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Original Article

Risankizumab differentially modulates circulating T-cell populations in psoriasis according to autoreactivity status

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ABSTRACT

Background: In a recent paper, our group described that the presence of double autoreactivity to both LL37 and ADAMTSL5 autoantigens in psoriatic patients decreased the clinical responses to risankizumab, but how this influences the changes in the peripheral inflammatory T-cell populations is still unknown.
Objective: This study aims to evaluate how risankizumab modulates the circulating inflammatory T-cell populations in psoriatic patients and, specifically, in autoreactive subjects.
Methods: The presence of LL37- and ADAMTSL5-reactive circulating T-cells was assessed in a cohort of 142 psoriatic patients, and 87 demonstrated autoreactivity at baseline. Patients were treated with risankizumab for 52 weeks, and specific T-cell populations were analyzed at different timepoints.
Results: The frequency of $Ki67^+CD4^+$, $Ki67^+CD8^+$ T-cells, $CD8^+IL\text{-}17^+$ and $CD8^+IL\text{-}22^+$ T-cells showed a positive correlation with baseline PASI and decreased with treatment. Notably, $CD8^+IL\text{-}17^+$ T-cells decreased both in single-LL37 and single-ADAMTSL5-reactive subjects, but not in subjects that showed autoreactivity to both autoantigens. LL37 autoreactivity of $CD4^+$ and $CD8^+$ T-cells decreased with treatment, but not for $CD4^+$ in double-reactive subjects. While Treg frequency negatively correlated with baseline PASI and increased within 16 weeks of treatment, significantly decreasing the $IL\text{-}17^+CD4^+$/Treg ratio over time, Treg modulation was not evident in double-reactive subjects.

Interestingly, the subpopulations of $CD8^+$MAIT $IL\text{-}17^+$ and $CD3^+$MAIT $IL\text{-}22^+$ cells, involved also in psoriatic arthritis, decreased in treated subjects following IL-23 inhibition.
Conclusion: Rizankizumab efficiently decreases the circulating inflammatory T-cell populations and modulates Tregs' plasticity in single-LL37- or single-ADAMTSL5-reactive subjects, but not in double-reactive subjects.

1. Introduction

Psoriasis is a chronic immune-mediated disease with features of T-cell–driven autoimmunity, where T-cell activation and the production of specific inflammatory cytokines play a central role in the disease pathogenesis [1,2].

While the adaptive immune response remains the primary driver of psoriatic inflammation, components of the innate immune system, including keratinocytes and dendritic cells, may contribute to disease initiation by activating NF-κB and type I interferon pathways. Moreover, genetic variants in innate immune genes such as CARD14, IFIH1 (MDA5), and DDX58 (RIG-I) have been associated

Abbreviations: IFN, interferon; HD, healthy donor; IL, interleukin; MAIT, Mucosal-Associated Invariant T cells; PASI, psoriasis area and severity index; P0, psoriatic patients at time 0; Th17, T helper 17 cells; TNF, tumor necrosis factor; Tregs, Regulatory T-cells

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with enhanced inflammatory signaling, potentially amplifying and sustaining chronic skin inflammation [3].

In psoriatic lesional skin and in the blood of severe psoriatic patients, high levels of inflammatory cytokines, including interleukin (IL)-17, IL-22, IL-23, IL-21, IFN-γ, and TNF-α, have been demonstrated [4–6]. IL-23 and IL-17 play a crucial role in chronic inflammation [1–3] and represent the target of selective biologic drugs for moderate-to-severe psoriasis. IL-23, binding to the IL23Rα, induces the terminal differentiation of $CD4^+$IL-17^+ [7] and modulates the secretion of important effector cytokines, including IL-17 and IL-22, from different cellular sources [8,9]. Risankizumab, an anti-IL-23 monoclonal antibody that efficiently blocks the binding of IL-23 to IL-23Rα, has proven to be a highly effective treatment, achieving high rates of PASI100 in both clinical trials [10,11] and in real-world studies [12].

Different T-cell populations contribute to psoriasis pathogenesis. $CD8^+$ T-cells are considered the ultimate effectors in psoriatic skin inflammation [1,13], but $CD4^+$ T-cells are also crucial [14,15]. A subset of the IL-17A-producing $CD8^+$ T cell population in psoriatic lesional skin is represented by the Mucosal-Associated Invariant T (MAIT) cells [16,17], which display innate-like effector functions upon activation. Interestingly, MAITs, which are regulated by IL-23, have been found in the synovial fluid of patients affected by psoriatic arthritis [18], suggesting a pivotal role in the development of psoriatic arthritis. Regulatory T cells (Treg), on the other hand, have strong anti-inflammatory functions, and an imbalance of the $CD4^+$IL-17^+/Treg ratio has been observed in moderate-to-severe psoriasis [19,20].

In up to two-thirds of moderate-to-severe patients, an autoimmune response to skin autoantigens contributes to psoriasis pathogenesis [21–23]. To date, at least four psoriasis-associated autoantigens have been described: self-nucleic acid complexes of the cathelicidin antimicrobial peptide (LL37) [21], the melanocytic antigen ADAMTS-like protein 5 (ADAMTSL5) [22], the lipid antigen PLA2G4D [24], and keratin 17 [25]. The autoantigens LL37 and ADAMTSL5 are presented to $CD8^+$ T-cells mainly via HLA-C*06:02 and to $CD4^+$ T-cells via different class II HLAs [23]. LL37 autoreactive T-cells can have either a $CD8^+$ or $CD4^+$ phenotype and are rare in healthy controls [21]. Little is known about the role of the T-cell autoreactivity in influencing the response to treatment. In a recent work [26], we described that the presence of reactive circulating T-cells to both LL37 and ADAMTSL5 autoantigens was associated with a suboptimal clinical response to risankizumab; on the contrary, the presence of reactive T-cells to either LL37 or ADAMTS5 did not influence the effectiveness of the drug [26]. While in our previous paper, we focused on the clinical efficacy of risankizumab, differentiating the patients based on autoreactivity status, with the present study, we explored the underlying changes in the circulating T-cell populations that can justify the differences in effectiveness.

We aimed to investigate how risankizumab treatment modulates distinct inflammatory T-cell populations involved in psoriasis pathogenesis, including IL-17A (hereafter referred to as IL-17)- and IL-22-producing T cells, circulating LL37- and/or ADAMTSL5-autoreactive T cells, Tregs, and MAITs, according to the patient's autoreactivity status.

2. Materials and methods

2.1. Study population

This study is conducted in accordance with the Note for Guidance on Good Clinical Practice (Humanitas ICH Harmonized Tripartite Guideline E6(R1); the general guideline indicated in the Declaration of Helsinki, and all applicable regulatory requirements. The study was approved by the Humanitas ICH Ethic Committee (Institutional Review Board, EUDRA 2019–004250–28) as in Favaro et al., 2024 [26]. Written informed consent was obtained from all participants before enrolment. Patients affected by moderate-to-severe psoriasis (PASI > 10 and BSA > 10 %) for at least 6 months were enrolled in one year (April 2021-April 2022) (n = 142). All patients received risankizumab treatment as per protocol. Blood samples were collected at baseline and tested for reactivity to LL37 and ADAMTSL5. PASI was collected at baseline and at every time point until week 52.

2.2. PBMC isolation and analysis

Peripheral blood was collected after venipuncture in heparin-containing tubes from enrolled patients and controls after informed consent (EUDRA 2019–004250–28 ICH). PBMCs were isolated and stimulated as previously described [26]. The LL37 or ADAMTSL5 reactivity of $CD4^+$ and $CD8^+$ cells was expressed by Stimulation Index (SI) of proliferation, which was calculated as the ratio of $Ki67^+$ cells in treated samples compared with control after 6 days of in vitro culture. A subject was considered autoreactive if either $CD4^+$ or $CD8^+$ stimulation demonstrated an SI > 2. We also verified the expression of the marker CD25 in combination with $Ki67^+$ on the PBMCs of a subset of reactive subjects (n = 20). We assessed that cells that respond to the antigen stimulation with proliferation ($Ki67^+$) are indeed cells that express CD25 ($CD25High^+$, as indicated in Suppl. Fig. 1).

