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Autoreactivity to self-antigens LL37 and ADAMTSL5 influences the clinical response to risankizumab in psoriatic patients

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ABSTRACT

The autoantigens LL37 and ADAMTSL5 contribute to induce pathogenetic T-cells responses in a subset of psoriatic patients. Whether the presence of LL37-and/or ADAMTS5-reactive T-cells influences the clinical response to treatment is still unknown.

The aim of the study is to evaluate the clinical responses to the anti-IL-23 risankizumab in LL37 and/or ADAMTSL5-reactive patients in comparison with non-reactive ones and to assess whether genetics (HLA-Cw06.02) or BMI influences the response to treatment. Patients were screened at baseline for the presence of circulating LL37 or/and ADAMTSL5-reactive T-cells and were treated as per protocol with risankizumab. Effectiveness data (PASI scores) were collected at weeks 4, 16, 28, 40 and 52. Data were also analyzed based on HLA-Cw06.02 status and BMI.

The overall response to treatment of patients with autoreactivity to LL37 or ADAMTSL5 did not differ compared to the non-reactive cohort as measured as PASI75/90/100 at different time points; however, subjects that had autoreactive T-cells to both LL37 and ADAMTS5 demonstrated suboptimal response to treatment starting at week16. HLA-Cw06:02^{+} patients demonstrated faster response to risankizumab at week 4 compared to HLA-Cw06:02^{-}. Additionally, the response to treatment was influenced by the BMI with slower responses seen in overweight and obese patients at week 4 and week16.

In conclusion, while the presence of either LL37-and ADAMTS5-reactive circulating T-cells do not influence the clinical response to risankizumab, the presence of the double reactivity to both LL37 and ADAMTS5 decreases the clinical responses. Moreover, we evidenced that HLA-Cw06^{+} respond faster to IL-23 inhibition and that BMI, associated to autoreactivity, can influence the speed in response.

1. Introduction

Psoriasis is a chronic inflammatory skin disease affecting 2–3% of individuals in Europe and 4 % in USA [1]. It is mediated by the activation of T-cells that produce pathogenetic cytokines, such as IL-17, IL-22, IL-23, IL-21, INF-γ [1–3]. Some of these key cytokines, namely TNF-α, IL-23, and IL-17 are the target of selective biologic drugs for moderate-to-severe psoriasis [4,5]. Psoriasis is also considered to have an autoimmune base and to be triggered by autoantigens that sustain the psoriatic inflammation, the most frequent being LL37 and ADAMTSL5 [6–8]. LL37-specific T-cells were detected in up to two-thirds of moderate-to-severe psoriasis patients [7], but whether the presence of specific autoreactive T-cells influences the clinical response is still not clear. Among the most recently developed anti-IL-23 therapeutic antibodies, risankizumab has been introduced in US and Europe for the treatment of moderate-to-severe psoriasis [4,5,9]. Risankizumab, not only demonstrated to induce a complete clearance (PASI100) in more than 50 % of patients, but also demonstrated long-term maintenance of

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the remission confirmed in real life studies [9–12]. With this study, we wanted to determine whether the presence of LL37-and/or ADMAMTSL5-reactive T-cells influences the clinical effectiveness of IL-23 inhibition by risankizumab. Additionally, we wanted to explore the effect of one of the most strongly associated psoriasis susceptibility alleles HLA-Cw06:02 [13] and of body mass index (BMI) [14] on the effectiveness of the treatment.

2. Material and methods

2.1. Study population

Patients affected by moderate-to-severe psoriasis (PASI>12 and BSA>10 %) for at least 6 months, attending the Dermatology Unit of the IRCCS Humanitas were enrolled in a one year period (April 2021–April 2022) (n = 142). Informed consent (EUDRA 2019-004250-28 IRCCS Humanitas Ethical Cometee) was signed by each study subject. The patients received risankizumab as per clinical guidelines at baseline, week 4, 16, 28, and 40 and their demographic characteristics, including BMI, are listed in Table 1. PASI was collected at each timepoint until week 52. All subjects were tested at baseline for the presence of HLA-Cw06 allele and for the presence of autoreactivity to LL37 and/or ADAMTSL5. Eighty-seven out of 142 demonstrated autoreactivity to LL37 and/or ADAMTSL5 (Stimulation Index of proliferation, SI > 2) and for 51 of them we collected data at each single time points. Three patients were excluded: one for pregnancy at w16 and two for positivity to tuberculosis at w4. Forty-two screened patients that resulted as non-reactive (SI < 2) to the autoantigens received risankizumab treatment as per clinical guidelines. Thirty-three healthy controls (HC) subject that did not present psoriasis or other dermatologic conditions were also enrolled and blood samples were tested for the presence of auto-antigen reactivity and genetic status (Table 1).

