoriasis was selected from the Dermatology Clinic of Ospedali Riuniti delle Marche database. This new group of patients has been recruited to investigate the relationship between certain miRNAs, identified through small-RNA sequencing, and the severity of psoriasis. They were selected based on the availability of blood samples and clinical data collected at the time of admission, using the same inclusion and exclusion criteria as the discovery group. The second cohort of patients was not followed up with risankizumab treatment; therefore, only the blood samples at baseline (T0) were used for some of the following analysis. Anthropometric and clinical data for the 11 patients are summarized in Table 2.

RNA Isolation and Mature microRNA Expression by RT-qPCR

Blood samples from the first and second cohorts of patients at T0 were centrifuged within 2 h of collection at 1800 RPM for 10 min to separate plasma, which was then aliquoted and frozen at – 80 °C. Total RNA was isolated from 100 µl of plasma using the Norgen Biotek Kit (#37500, Thorold, ON, Canada) according to the manufacturer's instructions.

MiRNA expression was quantified by RT-qPCR using the TaqMan miRNA assay (catalog #4427012—Thermo Fisher Scientific) according to the manufacturer's protocol. Data were analyzed with Rotor Gene Q (Qiagen, Hilden, Germany). The plasma levels of circulating miRNAs were normalized to the mean of spiked-in miRNA cel-miR-39 using the $2^{-\Delta CT}$ method.

Small RNA-seq Analysis of Circulating miRNAs

RNA Extraction

RNA was purified from 300 µl of plasma from the first cohort of patients (T0 and T1) using the Maxwell RSC miRNA Plasma and Serum Kit (catalog no. AS1680, Promega, Madison, USA) and eluted in 50 µl of RNAse-free water.

Library Preparation and Sequencing

Small RNA sequencing libraries were prepared from 5 µl of plasma RNA using the QIAseq miRNA Library Kit (catalog no. 331505, Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quality and concentration of libraries were determined using the High Sensitivity DNA ScreenTape Analysis on the TapeStation 4150 system (Agilent Technologies, Santa Clara, CA, USA). Libraries were diluted to 1.5 pM and sequenced using the NextSeq 500/550 High Output Kit v2.5 75-cycle flow cell (Illumina, San Diego, CA, USA) on the NextSeq 500 platform (Illumina). Sequencing raw data (FASTQ) were analyzed using the QIAseq miRNA Primary Quantification pipeline via the GeneGlobe Data Analysis Center.

Raw counts were normalized using the DESeq2 bioconductor package. MiRNAs with normalized expression > the 40th percentile in at least one sample were selected as expressed. Data analysis was performed using the DESeq2 1.26.0 Bioconductor package within the R version 4.2.1 environment. Differentially expressed miRNAs were identified using a fold change ≥ 1.5 and an adjusted p value < 0.10. The volcano plot was created using the EnhancedVolcano

Table 2 Baseline demographic and clinical characteristics of the patients of the second cohort

Variables	*N* = 11
Age (years)—*m* (DS)	51.2 ± 10.1
Gender (M)—*n* (%)	9 (81.8)
Weight (kg)—*m* (DS)	82.1 ± 12.4
BMI (kg/m^2)—*m* (DS)	26.2 (6.1)
Smokers—*n* (%)	5 (45.5)
Comorbidities—*n* (%)	
Dyslipidemia	1 (9.1)
Hypertension	2 (18.2)
Diabetes mellitus type II	1 (9.1)
Atopic dermatis	2 (18.2)
Anxiety and depressive disorders	1 (9.1)
Thyroiditis	1 (9.1)
Age of onset of psoriasis (years)—*m* (SD)	31.5 ± 12.3 (9–56)
Duration of psoriasis (years) *m* (SD)	14.2 ± 12 (3–45)
Familiarity *n* (%)	3 (27.3)
Patients with special sites involvement (%)	
Two or more sites	3 (27.3)
Facial lesions	1 (9.1)
Palmoplantar lesions	1 (9.1)
Scalp lesions	4 (36.4)
Genital lesions	1 (9.1)
Previous biologic tratment *n* (%)	
No	10 (83.3)
Yes	2 (16.7)
1	1 (8.3)
2	0
3 or more	1 (8.3)
PASI *m* (DS)	18.2 ± 15.8 (4–36.1)
BSA *m* (%) (DS)	26.1 ± 0.4 (4–62)
PGA *m* (DS)	3.2 ± 0.4 (3–4)
DLQI *m* (DS)	8.1 ± 3.5 (2–16)

Data are presented as the mean ± standard deviation (SD), with or without the range in parentheses, or as the count (number of patients) with the percentage in parentheses

BMI body mass index, *PASI* psoriasis area severity index, *BSA* body surface area, *PGA* physician global assessment, *DLQI* dermatology life quality index

Bioconductor package, and the heatmap was generated using the pheatmap package.