Immunostaining was performed with Zombie Aqua™ Fixable Viability Kit (Biolegend 77143), and human surface markers α-CD3(UCHT1)-PERCPCY5.5(Biolegend-300430), α-CD4(RPA-T4)–FITC(Biolegend-300538), α-CD8(RPA-T8)-BV421(BD-562428) and/or α-CD25(2A3)-BV421(BD-564033); internal marker: α-Ki67(B56) AF700 (BD-561277), α-IL-17A (BL168)-PE (Biolegend-B255234), α-IL-22 (22URTI)-PE-Cy7 (e-bioscience-25–7229–42) and α-FOXP3(259D/C7)-AF647 (BD-560045) after FOXP3/Transcription Factor Fixation/Permeabilization (e-bioscience-00–5521–00). MAIT staining panel: α-CD3(UCHT1)-PERCPCY5.5(Biolegend-300430), α-CD4(OKT4)-BV650 (Biolegend 317436) α-TCRVα7.2-FITC(Miltenyi 130–123–685) α-CD161(HP-3G10)-BV605(Biolegend 339916) α-IL-17A (BL168)-PE (Biolegend-B255234), α-IL-22 (22URTI)-PE-Cy7 (e-bioscience-25–7229–42) and α-IL23R-PE (R&D Bio-techne FAB-14001P-100). To test ILs, cells were treated for 2 h (37°C and 5 % CO_2) with Cell activation cocktail w/o Brefeldin (423302 Biolegend) and Golgi plug (555029 BD) in complete medium. All stained-cell samples were acquired on a BD-LSR-Fortessa and analyzed with DIVA software (BD). The gating strategy is described in Suppl. Fig. 2 and Suppl. Fig. 3A-B.

2.3. Statistical analysis

Continuous variables are presented as mean, SEM or Min to Max boxes. Discrete variables were summarized as frequencies and percentages. Data were compared by *t*-test (unpaired or paired *t*-test with Welch's correction) for single comparison, multiple *t*-test or ANOVA for multiple comparisons, non-normal distributions were tested using Kruskal-Wallis or Friedman test, and uncorrected Dunn's test was applied. R is calculated as Pearson correlation coefficient. GraphPad Prism 7.1 Software was used to analyze data with the appropriate statistical test. The significance threshold was defined as $P < 0.05$ (2-sided).

3. Results

3.1. Study population and ***autoreactivity*** frequencies

As described in our previously published clinical work [26], one hundred forty-two patients affected by moderate-to-severe psoriasis **(PASI > 10,** BSA > 10 %) were screened at baseline for the presence of circulating LL37 and ADAMTSL5 autoreactive T-cells (SI > 2). Their demographic and genetic characteristics are listed in Suppl. Table 1. Thirty-three healthy controls (HD) with no personal history of psoriasis or other dermatological diseases were included in the study for comparison (Suppl. Table 1 and Favaro et al., 2024 [26]). A total of 87 out of 142 (61 %)

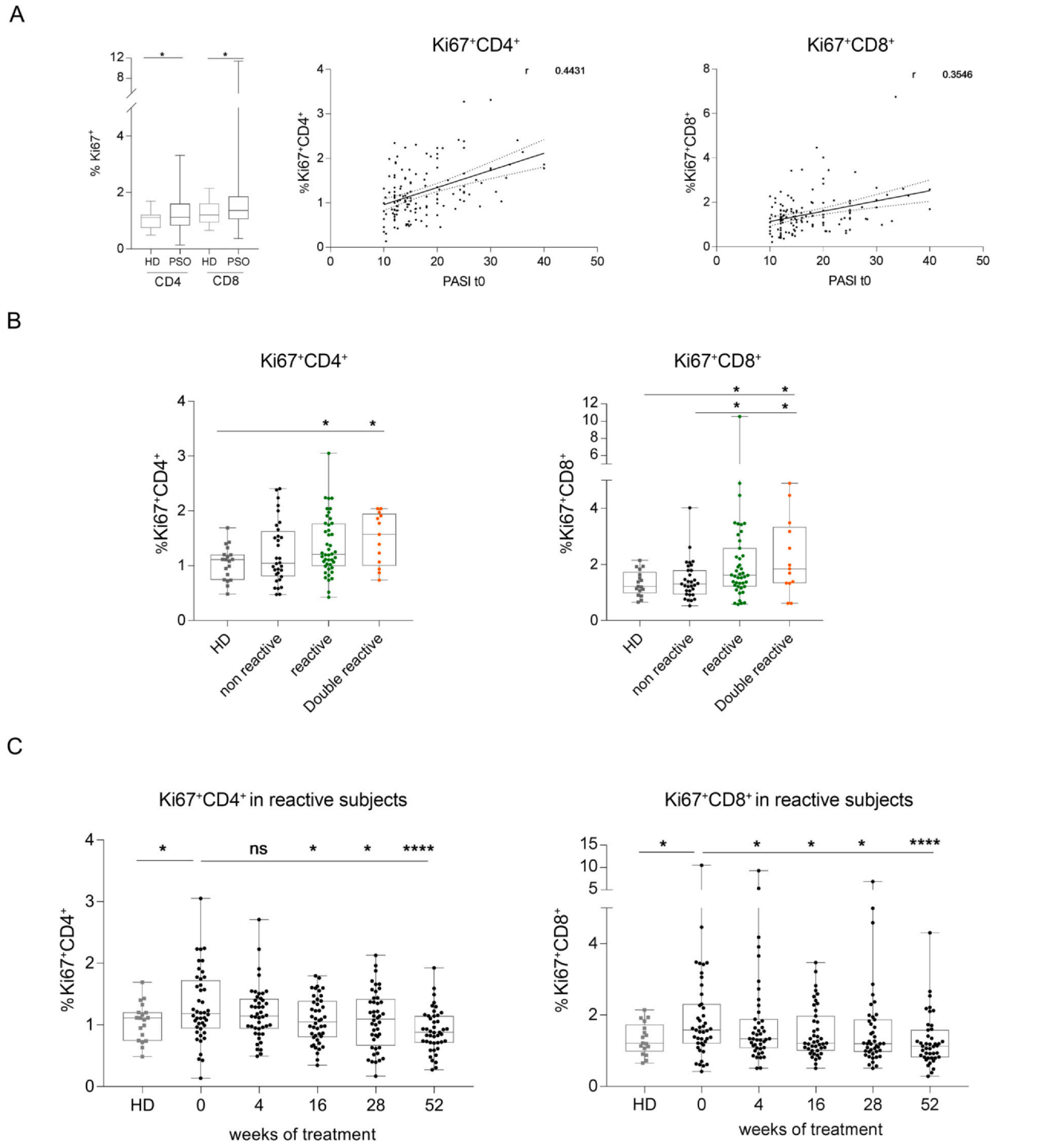

Fig. 1. The frequencies of circulating Ki67$^+$CD4$^+$ and CD8$^+$ T-cells correlated with baseline PASI and decreased with treatment. A. The frequencies of Ki67$^+$CD4$^+$ and Ki67$^+$CD8$^+$ were significantly higher in psoriatic patients compared to healthy donors (HD) (Ki67$^+$CD4$^+$: 1.26 % ± 0.05 vs 1.05 % ± 0.07 respectively; Ki67$^+$CD8$^+$: 1.71 % ± 0.12 vs 1.29 % ± 0.11 respectively, Welch *t*-test p = 0.018 and p = 0.0134, respectively). The percentage of Ki67$^+$CD4$^+$ and Ki67$^+$CD8$^+$ T-cells showed statistically significant Pearson correlation with PASI (r = 0.44, p < 0.0001 and r = 0.35, p < 0.0001, respectively). Linear regression represented with a 95 % Confidence Interval. B. Frequencies of circulating Ki67$^+$CD4$^+$ and Ki67$^+$CD8$^+$ in psoriatic patients at baseline in the three different autoreactivity groups: non-reactive (black), reactive to LL37 and/or ADAMTSL5 (green), and double reactive to both LL37 and ADAMTSL5 (orange). Minimum to Maximum representation, One-way ANOVA Kruskal-Wallis, uncorrected Dunn's test; significant threshold p < 0.05 two-sided. C. Risankizumab treatment decreased the proliferation of circulating CD4$^+$ and CD8$^+$ T-cells in reactive subjects. Data represented as Minimum to Maximum graph, One-way ANOVA: Friedman test with Uncorrected Dunn's test applied; significant threshold p < 0.05 two-sided.HD, healthy donors; PSO, psoriatic patients; t0, baseline. * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001; ns, non-significant.

psoriatic patients were found to be autoreactive: 31 (22 %) had a positive SI for LL37, 37 (26 %) for ADAMTSL5, and 19 patients showed a positive SI for both (double reactive, 13 %). Autoreactivity frequency was significantly higher in psoriatic patients (87/142) compared to HD (13/33; p = 0.022). Reactive patients were sampled at all specific timepoints (weeks 4, 16, 28, and 52). Three patients were excluded from the study (one for pregnancy at week 16 and two for positivity to TB-quantiferon at week 4).

