Low-Dose IL-2 for Treating Moderate to Severe Alopecia Areata: A 52-Week Multicenter Prospective Placebo-Controlled Study Assessing its Impact on T Regulatory Cell and NK Cell Populations

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Low dose IL-2 for treating moderate to severe alopecia areata. A 52 weeks multicenter prospective placebo-controlled study assessing its impact on T regulatory cells and natural killer populations

Short title: Low dose IL-2 for treating alopecia areata

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To the Editor, the treatment of severe Alopecia areata (AA) remains highly challenging. A breakdown of immune privilege of the hair follicle resulting in the development of an admixed immune of antigen presenting cells (APCs), CD4+ and CD8+ T lymphocytes infiltrate is hypothesized to represent an important driver of AA(Pratt et al., 2017). A deficiency in T regulatory cells (Tregs) might facilitate the occurrence of this immune privilege breakdown. A growing corpus of data in animal models but also from blood and skin in AA patients emphasized the likely key role of Tregs in AA pathomechanisms(Conteduca et al., 2014, Hamed et al., 2019, McElwee et al., 2005, Petukhova et al., 2010, Shin et al., 2013, Tembhre and Sharma, 2013). Interleukin-2 (IL-2) is essential for Tregs homeostasis(Zorn et al., 2006). Low dose IL-2 treatment results in Treg recovery and concomitant clinical improvement in patients with HCV-induced vasculitis, Graft-Versus-Host disease, lupus and auto-immune thrombopenia(He et al., 2016, Koreth et al., 2011, Saadoun et al., 2011, Zhang et al., 2018). Conversely, low-dose IL-2 failed to improve type 1 diabetes while efficiently increasing the Tregs population(Hartemann et al., 2013). Using a similar approach, we reported a partial hair regrowth in 4 out of 5 patients with severe AA(Castela et al., 2014). We conducted a multicentric randomized placebo-controlled trial with a 52 weeks follow-up period in adult patients with severe AA of the scalp. The study was registered to the French Health Authorities (ANSM registration number: 150355A-42) and to the IRB Sud Méditerranée V (registration number: 15.039). Written inform consent was obtain for all the patients. Patients received a total of 4 cycles of subcutaneous low-dose IL-2 or saline serum. SALT score was used as primary criteria to assess the efficacy. Treg and NK peripheral populations analysis was carried out at baseline, final day of the 4^{th} cycle and at 1, 3, 6, and 12 months(additional methods in supplementary files).

A total of 43 patients were randomized, of whom 21 were assigned to receive low dose IL-2 and 22 placebo the. Nine patients did not complete the study. The flow diagram of the study

and the baseline characteristics of the population are presented in Supplementary Figure 1 and in Supplementary Table 1, respectively. SALT50 at 12 months after the end of the treatment was achieved in 14.3% in low dose IL-2 group vs 9.1% in placebo group(p=0.66) in the ITT analysis. The evolution of SALT score during treatment is presented in Table 1. No significant improvement was observed in both groups for body hairs and nails. The DLQI and the satisfaction of the patients did not change significantly between baseline and 6 and 12 months after the end of the treatment in both groups, without statistical difference between the two groups(Supplementary Tables 2 and 3). Adverse events encountered during the study are presented in Supplementary Tables 4 and 5. Flu-like syndrome was the most frequent adverse event in low dose IL-2 group, with 66.7% of patients presenting these symptoms at least once compared to 13.6% in placebo group(p=0.0005). Eosinophilia was observed in 8 patients(38.1%) in low dose IL-2 arm vs in none in placebo arm(p=0.0014). All side-effects were transient. The total Treg peripheral population(CD3+CD4+CD25++CD127low) was significantly increased in low dose IL-2 group at all time points compared to baseline; after the end of the last cycle(p=0.0063), and after 1 month(p<0.0001), 3 months(p<0.0001), 6 months(p=0.0012) and 12 months(p=0.084)(Figure 2a). Conversely, no significant variation of the total Treg population was observed in the placebo arm at any time point compared to baseline. Similarly, the Treg/CD8+ cells ratio was significantly increased at all time points in low dose IL-2 arm(p=0.0004, p<0.0001, p<0.0013, p<0.0001, p=0.0051 after the end of the last cycle, and after 1, 3, 6 and 12 months respectively)(Figure 2b). Interestingly, the increase of Tregs only involved the naive subpopulation(CD45RA+ CD197+)(p=0.005, p<0.0001, p=0.0123, p=0.0077, p=0.08 after the end of the last cycle, and after 1, 3, 6 and 12 months, respectively)(Figure 2c). Conversely, the relative percentage of central memory(CD45RA- CD197+)(Figure 2d), effector memory Tregs (CD45RA- CD197-)(Figure 2e) and CLA+ CCR4+ CCR10+ DR+ Tregs(Figure 2f) were decreased in the low dose IL-2 arm. Similarly, a