Quantitative RT-PCR of mRNA

Total RNA was isolated from 100 µl of whole blood from the first cohort (T0 and T1) using the Norgen Biotek Kit (#37500, Thorold, ON, Canada) according to the manufacturer's instructions. RNA quantity was determined using the Nanodrop ONE spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Total RNA was reverse-transcribed using the TAKARA Kit (PrimeScript™ RT reagent Kit with gDNA Eraser, catalog no. RR047A) following the manufacturer's instructions. qRT-PCR was performed in a Rotor-Gene Q (Qiagen) using TB Green™ Premix Ex Taq™ (catalog no. RR420A) in a 10-µl reaction volume. mRNA quantification was assessed using the $2^{-\Delta CT}$ method, with β-actin as an endogenous control.

Luminex Multiplex Assays

Multianalyte profiling was performed using the Luminex-100 system (Bioplex, BIORAD). Calibration microspheres and sheath fluid were purchased from BIORAD. Fluorescence data were analyzed with Bioplex manager software v.4.0. The Premixed Human Cytokine 8-Plex Panel (Biotechne) was used to analyze TNF-α, IL-8, IL-6, IL-12, IL-18, IL-1β, IL-17, and INF-α in serum from all patients (first at T0 and T1 and second cohort at T0), following the manufacturers' protocols.

Treg Population Analysis

Lymphocyte subpopulation analysis was performed using a six-color flow cytometer (FacsCanto II, Becton-Dickinson, Franklin Lakes, NJ) and BD FACSDiva software (BD Biosciences).

PBMCs from the first group of patients (T0 and T1) were stained with conjugated monoclonal antibodies (Becton-Dickinson). For extracellular staining, cells were incubated with CD3* APC-Cy7, CD4* FITC, CD25* APC, CD45RA* PE, and CD127* PE-Cy7. For intracellular staining, cells were incubated with FOXP3* PerCP-Cy5.5. After surface labeling, cells were fixed, permeabilized, and then incubated with FOXP3* PerCP-Cy5.5.

Cells were analyzed immediately, assessing viability using 7-AAD (BD Biosciences) and acquiring a minimum of 500,000 cells per tube. To avoid overestimation, CD3-positive cells with very high mean fluorescence intensity were gated out. CD3-positive and CD8-negative lymphocytes (CD4-positive T cells) were gated, and Tregs were identified as CD25-positive, FOXP3-positive, and CD127 low or negative cells.

Statistical Analysis and KEGG Pathway Analysis

Statistical analysis was performed using SPSS/Win (version 25.0, SPSS, Chicago, IL). Continuous variables with normal distribution were reported as mean ± standard deviation (SD). Dichotomous variables were reported as number and percent (%). Comparisons between continuous variables were performed using the *t* test, paired *t*-test, or Mann-Whitney test as appropriate; comparisons between dichotomous variables were performed using the chi-square test, McNemar test, or *Z* test as appropriate. Spearman's rho correlation test was used to estimate correlations between miRNA expression levels and clinical parameters. A p value <0.05 was considered statistically significant. KEGG pathway analysis was performed with the DNA intelligent analysis (DIANA)-mirPath online software suite to identify pathways potentially altered by deregulated miRNAs.

RESULTS

Patient Characteristics

Twelve subjects—9 men and 3 women—with moderate-severe plaque psoriasis who visited the Dermatology Clinic of the Università Politecnica delle Marche (UNIVPM)-Ospedali Riuniti delle Marche between January 2021 and July 2022 and met the inclusion criteria were recruited and included in the first group of patients.

None of the subjects had other variants of psoriasis (erythrodermic, suberitrodermic, pustular psoriasis, etc.) and psoriatic arthritis. At the time of dermatologic examination at baseline (T0), the following was found: the mean duration of disease was 15.6 (range 1–46, standard deviation [SD] 14) years; the mean age at diagnosis of the disease was 29.3 years; 25% of patients reported a positive family history of psoriasis; 10 out of 12 patients showed special site involvement (83.3%), including 3 with involvement of two or more special sites; 2 out of 12 patients (16.7%) had lesions on the face, 3/12 (25%) palmoplantar, 7/12 (58.3%) on the scalp, and 2/12 (25%) on the genital area.