2.2. PBMC isolation and analysis

Peripheral blood was collected after venipuncture in heparin-containing tubes from enrolled patients and donors after informed consent (EUDRA 2019-004250-28 ICH). PBMCs were isolated using Lympholyte®-H human Cell Separation Media and cryopreserved in FBS-10%DMSO in liquid nitrogen until use. Proliferation test was performed with $0.6x10^6$ cells/well in RPMI-1640 supplemented with heat inactivated 10 % AB human serum Merck (H3667) L-glut and Pen/Strep and seeded in 48 flat-bottom wells at 37 °C and 5 % CO_2, stimulated for 6 days with 10 μg/ml of antigenic peptide LL37, LL37 scramble (Innovagen SP-LL37, SP-LL37SCR) as control and ADAMTSL5 (57–67 VRSRRCLRL) peptide (Eurogentec or Sigma Aldrich peptide synthesis) the vehicle solution of 0.1 % DMSO was considered as non-treated (NT) well. One well is stimulated at day 2 or day 4 as positive control of activation with Staphylococcal enterotoxin B (SEB) (SIGMA S4881) 1 μg/ml. Immunostaining is 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) in PBS-2%FBS at 37 °C and after Foxp3/Transcription Factor Fixation/Permeabilization (e-bioscience 00-5521-00) α-Ki67(B56) (BD-561277) at 4 °C. All samples were acquired in a BD-LSR-Fortessa FACS and analyzed with DIVA software (BD). The gating strategy is described in (Suppl Fig. 1). LL37 Stimulation Index is calculated for CD4 and CD8 as ratio of percentage of $CD4^+Ki67^+$ or $CD8^+Ki67^+$ of treated sample LL37 against the respective percentage of LL37 scramble treated sample. ADAMTSL5 stimulation Index is calculated as ratio of percentage of $CD4^+Ki67^+$ or $CD8^+Ki67^+$ of treated sample ADAMTSL5 against the DMSO treated control.

Table 1
Demographic and genetic characteristics of the enrolled populations patients. N/A, UNK; unknown, non-applicable.

Characteristics	Total Tested Psoriatic Patients = 142	Enrolled Reactive Patients n = 51	Healthy controls n = 33
	(%) and mean ± sd	(%) and mean ± sd	(%) and mean ± sd
Male	101/142 (71.1)	37/51 (72.5)	17/33(51.5 %)
Female	41/142 (28.9)	14/51 (27.5)	16/33(48.5)
Mean age	50.21 ± 13.43	46.24 ± 13.41	34.90 ± 9.75
Duration of psoriasis	18,24 ± 14.62	16.96 ± 13,56	N/A
BMI	26.67 ± 4.56	26.81 ± 4.61	UNK
Mean PASI at baseline	15.9 ± 5,7	15.43 ± 5,4	N/A
Genetic HLA-Cw06+	50/142 (35,2)	20/51 (39,2)	2/33(6.06)
Mean onset age	29.82 ± 16.96	28.78 ± 17.15	N/A
Onset HLA-Cw06+	23.96 ± 15.59	22.35 ± 15.56	N/A
Onset HLA-Cw06-	33.08 ± 16.89	32.94 ± 17.08	N/A

2.3. Genotyping

20 ng of DNA extracted from 0.2 ml of blood (Qiagen 51104) was tested for the presence on a 3 % agarose gel of the PCR specific 304 bp product HLA-Cw*06:02, amplified with specific primer (HLA-Cw06F 5′-TACTACAACCAGAGCGAGGA-3'; HLA-Cw06R 5′-GGTCGCAGCCATACATCCA-3′ Bio-Rad Master Mix for PCR (1665009EDU). To confirm HLA-Cw*06:02 genotype and assess the heterozygosis or homozygosis state we tested four TaqMan allele-probes designed for SNPs: rs10484554 (C_29612773_30 Thermofisher), rs3130457 (C__2452188_10), rs6904246 (C_31338447_10) and rs7745906 (C_31338473_10) using 9 ng of DNA with Taqman GTX press master mix (4401892 Thermofisher) as described in Ref. [15]. The reactions run in QuantStudioTM 5–96 well 0.2 ml block and SNPs analysis is performed with QuantStudioTM Design and Analysis Software v1.43.

2.4. Statistical analysis

Continuous variables were presented as mean and standard deviation (SD) or mean and Standard Error for the Mean (SEM). Discrete variables were summarized as frequencies and percentages. Data were compared using *t*-test (unpaired *t*-test with Welch's correction) for single comparisons, multiple *t*-test or ANOVA for multiple comparisons. Non-normal distributions were tested using Kruskal-Wallis uncorrected Dunn's test or multiple *t*-test. For categorical variables, Fisher's exact test or χ^2-test was used to calculate P-value. To evaluate the impact of multiple independent variables on clinical outcomes, a multivariate linear regression was performed (Stata/SE 17.0 Software was used), considering parameters such as BMI, auto-reactivity status, and HLA-Cw06:02 status. GraphPad Prism 7.1 Software and Stata/SE 17.0 were used to analyze data with the appropriate statistical test. The significance threshold was defined as P < 0.05 (2-sided).