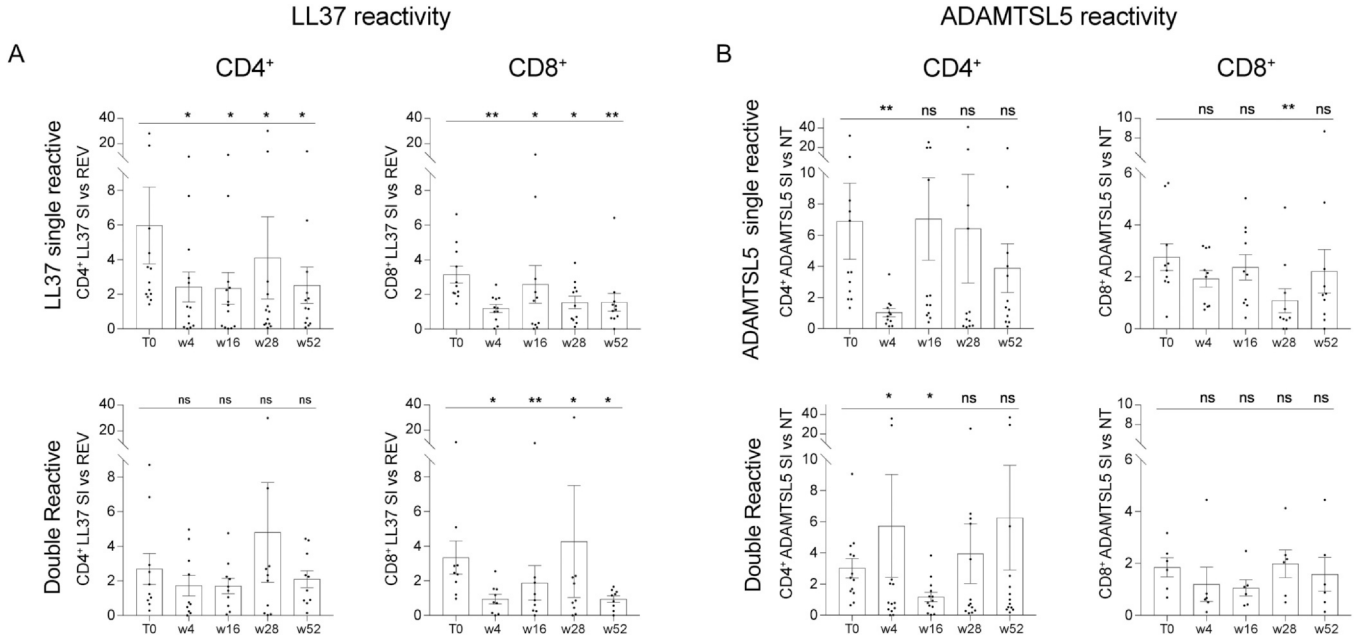

Fig. 2. Stimulation index of LL37 and ADAMTSL5 reactive T-cells consistently decreased after risankizumab treatment only in LL-37-reactive subjects. A. The changes in SI of the $CD4^+$ and $CD8^+$ LL37-reactive cells in LL37-reactive subjects and double-reactive subjects. **B.** The changes in SI of the $CD4^+$ and $CD8^+$ ADAMTSL5-reactive cells in ADAMTSL5-reactive and double-reactive subjects. Histograms with dispersed dots represent mean ± SEM; one-way ANOVA: Friedman test with Uncorrected Dunn's test applied; significant threshold $p < 0.05$ two-sided. NT, non-treated sample; REV, reverse peptide; SI, stimulation Index, T0, baseline. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, non-significant.

3.2. The frequency of peripheral proliferating $Ki67^+CD4^+$ and $Ki67^+CD8^+$ T-cells correlated with baseline PASI and decreased with risankizumab treatment

$Ki67^+$ is a marker of cell proliferation and is expressed in actively proliferating cells. To verify the proliferation rate of peripheral T-cells and its correlation with the disease activity, we analyzed the frequencies of circulating $Ki67^+CD4^+$ and $Ki67^+CD8^+$ T-cells at baseline. We found higher frequencies compared to HD ($Ki67^+CD4^+$: $p = 0.018$ and $Ki67^+CD8^+$: $p = 0.0134$, Fig. 1A) and a positive correlation with baseline PASI ($r = 0.44$, $P < 0.0001$ for $Ki67^+CD4^+$; and $r = 0.35$, $P < 0.0001$ for $Ki67^+CD8^+$, Fig. 1A), indicating a higher presence of activated and proliferating T-cells in severe patients.

Then we stratified the frequency of $Ki67^+$ cells based on autoreactivity status. We observed that antigen-reactive patients showed a higher frequency of $Ki67^+CD4^+$ and $Ki67^+CD8^+$ T-cells compared to HD (for overall reactive patients, $p = 0.042$ for $Ki67^+CD4^+$ and $p = 0.045$ for $Ki67^+CD8^+$, for double reactive $p = 0.021$ for $Ki67^+CD4^+$ and $p = 0.036$ for $Ki67^+CD8^+$), while no differences were observed between non-reactive and HD (Fig. 1B). The frequencies of $Ki67^+CD8^+$ T-cells in single reactive and double reactive subjects were also higher compared to non-reactive patients ($p = 0.044$ and $p = 0.045$ respectively, Fig. 1B), suggesting that reactive subjects have a higher systemic inflammatory burden compared to non-reactive subjects.

In reactive patients, risankizumab treatment decreased the circulating $Ki67^+CD4^+$ T-cells starting from week 16 to week 52, while $Ki67^+CD8^+$ T-cells decreased as early as week 4 (Fig. 1C). In non-reactive patients, we noticed that the frequency of $Ki67^+CD4^+$ T-cells was also reduced at week 52, while no significant changes were seen for $Ki67^+CD8^+$ T-cells (Suppl. Fig. 4). Overall, these results suggest that risankizumab strongly affects the $Ki67^+CD8^+$ T-cells population in reactive subjects, while the effect is weaker in non-reactive subjects (Suppl. Fig. 4).

3.3. LL37-autoreactivity was decreased by treatment, but less efficiently in double-reactive subjects

To assess the effect of anti-IL-23 treatment on circulating autoreactive LL37 T-cells, we evaluated the SI at different timepoints (weeks 4, 16, 28, and 52) for $CD4^+$ and $CD8^+$ T-cells. Risankizumab reduced the LL37-induced proliferation of $CD4^+$ T-cells and $CD8^+$ T-cells as early as week 4 until week 52 in patients that were LL-37-reactive only; on the contrary, in double-reactive patients, the LL37-induced SI was reduced only for $CD8^+$ T-cells but not for $CD4^+$ (Fig. 2A), indicating a more specific effect of risankizumab on $CD8^+$ T-cells.

3.4. ADAMTSL5-autoreactivity was less impacted by IL-23 inhibition

In single ADAMTSL5-reactive patients, the SI for ADAMTSL5-stimulated $CD4^+$ T-cells was strongly decreased only at week 4 (Fig. 2B) and in double-reactive subjects only at week 4 and week 16. ADAMTSL5-reactive $CD8^+$ T-cells demonstrated more inconsistent responses: the SI for single ADAMTSL5-reactive patients was reduced only at week 28, while no changes were seen for double-reactive patients over time. These results suggest that risankizumab treatment does not affect the reactivity of $CD8^+$ T-cells in ADAMTSL5-reactive subjects.