Regarding previous therapies, all patients had unsuccessfully performed treatments with disease-modifying antirheumatic drugs (DMARDs): 4/12 patients (33.3%) with acitretin, 7/12 with cyclosporine (58.3%), 3/12 (25%) with methotrexate, and 1/12 (8.3%) with apremilast. Of the 12 patients in the study, 10 (83.3%) were naïve to biologic therapy, while for the remaining 2 (16.7%), therapy with at least one biologic drug was unsuccessful. Of the latter, one (8.3%) failed therapy with adalimumab and apremilast, and one (8.3) with adalimumab, certolizumab, and etanercept. In patients observed in T0 (pre-risankizumab), the mean PASI score was 19.9 (3–33.2, SD 14.8). Seven out of 12 patients (58.3%) had a PASI score between 10 and 20, corresponding to moderate psoriasis, while 4/12 (33%) had a PASI score > 20, corresponding to severe psoriasis. Mean BSA was 27.8% (3–75, SD 0.2), mean PGA was 3.3 (3–4, SD 0.5), and mean DLQI was 7.1 (2–15, SD 3.6). Baseline

Table 3 PASI, PGA, BSA, and DLQI in patients pre-risankizumab (T0) and after 1-year risankizumab treatment (T1)

	T0	T1	*p* value
PASI *m* (DS)	19.9 ± 14.8 (3–33.2)	0.4 ± 0.8 (0–2)	**0.0007**
BSA *m* (%) (DS)	27.8 ± 0.2 (3–75)	0.4 ± 0.01 (0–2)	**0.0018**
PGA *m* (DS)	3.3 ± 0.5 (3–4)	0.25 ± 0.5 (0–1)	**0.0001**
DLQI *m* (DS)	7.1 ± 3.6 (2–15)	0.25 ± 0.5 (0–1)	**0.00008**

Significant *p*-value are shown in bold

demographic and clinical characteristics of patients are shown in Table 1.

The second group of patients had similar demographic and clinical characteristics compared to the first group and are summarized in Table 2.

The Effectiveness of Risankizumab Treatment in Psoriasis Resolution

Patients were treated with risankizumab 150 mg subcutaneously at day-0, after 4 weeks, and every 12 weeks thereafter.

After 1 year of treatment with risankizumab (T1), all patients achieved PASI 50, or a PASI improvement of 50% following treatment, and PASI 75 (75% improvement); 11/12 patients (91.7%) achieved PASI 90 (90% improvement), and 9/12 patients (75%) achieved PASI 100, reaching complete remission of symptoms.

We observed a significant reduction of PASI, BSA, DLQI, and PGA at T1 compared to T0: mean PASI score at T1 was 0.4 (0–2, SD 0.8; $p<0.0007$), mean BSA was 0.4% (0–2, SD 0.01; $p<0.0018$), mean PGA was 0.25 (0–1, SD 0.5; $p<0.0001$), and mean DLQI was 0.25 (0–1, SD 0.5; $p<0.00008$). The results are shown in Table 3 and Fig. 1. No adverse events were reported during follow-up.

Analysis of Whole-blood Pro-inflammatory Cytokine Expression and Treg Lymphocyte Population

Psoriasis not only affects the skin but is also associated with chronic systemic inflammation, driven by persistently elevated levels of immune mediators and impaired Treg regulatory function.

The percentage of the Treg population (CD4+ CD25+ FOXP3+), selected from the CD4+ CD25+ lymphocyte population, was evaluated by flow cytometry. We found that Treg population increases significantly ($p=0.025$) after 1 year of risankizumab treatment (Fig. 2A).

In addition, we measured gene expression levels of three major pro-inflammatory cytokines, interleukin 1β (IL-1 β), interleukin 23 (IL-23), and interleukin 8 (IL-8) in whole blood of patients with psoriasis at T0 and T1. Following treatment with anti-IL-23 therapy, we observed that IL-1β, IL-23, and IL-8 expression levels were significantly reduced compared to T0 (Fig. 2B–D).

MicroRNA Profiling from Plasma of Psoriatic Patients Treated with Risankizumab

Small RNA-seq was performed on plasma samples from 12 psoriasis patients before (T0) and after 1 year of risankizumab treatment (T1). Figure 3A shows a volcano plot of differentially expressed miRNAs post-treatment, selecting those with a T1/T0 fold change of at least 1.5 and an adjusted *p* value ≤ 0.10. Figure 3B displays a bar plot with 9 upregulated miRNAs (green) and 15 downregulated miRNAs (red). The heatmap in Fig. 3C reveals the expression of each miRNA in individual patients at T0 and T1.