significant increase of the absolute number of naïve Tregs was only observed in IL-2 group at all time points while no significant variation was observed in the other sub-type of Tregs. The total NK cells transiently increased in the IL-2 arm but only at the end of the treatment, but the variation did not reach statistical significance(p=0.08). The NK cells count then returned to levels comparable to baseline(Suppl. Figure 2a). Analysis of CD158(KIR) and CD314(NKG2D) markers showed that the NK 158+/314- subpopulation corresponded to the subset the most influenced by low-dose IL-2 with a significant decrease at the end of the treatment(p=0.036) while NK 158-/314+ population is increased at the end of the treatment but without reaching a statistically significant level(p=0.23)(Suppl. Figure 2b-e).

Despite encouraging results in the pilot study we initially conducted(Castela et al., 2014), the results of this large prospective randomized placebo-controlled trial did not further support the efficacy of low-dose IL-2 in treating severe AA, at least with the type and regimen of IL-2 used in this study. The analysis of Tregs in the blood of patients during and after treatment showed that low dose IL-2 is likely to elicit a proliferation or a recruitment of Tregs. However, despite this significant increase of peripheral Tregs, the treatment failed to significantly stimulate hair regrowth. The mild and transient increase of NK does not support a significant involvement of NK cells in the failure of low dose IL-2 treatment. The observed limitation of the overall increase of Tregs to the naive subset with no expansion of the effector and memory populations with skin homing capabilities probably explains, at least partially, the lack of clinical efficacy in AA patients. New generation of long-lived IL-2 that are more specific for Tregs are currently developed and tested in early clinical stages for several auto-immune and inflammatory disorders(Peterson et al., 2018). Ours results emphasize the importance for a more specific characterization of the different subsets of Tregs, and the necessity of assessing if the expanded Treg express tissue homing markers in the setting of organ-specific diseases.

Conflict of interest: Novartis provided Proleukin at a discounted price for this study

Data availably statement: The raw data are available upon reasonable request to Dr Eric Fontas (fontas.e@chu-nice.fr) for research purposes only.

REFERENCES

Castela E, Le Duff F, Butori C, Ticchioni M, Hofman P, Bahadoran P, et al. Effects of low-dose recombinant interleukin 2 to promote T-regulatory cells in alopecia areata. JAMA Dermatol 2014;150(7):748-51.

Conteduca G, Rossi A, Megiorni F, Parodi A, Ferrera F, Tardito S, et al. Single nucleotide polymorphisms in the promoter regions of Foxp3 and ICOSLG genes are associated with Alopecia areata. Clin Exp Med 2014;14(1):91-7.

Hamed FN, Astrand A, Bertolini M, Rossi A, Maleki-Dizaji A, Messenger AG, et al. Alopecia areata patients show deficiency of FOXP3+CD39+ T regulatory cells and clonotypic restriction of Treg TCRbeta-chain, which highlights the immunopathological aspect of the disease. PloS one 2019;14(7):e0210308.

Hartemann A, Bensimon G, Payan CA, Jacqueminet S, Bourron O, Nicolas N, et al. Low-dose interleukin 2 in patients with type 1 diabetes: a phase 1/2 randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol 2013;1(4):295-305.

He J, Zhang X, Wei Y, Sun X, Chen Y, Deng J, et al. Low-dose interleukin-2 treatment selectively modulates CD4(+) T cell subsets in patients with systemic lupus erythematosus. Nat Med 2016;22(9):991-3.

Koreth J, Matsuoka K, Kim HT, McDonough SM, Bindra B, Alyea EP, 3rd, et al. Interleukin-2 and regulatory T cells in graft-versus-host disease. The New England journal of medicine 2011;365(22):2055-66.

