

# **Predictive role of cutaneous lymphocyte-associated antigen (CLA) in TNF- $\alpha$ inhibitor treatment of psoriasis**

Thesis of doctoral (PhD) dissertation

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## **Introduction**

Due to the advanced scientific knowledge psoriasis, as a member of the immune-mediated inflammatory disease (IMID) group, has been re-defined in the recent years. It has been proven that by causing systemic inflammation psoriasis is not a disease solely confined to the skin. Now it is considered to be a complex disorder with several co-morbidities providing both physical as well as mental burden to the affected patients and reducing their life expectancy and the quality of life. Biologic response modifiers selectively targeting certain steps of the immune-pathogenesis have revolutionized the treatment of severe psoriasis. Due to specific inhibition of the immune system they have significantly improved the therapeutic risk-benefit ratio, when compared to previous treatment modalities. In spite of the undoubted effectiveness we have to keep in mind some aspects of the applicability of biologics. In 20 to 30 percent of the patients starting TNF- $\alpha$  inhibitor biological therapy we have to interrupt treatment in 2 years and change to another (often another TNF- $\alpha$  inhibitor) biologic agent. This becomes necessary mostly because of the loss of effectiveness and to a lesser extent, because of the therapy's possible side effects. On the other hand, high costs of the drugs financially restrict the overall availability. Preventing unnecessary complications of biologics and financial considerations are the main causes why we urgently need reliable predictive biomarkers of long-term effectiveness of anti-TNF- $\alpha$  therapy.

In the last few years we performed a study involving our own patients which aimed to collect data on the predictive role of cutaneous lymphocyte-associated antigen (CLA) in TNF- $\alpha$  inhibitor treatment of psoriasis. Accordingly, we thoroughly investigated the role of this skin-specific homing molecule under physiologic conditions, in certain dermatologic diseases and especially in the pathogenesis of psoriasis. Our applied study methods, the results of the investigation and our conclusions are summarized below.

### **Aims of the study**

Due to the lack of an accepted biomarker our primary aim was to study the possibilities of predicting long-term effectiveness of TNF- $\alpha$  inhibitors in psoriasis. The thorough examination of the pathomechanism led to our hypothesis that CLA as a skin-specific homing marker of pathogenic T-lymphocytes and consequently, a key molecule in the pathogenesis might be a suitable predictive marker of biological treatment. We aimed to answer the following questions:

1. Is there a difference between healthy, non-psoriatic adults and severe psoriatic patients before TNF- $\alpha$  inhibitor therapy in peripheral lymphocyte CLA expression?
2. Do peripheral initial CLA expression rates differ significantly in the responder and relapsing patient groups before starting anti-TNF- $\alpha$  treatment?
3. Is there a significant difference between CLA tendencies of these two patient populations during the induction treatment period (first 6 weeks)?
4. Do changes of the PASI values (representing clinical response) correlate with changes of CLA expression measured during the initial treatment phase?
5. Based on the answers to the above questions, can we consider peripheral lymphocyte CLA expression monitored in the induction treatment period as an acceptable predictive biomarker regarding long-term effectiveness of TNF- $\alpha$  inhibitor therapy of severe psoriatic patients?

## Methods

### Patients

38 patients with chronic, large plaque psoriasis were involved into the study. Inclusion criteria corresponded to those of initiation of TNF- $\alpha$  inhibitor therapy. Regarding gender, age, disease duration, co-morbidity status and previous medications no criteria were defined at all. Based on the random selection the study population consisted of 29 men and 9 women (median age: 43.7, from 20 to 68, years). The control group with 5 additional, non-psoriatic healthy adults served to compare initial CLA expression values. Approval of the regional ethical committee was obtained. (No. 78/2011). During the study process we complied with the principles of Helsinki Declaration. Patients were informed about aims and details of the investigation and gave written informed consent. Requested investigations were performed before starting therapy and we found no contraindication of treatment in any cases. Anti-TNF- $\alpha$  agents in their standard doses were initiated according to the S3 European Protocol. 14 patients got infliximab, while 10 adalimumab and the remaining 14 etanercept in monotherapy.