DIANA-miRpath v.3 analyzed the 9 upregulated and 15 downregulated miRNAs, identifying

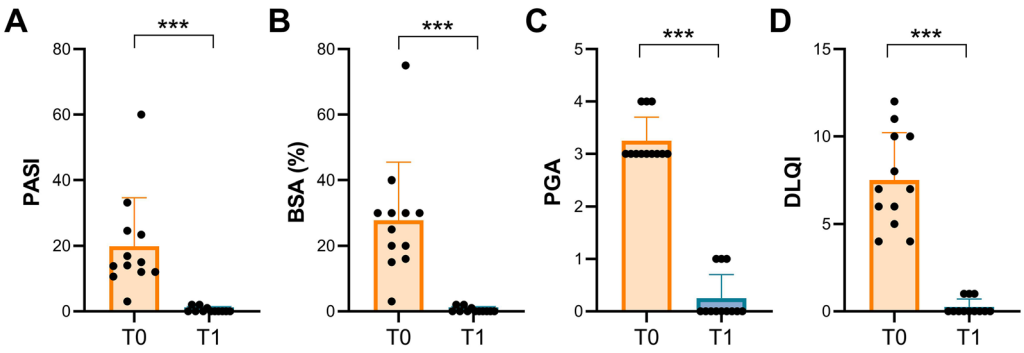

Fig. 1 Changes in PASI (**A**), BSA (**B**), PGA (**C**), and DLQI (**D**) after 1 year of treatment (T1) compared to baseline (T0). ***$p < 0.001$

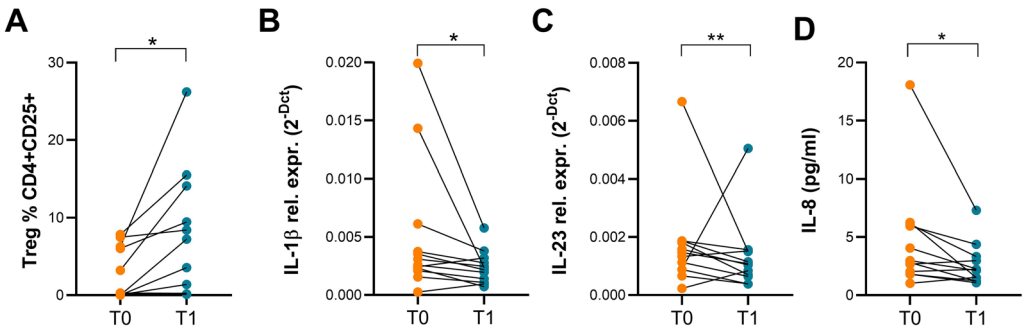

Fig. 2 Percentage of Treg CD4+ CD25+ population (**A**), IL-1β (**B**), and IL-23 (**C**) expression and IL-8 (**D**) concentration level in whole blood of T0 and T1 patients. *$p < 0.05$; **$p < 0.01$

mRNA targets and involved pathways. Supplementary Table 1 lists 14 pathways regulated by upregulated miRNAs, with the most enriched pathway related to extracellular matrix and membrane receptor interactions. Notable pathways include focal adhesions and PI3K-Akt signaling. Supplementary Table 2 shows 136 pathways significantly regulated by downregulated miRNAs, such as adherens junctions, mucin biosynthesis, and TGF-β signaling.

The 24 miRNAs significantly affected by risankizumab were correlated with disease severity indices in the 12 patients at T0. Among downregulated miRNAs, miR-190a-5p correlated positively with PASI (r=0.690; p=0.013) and BSA (r=0.595; p=0.041), while miR-148b-5p correlated positively with BSA (r=0.834; p=0.001). Among upregulated miRNAs, miR-200a-3p showed a negative correlation with PASI (r=0.620; p=0.032) (Fig. 4).

RT-qPCR Analysis and Investigation of Circulating Cytokines by Multiplex Assay in a Second Cohort of Patients with Psoriasis

As a further proof of their involvement in psoriasis pathogenesis, we evaluated the expression of the three miRNAs that displayed significant correlations with clinical characteristics of psoriasis (miR-200a-3p, miR-190a-5p and miR-148b-5p) in a second cohort of 23 patients with psoriasis using RT-qPCR. Our analysis confirmed the negative correlation between miR-200a-3p expression level and PASI index (Fig. 5A).