3. Results

3.1. Frequency and level of autoreactivity to LL37 and ADAMTSL5 were higher in psoriatic patients and HLA-Cw06:02 positivity was associated with a higher proliferation index (SI) in LL37-reactive patients

In the 142 screened patients the reactivity to antigens LL37 or ADAMTSL5, defined as proliferation index SI > 2, was statistically associated with psoriasis (87/142) $\chi^2 = 5.23$ P = 0.022 compared to HC (13/33). The 87 reactive patients for the autoantigens resulted in 31 (22 %) positive for LL37, 37 (26 %) for ADAMTSL5, and 19 patients shown a positive SI for both the antigens (13 %). HLA-Cw06:02 allele was strongly associated with psoriasis and was present in 35 % of psoriatic

patients (50/142) $\chi^2 = 10.89$ P = 0.001 (two homozygotes) compared to 6 % (2/33) in HC. HLA-Cw06:02$^+$ subjects also demonstrated a statistically significant early onset of psoriasis compared to HLA-Cw06$^-$ with a mean age of onset of 23.96 ± 15.59 compared to 33.08 ± 16.89 (P = 0.0023) confirming previous reports in the literature [14] (Table 1).

Overall, compared to HC, psoriatic patients demonstrated to have a higher SI for both CD4$^+$ and CD8$^+$ T-cells when stimulated with LL37 (SI CD4 1.95 ± 3.76 vs HC 0.93 ± 0.66 P = 0.003; CD8 SI 1.56 ± 1.84 vs HC 0.77 ± 0.94 P = 0.0007) and ADAMTSL5 (SI CD4 2.72 ± 6.71 vs HC 1.42 ± 1.26 P = 0.033; CD8 SI 2.18 ± 4.05 vs HC 1.88 ± 1.62 P = 0.015) (Fig. 1A–B). No significant differences among psoriatic patients based on HLA-Cw06 status were seen for LL37 or ADAMTLS5 reactivity (Fig. 1A–B).

Interestingly, analyzing the reactive patients, we observed that among LL37-reactive patients, HLA-Cw06:02$^+$ subjects demonstrated a higher SI compared to HLA-Cw06$^-$ for both CD4$^+$ (SI 4.37 ± 7.68 and 1.38 ± 1.28 P = 0.02) and CD8$^+$ (SI 3.38 ± 4.41 and 1.56 ± 1.81 P = 0.04) stimulated T-cells, while no differences were observed in the ADAMTLS5-reactive subjects when stimulated with ADAMTSL5 antigen (Fig. 2).

3.2. The presence of double reactivity to both LL37 and ADAMTSL5 influences the clinical response to risankizumab and HLA-Cw06:02$^+$ patients demonstrated a faster response to treatment

We evaluated the effectiveness of risankizumab as PASI 75, 90 and 100 at different time points and how the autoreactivity status influenced the clinical response. Risankizumab demonstrate high effectiveness on both single reactive (SI > 2 for LL37 or ADAMTSL5) and non-reactive (SI < 2) patients' population and no statistically significant differences were seen between the two groups except for PASI100 at w52 (Table 2 and Supplementary Table 1). However, when considering the effectiveness in double-reactive patients (SI > 2 for LL37 and ADAMTSL5), we found a strong statistically significant difference compared to single reactive subjects (SI > 2 for LL37 or ADAMTSL5 only) (Fig. 3A, Table 2 and Supplementary Table 1). Differences in PASI90 and PASI100 were evident as early as week 16 and at week 28 for PASI75 (Fig. 3A–Table 2).

When we considered the effect of the specific autoreactivity on PASI reduction, patients that were double reactive demonstrated a strong statistically significant decrease of effectiveness starting at week 16 until week 52 compared with single reactive and non-reactive patients. (Fig. 3B).

We also assessed if the presence of the HLA-Cw06:02 allele could influence risankizumab effectiveness. At week 4, HLA-Cw06:02$^+$

