

Original Article

Real-world effectiveness of a topical dihydroavenanthramide D formulation for chronic pruritus: A multicenter clinical study with molecular evidence of pruritogenic pathway modulation

Simone Ribero¹, Mario Vaccaro², Francesco Borgia², Carlo Mattozzi³, Edoardo Zattra⁴, Alessandra Mattiucci⁵ and Piercarlo Minoretti^{6*}

¹Section of Dermatology, Department of Medical Sciences, University of Turin, Turin, Italy; ²Section of Dermatology, Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy; ³Private Practice, Marsala, Italy; ⁴Private Practice, Bassano del Grappa, Italy; ⁵Private Practice, Brescia, Italy; ⁶Studio Minoretti, Oggiono, Lecco, Italy

*Corresponding author: scientific.direction@studiominoretti.it

Abstract

Chronic pruritus is a prevalent and clinically burdensome condition that substantially impairs quality of life and is sustained by complex interactions between neuro-immune signaling and epidermal barrier dysfunction. Central mediators implicated in this pruritogenic network include interleukin-4 (IL-4), interleukin-13 (IL-13), interleukin-31 (IL-31), substance P encoded by *TAC1*, and nerve growth factor (NGF). The aim of this study was to evaluate the real-world effectiveness of a topical formulation containing dihydroavenanthramide D (DHA_vD), a neurokinin-1 receptor inhibitor, in combination with barrier-repairing agents for reducing pruritus severity in patients with chronic pruritus of diverse etiologies. A supportive molecular sub-study was also performed to determine whether clinical improvement was accompanied by modulation of cutaneous pruritogenic gene expression. A prospective, single-arm, multicenter clinical study was conducted in 1,000 patients with chronic pruritus of diverse etiologies. The formulation was applied twice daily for 14 days, and pruritus severity was assessed using the 12-Item Pruritus Severity Scale (12-PSS; range 3–22). In a molecular sub-study, 20 patients with chronic idiopathic pruritus underwent paired skin biopsies from symptomatic areas before and after treatment, and cutaneous mRNA expression of *IL4*, *IL13*, *IL31*, *TAC1*, and *NGF* was quantified by qRT-PCR. The mean 12-PSS score decreased from 11.37±3.71 at baseline to 5.64±2.87 at day 14 ($d=-1.72$; $p<0.001$), and 55.4% of participants achieved a ≥50% reduction in pruritus severity. The reduction in symptom severity was consistent across etiological subgroups ($p=0.787$). A clinically meaningful reduction was also observed among patients treated with the cream as monotherapy, with 12-PSS scores decreasing from 10.44±3.78 to 5.06±2.62 ($d=-1.53$; $p<0.001$). In the molecular sub-study, significant downregulation of all five target genes was observed, with effect sizes ranging from $d=-0.62$ for *IL4* ($p=0.013$) to $d=-2.64$ for *NGF* ($p<0.001$). In conclusion, topical DHA_vD combined with barrier-repairing agents was associated with clinically meaningful real-world reduction in chronic pruritus severity and supportive molecular evidence of multi-target pruritogenic pathway modulation. These findings warrant confirmation in randomized vehicle-controlled trials.

Keywords: Chronic pruritus, dihydroavenanthramide D, gene expression, substance P, nerve growth factor



Introduction

Chronic pruritus, defined as itch persisting for more than six weeks, affects approximately one in five individuals over a lifetime and is associated with substantial reductions in quality of life [1]. This condition can arise from dermatological, systemic, neuropathic, and psychogenic etiologies, although in many cases a single predominant cause cannot be identified [2]. Beyond persistent itch, chronic pruritus frequently disrupts sleep, concentration, emotional well-being, and daily functioning, reinforcing its broader clinical and psychosocial burden. Chronic pruritus is currently conceptualized not merely as a symptom of skin disease, but as a distinct condition sustained by a complex bidirectional interplay between the cutaneous immune system and the peripheral nervous system, collectively referred to as the neuro-immune axis [3].