### Monitoring of clinical responsiveness

Clinical state of the patients was described by calculated PASI (Psoriasis Area and Severity Index) values. The PASI index was documented before therapy initiation, then at the end of weeks 12 and 24, referring to initial and long-term therapeutic response, respectively. PASI determination was always done by the same doctor. Effectiveness of the treatment was judged individually, based on the initial clinical picture and the observed changes during the 24-week-long study period.

#### Flow cytometric measurement of peripheral CLA expression

CLA expression was measured in peripheral blood samples collected once from healthy controls as well as from psoriatic patients before starting therapy and at the end of the 2<sup>nd</sup> and 6<sup>th</sup> weeks. Red blood cells were eliminated from the samples by using FACS Lysing Solution (PharMingen International, BD Biosciences, San Jose, CA, USA). Cell phenotype analysis was performed by using a 3-color staining system; the applied monoclonal antibodies were preconjugated with fluorescein isothiocyanate (FITC), phycoerythrin (RPE) or RPE cyanin 5 (RPE-CY5). For the purpose of identifying CLA<sup>+</sup> lymphocytes and CD4<sup>+</sup>CLA<sup>+</sup> as well as CD8<sup>+</sup>CLA<sup>+</sup> T-cell subsets we applied following monoclonal antibodies: anti-human CLA (HECA 452, FITC; PharMingen International, BD Biosciences, San Jose, CA, USA), anti-human CD4 (RPE-CY5; Dako, Glostrup, Denmark), anti-human CD8 (RPE; Dako, Glostrup, Denmark) and specific isotype controls (Rat IgM  $\kappa$ -FITC, PharMingen International, BD Biosciences, San

Jose, CA, USA; Mouse IgG1 RPE and Mouse IgG1 RPE-Cy5, Dako, Glostrup, Denmark). All antibodies were pretitrated. CLA expression of circulating lymphocytes was measured by a bench-top flow cytometer (FACSCalibur, PharMingen International, BD Biosciences, San Jose, CA, USA) and the results were evaluated by the program CellQuest Pro (PharMingen International, BD Biosciences, San Jose, CA, USA). 100 thousand events were measured in all samples. We identified the different cell populations by the typical flow cytometric dot-plot graphics. Forward scatter (FSC) of the laser light gave information on cell size, while side scatter described the granularity and morphologic characteristics of the cells analyzed. The fluorochrome staining served to detect the evoked fluorescent light and consequently, to examine the target parameters. CLA expression was determined by gating for the lymphocyte population and comparing CLA<sup>+</sup> lymphocytes to the total white blood cell number (WBC)/the whole lymphocyte subpopulation (CLA tot%, CLA lymph%) as well as CLA<sup>+</sup>CD8<sup>+</sup> and CLA<sup>+</sup>CD4<sup>+</sup> T-lymphocyte subsets to WBC (CLA CD8<sup>+</sup> Tlymph%, CLA CD4<sup>+</sup> Tlymph%). Control measurements were subtracted from calculated CLA values in order to gain real expression values.

In order to examine the independent predictive role of CLA, we compared some clinical factors in the two patient groups with opposite long-term clinical outcome which have been reported to potentially modulate effectiveness of TNF- $\alpha$  inhibitors, eg. disease severity before starting

therapy (initial PASI), disease duration, joint involvement and previous administration of TNF- $\alpha$  inhibitors.

### Statistical analysis

PASI and CLA values were analyzed by paired Student's t-test and Wilcoxon matched pairs signed rank test using the Statistica 7.0 software (StatSoft, Inc., 1984-2004, Tulsa, USA). An error probability lower than 5% ( $p < 0.05$ ) was considered significant.

PASI changes of the whole patient group between weeks 12 and 24 (representing long-term clinical responsiveness) were compared with CLA changes of the initial treatment period (CLA week 0 vs. week 2; CLA week 0 vs. week 6; CLA week 2 vs. week 6) by using Pearson's correlation analysis. Results were also accepted as significant when  $p < 0.05$ .