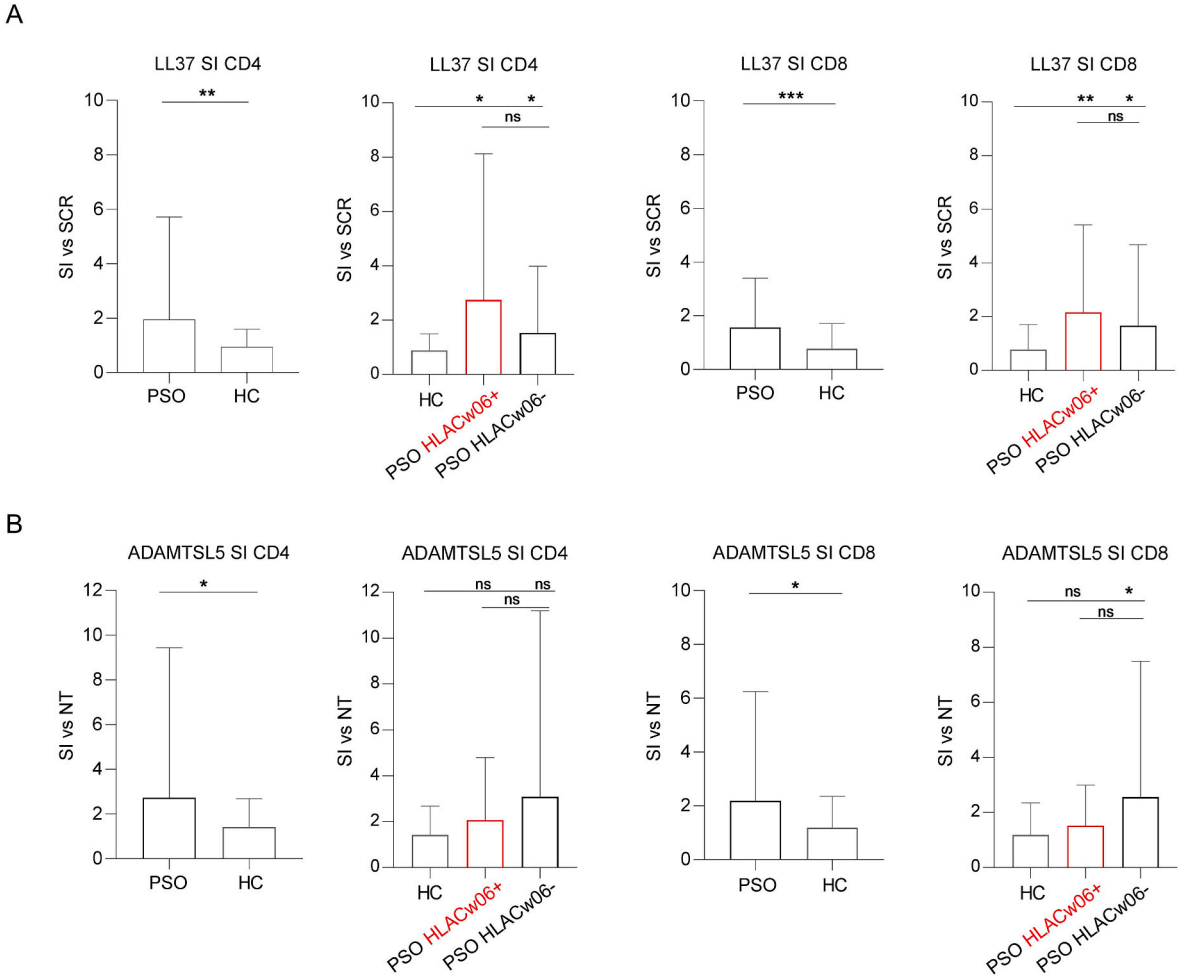

Fig. 1. Reactivity of CD4$^+$ and CD8$^+$ T-cells to LL37 and ADAMTSL5 antigens in enrolled patients.Histograms representing the distribution of value as mean and standard deviation of T-cells (CD4$^+$ and CD8$^+$) stimulation index of proliferation (SI) after antigen stimulation. **A.** LL37 stimulation index of proliferation in 142 psoriatic (PSO) patients and in psoriatic patients tested for HLA-Cw06 positivity: PSO HLA-Cw06$^+$ (red) and PSO HLA-Cw06$^-$ (black), for CD4$^+$ and CD8$^+$ in comparison with HC. **B.** ADAMTSL5 stimulation index of proliferation in 142 psoriatic (PSO) patients and in psoriatic patients tested for HLA-Cw06 positivity, CD4$^+$ and CD8$^+$ in comparison with HC. Unpaired T-test with Welch's correction (P value < 0.05 set as significance threshold, 2-sided). SCR is scramble for LL37 (SP-LL37SCR); NT, non-treated well.