Several mediators occupy central positions within the neuro-immune axis and have emerged as therapeutic targets [3,4]. The Th2 cytokines interleukin-4 (IL-4) and interleukin-13 (IL-13) signal through shared receptor subunits (IL-4R α /IL-13R α 1) on keratinocytes and sensory neurons, promoting barrier disruption and direct sensitization of pruriceptive nerve fibers [5,6]. Importantly, IL-4 and IL-13 also drive expression of interleukin-31 (IL-31) [7], a Th2 cell-derived cytokine that acts on sensory neurons of the dorsal root ganglia through the IL-31 receptor A (IL-31RA), which colocalizes with transient receptor potential vanilloid 1 (TRPV1) channels on pruriceptive C-fibers [8]. On the neurogenic side of the axis, substance P – encoded by the tachykinin precursor 1 gene (*TAC1*) – is a neuropeptide released by cutaneous C-fibers that activates the neurokinin-1 receptor (NK1R) on mast cells, triggering degranulation and release of histamine and pro-inflammatory mediators [9]. This mechanism creates a neurogenic amplification loop that perpetuates both inflammation and itch [10]. In parallel, nerve growth factor (NGF), acting through the high-affinity tropomyosin receptor kinase A (TrkA), promotes sprouting and sensitization of intraepidermal nerve fibers, upregulates TRPV1 expression, and lowers the activation threshold of pruriceptors, thereby contributing to itch chronification [11,12]. Beyond these mediators, impairment of the epidermal permeability barrier is itself a recognized driver of pruritogenesis [13,14]. Barrier dysfunction is associated with Th2 polarization and release of pruritogenic cytokines, including IL-31, and sustains peripheral nerve sensitization [15,16]. More specifically, perturbation of the stratum corneum exposes keratinocytes to environmental insults and proteolytic stimuli, triggering release of epithelial-derived alarmins that prime cutaneous immune cells toward type 2 polarization [15]. The resulting type 2 cytokine milieu – characterized by IL-4, IL-13, and IL-31 – sustains a pruritogenic state through direct activation of sensory neuron IL-31 receptor A/oncostatin M receptor β heterodimers and through indirect effects on barrier-related gene expression that further compromise epidermal integrity [17]. This bi-directional coupling between barrier impairment and type 2 inflammation defines the epidermal component of the itch circuit, complementing the neurogenic component described above. The interplay between barrier function and neuro-immune signaling suggests that topical formulations combining a direct pruritogenic mediator antagonist with barrier-repairing agents may address multiple nodes of the itch circuit simultaneously.

One candidate for such a combined approach is dihydroavananthramide D (DHA ν D), a synthetic polyphenol that inhibits substance P-mediated mast cell degranulation *in vitro* by acting as an NK1R inhibitor [17]. These pharmacological properties suggest that DHA ν D, when incorporated into a barrier-repairing vehicle, may target both the neurogenic and the epidermal components of the itch circuit [18]. This dual mechanism is particularly relevant because chronic pruritus often persists through reciprocal crosstalk between impaired barrier function, immune activation, and peripheral nerve sensitization. To date, however, whether these effects translate into measurable changes in pruritogenic gene expression in the skin of patients with chronic pruritus has not been determined. Therefore, this study was conducted to assess the real-world effectiveness of a topical DHA ν D-containing formulation with barrier-repairing agents in a multicenter clinical cohort of patients with chronic pruritus of diverse etiologies. To provide supportive mechanistic evidence, a molecular sub-study using paired skin biopsies was also performed to evaluate changes in cutaneous mRNA expression of *IL4*, *IL13*, *IL31*, *TAC1*, and *NGF* after 14 days of topical application.

Methods

Study product

The study product (Xeronorm Crema; Bionativa Spa, Barberino Tavarnelle, Italy) was a commercially available topical cream containing DHA_vD (~1% w/w) as the active antipruritic mediator antagonist. The formulation also contained a papain-based complex with natural moisturizing factors (NMF; ~7% w/w), intended to support physiological desquamation and stratum corneum hydration, and a heteropolysaccharide film (~5% w/w) composed of microfluidized xanthan gum, glucose, and carrageenan, designed to form a continuous occlusive layer on the skin surface. Additional excipients included glycerol, tocopheryl acetate, medium-chain triglycerides, and a mild surfactant system. Within the formulation, three components are functionally directed at restoration of epidermal barrier integrity. The papain-NMF complex (~7% w/w) provides natural moisturizing factors that re-establish stratum corneum osmotic balance and promote gentle physiological desquamation, supporting renewal of the cornified envelope. The heteropolysaccharide film (~5% w/w) forms a continuous occlusive layer that reduces transepidermal water loss and creates a humid microenvironment conducive to barrier reconstitution. Glycerol, in combination with tocopheryl acetate and medium-chain triglycerides, contributes additional humectant and lipophilic emollient activity, supplementing stratum corneum lipids and supporting the integrity of the intercellular lipid lamellae. Together, these components constitute the barrier-repairing complement of the formulation.

Study design and patients

A prospective, single-arm, multicenter, real-world clinical study was conducted across five dermatology centers in Italy in 2025. Adult patients aged ≥ 18 years with chronic pruritus of any duration and etiology were consecutively enrolled at each participating center. The exclusion criteria were inability to provide written informed consent, severe psychiatric disorders, pregnancy, and lactation. The study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board. Written informed consent was obtained from all participants before enrollment.

Sample size and sampling

No formal a priori sample size calculation was performed, consistent with the descriptive, real-world nature of the study. A target enrollment of 1,000 patients was pre-specified to provide adequate statistical precision for the primary effectiveness endpoint and to support planned subgroup analyses across the main etiological categories of chronic pruritus. Patients were enrolled consecutively at each participating center until the target sample size was reached.