Then, we measured a panel of 8 pro-inflammatory cytokines (TNF-α, IL-8, and IL-6, IL-12,

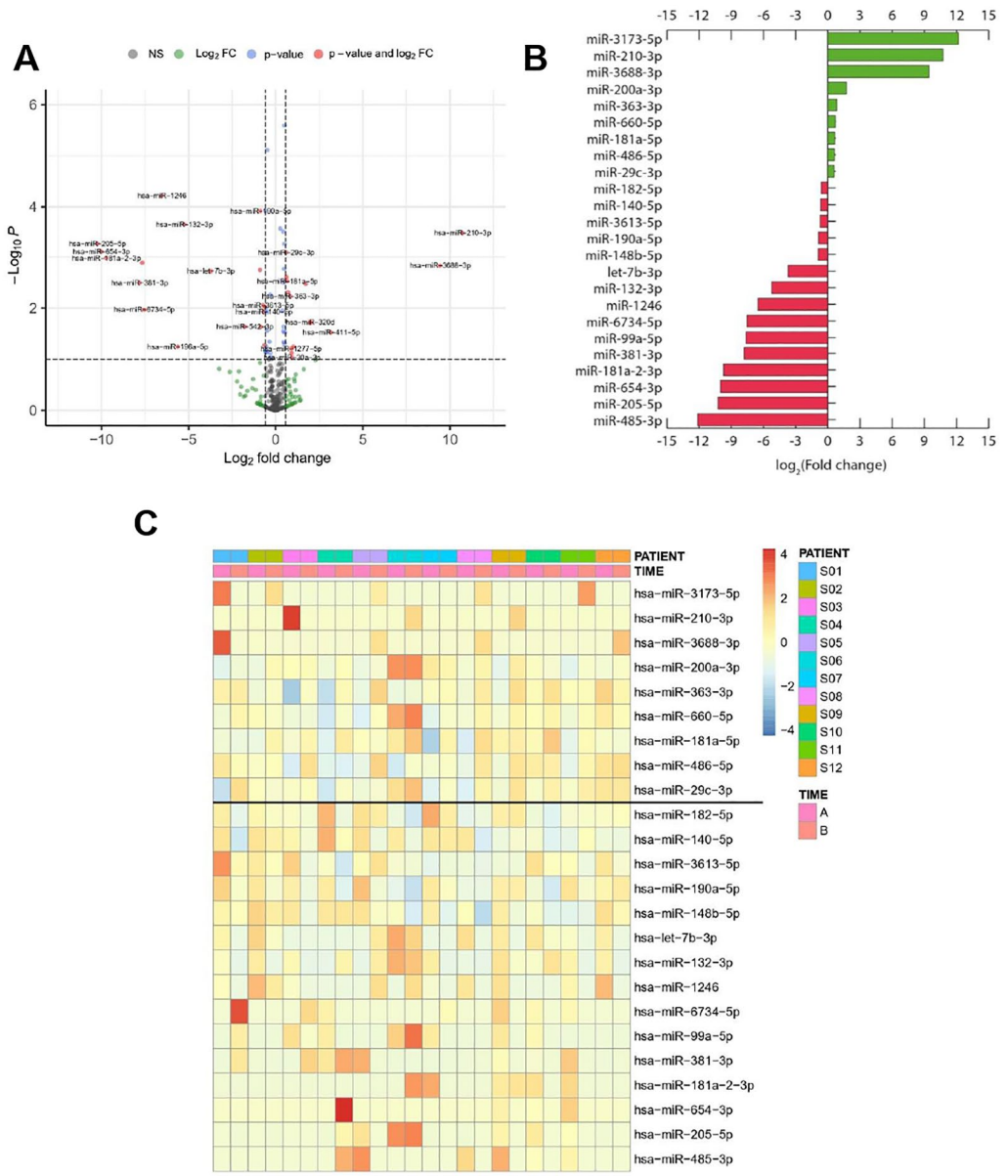

Fig. 3 **A** Volcano plot of up- and downregulated miRNAs after treatment with risankizumab. **B** Bar graph of the 9 upregulated miRNAs (in green) and the 15 downregulated miRNAs (in red). **C** Heatmap of the expression of each miRNA in individual patients at T0 and T1

IL-18, IL-1α, IL-17 and INF-γ) in serum samples of the 23 patients with psoriasis through a multiplex immunoassay and correlated the circulating cytokines concentration levels with the indices of disease severity at T0 condition. We found that only the INF-γ showed a significant and positive correlation with PASI index (Fig. 5B).