3.5. $CD8^+IL\text{-}17^+$ and $CD8^+IL\text{-}22^+$ T-cells decreased with risankizumab treatment, but $CD8^+IL\text{-}17^+$ T-cells did not decrease in double-reactive subjects

IL-17 and IL-22 are key pathogenetic cytokines in psoriasis, therefore, we evaluated the changes in circulating $CD8^+IL\text{-}17^+$ and $CD8^+IL\text{-}22^+$ over time. The frequency of $CD8^+IL\text{-}17^+$ in the peripheral blood of psoriatic patients was higher compared to HD ($p = 0.0026$) and positively correlated with PASI at baseline in severe subjects (PASI≥15, $r = 0.44$, $p = 0.0002$, Fig. 3A). Similarly, the frequency of $CD8^+IL\text{-}22^+$ was also higher in psoriatic patients compared to HD

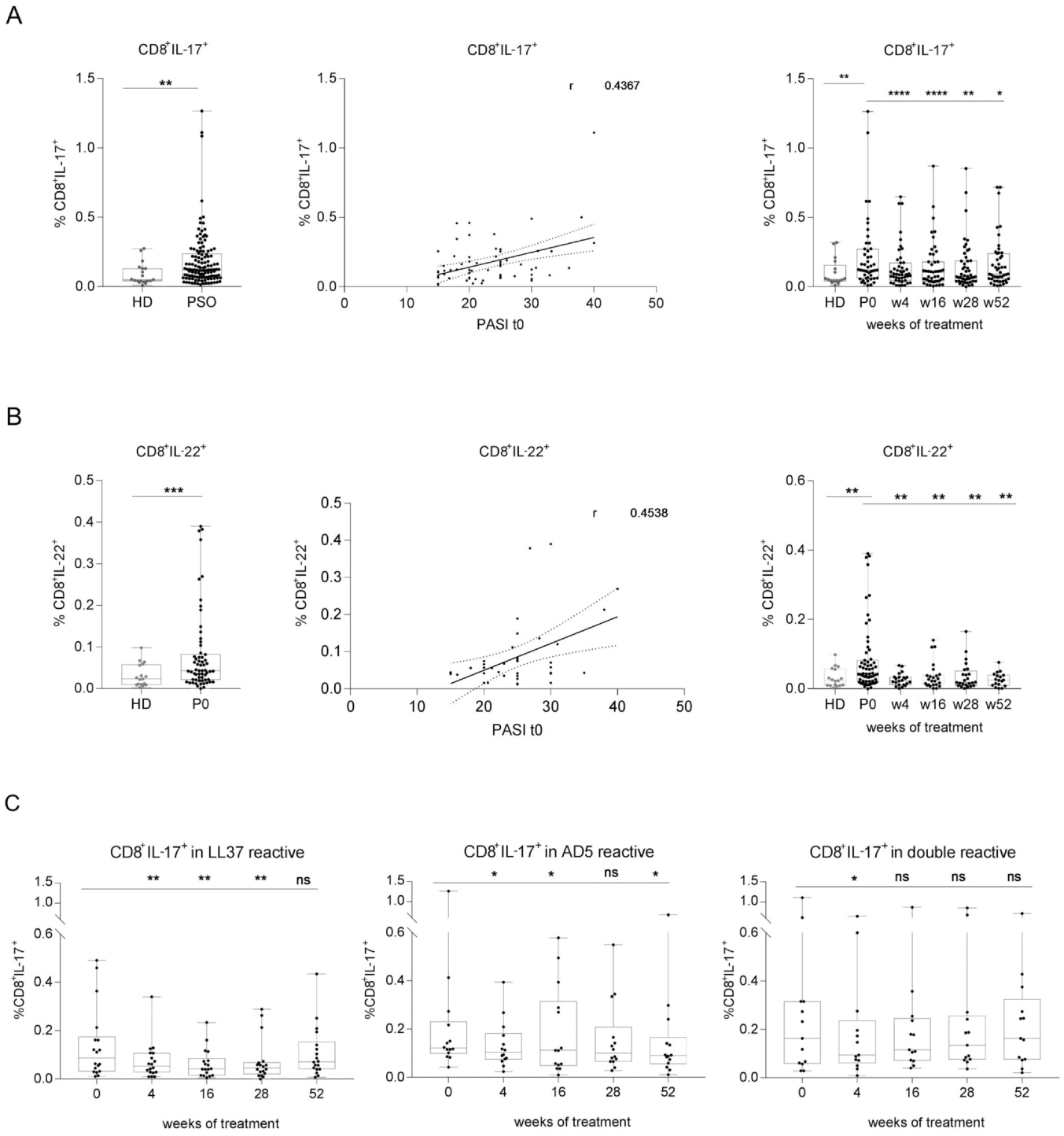

Fig. 3. Circulating pathogenic $CD8^+IL\text{-}17^+$ and $CD8^+IL\text{-}22^+$ T-cells correlated with PASI at baseline and decreased after treatment. **A.** $CD8^+IL\text{-}17^+$ frequency was higher in psoriatic patients compared to healthy donors (0.19 ± 0.19 vs 0.09 ± 0.08, p = 0.0026), correlated with PASI in severe patients (PASI≥15, Pearson correlation r = 0.44; p = 0.0002, n = 67) and decreased with treatment. **B.** $CD8^+IL\text{-}22^+$ frequency was higher in psoriatic patients compared to healthy donors (0.08 ± 0.08 vs 0.03 ± 0.03, p = 0.0006), correlated with PASI in severe psoriatic patients (PASI≥15, Pearson correlation r = 0.45; p = 0.0042, n = 38) and decreased with treatment. **C.** In the subpopulation of reactive subjects, $CD8^+IL\text{-}17^+$ decreased with treatment in single LL37- and single ADAMTSL5-reactive patients but not in double reactive oneswith the exception of a significant initial decrease at week 4. Minimum to Maximum dot representation, Mann Whitney test, or in multiple analyses,Friedman test or Kruskall-Wallis test are applied with Uncorrected Dunn's test; significant threshold P < 0.05 two-sided. AD5, ADAMTSL5 antigen. AD5, ADAMTSL5; HD, healthy donors; PSO, psoriatic patients; P0, psoriatic patients at baseline; t0, baseline. * p < 0.05; ** p < 0.01; *** p < 0.001; ns, non-significant.

(p = 0.0006, Fig. 3B) and positively correlated with PASI at baseline in severe subjects (PASI≥15, r = 0.45, p = 0.0042). The rates of $CD8^+IL\text{-}17^+$ and $CD8^+IL\text{-}22^+$ consistently decreased after treatment from week 4 until week 52 (Fig. 3A-B). The subpopulation of proliferating $Ki67^+CD8^+IL\text{-}17^+$ also significantly decreased over time indicating that IL-23 inhibition reduces their proliferation (Suppl. Fig. 5). Analyzing the changes in the frequency of circulating $CD8^+IL\text{-}17^+$ T-cells in the three different autoreactive populations, we observed a significant decrease in single LL37-reactive subjects starting from week 4 to week 28 and in single ADAMTSL5-reactive subjects at week 4, week 16 and week 52 (Fig. 3C). In double-reactive patients $CD8^+IL\text{-}17^+$ T-cells significantly decreased only at week 4 and no

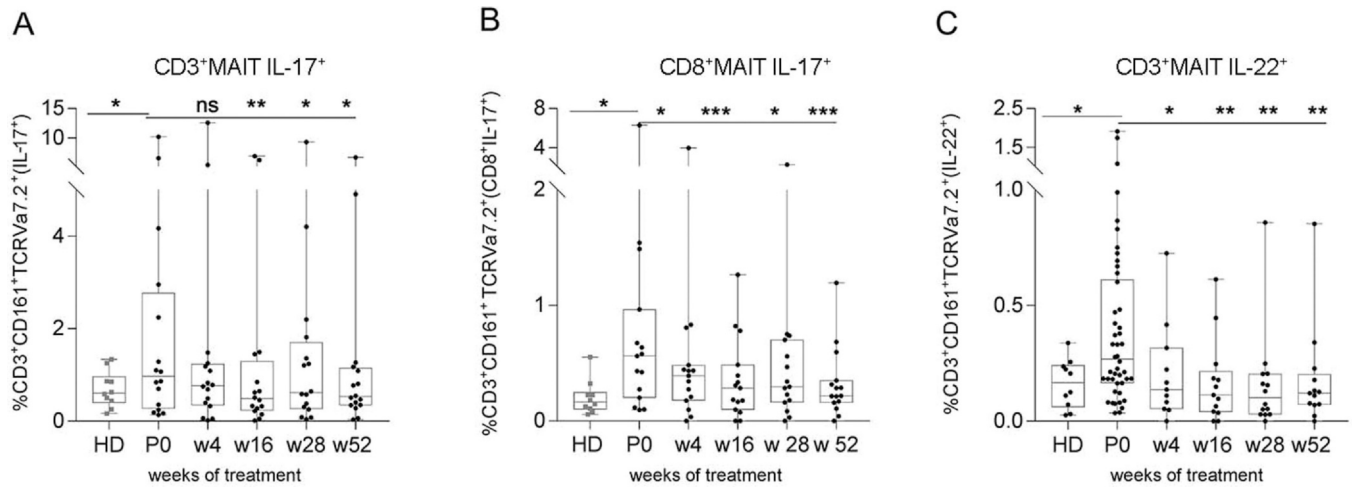

Fig. 4. Reduction of circulating CD3$^+$MAIT-IL-17$^+$, CD8$^+$MAIT-IL-17$^+$ and CD3$^+$MAIT-IL-22$^+$ with risankizumab treatment. A. Circulating CD3$^+$MAIT-IL-17$^+$ were significantly higher at baseline in psoriatic subjects compared to HD (1.74 ± 1.81 vs 0.69 ± 0.39, respectively, p = 0.0125), and their frequency significantly decreased with treatment starting from week 16 until week 52. **B.** CD8$^+$MAIT-IL-17$^+$ were significantly higher at baseline in psoriatic subjects compared to HD (0.73 ± 0.93 vs 0.20 ± 0.15, respectively, p = 0.0009), and their frequency significantly decreased with treatment from week 4 until week 52. **C.** Circulating CD3$^+$ MAIT-IL-22$^+$ were significantly higher at baseline in psoriatic subjects compared to HD (p = 0.035), and their frequency decreased with treatment starting from week 4 until week 52. Data represented as Minimum to Maximum graph, Mann Whitney or Friedman test with Uncorrected Dunn's test applied; significant threshold p < 0.05 two-sided. HD, healthy donors; P0, psoriatic patients at baseline. * p < 0.05; ** p < 0.01; *** p < 0.001; ns, non-significant.

changes were seen at later timepoints compared to baseline (Fig. 3C).