Treatment

The study cream was applied by patients to the affected skin areas twice daily for 14 consecutive days. Concomitant antipruritic treatments, if used, were recorded at baseline and were not discontinued or modified during the observation period, in line with the pragmatic, real-world design. The 14-day evaluation period was selected on pragmatic clinical grounds, as this timeframe reflects when patients with chronic pruritus would be expected to perceive a meaningful benefit from a topical antipruritic intervention. In routine dermatological practice, absence of perceived improvement after this period would typically prompt treatment re-evaluation or escalation.

Outcome assessment

The primary outcome was the change in pruritus severity from baseline to day 14, assessed using the 12-Item Pruritus Severity Scale (12-PSS) [21]. The 12-PSS is a patient-reported questionnaire developed by Reich *et al.* to provide a multidimensional assessment of pruritus severity. It includes 12 items across five conceptual domains: pruritus intensity, pruritus extent, pruritus duration, the effect of pruritus on concentration and psychological status, and scratching as a behavioral response to itch stimuli. Individual items are scored using ordinal categorical scales, and the total score ranges from 3 to 22, with higher scores indicating more severe pruritus. The instrument has shown strong internal consistency (Cronbach's $\alpha=0.81$), satisfactory test-retest

reliability (ICC=0.72), and significant convergent validity with the Visual Analogue Scale for itch and the Dermatology Life Quality Index [21].

At each participating center, the 12-PSS was administered by the treating dermatologist during in-person visits. Assessments were performed at baseline (day 0) and day 14 using the same procedure. Pre-specified secondary outcomes included the proportion of patients achieving a $\geq 50\%$ reduction in 12-PSS score from baseline, defined as the responder rate; the consistency of treatment effects across etiological subgroups; and the consistency of treatment effects between patients receiving and not receiving concomitant antipruritic therapies.

Molecular study assessing gene expression

Twenty adult patients (≥ 18 years) with chronic idiopathic pruritus (duration ≥ 6 weeks) were enrolled. Chronic idiopathic pruritus was defined as itch in the absence of identifiable primary cutaneous lesions or known systemic causes [19]. Patients were excluded if they had active inflammatory dermatoses (atopic dermatitis, psoriasis, urticaria, or eczema), pruritus of known systemic origin (hepatic, renal, hematologic, endocrine, or paraneoplastic), pregnancy or lactation, use of topical or systemic corticosteroids within 4 weeks, systemic antihistamines within 2 weeks, ongoing immunosuppressants or biologics, regular application of topical cosmetic or dermatologic products with claimed barrier-repairing, emollient, or anti-inflammatory properties within 2 weeks before enrollment, or contraindications to skin biopsy.

Paired 4-mm punch biopsies were obtained from a symptomatic skin area (volar forearm) at baseline (day 0) and after 14 days of twice-daily application of the study cream. All paired biopsies were taken from the same anatomical region (the volar forearm) across the cohort, so that regional variations in skin thickness, stratum corneum permeability, and intraepidermal nerve fiber density were standardized between participants. The post-treatment biopsy was taken from a clinically symptomatic region located within 1.5 cm of the baseline biopsy site, so as to maximize within-subject comparability of the cutaneous microenvironment between the two time points. Biopsies were performed under local anesthesia (2% lidocaine without epinephrine). Written informed consent was obtained from all participants before enrollment.

RNA extraction and gene expression analysis

Each biopsy was placed in 300 μ L of RNeasy Lysis Buffer (Qiagen, Crawley, UK) and stored at -80°C until processing. Total RNA was extracted with the RNeasy Mini Kit (Qiagen, Crawley, UK) following the manufacturer's instructions, including an on-column DNase I digestion step to remove residual genomic DNA contamination. RNA integrity was qualitatively assessed by denaturing agarose gel electrophoresis through visualization of intact 28S and 18S ribosomal RNA bands, with a 28S/18S intensity ratio of approximately 2:1 considered indicative of preserved integrity. RNA concentration and purity were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA); samples were considered suitable for downstream analysis when the A260/A280 absorbance ratio was between 1.8 and 2.1 and the A260/A230 ratio was ≥ 1.8 . All extracted samples met the pre-specified quality criteria and were therefore included in the downstream analyses. Reverse transcription was performed on 1 μ g of total RNA using the iScript cDNA Synthesis Kit (BioRad, Hercules, CA, USA); cDNA samples were then stored at -20°C until analysis.

Gene expression was quantified by qRT-PCR on a BioRad iQ5 Cyclor (BioRad). Reactions were carried out in a total volume of 25 μ L containing 40 ng of cDNA, 400 nM each of forward and reverse primers, and iQ SYBR Green Supermix (BioRad). The thermal cycling protocol comprised an initial denaturation step at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 15 s, and extension at 72°C for 30 s.