Differential Expression of miRNAs in the Older Patient Subgroup

Psoriasis is increasing among elderly patients, and this will pose a significant challenge for physicians [18, 19]. Discovering novel biomarkers could aid in disease monitoring and identifying the most effective treatment. In this framework,

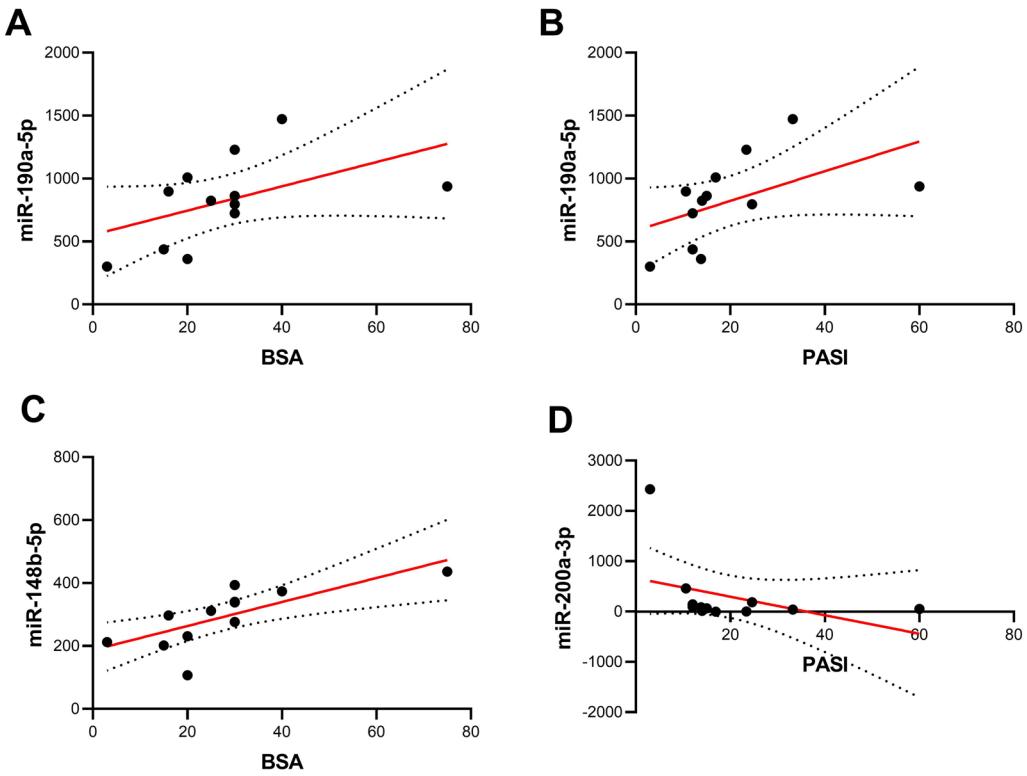

Fig. 4 **A**, **B** miR-190a-5p-positive correlation with BSA ($r = 0.595$; $p = 0.041$) and PASI ($r = 0.690$; $p = 0.013$); **C** miR-148b-5p-positive correlation with BSA ($r = 0.834$; $p = 0.001$) and miR-200a-3p-negative correlation with PASI ($r = 0.620$; $p = 0.032$) (**D**)

the expression of the three main deregulated miRNAs which correlated with clinical parameters of disease severity at T0 was studied in the same patients (second cohort) divided into two groups according to age: patients < 60 years old [mean age: 43.6 (± 10.4)], defined as the "adult group," and patients > 60 years old [mean age: 68.5 (± 7.6)], defined as the "elderly group." Our findings showed a significant difference in miR-148b-5p expression between the two groups, with the elderly group exhibiting the lower level of miR-148b-5p expression (Table 4).

DISCUSSION

Psoriasis is a complex, multifactorial disease characterized by immune-mediated inflammation and erythematous-desquamative skin lesions that can occur locally or extend throughout the body. It primarily affects the skin but can also involve other organs, including the joints, classifying it as a systemic disease [13].

In recent years, there has been a significant "revolution" in psoriasis treatments, transitioning from the use of broad-spectrum immunosuppressants (such as cyclosporine, methotrexate, or similar drugs) to biologic therapies. The latest biologic therapies, including drugs targeting interleukin 17 (IL-17) and interleukin 23 (IL-23), were developed following advancements in understanding the mechanisms underlying psoriatic plaque formation and maintenance [13]. IL-23, in particular, is now recognized as a key player in the pathogenesis of cutaneous and systemic joint inflammation by promoting the release of cytokines like IL-17, IL-22, and TNF-α [18].