3.6. Circulating MAIT-IL-17$^+$ cells and MAIT-IL-22$^+$ cells were reduced by risankizumab treatment

A subgroup of IL-17-producing CD8$^+$ T-cell population in psoriatic lesional skin is represented by MAITs (CD3$^+$TCRVα7.2$^+$CD161$^+$), which constitute the largest circulating innate-like αβT-cell community in human adults, characterized by a semi-invariant TCRVα7.2 receptor and by MHC-related protein-1 MR1 restricted affinity toward microbial metabolites [16]. The frequency of circulating CD3$^+$MAIT-IL-17$^+$ cells was higher in psoriatic patients compared to HD (p = 0.0292) and it decreased with treatment starting from week 16 (Fig. 4A); the CD8$^+$MAIT-IL-17$^+$ subpopulation was also higher at baseline compared to HD (p = 0.0009) and it strongly decreased as early as at week 4 until week 52 (Fig. 4B). Similarly, the CD3$^+$MAIT population that express IL-22 (CD3$^+$ MAIT-IL-22$^+$) was higher in psoriatic subjects compared to HD (p = 0.035 vs HD) and decreased with treatment (Fig. 4C). This is possibly due to the direct effect of anti-IL23 on MAITs. We verified the expression of IL23R on MAITs in psoriatic patients and found that it is present with a frequency of 1.43 % ± 1.29 in circulating CD3$^+$MAITs and with a frequency of 0.88 % ± 0.73 of CD3$^+$CD8$^+$MAITs. These frequencies are very similar to the rate of IL-17-producing CD3$^+$MAITs and CD8$^+$MAITs cells, respectively (CD3$^+$MAIT-IL-17$^+$: 1.47 % ± 1.64; CD3$^+$CD8$^+$MAIT-IL-17$^+$: 0.95 % ± 0.97, Suppl. Fig. 6), suggesting that there is a specific IL-17-producing MAIT subpopulation that expresses IL-23R and responds to IL-23 inhibition.

3.7. Treg frequency was lower in severe psoriatic patients and increased with treatment in single-reactive patients, but not in double-reactive ones

Treg cells act as suppressors of inflammation and an effective treatment in psoriatic patients induces their upregulation [27]. We assessed the frequency of circulating Treg (CD4$^+$CD25$^+$FOXP3$^+$ T-cells) at baseline and after anti-IL-23 treatment with risankizumab. For the more severe psoriatic patients (PASI≥15, n = 46), we found a negative correlation (r = -0.48, p = 0.0007) between Treg frequency and baseline PASI (Fig. 5A).

The frequency of circulating Tregs in the different categories of reactive patients at baseline showed a significant difference only between double-reactive and LL-37-reactive subjects, with double-reactive showing higher values (Fig. 5B). This result was independent of the PASI score of the patients (Suppl. Fig. 7). Matched data collected after treatment indicated an increase in Treg in all-reactive (LL37 or ADAMTSL5) and single LL37-reactive patients at week 4 and week 16 (Fig. 5C) and at week 4 in single ADAMTSL5-reactive ones. Interestingly, double-reactive subjects did not show any variation in the frequency of the Treg population over time (Fig. 5C). We also analyzed these data considering patients based on the presence of the HLA-C*06:02 alleles and we found that only in HLA-C*06:02$^+$subjects there was a statistically significant increase in Tregs compared to baseline, but not in HLA-C*06:02$^-$ (Suppl. Fig. 8).

3.8. The CD4$^+$IL-17$^+$ population and the CD4$^+$IL-17$^+$/Treg ratio decreased with treatment

In psoriatic patients, an imbalance between the CD4$^+$IL-17$^+$ and Treg ratio has been described [28]. CD4$^+$IL-17$^+$ frequency was higher in the psoriatic cohort at baseline (p = 0.0032) compared to HD and decreased at week 4 and week 16 (Fig. 6A). This result was associated with a strong decrease in the frequency of the proliferating counterpart (Ki67$^+$CD4$^+$IL-17$^+$), at every time point compared to the baseline (Fig. 6B).

The CD4$^+$IL-17$^+$/Treg ratio can be considered an indicator of the inflammatory status in psoriatic patients. We observed a positive correlation (r = 0.445; p = 0.001) between the CD4$^+$IL-17$^+$/Treg ratio and PASI in severe psoriatic patients (PASI≥15), indicating a prominent inflammatory environment (Fig. 6C). When we analyzed the changes in the CD4$^+$IL-17$^+$/Treg ratio over time, we observed a significant reduction at week 4, 16, and week 52, (Fig. 6D) according to the decrease of CD4$^+$IL-17$^+$ T-cells and the increase of Treg (Fig. 5C), suggesting the restoration of a less inflammatory environment.

Increasing evidence shows that Tregs are a heterogeneous population. In particular, an interesting Treg subpopulation expressing IL-17 and RoRγT (IL-17$^+$ Treg) has been described [29]. IL-17$^+$Treg population is characterized by high plasticity and by the ability to