The primer pairs used were as follows: *IL4* forward, 5'-CGAGTTGACCGTAACAGACAT-3'; *IL4* reverse, 5'-CGTCTTTAGCCTTTCCAAGAAG-3'; *IL13* forward, 5'-GGAGCTGGTCAACATCACCC-3'; *IL13* reverse, 5'-CGTTGATCAGGGATTCCAGG-3'; *IL31* forward, 5'-TCGAGGAATTACAGTCCCTCTC-3'; *IL31* reverse, 5'-TGTCGAGGTGCTCTATGATCTC-3'; *TAC1* forward, 5'-ACTGTCCGTCGAAAATCC-3'; *TAC1* reverse, 5'-ACTGCTGAGGCTTGGGTCTC-3'; *NGF* forward, 5'-AGCGGCGATAGCTGCACGCTGGCG-3'; *NGF* reverse, 5'-CAGATCCTGAGTGTCTGCAGCTTCAC-3'. Glyceraldehyde-3-phosphate dehydrogenase gene

(*GAPDH*) served as the reference gene for normalization, with forward and reverse primers of 5'-GAAGGTGAAGGTCGGAGTC-3' and 5'-GAAGATGGTGATGGGATTTC-3', respectively. Fluorescence signals were processed with Bio-Rad iQ5 Optical System Software Version 2.0, and relative mRNA levels at each time point were expressed as $2^{-\Delta CT}$ values, where ΔCT represented the difference between the cycle threshold of the target gene and that of *GAPDH*. Pre-post changes were evaluated by comparing paired $2^{-\Delta CT}$ values statistically [20].

Statistical analysis

For the clinical study, pre-post comparison of the 12-PSS score was assessed by the paired *t*-test. Subgroup analyses by etiological category, sex, and age group were performed using the Kruskal-Wallis and Mann-Whitney *U* test. Correlations were analyzed with Spearman's ρ . Multiple linear regression was performed with the absolute change in 12-PSS score from baseline to day 14 as the dependent variable. Multivariable logistic regression was performed with responder status (defined as a $\geq 50\%$ reduction in 12-PSS score from baseline) as the dependent variable. Both models included baseline 12-PSS score, age, sex, and pruritus duration as candidate predictors. Linear regression coefficients are reported as unstandardized β values with 95% confidence intervals, and logistic regression results as odds ratios with 95% confidence intervals. To address potential confounding by concomitant antipruritic therapies, an additional subgroup analysis comparing monotherapy and concomitant therapy patients was performed using the Mann-Whitney *U* test for between-group comparisons of Δ values and the chi-square test for responder rates. Analysis of covariance (ANCOVA) was used to adjust between-group differences in Δ for baseline 12-PSS score, age, and sex.

For the molecular sub-study, the distribution of within-subject differences was assessed by the Shapiro-Wilk test. Pre-post-comparisons were performed using the paired Student's *t*-test for normally distributed variables and the Wilcoxon signed-rank test otherwise. Effect sizes were estimated using Cohen's *d* for paired data – calculated as the mean within-subject difference divided by the standard deviation of within-subject differences – with 95% confidence intervals (CIs) for the mean difference. Given the exploratory nature of the sub-study and the small sample size, no correction for multiple comparisons was applied. All analyses were performed with SPSS 20.0 (IBM, Armonk, NY, USA), and two-sided *p*-values < 0.05 were regarded as statistically significant.

Results

Patient baseline characteristics

The baseline characteristics of the 1,000 patients enrolled in the multicenter clinical study are summarized in **Table 1**. The cohort comprised 438 men and 562 women with a mean age of 47.9 ± 22.2 years (range 18–95 years). Mean pruritus duration was 11.1 ± 10.4 months. The mean 12-PSS score decreased from 11.37 ± 3.71 at baseline to 5.64 ± 2.87 at day 14, yielding a mean reduction of 5.73 ± 3.32 points (paired $t = 54.52$; $p < 0.001$; Cohen's $d = -1.72$). A total of 554 patients (55.4%) met the responder criterion ($\geq 50\%$ score reduction) (**Table 1**).

Table 1. Baseline characteristics of the patients included in clinical study (n=1,000)

Characteristic	Frequency (%)
Age, years (mean $\pm$ SD)	47.9 $\pm$ 22.2
Age range, years	18–95
Men/women	438/562
Pruritus duration, months (mean $\pm$ SD)	11.1 $\pm$ 10.4
Pruritus etiology	
Atopic dermatitis	436 (43.6)
Metabolic conditions	98 (9.8)
Renal pruritus	53 (5.3)
Hepatic pruritus	32 (3.2)
Other/mixed	381 (38.1)
Concomitant therapy	
None	452 (45.2)
Antihistamines	156 (15.6)
Topical corticosteroids	116 (11.6)

Characteristic	Frequency (%)
Multiple agents	215 (21.5)
Other	61 (6.1)
Baseline 12-Item Pruritus Severity Scale (mean±SD)	11.37±3.71
12-PSS: 12-Item Pruritus Severity Scale (range 3–22)	

Effectiveness of DHAvD-containing formulation on disease severity

The reduction in pruritus severity was consistent across etiological subgroups (**Table 2**). After 14 days of treatment, significant decreases in 12-PSS scores were observed in patients with atopic dermatitis, metabolic pruritus, renal pruritus, hepatic pruritus, and other or mixed etiologies. The mean reduction ranged from 5.59 to 5.77 points across subgroups, indicating a comparable magnitude of improvement. No significant difference in treatment-associated reduction was observed across etiological categories (Kruskal-Wallis test, $p=0.787$), suggesting that the clinical benefit was not restricted to a specific underlying cause of chronic pruritus.