McElwee KJ, Freyschmidt-Paul P, Hoffmann R, Kissling S, Hummel S, Vitacolonna M, et al. Transfer of CD8(+) cells induces localized hair loss whereas CD4(+)/CD25(-) cells promote systemic alopecia areata and CD4(+)/CD25(+) cells blockade disease onset in

the C3H/HeJ mouse model. The Journal of investigative dermatology 2005;124(5):947-57.

Peterson LB, Bell CJM, Howlett SK, Pekalski ML, Brady K, Hinton H, et al. A long-lived IL-2 mutein that selectively activates and expands regulatory T cells as a therapy for autoimmune disease. J Autoimmun 2018;95:1-14.

Petukhova L, Duvic M, Hordinsky M, Norris D, Price V, Shimomura Y, et al. Genome-wide association study in alopecia areata implicates both innate and adaptive immunity. Nature 2010;466(7302):113-7.

Pratt CH, King LE, Jr., Messenger AG, Christiano AM, Sundberg JP. Alopecia areata. Nat Rev Dis Primers 2017;3:17011.

Saadoun D, Rosenzwajg M, Joly F, Six A, Carrat F, Thibault V, et al. Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis. The New England journal of medicine 2011;365(22):2067-77.

Shin BS, Furuhashi T, Nakamura M, Torii K, Morita A. Impaired inhibitory function of circulating CD4+CD25+ regulatory T cells in alopecia areata. J Dermatol Sci 2013;70(2):141-3.

Tembhre MK, Sharma VK. T-helper and regulatory T-cell cytokines in the peripheral blood of patients with active alopecia areata. The British journal of dermatology 2013;169(3):543-8.

Zhang J, Ruan Y, Xu X, Wang H, Tao Q, Lu J, et al. Therapeutic potential of low-dose IL-2 in immune thrombocytopenia: An analysis of 3 cases. Cytometry B Clin Cytom 2018;94(3):428-33.

Zorn E, Nelson EA, Mohseni M, Porcheray F, Kim H, Litsa D, et al. IL-2 regulates FOXP3 expression in human CD4+CD25+ regulatory T cells through a STAT-dependent

mechanism and induces the expansion of these cells in vivo. Blood 2006;108(5):1571-9.

Table 1. Evolution of the SALT score

	W0 (n=22)	**W3 (n=22)**	**W6 (n=22)**	**W9 (n=22)**	**1 month follow-up (n=22)**	**3 months follow-up (n=22)**	**6 months follow-up (n=22)**	**12 months follow-up) (n=12)**
PLACEBO median. (IQR)	84 (56.6-100)	81.9 (61.9-100)	81 (68.4-100)	80.1 (67.4-100)	91.5 (44.8-100)	93 (44.8-100)	82.9 (48.2-100)	72.2 (44-100)
	W0 (n=21)	**W3 (n=21)**	**W6 (n=21)**	**W9 (n=21)**	**1 month follow-up (n=21)**	**3 months follow-up (n=21)**	**6 months follow-up (n=21)**	**12 months follow-up) (n=21)**
Low dose IL-2 median (IQR)	100 (76.4-100)	100 (76.4-100)	100 (76.4-100)	100 (69.5-100)	100 (61.2-100)	100 (64.4-100)	100 (90-100)	96.8 (70.3-100)

W0: Baseline; W3, W6 and W9: weeks 3, 6 and 9, respectively after the onset of treatment. IQR (Interquartile Range). Data are imputed using Last Observation Carried Forward (LOCF)