## Results

### Clinical responsiveness

All the 38 involved patients presented with severe plaque type psoriasis and accordingly, with high PASI values. Mean initial PASI of the whole patient group was 24.34. In the first 12 weeks of treatment all patients showed a



remarkable improvement of skin symptoms with a significant decrease of the PASI values. As a sign of the overall excellent initial responsiveness every patient achieved PASI75 clinical improvement (mean 90% PASI improvement). Regarding long-term effectiveness, at the end of the 24<sup>th</sup> week psoriatic skin symptoms of 32/38 patients showed a long-lasting regression. These “responder” patients maintained PASI75 response with a significantly reduced mean PASI of 1.14 SD  $\pm$  1.66. All of the 20/32 responder patients, who achieved PASI90 improvement till the 12<sup>th</sup> week, maintained this result till the end of week 24. That time we found a total of 30/32 responders with PASI90. On the contrary, there was a significant clinical relapse to observe in the remaining 6/38 patients with reappearance of large psoriatic plaques. These “relapsing” patients lost initial PASI75 between weeks 12 and 24 and showed <60% PASI improvement (PASI60) at the end of the 24<sup>th</sup> week when compared to initial values. At the end of the study period their average PASI was significantly re-increased and calculated 13.10 SD  $\pm$  2.56. (Figure 1)

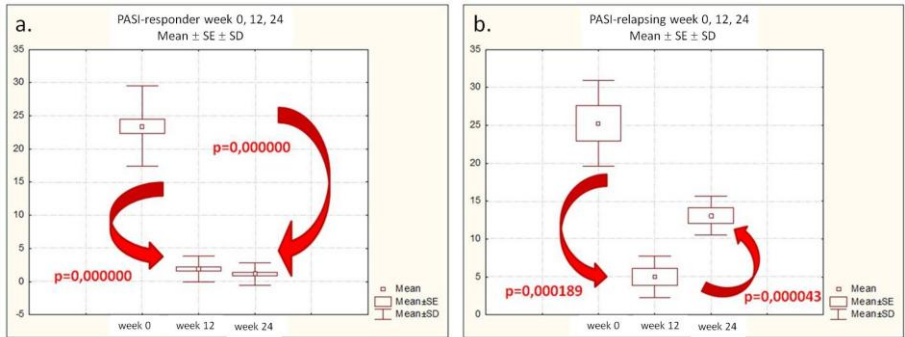


Figure 1. Changes of the PASI values referring to clinical responsiveness in the a.) responder and b.) relapsing patient groups. (with p values of paired Student's t-test)

### Changes of peripheral CLA expression

In the first 6 weeks of initial treatment period an increasing tendency of peripheral CLA expression was found among responders. All the expression rates (CLA tot%, CLA lymph%, CLA CD8+ Tlymph%, CLA CD4+ Tlymph%) were significantly ( $p=0.000035$ ;  $p=0.034604$ ;  $p=0.035310$ ;  $p=0.000037$ , respectively) increasing in the first 2 weeks. Between the 2<sup>nd</sup> and 6<sup>th</sup> weeks a further increase of CLA was detected which proved to be significant only in case of CLA CD8+ Tlymph% ( $p=0.022634$ ). (Figures 2-5) In the relapsing patient group an initial moderate increase till the 2<sup>nd</sup> week was followed by a significant decline between weeks 2 and 6 (CLA tot%,  $p=0.034552$ ; CLA lymph%,  $p=0.012539$ ). (Figures 6-7) The initial peripheral CLA expression values

before starting TNF- $\alpha$  inhibitor therapy were remarkably lower in the responder group compared to the relapsing patients; however, the difference was not significant. Initial CLA values of psoriatic patients didn't significantly differ from CLA expression of the healthy controls (except for CLA CD4+ Tlymph% that was significantly higher among controls compared to responders and the whole psoriatic patient group;  $p=0.020716$  and  $p=0.035284$ , respectively).