Table 2. Reduction in 12-Item Pruritus Severity Scale scores across chronic pruritus etiologies

Etiology	n	12-Item Pruritus Severity Scale score, mean±SD			p-value
		Baseline	Day 14	Δ	
Atopic dermatitis	436	11.52±3.68	5.75±2.88	5.77±3.33	<0.001
Metabolic	98	11.12±3.74	5.42±2.85	5.70±3.34	<0.001
Renal	53	11.28±3.82	5.58±2.92	5.70±3.38	<0.001
Hepatic	32	11.09±3.65	5.50±2.78	5.59±3.25	<0.001
Other/mixed	381	11.22±3.72	5.52±2.84	5.70±3.30	<0.001

Kruskal-Wallis test across etiological categories: $H=1.72$, $p=0.787$ (no significant difference)

Spearman correlation analysis showed a weak negative association between age and absolute 12-PSS score reduction ($\rho=-0.129$, $p<0.001$) (**Table 3**). In multiple linear regression, baseline 12-PSS score was the strongest predictor of absolute score reduction ($\beta=0.605$, 95%CI: 0.564–0.645, $p<0.001$), indicating greater improvement among patients with higher baseline pruritus severity. Younger age ($\beta=-0.020$, $p<0.001$) and shorter pruritus duration ($\beta=-0.022$, $p=0.002$) were also independently associated with greater score reduction, whereas sex was not a significant predictor ($\beta=0.217$, $p=0.160$). The model explained 47.5% of the variance in absolute score reduction ($R^2=0.475$; $F=224.2$; $p<0.001$) (**Table 3**).

Table 3. Multiple linear regression analysis of predictors of absolute reduction in 12-Item Pruritus Severity Scale score (n=1,000)

Predictor	Unstandardized regression coefficient (β)	Standard error (SE)	t	95% confidence interval (95%CI)	p-value
Age (years)	-0.020	0.003	-5.904	-0.027 to -0.014	<0.001
Sex (female vs male)	0.217	0.154	1.408	-0.085 to 0.519	0.160
Baseline 12-PSS score	0.605	0.021	29.313	0.564 to 0.645	<0.001
Pruritus duration (months)	-0.022	0.007	-3.046	-0.037 to -0.008	0.002

Model fit: $R^2=0.475$, $F(4, 995)=224.2$, $p<0.001$

12-PSS: 12-Item Pruritus Severity Scale

Multivariable logistic regression confirmed these findings for the binary responder outcome ($\geq 50\%$ reduction in 12-PSS score at day 14) (**Table 4**). A higher baseline 12-PSS score was independently associated with a greater probability of achieving a $\geq 50\%$ score reduction (OR=1.089 per point, 95%CI: 1.051–1.128, $p<0.001$). Younger age (OR=0.983 per year, $p<0.001$) and shorter pruritus duration (OR=0.977 per month, $p<0.001$) were also independently associated with responder status, whereas sex was not a significant predictor (OR=1.149, $p=0.295$).

Table 4. Multivariable logistic regression analysis of predictors of responder status, defined as $\geq 50\%$ reduction in 12-Item Pruritus Severity Scale score (n=1,000)

Predictor	Odds ratio (OR)	95% confidence interval (95%CI)	p-value
Age (per year)	0.983	0.978 to 0.989	<0.001
Sex (female vs male)	1.149	0.886 to 1.492	0.295

Predictor	Odds ratio (OR)	95% confidence interval (95%CI)	p-value
Baseline 12-Item Pruritus Severity Scale (per point)	1.089	1.051 to 1.128	<0.001
Pruritus duration (per month)	0.977	0.964 to 0.989	<0.001