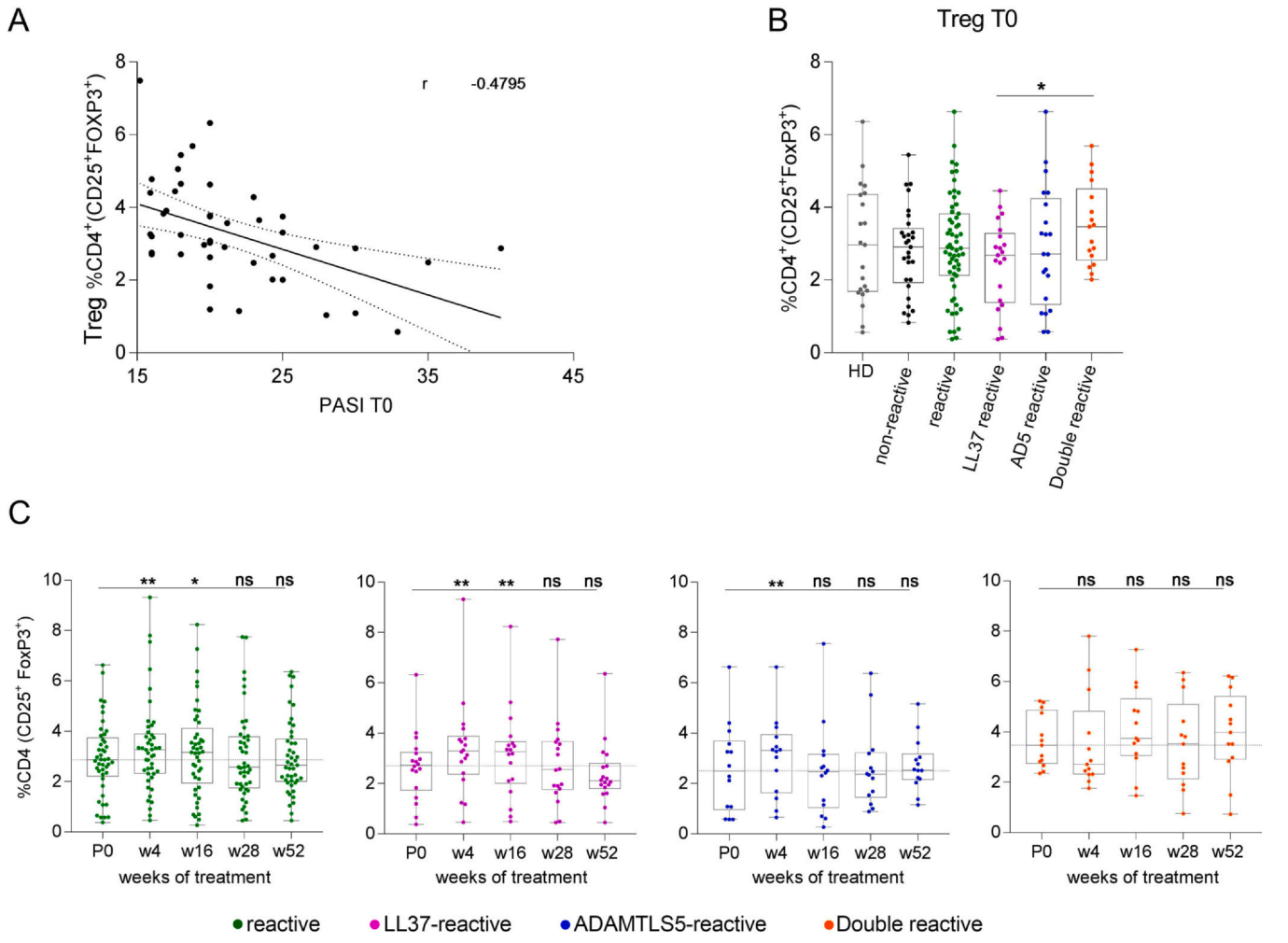

Fig. 5. Tregs frequency negatively correlated with PASI and was modulated by treatment in single LL37- and ADAMTSL5-reactive patients, but not in double-reactive ones. **A.** Treg frequency negatively correlated with baseline PASI in severe patients (PASI≥15; r = -0.479,p = 0.0007; n = 46). Linear regression with 95 % Confidence Interval Pearson correlation. **B.** Treg frequency at baseline in patients subdivided into categories by antigen reactivity. The only significant difference in the frequency of circulating Tregs at baseline was seen for double-reactive subjects compared to single LL37-reactive (p = 0.039), with double-reactive showing the higher value. **C.** Changes after treatment in the frequency of Tregs in patients subdivided into categories by antigen reactivity. B-C: Data represented as Minimum to Maximum graph, Friedman test with Uncorrected Dunn's test applied; significant threshold P < 0.05 two-sided. HD, healthy donors; P0, psoriatic patients at baseline; T0, baseline. * p < 0.05; ** p < 0.01; *** p < 0.001; ns, non-significant.

switch phenotype towards Treg in the presence of IL-7, IL-15, or IL-35 or towards Th17 in the presence of IL-23 or IL-6. We investigated the IL-17$^+$Treg population changes compared to the Treg changes, and monitored the IL-17 Treg/Treg ratio. We found that, in single LL37-reactive patients, the ratio IL-17$^+$Treg/Treg decreased during treatment, while in double-reactive patients, no significant change was evident (Suppl. Fig. 9).

4. Discussion

Our study gives a significant contribution to understanding the effect of IL-23 inhibition through risankizumab on different pathogenic T-cell populations, especially in auto-reactive subjects. In a previous article [26], we demonstrated that single autoreactivity for either LL37 or ADAMTSL5 does not affect the effectiveness of anti-IL23 treatment, while double reactivity to both LL37 and ADAMTSL5 decreases the response to treatment. Consistent with these findings, risankizumab effectively modulated pathogenic T-cell populations in single-reactive patients, whereas in double-reactive individuals, these populations exhibited a distinct modulation pattern compared to single-reactive subjects.

In single-reactive subjects, risankizumab reduced the frequencies of the circulating CD8$^+$IL-17$^+$ and CD8$^+$IL-22$^+$ cells, increased the Treg population in the first 16 weeks of treatment (in particular in HLA-C*06:02$^+$ subjects), and restored the IL-17$^+$CD4$^+$/Treg balance. Risankizumab also significantly reduced the proliferation of auto-reactive T-cells to the autoantigen LL37 in LL37-single-reactive subjects. On the contrary, double-reactive subjects demonstrated differences in their pathogenetic populations at baseline and in their changes after treatment. First of all, while both single-reactive and double-reactive subjects demonstrated higher frequencies of peripheral proliferating Ki67$^+$CD4$^+$ and Ki67$^+$CD8$^+$ T-cells at baseline compared to HD, double-reactive subjects demonstrated higher frequencies of Ki67$^+$CD8$^+$ T-cells also compared to LL37-single-reactive. Secondly, in double reactive subjects, the CD8$^+$IL-17$^+$ population decreased only at week 4 but not at later timepoints. Thirdly, the autoreactive CD4$^+$ and CD8$^+$ T-cells did not consistently decrease after treatment, with the exception of LL37-reactive CD8$^+$ T-cells. ADAMTSL5-autoreactive CD4$^+$ and CD8$^+$ T-cell populations demonstrated inconsistent changes in their frequency as well, suggesting that in double-reactive and ADAMTSL5-reactive subjects, IL-23 inhibition induced by risankizumab does not significantly impact the autoreactivity. Finally, Treg cells are not modulated with treatment in double-reactive subjects, and, interestingly, their frequency was higher at baseline compared to the single LL37-reactive subjects, and this result was PASI-independent.