Comparing the clinical factors that potentially modulate treatment response we made the following observations: There was no significant difference between responder and relapsing patients regarding initial disease severity (mean PASI of responders:  $23.42 \text{ SD} \pm 6.06$ ; mean PASI of relapsing patients:  $25.25 \text{ SD} \pm 5.69$ ). Average disease duration was also similar:  $17.23 \text{ SD} \pm 7.68$  years among responders and  $23.50 \text{ SD} \pm 18.15$  years in the relapsing group. 18/32 responder and 5/6 relapsing patients had psoriatic arthritis. 3/32 responder and 1/6 relapsing patients were not biologic therapy naive, in their cases a new anti-TNF- $\alpha$  agent was initiated following the loss of effectiveness of a previous TNF- $\alpha$  inhibitor.

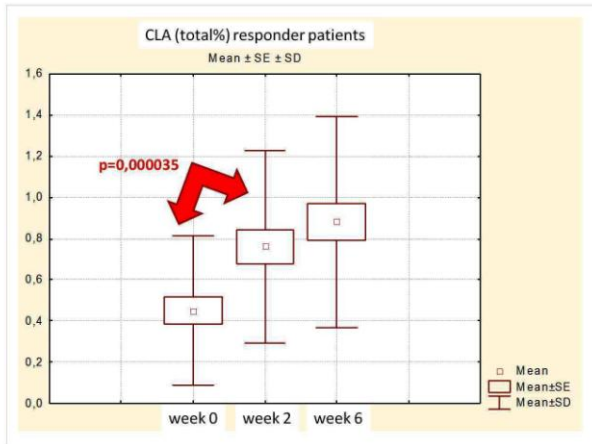


Figure 2. Changes of CLA expression (CLA tot%) of the responder group during the initial treatment period (weeks 0, 2, 6). (paired Student's t-test)

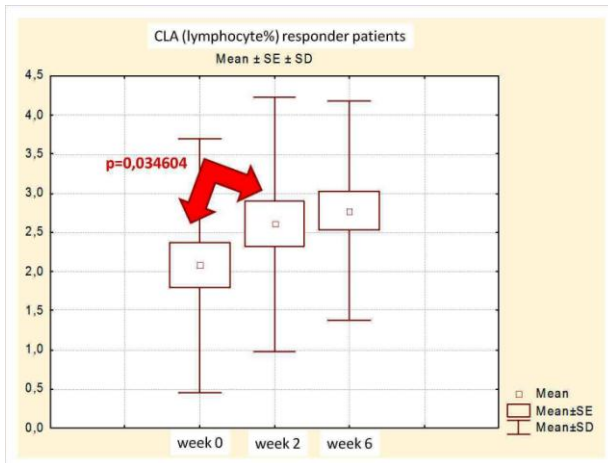


Figure 3. Changes of CLA expression (CLA lymph%) of the responder group during the initial treatment period (weeks 0, 2, 6). (paired Student's t-test)

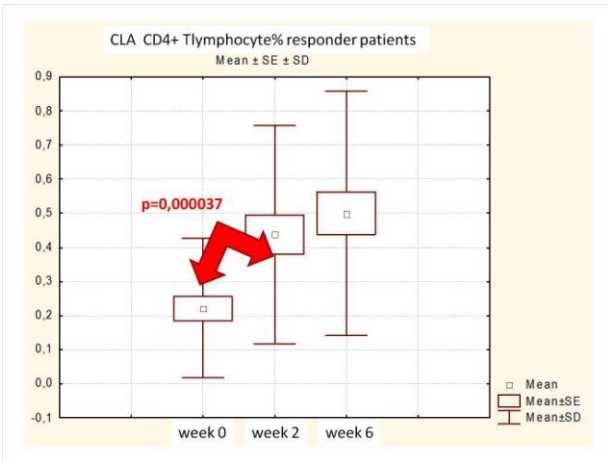


Figure 4. Changes of CLA expression (CLA CD4+ Tlymph%) of the responder group during the initial treatment period (weeks 0, 2, 6). (paired Student's t-test)