Treatment effect according to concomitant therapy use

Given that approximately half of the participants were using concomitant antipruritic therapies (Table 1), an additional subgroup analysis was performed to assess whether the observed clinical effect was attributable to the study cream itself rather than to co-administered treatments. Among the 452 patients who used the cream as monotherapy, 12-PSS scores decreased from 10.44 ± 3.78 at baseline to 5.06 ± 2.62 at day 14, yielding a mean reduction of 5.38 ± 3.51 points (paired $t=32.58$, $p<0.001$; Cohen's $d=-1.53$). The responder rate ($\geq 50\%$ score reduction) was 57.7% in this subgroup, comparable to the 53.6% observed in the 548 patients receiving the cream alongside at least one concomitant therapy (chi-square $p=0.218$). Pairwise comparisons against each individual concomitant therapy category showed no significant difference in Δ between monotherapy and antihistamines, topical corticosteroids, systemic corticosteroids, topical calcineurin inhibitors, systemic immunosuppressants, or biologics (Table 5). Patients receiving multiple concomitant agents showed a larger Δ than monotherapy (6.49 vs 5.38 points, $p<0.001$), an effect attributable to their higher baseline severity (13.02 vs 10.44). To control for unbalanced baseline characteristics between subgroups, an ANCOVA model was fitted with Δ as the dependent variable and concomitant therapy status, baseline 12-PSS score, age, and sex as covariates ($R^2=0.480$). After adjustment, the between-group difference in Δ was 0.54 points in favor of the monotherapy subgroup (95%CI 0.23–0.85, $p<0.001$), confirming that the apparent advantage of the concomitant therapy subgroup in the unadjusted analysis was attributable to its higher baseline severity rather than to a synergistic effect of co-administered antipruritic agents.

Table 5. Treatment effect by concomitant therapy category

Therapy category	n	12-Item Pruritus Severity Scale score, mean $\pm$ SD			p vs Mono*
		Baseline	Day 14	Δ	
Cream alone (monotherapy)	452	10.44 $\pm$ 3.78	5.06 $\pm$ 2.62	5.38 $\pm$ 3.51	—
Antihistamines	156	11.65 $\pm$ 3.16	5.85 $\pm$ 2.36	5.81 $\pm$ 2.93	0.051
Topical corticosteroids	116	11.11 $\pm$ 3.81	5.53 $\pm$ 2.79	5.58 $\pm$ 3.17	0.468
Systemic corticosteroids	11	12.64 $\pm$ 4.90	6.91 $\pm$ 3.51	5.73 $\pm$ 3.38	0.615
Topical calcineurin inhibitors	9	12.78 $\pm$ 3.15	6.11 $\pm$ 2.42	6.67 $\pm$ 2.65	0.144
Systemic immunosuppressants	16	12.25 $\pm$ 3.17	6.31 $\pm$ 2.57	5.94 $\pm$ 2.17	0.290
Biologics	25	11.40 $\pm$ 4.53	6.08 $\pm$ 3.74	5.32 $\pm$ 2.41	0.696
Multiple agents	215	13.02 $\pm$ 3.11	6.53 $\pm$ 3.35	6.49 $\pm$ 3.39	<0.001

* Analyzed using Mann-Whitney U test comparing Δ in each concomitant therapy group with Δ in the monotherapy reference group. Mono: monotherapy.

Changes in cutaneous gene expression

Twenty patients (12 men and 8 women; mean age 45.5 ± 11.4 years) were enrolled and all completed the study protocol. No adverse events related to the study product or biopsy procedures were recorded. After 14 days of twice-daily application of the cream, statistically significant reductions were observed in the mRNA expression of all five target genes in paired skin biopsies obtained from symptomatic areas (Table 6).

Table 6. Cutaneous gene expression before and after 14 days of treatment (n=20)

Gene	Gene expression (mean $\pm$ SD) ^a		Δ	p-value	Cohen's d ^b	95%CI of Δ
	Baseline	Day 14				
<i>IL4</i>	3.41 $\pm$ 0.96	2.89 $\pm$ 1.51	-0.52	0.013*	-0.62	-0.91 to -0.12
<i>IL13</i>	3.84 $\pm$ 0.82	3.01 $\pm$ 1.25	-0.83	0.002*	-0.82	-1.30 to -0.35
<i>IL31</i>	2.89 $\pm$ 0.72	2.20 $\pm$ 0.56	-0.69	<0.001*	-2.15	-0.84 to -0.54
<i>TAC1</i>	2.91 $\pm$ 0.66	2.38 $\pm$ 0.49	-0.53	<0.001*	-1.59	-0.69 to -0.37
<i>NGF</i>	4.31 $\pm$ 0.82	3.53 $\pm$ 0.88	-0.79	<0.001**	-2.64	-0.93 to -0.65

Δ , mean within-subject change; CI, confidence interval; *IL4*, interleukin-4 gene; *IL13*, interleukin-13 gene; *IL31*, interleukin-31 gene; *TAC1*, tachykinin precursor 1 gene; *NGF*, nerve growth factor gene. All comparisons were performed with paired Student's t -tests, unless otherwise specified.

^a Expressed as $2^{-\Delta CT}$ relative to *GAPDH*

^b Negative Cohen's d values indicate a reduction in gene expression from baseline to day 14

* Analyzed using paired Student's t -test

** Analyzed using Wilcoxon signed-rank test

Effect sizes ranged from a moderate decrease for *IL4* ($d=-0.62$; $p=0.013$) to a large reduction for *NGF* ($d=-2.64$; $p<0.001$). The Shapiro-Wilk test indicated deviation from normality for within-subject *NGF* differences ($p=0.015$); accordingly, the pre-post comparison for this gene was performed with the Wilcoxon signed-rank test. All other variables met the normality assumption and were analyzed with paired Student's *t*-tests.