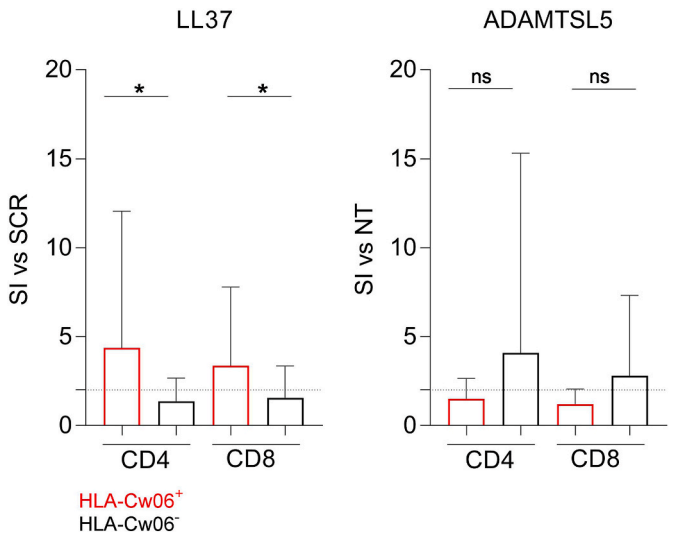

Fig. 2. HLA-Cw06:02 influence the reactivity of $CD4^+$ and $CD8^+$ T-cells to LL37 antigen. Histograms representing the distribution of value as mean and standard deviation of T-cells ($CD4^+$ and $CD8^+$) SI after antigen stimulation. Analyzing the 51 reactive patients, LL37 stimulation is significant higher both in $CD4^+$ and in $CD8^+$ T-cells in the HLA-Cw06 positive (red) while no significant difference was detected with ADAMTSL5 stimulation. Unpaired T-test with Welch's correction (P value < 0.05 set as significance threshold, 2-sided). SI, stimulation index; SCR is scramble for LL37 (SP-LL37SCR); NT, non-treated well.

Table 2
Percentage of double (LL37 and ADAMTSL5), single (LL37 or ADAMTSL5) reactive SI > 2 and non-reactive (SI < 2) patients that reached PASI75, 90, 100 at the weeks indicated. Fisher's exact test P values were calculated between single-reactive and non-reactive subjects and between double-reactive and single reactive subjects. Significant p-values ($p < 0.05$) are indicated in bold: they were nonsignificant between single-reactive and non-reactive at the different weeks, while they were significant between double-reactive and single reactive at different time-points (see also Supplementary Table 1 and Fig. 3A).

Double Reactive (SI > 2) LL37 and ADAMTSL5					
week	4	16	28	40	52
% (pz PASI/ tot pz)					
PASI 75	16.67(2/12)	**83.33 (10/12)**	**75.00(9/12)**	**75.00(9/12)**	**75.00(9/12)**
PASI 90	0.00(0/12)	**41.67(5/12)**	75.00(9/12)	**66.67(8/12)**	**58.33(7/12)**
PASI 100	0.00(0/12)	**16.67(2/12)**	**33.33(4/12)**	41.67(5/12)	**41.67(5/12)**
Single Reactive (SI>2) LL37 or ADAMTSL5					
week	4	16	28	40	52
% (pz PASI/ tot pz)					
PASI 75	24.32(9/37)	97.22 (35/36)	100.00 (36/36)	100.00 (36/36)	100.00 (36/36)
PASI 90	2.7(1/37)	80.56 (29/36)	94.44(34/36)	94.44(34/36)	97.22(35/36)
PASI 100	0.00 (0/37)	63.89 (23/36)	69.44(25/36)	72.22(26/36)	86.11(31/36)
Non-reactive (SI<2)					
week	4	16	28	40	52
% (pz PASI/ tot pz)					
PASI 75	27.27(6/22)	95.12 (39/41)	95.24(40/42)	95.24(40/42)	92.86(39/42)
PASI 90	4.55(1/22)	80.49 (33/41)	90.48(38/42)	88.10(37/42)	85.71(36/42)
PASI 100	0.00	41.46 (17/41)	57.14(24/42)	64.29(27/42)	59.52(25/42)

(heterozygotes and homozygotes) subjects demonstrated a statistically greater PASI reduction compared to HLA-Cw06:02$^-$ patients ($P = 0.009$), while no differences were seen at later time points (Fig. 3C). This suggests a faster response to IL-23 inhibition for HLA-Cw06$^+$ subjects.

3.3. The influence of BMI and auto-reactivity on the clinical response to IL-23 inhibition

We then evaluated the influence of BMI on the clinical response to risankizumab in autoreactive patients. Using a multivariate analysis, we assessed that BMI did not influence the clinical response to treatment at any timepoint (Table 3). On the opposite, using the same analysis, the double reactivity to both LL37 and ADAMTSL5 demonstrated to reduce the clinical response compared to single LL37-and ADAMTSL5-reactive patients at week 16, 28, 40 and 52, similarly to what we reported in the previous 3.2 paragraphs (Table 3).

However, we noted that the only 3 patients that were considered non-responders (failure to achieve PASI75 at week 28) were double-reactive and normal-weight patients. We considered that in these three patients, the resistance to biologic treatment was due mainly to the double-reactivity status. For this reason, we also performed a univariate analysis considering only BMI excluding these three non-responder normal-weight patients. In this analysis, overweight and obese patients demonstrated a lower PASI reduction compared to normal-weight patients at week 4 in both the LL37-or ADAMTSL5-reactive patients (normal weight vs obese $P < 0.0001$, normal weight vs overweight $P = 0.0008$) and non-reactive one (normal weight vs obese $P = 0.018$ normal weight vs overweight $P = 0.0011$, Fig. 4). LL37-or ADAMTS5-reactive patients showed a significant difference in PASI reduction also at week 16 in overweight and obese patients compared to normal weight (normal weight vs obese $P = 0.019$ and normal weight vs overweight $P = 0.042$). From these data, it appears that higher BMI can slower the clinical response, especially in reactive patients.