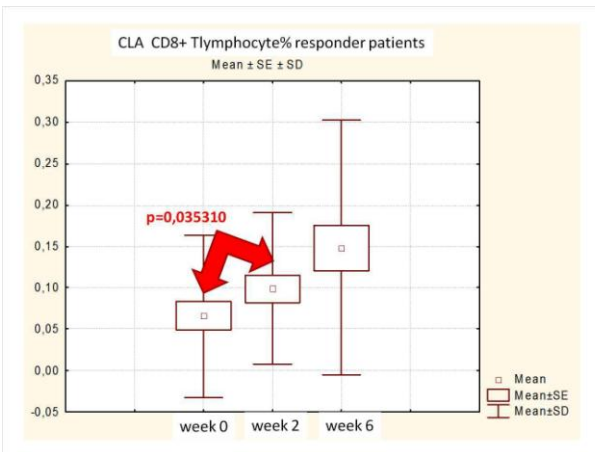


Figure 5. Changes of CLA expression (CLA CD8+ Tlymph%) of the responder group during the initial treatment period (weeks 0, 2, 6). (paired Student's t-test)

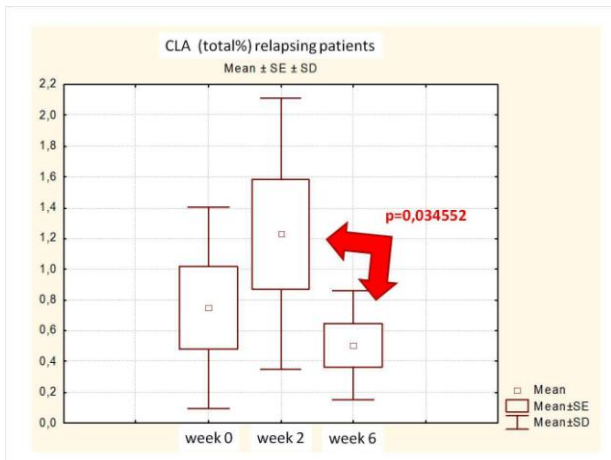


Figure 6. Changes of CLA expression (CLA tot%) of the relapsing group during the initial treatment period (weeks 0, 2, 6). (paired Student's t-test)

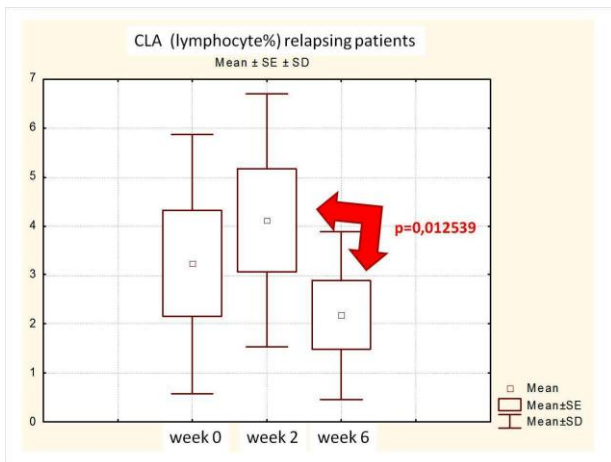


Figure 7. Changes of CLA expression (CLA lymph%) of the relapsing group during the initial treatment period (weeks 0, 2, 6). (paired Student's t-test)

## Results of Pearson's correlation analysis

Pearson's correlation analysis described a significantly negative, moderate relationship (Pearson's correlation coefficient  $r$ : 0.3-0.49;  $p$  value $<$ 0.05) between PASI 12-24 (change of the values referring to long-term clinical responsiveness) and CLA changes observed during the induction treatment period (CLA 0-6, CLA 2-6). This result could confirm our observation (based on the opposite CLA tendencies of responder and relapsing patients) that there is an obvious correlation between long-term disease course (therapeutic response) and CLA changes of the initial treatment period.