Discussion

The present study showed that twice-daily topical application of a cream formulation containing DHA_vD and barrier-repairing agents for 14 days was associated with clinically meaningful reductions in pruritus severity in a large cohort of patients with chronic pruritus of diverse etiologies, with consistent responses across etiological subgroups. This clinical improvement was supported by molecular evidence showing significant downregulation of *IL4*, *IL13*, *IL31*, *TAC1*, and *NGF* mRNA in paired biopsies of symptomatic skin from patients with chronic idiopathic pruritus. Overall, these findings suggest that the formulation may attenuate shared neuro-immune and barrier-related pathways involved in chronic pruritus, rather than acting solely through disease-specific inflammatory mechanisms.

Baseline pruritus severity was the strongest predictor of treatment response, and higher baseline 12-PSS scores increased the probability of achieving responder status (OR=1.089 per point). This finding suggests a possible floor effect among patients with milder symptoms, while supporting a clinically relevant benefit among those with greater symptomatic burden. Subgroup analysis further showed that the magnitude of pruritus reduction among patients using the study cream as monotherapy ($n=452$; mean reduction= 5.38 ± 3.51 points; Cohen's $d=-1.53$) was comparable to that observed among patients receiving concomitant antipruritic therapies after adjustment for baseline severity. This supports a treatment effect that was largely independent of co-administered agents.

The formulation contains a papain-based exfoliating complex, which should be considered in relation to protease-activated receptor 2 (PAR-2) signaling in chronic pruritus. Papain is an authorized cosmetic ingredient in the European Union and is widely used in topical products intended for application to both intact and partially compromised skin. In principle, proteases, including papain, may activate PAR-2 on epidermal keratinocytes and cutaneous sensory nerve endings. In the study formulation, however, papain is incorporated in a complexed form with natural moisturizing factors, and the complex is designed to promote gentle physiological desquamation rather than aggressive surface proteolysis. Consistent with this intended mode of action, no patient in either study discontinued treatment because of skin irritation or paradoxical worsening of pruritus, and no product-related adverse events were recorded in the etiologically diverse cohort of 1,000 patients, which included a substantial proportion of patients with atopic dermatitis. These tolerability findings are reassuring, although they cannot exclude subclinical PAR-2-mediated signaling. Dedicated controlled studies of papain-based exfoliating complexes in severely barrier-disrupted skin therefore remain warranted.

The downregulation of *TAC1* mRNA in treated skin biopsies is mechanistically consistent with the established pharmacology of DHA_vD, which interacts with NK1R and inhibits downstream mast cell degranulation [17]. Notably, NK1R antagonism by DHA_vD may attenuate not only substance P-mediated mast cell activation, but also the positive feedback loop through which mast cell-derived mediators stimulate further neuropeptide release from sensory nerve endings [22]. Systemic NK1R antagonists have shown antipruritic efficacy in clinical trials [23], and the present findings suggest that topical targeting of the same pathway may produce measurable molecular and clinical effects.

The observed decrease in cutaneous *IL31* mRNA expression may reflect attenuation of Th2-mediated inflammation secondary to restoration of the epidermal barrier [24]. Barrier dysfunction promotes Th2 polarization and IL-31 production through keratinocyte-derived alarmins [15], and the formulation's NMF-enriched exfoliating complex, by promoting physiological desquamation and stratum corneum hydration, may interrupt this outside-in signaling cascade. This interpretation is consistent with evidence that barrier repair reduces cutaneous inflammation and associated cytokine expression [25]. The moderate reductions in *IL4* and *IL13* mRNA alongside *IL31* suggest that the formulation may attenuate not only terminal

effector signaling at the IL-31/IL-31RA level but also the upstream type 2 inflammatory milieu that sustains pruritogenic gene transcription [26]. The smaller effect sizes for *IL4* and *IL13* relative to the three established pruritogenic targets are consistent with the topical mode of action, which is expected to exert its strongest effects on mediators most directly coupled to barrier function and local neuro-immune circuits [27].

The downregulation of *NGF* mRNA in treated skin carries potential implications for peripheral nerve sensitization in chronic pruritus. NGF promotes intraepidermal nerve fiber sprouting, upregulates TRPV1 expression, and lowers the activation threshold of pruriceptors [28]. The concurrent reduction of *NGF* mRNA alongside *TAC1* and *IL31* is unlikely to reflect a single mechanism, given that IL-31, substance P, and NGF operate through largely independent receptor systems – i.e., IL-31RA/oncostatin M receptor subunit beta [26], NK1R [23], and TrkA [29], respectively. This pattern instead supports the hypothesis that the multi-component formulation addresses different pruritogenic pathways simultaneously (**Figure 1**).