4. Discussion

Patients' stratification based on the predictivity of response to treatment is fundamental in the current biologic treatment landscape. Early or even pre-treatment identification of responders and non-responders to specific drugs can avoid treatment failures and maximize the balance between costs and effectiveness of therapy. According to a recent multicenter real-world study, bio-naïve patients and those with a short disease history (≤2 years) are more likely to achieve better clinical outcomes to risankizumab [16]. Moreover, there is growing evidence that early treatment with the most suitable drug can impact the history of the disease and prevent the onset of comorbidities such as psoriatic arthritis [17]. Thus, knowing how the presence of variables such as auto-reactivity status and genetic predisposition (HLA-Cw06:02) can influence the response to treatment is paramount.

The frequency of autoreactivity in our cohort was 22 % for LL-37, 26 % for ADAMTLS5 and 13 % for the double reactivity for both LL37 and ADAMTLS5. Unsurprisingly, the entire cohort (reactive and non-reactive) of psoriatic patients demonstrated a higher reactivity to both LL37 and ADAMTSL5 compared to HC in the $CD4^+$ and $CD8^+$ T-cell compartments, confirming their pathogenetic role in psoriasis [6,7].

HLA-Cw06 is strongly associated with psoriasis, and in particular with Type I psoriasis [18,19]. While HLA-Cw06 status did not influence the reactivity of $CD4^+$ and $CD8^+$ T-cells to the autoantigens in the whole cohort of psoriatic patients, in LL-37-reactive patients only, HLA-Cw06$^+$ subjects demonstrated a higher proliferation of $CD4^+$ and $CD8^+$ compared to HLA-Cw06$^-$ subjects. Previous studies demonstrated that LL37 binds to HLA-Cw06:02 and is presented to $CD8^+$ T-cells [7,20], thus, the higher SI obtained is this cohort is probably sustained by a higher T-cells activations in these predisposed individuals. Interestingly HLA-Cw06$^+$ also influenced the speed of the response to anti-IL-23 treatment, with HLA-Cw06$^+$ patients achieving a higher PASI