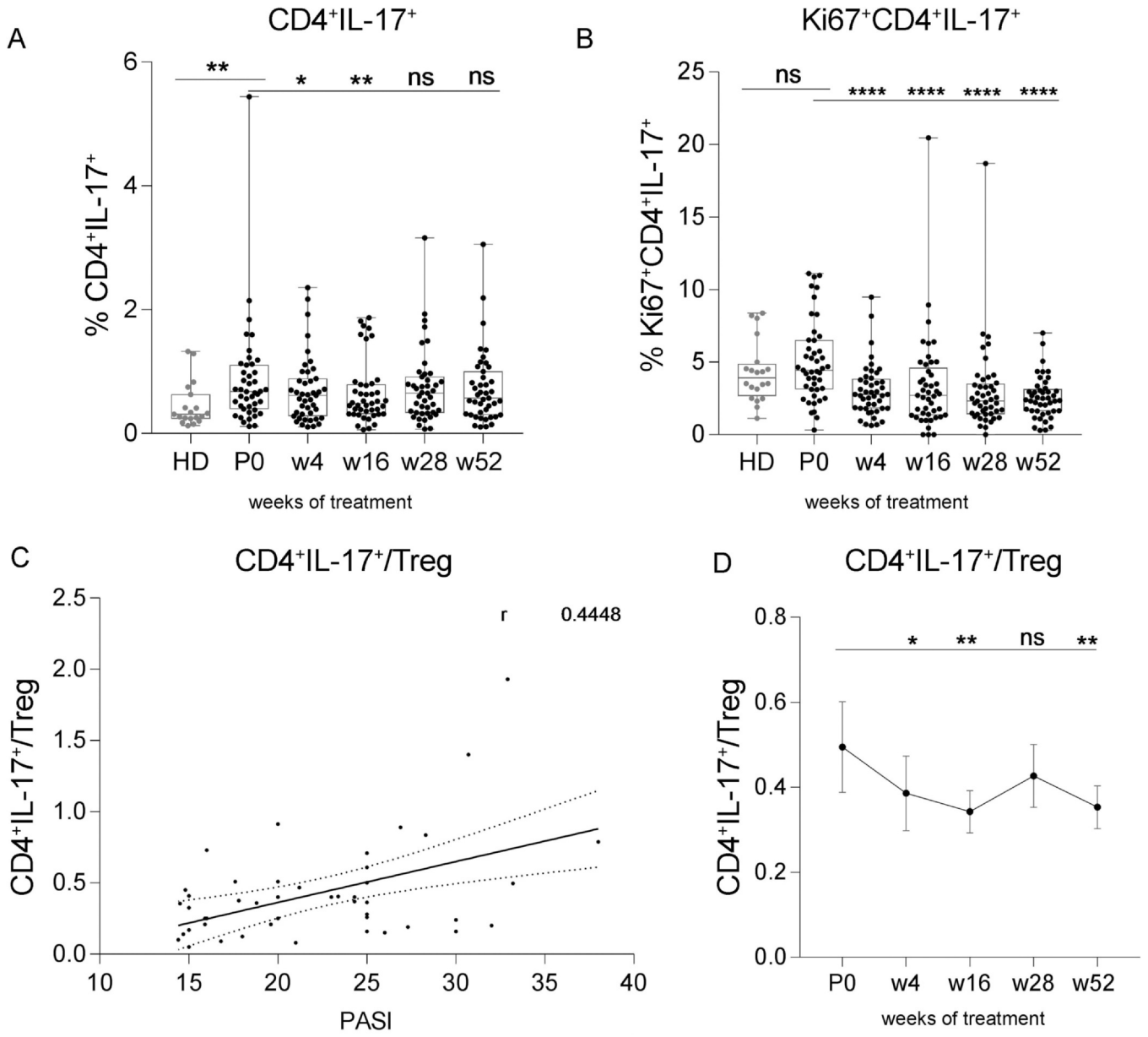

Fig. 6. $CD4^+IL\text{-}17^+$ population and the $CD4^+IL\text{-}17^+$/Treg ratio decreased with risankizumab treatment. **A.** $CD4^+IL\text{-}17^+$ frequency was higher in psoriatic patients compared to HD (0.85 ± 0.84 vs 0.45 ± 0.36, respectively, p = 0.0032) and decreased with treatment at week 4 (p = 0.044) and week 16 (p = 0.0096). Minimum to Maximum representation Mann Whitney or Friedman test with Uncorrected Dunn's test applied; significant threshold p < 0.05 two-sided. **B.** The proliferating $Ki67^+CD4^+IL\text{-}17^+$ population strongly decreased at every time point compared to the baseline. Minimum to Maximum representation Mann Whitney or Friedman test with Uncorrected Dunn's test applied; significant threshold p < 0.05 two-sided. **C.** The $CD4^+IL17^+$/Treg ratio negatively correlated with baseline PASI (r = 0.445; p = 0.0011) in the more severe patients (PASI≥15, n = 52). Linear regression with 95 % Confidence Interval Pearson correlation. **D.** The $CD4^+IL\text{-}17^+$/Treg ratio decreased in psoriatic patients at each time point except for week 28. Mean with SEM connected graph Friedman test with Uncorrected Dunn's test applied; significant threshold P < 0.05 two-sided. HD, healthy donors; P0, psoriatic patients at baseline. * p < 0.05; ** p < 0.01; *** p < 0.001; ns, non-significant.

Our results suggest that the immunological profile of double-reactive patients differs from that of single-reactive and non-reactive individuals, potentially contributing to the distinct clinical response to treatment previously observed in this subgroup. While our findings support a differential behavior of T-cell populations in double-reactive subjects, the underlying mechanisms remain speculative. We hypothesize that in double-reactive patients, the standard dose of risankizumab may be insufficient to induce sustained modulation of pathogenic T-cell subsets. Alternatively, IL-17 production in this group may be maintained through IL-23-independent pathways. These proposed mechanisms are not directly addressed by our study and are interpretations that underscore the need for future mechanistic studies to explore these hypotheses and clarify the distinct immunopathogenic features of this subgroup.

In our study, we also found an interesting decrease in the frequency of circulating MAIT cells and verified the expression of IL23R on their surface. Multiple studies have demonstrated that MAITs express IL-23R, are functionally regulated by IL-23 signaling [18,30,31], and contribute as a source of IL-17 within the circulating $CD8^+$ T-cells [17,32,33]. We found that risankizumab reduced the circulating MAITs that produce IL-17 or IL-22 as early as week 4, suggesting a direct inhibitory effect on these pathogenic IL23R-expressing cells. MAIT cells have been detected in the synovial fluid of patients with psoriatic arthritis [18]. In a previous study, their

enrichment in the joint, coupled with reduced frequencies in peripheral blood, suggested antigen-driven recruitment mediated by CXCR3–CXCL10 interactions and the acquisition of an activated, tissue-resident phenotype [33]. Interestingly, recent evidence suggests that anti-IL-23 treatment could reduce the incidence of PsA in psoriatic patients [22]. Our findings on MAIT cells support their involvement in a potential mechanism that may reduce the risk of developing psoriatic arthritis.

Our study provides valuable insights into the modulation of T-cell populations associated with clinical response to risankizumab, although it is not without limitations. As previously mentioned, it represents a descriptive analysis of the changes in the circulating pathogenetic T-cell populations; thus, the involved mechanisms and the effector functions of the cells can only be speculated. Furthermore, given the systemic nature of the disease, our project focused on the changes in the blood T-cell populations, while changes in the skin compartment were not assessed. In patients with more severe disease, immunological alterations in peripheral blood reflect those occurring within lesional skin, supporting the use of circulating immune signatures to monitor disease activity [34]. Thus, blood-based analyses offer complementary information by capturing systemic immune activity [35]. Nonetheless, integrating skin data would help clarify how risankizumab affects immune shifts over time, providing deeper insight into the impact of IL-23 inhibition on both tissue-resident and circulating immune cells. Finally, although we confirmed the disease specificity of LL37 reactivity by analyzing a small group of atopic dermatitis patients (Suppl. Fig. 10), the inclusion of an additional control group with a different inflammatory skin condition involving tissue remodeling would have further reinforced our findings.

In conclusion, while risankizumab efficiently modifies key pathogenic T-cell populations in single LL37- or single ADAMTSL5-reactive subjects, promoting an anti-inflammatory phenotype, it is less effective in reducing systemic inflammation in double-reactive subjects.

CRediT authorship contribution statement

Rebecca Favaro: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – review & editing, Visualization, Supervision, Project administration. **Paola Facheris**: Conceptualization, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Alessandra Formai**: Methodology, formal analysis, Investigation. **Luigi Gargiulo**: Investigation, Resources, Visualization. **Luciano Ibba**: Investigation, Resources, Visualization. **Giovanni Fiorillo**: Investigation, Resources, Visualization. **Roberta Valeria Latorre**: Methodology, Formal analysis, Investigation. **Jessica Avagliano**: Methodology, Formal analysis, Investigation. **Alessandra Narcisi**: Conceptualization, Investigation, Visualization. **Gianpiero Girolomoni**: Conceptualization, Investigation, Visualization. **Santo Raffaele Mercuri**: Conceptualization, Investigation, Visualization. **Antonio Costanzo**: Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Ethic statements

This study is conducted in accordance with the Note for Guidance on Good Clinical Practice (Humanitas ICH Harmonized Tripartite Guideline E6(R1); the general guideline indicated in the Declaration of Helsinki and all applicable regulatory requirements. EUDRA 2019–004250–28 approved by Humanitas ICH Ethic Committee. Written informed consent was obtained from all participants prior to enrolment.

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This project was founded by Abbvie: A20–034 Psoriasi_Risa Exploring the Effects of IL-23 Inhibition by risankizumab on Psoriasis Autoimmunity.

Data Availability

All the data are available upon request.

Declaration of Competing Interest

R. Favaro A. Formai, R. V. Latorre, J. Avagliano, have no conflict of interest to declare. G. Fiorillo has served as advisory board member for Novartis. P. Facheris has served as a consultant for Eli Lilly and as a speaker for UCB, Abbvie, and Pfizer. L. Gargiulo has served as advisory board member for Abbive, Almirall, Amgen, Eli-Lilly, Leo-Pharma, Sanofi, Novartis, UCB. I. Ibba served as consultants for Almirall. A. Narcisi has served on advisory boards, received honoraria for lectures and research grants from Almirall, Abbvie, Leo Pharma, Celgene, Eli Lilly, Janssen, Novartis, Sanofi Genzyme, Amgen and Boehringer Ingelheim. G. Girolomoni served as consultant and/or speaker for AbbVie, Almirall, Bristol-Meyers Squibb (BMS), Eli-Lilly, LeoPharma, Novartis, Pfizer, Samsung, Sanofi. S. R. Mercuri has served as advisory board member, consultant and speaker for Abbvie, Almirall, Amgen, Leo-Pharma, Eli-Lilly, Janssen, Novartis, Pfizer, Bristol-Meyer Squibb, Sanofi Genzyme, UCB. A. Costanzo has served as an advisory board member, consultant and has received fees and speaker's honoraria or has participated in clinical trials for Abbvie, Almirall, Biogen, LEO Pharma, Eli-Lilly, Janssen, Novartis, Pfizer, Sanofi Genzyme and UCB.

Declaration of Generative AI and AI-assisted technologies in the writing process

Statement: During the preparation of this work the author(s) did not use any tools of Generative AI.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jdermsci.2025.08.002.