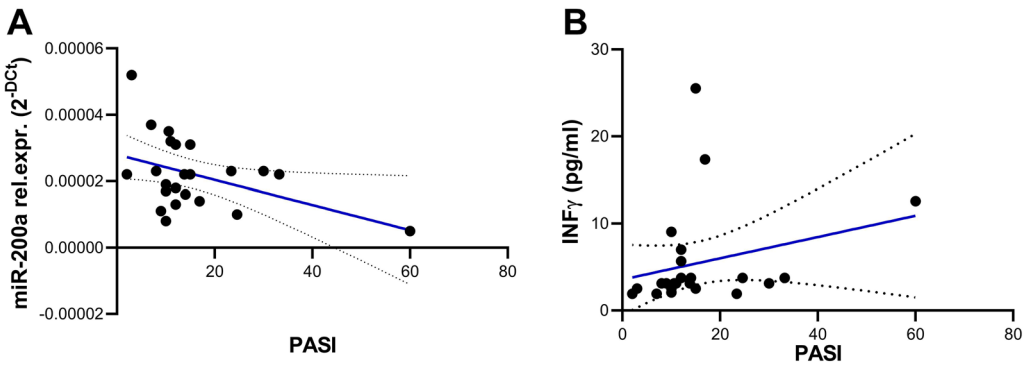

Fig. 5 **A** MiR-200a-3p negative correlation with PASI ($r = 0.186$; $p = 0.038$). **B** Positive correlation between circulating INF-γ and PASI ($r = 0.447$; $p = 0.042$)

Table 4 miR-148b, miR-190a, and miR-200a differential expression in patients < 60 years old and patients > 60 years old

	Patients < 60 years ($n = 15$)	Patients ≥ 60 years ($n = 8$)	p value
Age (years)	43.6 (± 10.4)	68.5 (± 7.6)	**0.0001**
miR-148b (rel.expr.)	3.13E−04 (± 1.42E−04)	2.06E−04 (± 1.02E−04)	**0.05**
miR-190a (rel.expr.)	3.10E−05 (± 2.67E−05)	2.91E−04 (± 1.50E−05)	0.832
miR-200a (rel.expr.)	2.47E−05 (± 1.08E−05)	1.81E−05 (± 8.03E−06)	0.381

Significant p-value are shown in bold

IL-23 also plays a role in disease relapse by reactivating specific memory T cells (tissue-resident memory T cell, TRM) in the skin, correlating with the recurrence of psoriatic disease after periods of quiescence [19]. Therefore, the use of anti-IL-23 therapies demonstrates efficacy not only in managing clinical symptoms but also in reducing relapse rates [20].

Furthermore, regulatory T cells (Tregs), crucial for maintaining immune balance and suppressing autoimmune responses, exhibit impaired suppressive capacity in psoriasis due to dysregulation with Th-17 cells and TRM. Targeting IL-23 with biologic therapies holds promise in restoring this imbalance [3, 21].

To better understand the mechanisms underlying the clinical efficacy of anti-IL-23 treatment in psoriasis, our study evaluated changes in circulating microRNAs (miRNAs), levels of pro-inflammatory cytokines, and Treg percentages. This two-phase study included a first cohort to identify miRNAs associated with clinical response to biologic therapy and a second group to correlate miRNA levels with disease severity indices at baseline. All psoriasis patients treated with risankizumab showed significant improvement in clinical symptoms, reflected in advancements across all assessment indices (PASI, BSA, PGA, DLQI) after 1 year of treatment.

Using small RNA-seq analysis of 12 plasma samples collected before (T0) and after 1 year (T1) of treatment, we identified 24 miRNAs significantly modulated at T1 compared to T0. Pathway analysis using DIANA-miRpath v.3 revealed these miRNA targets were involved in adhesion, migration, and proliferation pathways, suggesting their potential role in psoriasis pathogenesis and progression.

Subsequently, we focused on three miRNAs, i.e., miR-200a-3p, miR-190a-5p and miR-148b-5p, which displayed correlations with the main indices of disease severity. To confirm these findings, we assessed their levels in plasma samples of a second group composed of

the 12 patients of the first cohort and a second cohort of 11 patients with psoriasis at baseline. In this group of patients only a negative correlation between miR-200a-3p expression level and PASI index was observed, suggesting a potential involvement in the pathogenesis of psoriasis.

In our study, miR-200a-3p levels were found to be increased after treatment, and a negative correlation was demonstrated between miR-200a-3p level and PASI value at baseline. Collectively, our data demonstrated that patients with the most clinically evident disease phenotype had the lowest circulating levels of miR-200a.

MiR-200a-3p appears to be closely related to Th17 cells in psoriasis. Indeed, in a study conducted by Wang et al., a strong positive correlation between miR-200a expression and Th17/Treg ratio, Th17 percentage, IL-23 and IL-17 levels, and RORγt mRNA expression, as well as PASI score, was suggested. On the other hand, there was an inverse relationship between miR-200a and Treg percentage, FoxP3 level, and TGF-β mRNA expression [22]. The crucial role of miR-200a in promoting Th17 cell differentiation and suppressing Treg cell differentiation was also confirmed by a study on multiple sclerosis [23].