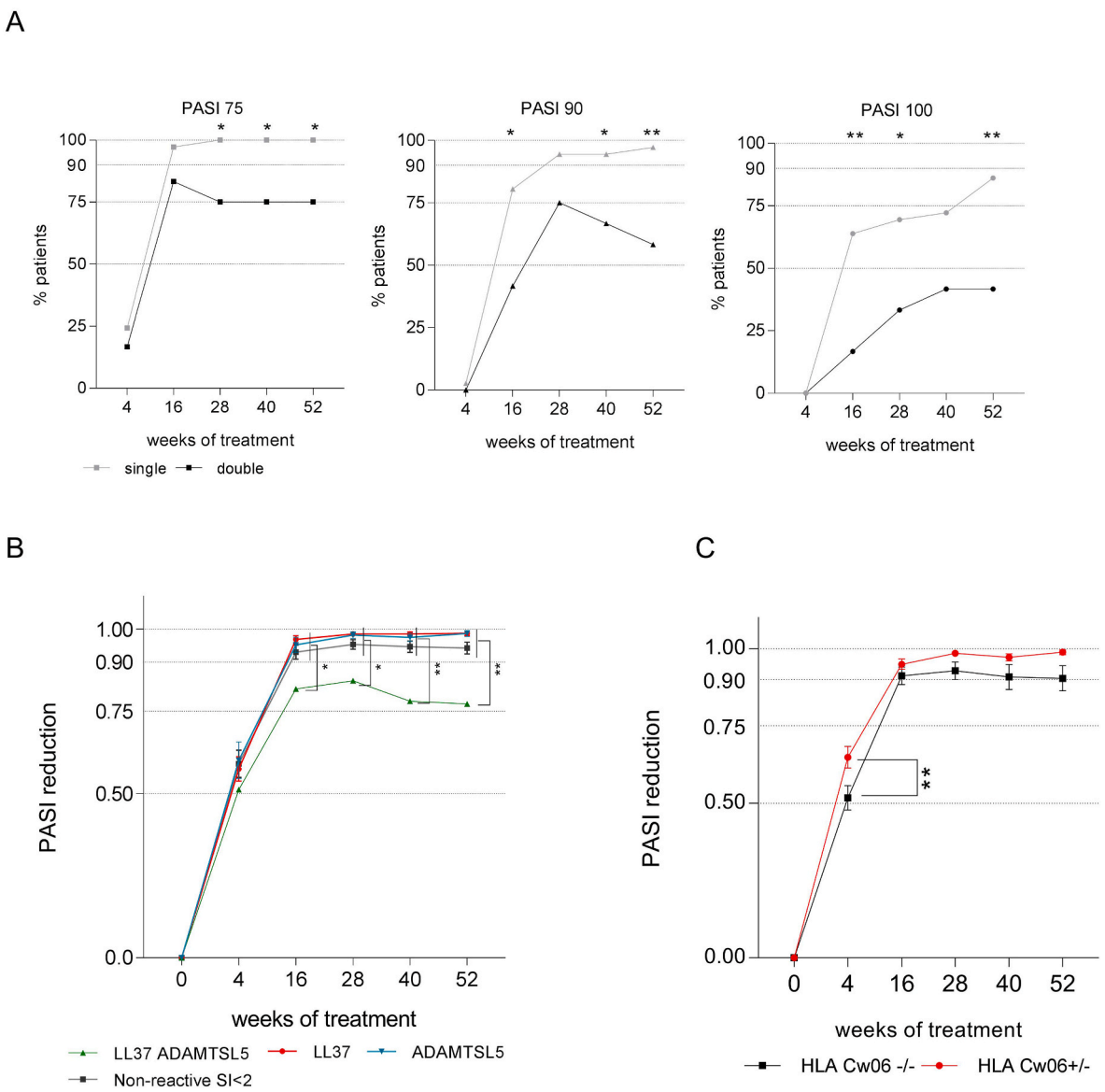

Fig. 3. Effectiveness of risankizumab is modulated by LL37 and ADAMTSL5 reactivity and by HLA-Cw06 status. A. Effectiveness of risankizumab expressed as percentage of patients that reach PASI75, PASI90 and PASI100 in single-reactive (LL37 or ADAMTSL5 SI > 2, light gray) vs double-reactive subjects (LL37 and ADAMTSL5 SI > 2, dark gray). Fisher's exact test calculated as two-tailed p-values (Supplementary Table 1). Significant p-values (*) $p < 0.05$, (**), $p < 0.01$. **B.** Effectiveness of risankizumab expressed as % of PASI reduction in all four categories: LL37-reactive only (red), ADAMTSL5-reactive only (blue), double LL37-and ADAMTSL5-reactive (green) and non-reactive (gray). Double-reactive subjects demonstrated a significant lower PASI reduction at all timepoints compared to LL37-reactive only (p-values at week 16, week 28, week 40 and week 52 were P = 0.010, 0.015, 0.0006 and 0.0003, respectively), ADAMTSL5-reactive only (P values at week 16, week 28, week 40 and week 52 were P = 0.048, 0.042, 0.005 and 0.002, respectively) and non-reactive patients (P values at week 16, week 28, week 40 and week 52 were P = 0.027 and 0.029, 0.001 and 0.009, respectively). P-values were calculated as Multiple T test without correction.**C.** Effectiveness of risankizumab expressed as %PASI reduction based on genetic status: HLA-Cw06:02^{+} (red) vs HLA-Cw06:02^{-} (black).

Table 3
Effect of BMI, HLA-Cw06 and reactivity to LL37 and ADAMTSL5 on the PASI variation at the different timepoints of risankizumab treatment. Multivariate regression data analysis was used to evaluate the role of each independent variable. Starting at week 16 the double auto-reactivity strongly modulate the efficacy of the treatment, while no effect of BMI or HLA-Cw06:02 or single reactivity to LL37 or ADAMTSL5 was noted.

	BMI		HLACw06		Double Auto-reactivity			
					versus LL-37		versus ADAMTSL5	
	Coefficient	p-value	Coefficient	p-value	Coefficient*	p-value*	Coefficient°	p-value°
Week 4	−0.05	0.20	0.12	0.06	0.03	0.64	0.08	0.26
Week 16	−0.02	0.41	0.01	0.76	**0.15**	**0.002**	**0.13**	**0.006**
Week 28	0.03	0.18	0.05	0.15	**0.13**	**0.006**	**0.14**	**0.003**
Week 40	0.05	0.098	0.06	0.25	**0.18**	**0.005**	**0.19**	**0.003**
Week 52	0.05	0.08	0.08	0.11	**0.19**	**0.003**	**0.21**	**0.001**