## Conclusions

Based on the performed examinations we gave the following answers to our questions:

- 1.No significant difference could be observed between CLA expression of healthy adults and initial expression values of psoriatic patients (except for the CLA+CD4+ T-lymphocyte analysis where expression values were significantly higher among controls than among responders or in the whole psoriatic patient group).

2. Initial peripheral CLA expression of relapsing patients (showing a remarkable disease relapse during long-term therapy) was markedly but not significantly higher than initial values of responder patients (giving a long-lasting response to TNF- $\alpha$  inhibitors). In the background of this phenomenon we suggested the possibility of a hidden provoking infectious focus among relapsing patients.
3. Peripheral CLA tendencies of the two patient groups were significantly different during the initial treatment period. In the first 2 weeks of therapy peripheral CLA expression was significantly increasing in the responder group. Between weeks 2 and 6 there was a further increase to note that proved to be also significant regarding CLA CD8+ Tlymph%. In the relapsing group initial moderate increase of CLA between weeks 0 and 2 was followed by a significant decline (CLA tot%, CLA lymph%) till the 6<sup>th</sup> week.
4. Pearson's correlation analysis showed significant negative, moderate correlation between PASI changes from week 12 to week 24 (referring to long-term clinical response) and CLA changes of the initial treatment period (CLA week 0 vs. week 6, CLA week 2 vs. week 6). These results suggest opposite changes of PASI and CLA to be dependent variables.
5. In summary, based on our results we drew the following conclusion:  
CLA as a skin-specific homing molecule of T-lymphocytes can



serve as a potential reliable and easily accessible predictive biomarker of TNF- $\alpha$  inhibitors' long-term effectiveness in severe psoriasis.

Clinical background analysis (examination of potentially modulating factors: eg. disease severity and duration) of the involved patients further confirmed the independent predictive role of CLA. As still there is no accepted predictive marker available, we suggest our results to be primarily of special importance in establishing a novel adequate method that is expected to reliably predict the long-term outcome of anti-psoriatic TNF- $\alpha$  inhibitor therapy. For the purpose of predicting long-lasting remission or the possibility of a clinical relapse we propose to measure peripheral CLA expression during the induction treatment phase (weeks 0, 2 and 6). Potential limitations of our investigation include the relatively small patient numbers, especially of the relapsing group. However, this latter corresponded to the well-known distribution of TNF- $\alpha$  inhibitor therapy effectiveness. Furthermore, we measured CLA only in the peripheral circulation without examining possible parallel changes in the skin. In the future we aim to further confirm and complete our primary observations with more patients involved and novel strategies (mentioned above) applied. The continuously growing knowledge on the pathogenesis and the molecular background of therapeutic responsiveness could undoubtedly contribute to revealing the mechanisms that influence the CLA tendencies we observed in our study groups.

## Publications closely related to thesis

### *Scientific articles*

1. Jókai H, Marschalkó M, Csomor J, Szakonyi J, Kontár O, Barna G, Kárpáti S, Holló P. (2012) Tissue-Specific homing of immune cells in malignant skin tumors. *Pathol Oncol Res*, 18: 749-759. **IF:1.366**
2. Jókai H, Szakonyi J, Kontár O, Barna G, Kárpáti S, Holló P. (2013) Cutaneous lymphocyte-associated antigen as a novel predictive marker of TNF-alpha inhibitor biological therapy in psoriasis. *Exp Dermatol*, 22: 221-223. **IF:3.543**
3. Ferran M, Romeu ER, Rincón C, Sacrista M, Giménez Arnau AM, Celada A, Pujol RM, Holló P, Jókai H, Santamaria-Babí LF. (2013) Circulating CLA+T lymphocytes as peripheral cell biomarkers in T-cell-mediated skin diseases. *Exp Dermatol*, 22: 439-442. **IF:3.543**

## Publications not related to thesis

### *Scientific articles*

1. Holló P, Jókai H. (2011) Előzetesen TNF-alpha gátló terápiában részesülő beteg ustekinumab kezelése. *Bőrgyógy Venereol Sz*, 87: 219-221.