Although our result appears to contradict the existing literature, it is worth mentioning that previous studies were performed in ex vivo samples, while our study was carried out on plasma samples. Therefore, the mechanisms underlying miR-200a intracellular expression and release into the bloodstream deserve further investigation.

Notably, the cells release various types of extracellular vesicles (EVs), which include cellular miRNAs among other components [24]. This leads to the possibility of a different level of circulating and cellular miR-200a as a result of differential EV loading.

The increasing prevalence of psoriasis among older patients will pose a significant challenge for physicians in the new millennium. Elderly individuals may experience drug-induced or drug-aggravated psoriasis, particularly if they are receiving polypharmacy or have not responded well to traditional treatments [25].

In this framework, we evaluated the differential expression of the three miRNAs, miR-200a-3p, miR-190a-5p, and miR-148b-5p, in the subgroup of older patients. We observed a significantly higher expression level of miR-148b-5p in the adult compared to the elderly group.

MiR-148b-5p is classified as a member of the miR-148/-152 family and has been connected to B and T cell differentiation, epigenetic mechanisms, tumorigenesis, and biologic processes like adipogenesis [26]. Studies analyzing its implications at the level of neuronal tissue noted that it would appear that a blockade of miR-148b can induce a process of neuroprotection through Wnt/β-catenin signaling [22]. Additional studies are necessary to uncover the involvement of miR-148b-5p in the pathogenesis of psoriasis in the elderly population.

Evaluating Tregs, we confirmed an increase in circulating Tregs after treatment in all psoriatic patients. Given the role of IL-23 in converting Tregs to Th-17 cells, anti-IL-23 therapy restores functional Treg levels that modulate inflammation [27].

Additionally, our analysis of pro-inflammatory cytokines showed reduced IL-23, IL-1β, and IL-8 levels in whole blood after risankizumab therapy, confirming the drug's efficacy in reducing systemic inflammation associated with psoriasis. IL-8, particularly expressed in the stratum granulosum, plays a crucial role in psoriasis by attracting polymorphonuclear cells and stimulating angiogenesis and keratinocyte mitogenesis.

Finally, a significant positive correlation between serum INF-γ and PASI index underscores the biologic impact of these cytokines on disease severity [28].

Our study has some limitations that should be discussed. First, it is important to consider the restricted nature of the sample size. Second, as all the patients responded to risankizumab treatment, we were unable to assess the ability of circulating miRNAs to predict disease remission after treatment.

Further studies on larger cohorts are required to define the role of these miRNAs in the pathogenesis and development of psoriasis.

CONCLUSIONS

The analysis of circulating miRNAs involved in pathogenetic disease processes and their changes after therapy can be extremely useful in increasing knowledge of psoriasis and its treatment.

Particularly, miR-200a-3p levels increased after risankizumab treatment and negatively correlated with PASI value at baseline. Thus, miR-200a-3p might be considered a good biomarker of disease activity.

In contrast, miR-148b-5p, which we observed to be significantly reduced in older patients with psoriasis compared to the adult ones, was found to be associated with neurologic disease, B and T cell differentiation, epigenetic mechanisms, and tumorigenesis. These findings might indicate that when treating psoriatic patients, their age should be considered as it might impact their response to therapy or the disease progression.

Finally, the effect of risankizumab on the reduction of systemic inflammation is demonstrated by the increase in Treg population and reduction of the inflammatory cytokines IL-1β and IL-8 expression level after risankizumab therapy.

In conclusion, further investigations on miRNA-associated regulatory mechanisms involved in psoriasis pathogenesis and development are mandatory.

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest. Anna Campanati is an Editorial Board member of Dermatology and Therapy. Anna Campanati was not involved in the selection of peer reviewers for the manuscript or any of the subsequent editorial decisions. Federico Diotallevi, Giulia Matacchione, Elena Marinelli Busilacchi, Nadia Viola, Ilaria Pace, Beatrice Fontana, Roberta Roncarati, Massimiliano Bonafè, Manuela Ferracin, Jacopo Sabbatinelli, and Fabiola Olivieri have nothing to disclose.

Ethics Approval. This study was performed in accordance with the Helsinki Declaration of 1964 and its later amendments. The ethics committee of UNIVPM approved the study protocol, and all enrolled participants provided written informed consent. The patients in this manuscript have given written informed consent to publication of their case details.

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