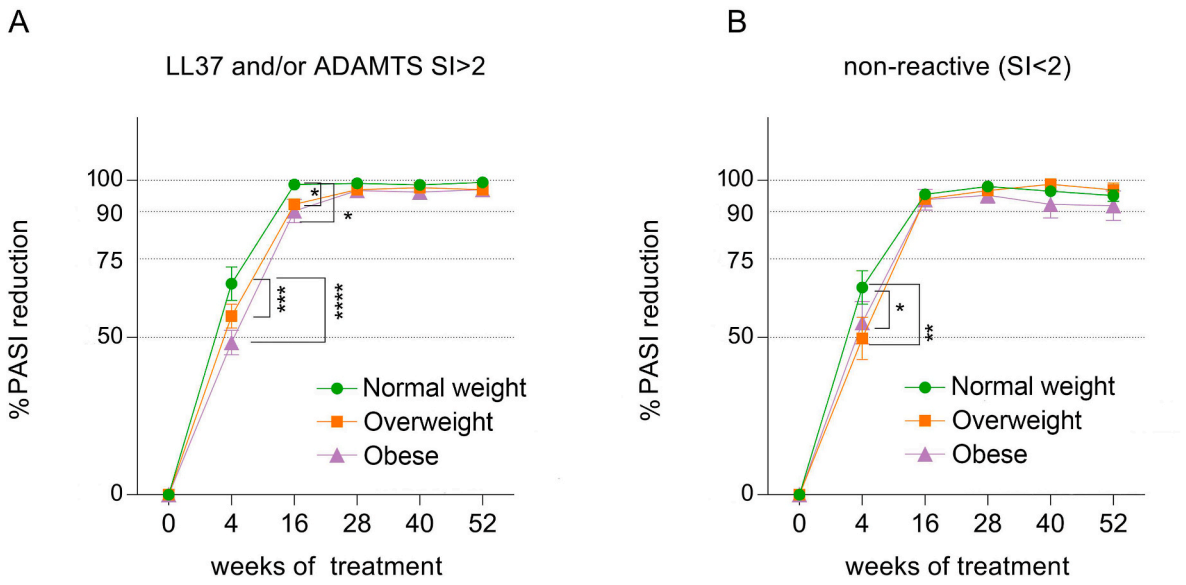

Fig. 4. Univariate analysis of the effect of BMI on risankizumab effectiveness in reactive and non-reactive patients. Overweight and obese patients show slower clinical responses in both reactive and non-reactive patients at week 4 and in reactive patients at week 16 compared to normal weight patients. Normal-weight patients is defined as BMI 18.5–24.99 (green), overweight is defined as a BMI 25–29.99 (orange), obese patients as BMI 30–40 (violet).

reduction at week 4. This is consistent with previous findings regarding IL-23 inhibition in HLA-Cw06^{+} subjects. A metanalysis demonstrated a modest superiority of the IL-12/23 inhibitor ustekinumab (IL-12/IL-23 inhibitor) in HLA-Cw06^{+} subjects compared to HLA-Cw06^{-} that reached a higher PASI75 at 6 months. A large observations study including 1326 subjects demonstrated that ustekinumab is preferable in HLA-Cw06^{+} psoriatic patients compared to adalimumab [21].

In line with the results observed in clinical trials [4,10] and real-world data [11], risankizumab demonstrated to be a very efficacious drug, reaching high rates of PASI90 and PASI100 in the entire psoriatic cohort. Patients showing autoreactivity for LL37 or ADAMTSL5 responded similarly to non-autoreactive patients. Interestingly, the presence of the double auto-reactivity to both LL37 and ADAMTSL5 decreased the response to treatment starting at week16 until week52. We supposed that the double-reactivity predispose patients to have higher inflammatory burden that leads to a suboptimal effectiveness of the inhibitory effect of IL-23, stabilizing treatment response around PASI75, compared to PASI90 and PASI100 that were reached by non-reactive or single-reactive patients.

Obese and overweight patients have often demonstrated suboptimal responses to biologic treatment [14]. In our study, we first observed no differences in the multivariate analysis, however, the combination of non-responsivity, double reactivity and normal weight in the only 3 non-responders patients could influence the result of the multivariate analysis. The univariate analysis, in fact, suggests that BMI can influence mainly the speed of response to treatment, but not the overall long-term response, especially in double-reactive subjects. In fact, lower PASI reductions were observed at week 4 and week16, while no differences were observed at later time points.

5. Conclusions

In our study, we demonstrated that the presence of LL37 or ADAMTS5 autoreactive T-cells does not influence the overall response to risankizumab, however, the double autoreactivity to both LL37 and ADAMTS5 reduces the effectiveness of risankizumab starting at week 16. Moreover, we found that HLA-Cw06^{+} subjects respond faster to risankizumab, while slower responses were observed in subjects with higher BMI.

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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.

CRediT authorship contribution statement

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

Declaration of Competing interest

R. Favaro A. Formai, L. R. V. Latorre, J. Avagliano, G. Fiorillo and S. R. Mercuri Declarations of interest: none. P. Facheris has served as a consultant for Eli Lilly. L. Gargiulo and L. Ibba has been a consultant 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, Amgen, Biogen, Boehringer-Ingelheim, Bristol-Meyers Squibb, Eli-Lilly, LeoPharma, Novartis, Pfizer, Samsung, Sanofi and 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, Lilly, Janssen, Novartis, Pfizer, Sanofi Genzyme and UCB-Pharma.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2024.103244.

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