References

[1] Y. Liang, M.K. Sarkar, L.C. Tsoi, J.E. Gudjonsson, Psoriasis: a mixed autoimmune and autoinflammatory disease, Curr. Opin. Immunol. 49 (2017) 1–8.
[2] J.E. Gudjonsson, A. Johnston, H. Sigmundsdottir, H. Valdimarsson, Immunopathogenic mechanisms in psoriasis, Clin. Exp. Immunol. 135 (2004) 1–8.
[3] J.L. Harden, J.G. Krueger, A.M. Bowcock, The immunogenetics of psoriasis: a comprehensive review, J. Autoimmun. 64 (2015) 66–73.
[4] I. Sieminska, M. Pieniawska, T.M. Grzywa, The immunology of Psoriasis-Current concepts in pathogenesis, Clin. Rev. Allergy Immunol. 66 (2024) 164–191.
[5] O. Arican, M. Aral, S. Sasmaz, P. Ciragil, Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity, Mediat. Inflamm. 2005 (2005) 273–279.
[6] M.A. Lowes, M. Suárez-Fariñas, J.G. Krueger, Immunology of psoriasis, Annu. Rev. Immunol. 32 (2014) 227–255.
[7] L. Zhou, Y. Wang, Q. Wan, F. Wu, J. Barbon, R. Dunstan, et al., A non-clinical comparative study of IL-23 antibodies in psoriasis, MAbs 13 (2021) 1964420.
[8] Y. Ru, X. Ding, Y. Luo, H. Li, X. Sun, M. Zhou, et al., Adverse events associated with Anti-IL-23 agents: clinical evidence and possible mechanisms, Front Immunol. 12 (2021) 670398.

[9] J.G. Krueger, P.M. Brunner, Interleukin-17 alters the biology of many cell types involved in the genesis of psoriasis, systemic inflammation and associated co-morbidities, Exp. Dermatol. 27 (2018) 115–123.
[10] K.B. Gordon, B. Strober, M. Lebwohl, M. Augustin, A. Blauvelt, Y. Poulin, et al., Efficacy and safety of risankizumab in moderate-to-severe plaque psoriasis (UltIMMa-1 and UltIMMa-2): results from two double-blind, randomised, placebo-controlled and ustekinumab-controlled phase 3 trials, Lancet 392 (2018) 650–661.
[11] K.B. Gordon, M. Lebwohl, K.A. Papp, H. Bachelez, J.J. Wu, R.G. Langley, et al., Long-term safety of risankizumab from 17 clinical trials in patients with moderate-to-severe plaque psoriasis, Br. J. Dermatol. 186 (2022) 466–475.
[12] R.G. Borroni, P. Malagoli, L. Gargiulo, M. Valenti, G. Pavia, P. Facheris, et al., Real-life effectiveness and safety of risankizumab in Moderate-to-severe plaque psoriasis: a 40-week multicentric retrospective study, Acta Derm. Venereol. 101 (2021) adv00605.
[13] J. Liu, H.W. Chang, Z.M. Huang, M. Nakamura, S. Sekhon, R. Ahn, et al., Single-cell RNA sequencing of psoriatic skin identifies pathogenic Tc17 cell subsets and reveals distinctions between CD8(+) t cells in autoimmunity and cancer, J. Allergy Clin. Immunol. 147 (2021) 2370–2380.
[14] B.J. Nickoloff, T. Wrone-Smith, Injection of pre-psoriatic skin with CD4+ t cells induces psoriasis, Am. J. Pathol. 155 (1999) 145–158.
[15] P. Hu, M. Wang, H. Gao, A. Zheng, J. Li, D. Mu, et al., The role of helper t cells in psoriasis, Front Immunol. 12 (2021) 788940.
[16] M.B.M. Teunissen, N.G. Yeremenko, D.L.P. Baeten, S. Chielie, P.I. Spuls, M.A. de Rie, et al., The IL-17A-producing CD8+ T-cell population in psoriatic lesional skin comprises mucosa-associated invariant t cells and conventional t cells, J. Invest Dermatol. 134 (2014) 2898–2907.
[17] M. Dusseaux, E. Martin, N. Serriari, I. Péguillet, V. Premel, D. Louis, et al., Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting t cells, Blood 117 (2011) 1250–1259.
[18] S.K. Raychaudhuri, C. Abria, A. Mitra, S.P. Raychaudhuri, Functional significance of MAIT cells in psoriatic arthritis, Cytokine 125 (2020) 154855.
[19] H.J. Koenen, R.L. Smeets, P.M. Vink, E. van Rijssen, A.M. Boots, I. Joosten, Human CD25highFoxp3pos regulatory t cells differentiate into IL-17-producing cells, Blood 112 (2008) 2340–2352.
[20] J. Kim, A. Moreno, J.G. Krueger, The imbalance between type 17 T-cells and regulatory immune cell subsets in psoriasis vulgaris, Front Immunol. 13 (2022) 1005115.
[21] R. Lande, E. Botti, C. Jandus, D. Dojcinovic, G. Fanelli, C. Conrad, et al., The antimicrobial peptide LL37 is a T-cell autoantigen in psoriasis, Nat. Commun. 5 (2014) 5621.
[22] A. Arakawa, K. Siewert, J. Stöhr, P. Besgen, S.M. Kim, G. Rühl, et al., Melanocyte antigen triggers autoimmunity in human psoriasis, J. Exp. Med 212 (2015) 2203–2212.
[23] L.L. Ten Bergen, A. Petrovic, A.K. Aarebrot, S. Appel, Current knowledge on autoantigens and autoantibodies in psoriasis, Scand. J. Immunol. 92 (2020) e12945.
[24] K.L. Cheung, R. Jarrett, S. Subramaniam, M. Salimi, D. Gutowska-Owsiak, Y.L. Chen, et al., Psoriatic t cells recognize neolipid antigens generated by mast cell phospholipase delivered by exosomes and presented by CD1a, J. Exp. Med 213 (2016) 2399–2412.
[25] M. Yunusbaeva, R. Valiev, F. Bilalov, Z. Sultanova, L. Sharipova, B. Yunusbayev, Psoriasis patients demonstrate HLA-Cw*06:02 allele dosage-dependent t cell proliferation when treated with hair follicle-derived keratin 17 protein, Sci. Rep. 8 (2018) 6098.
[26] R. Favaro, P. Facheris, A. Formai, L. Gargiulo, L. Ibba, G. Fiorillo, et al., Autoreactivity to self-antigens LL37 and ADAMTSL5 influences the clinical response to risankizumab in psoriatic patients, J. Autoimmun. 147 (2024) 103244.
[27] P. Quaglino, M. Ortoncelli, A. Comessatti, R. Ponti, M. Novelli, M. Bergallo, et al., Circulating CD4+CD25 bright FOXP3+ t cells are up-regulated by biological therapies and correlate with the clinical response in psoriasis patients, Dermatology 219 (2009) 250–258.
[28] L. Zhang, X.Q. Yang, J. Cheng, R.S. Hui, T.W. Gao, Increased Th17 cells are accompanied by FoxP3(+) treg cell accumulation and correlated with psoriasis disease severity, Clin. Immunol. 135 (2010) 108–117.
[29] H. Cui, N. Wang, H. Li, Y. Bian, W. Wen, X. Kong, et al., The dynamic shifts of IL-10-producing Th17 and IL-17-producing treg in health and disease: a crosstalk between ancient "Yin-Yang" theory and modern immunology, Cell Commun. Signal 22 (2024) 99.
[30] H. Wang, L. Kjer-Nielsen, M. Shi, C. D'Souza, T.J. Pediongco, H. Cao, et al., IL-23 costimulates antigen-specific MAIT cell activation and enables vaccination against bacterial infection, Sci. Immunol. 4 (2019).
[31] L. Camard, T. Stephen, H. Yahia-Cherbal, V. Guillemot, S. Mella, V. Baillet, et al., IL-23 tunes inflammatory functions of human mucosal-associated invariant t cells, iScience 28 (2025) 111898.
[32] A. Johnston, J.E. Gudjonsson, Psoriasis and the MAITing game: a role for IL-17A+ invariant TCR CD8+ t cells in psoriasis? J. Invest Dermatol. 134 (2014) 2864–2866.
[33] F. Penkava, M.D.C. Velasco-Herrera, M.D. Young, N. Yager, L.N. Nwosu, A.G. Pratt, et al., Single-cell sequencing reveals clonal expansions of pro-inflammatory synovial CD8 t cells expressing tissue-homing receptors in psoriatic arthritis, Nat. Commun. 11 (2020) 4767.
[34] N.J. Korman, Management of psoriasis as a systemic disease: what is the evidence? Br. J. Dermatol. 182 (2020) 840–848.
[35] P. Gjersvik, Biologics: targeting systemic inflammation in psoriasis, Br. J. Dermatol. 179 (2018) 247–248.