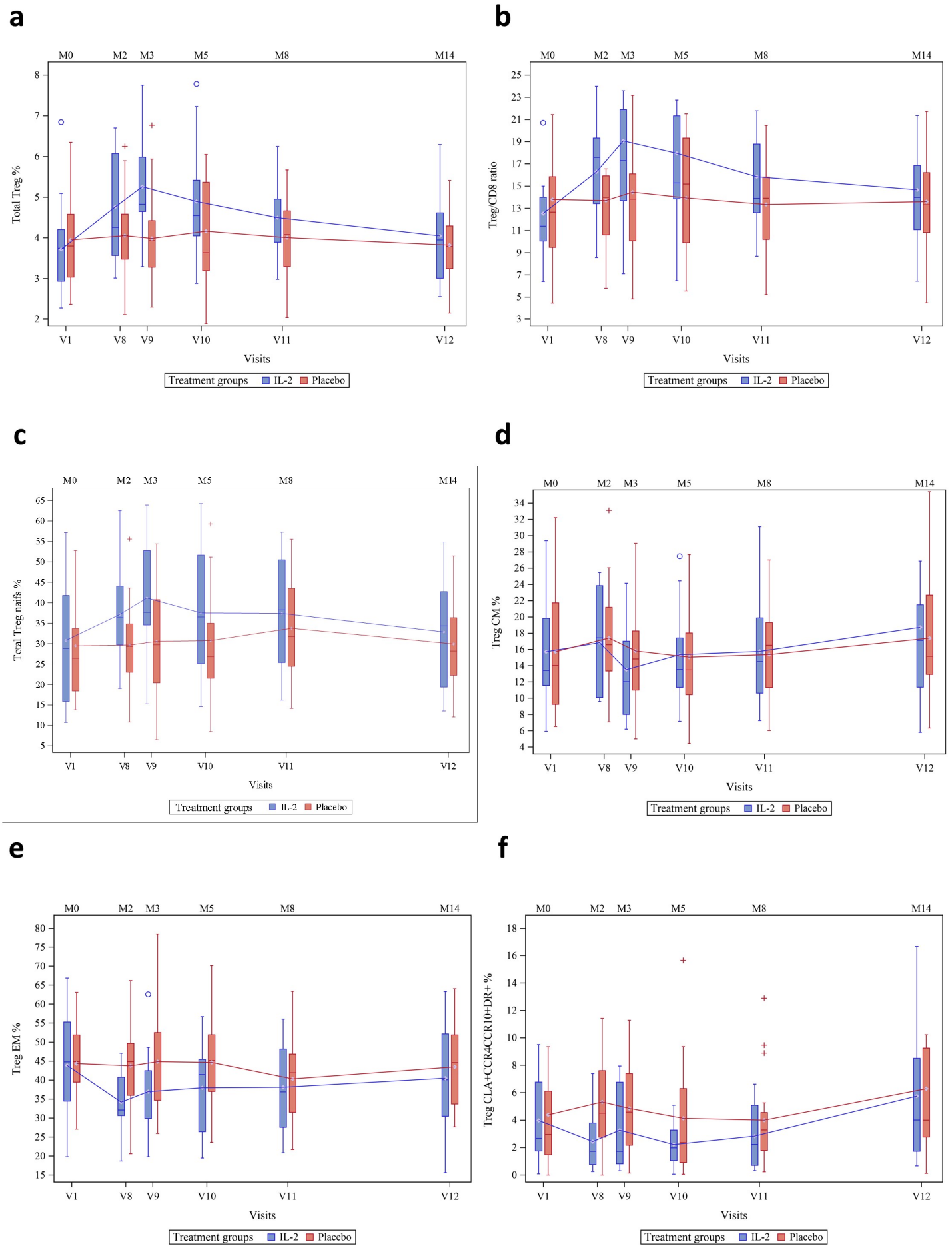
FIGURES LEGENDS

Figure 1. Monitoring of Treg populations over time

Flow cytometry analysis was performed at baseline (V1), at the end of the 4th cycle of treatment (V8), one month after the end of the cures (V9) and after 3 (V10), 6 (V11) and 12 months (V12).

(a) Percentage of total Tregs in the total lymphocyte population. (b) Tregs / CD8 ratio. (c) Naïve Treg subsets. (d) Central memory Tregs subsets. (e) Effector memory Tregs. (f) Activated Tregs with cutaneous homing markers.

Figure 1


a
M0 M2 M3 M5 M8 M14
Total Treg %
V1 V8 V9 V10 V11 V12
Visits
Treatment groups IL-2 Placebo
b
M0 M2 M3 M5 M8 M14
Treg/CD8 ratio
V1 V8 V9 V10 V11 V12
Visits
Treatment groups IL-2 Placebo
c
M0 M2 M3 M5 M8 M14
Total Treg naifs %
V1 V8 V9 V10 V11 V12
Visits
Treatment groups IL-2 Placebo
d
M0 M2 M3 M5 M8 M14
Treg CM %
V1 V8 V9 V10 V11 V12
Visits
Treatment groups IL-2 Placebo
e
M0 M2 M3 M5 M8 M14
Treg EM %
V1 V8 V9 V10 V11 V12
Visits
Treatment groups IL-2 Placebo
f
M0 M2 M3 M5 M8 M14
Treg CLA+CCR4CCR10+DR+ %
V1 V8 V9 V10 V11 V12
Visits
Treatment groups IL-2 Placebo