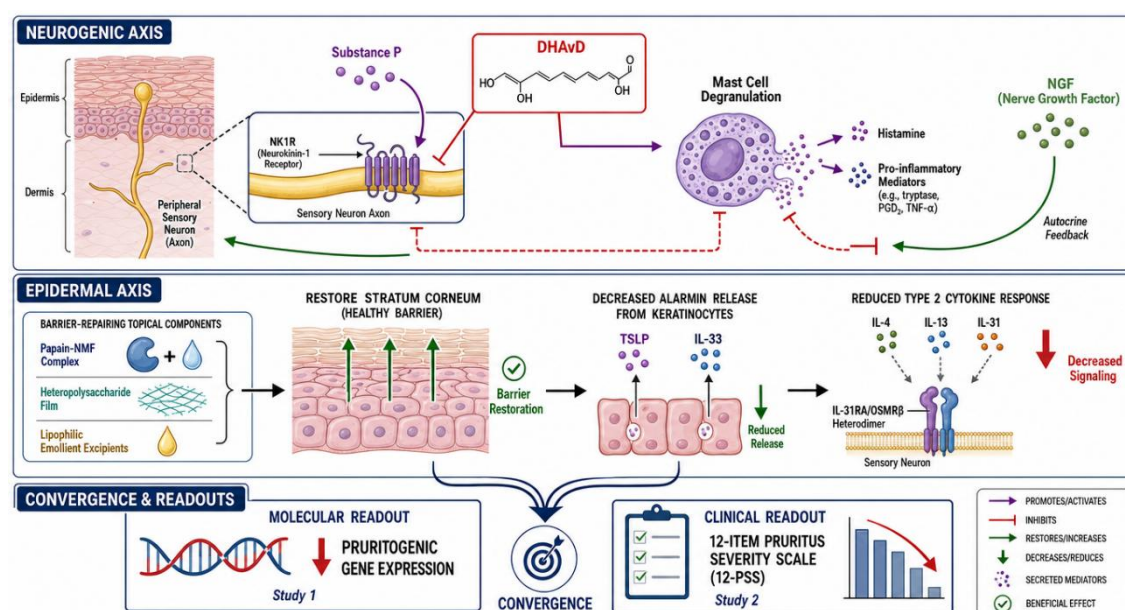


Figure 1. Schematic representation of the proposed mechanism of action of the topical formulation combining dihydroavenanthramide D (DHAvD) and barrier-repairing agents in chronic pruritus. In the neurogenic axis shown in the top panel, substance P released from cutaneous nerve endings binds the neurokinin-1 receptor (NK1R) expressed on peripheral sensory neuron axons and on mast cells, driving mast cell degranulation with release of histamine, tryptase, prostaglandin D₂ (PGD₂), and tumor necrosis factor-α (TNF-α), and engaging an autocrine NGF-mediated feedback loop that sustains peripheral nerve sensitization; DHAvD antagonizes NK1R and attenuates both downstream mast cell degranulation and NGF autocrine signaling. In the epidermal axis shown in the middle panel, the barrier-repairing topical components – the papain-NMF complex, the heteropolysaccharide film, and the lipophilic emollient excipients – support restoration of stratum corneum integrity, with consequent reduction of keratinocyte-derived alarmin release (TSLP, IL-33) and of the downstream type 2 cytokine response (IL-4, IL-13, IL-31), the latter signaling through the IL-31RA/oncostatin M receptor β (IL-31RA/OSMRβ) heterodimer on sensory neurons. As summarized in the bottom panel, the two axes converge on the readouts evaluated in the present work – reduction of pruritogenic gene expression in cutaneous biopsies and reduction of the 12-Item Pruritus Severity Scale (12-PSS) score in a multicenter cohort of patients with chronic pruritus of diverse etiology.

Our findings should be interpreted in the context of several limitations. First, the molecular sub-study was limited by a small sample size (n=20) and the absence of a vehicle-control arm. As a result, the contribution of emollient effects *per se* to the observed gene expression changes therefore cannot be excluded. Second, gene expression was measured at the mRNA level only, and protein-level assessments and functional assays would provide additional mechanistic depth. Third, the clinical study had an observational design that did not control for placebo or regression-to-the-mean effects. Although the large sample, the large effect size, and the

consistency across subgroups argue against a purely placebo-driven response, these findings require confirmation in randomized, vehicle-controlled trials. Fourth, and as a major limitation, the molecular and clinical studies were conducted on independent patient populations; direct correlation between individual gene expression changes and clinical response was therefore not possible. As a consequence, a causal relationship between the observed mRNA changes and the clinical reduction in pruritus severity cannot be inferred from the present design. Finally, the 14-day follow-up does not address long-term efficacy or potential gene expression rebound upon treatment discontinuation. Furthermore, dedicated controlled studies of the papain-based exfoliating complex in severely barrier-disrupted skin are advisable to fully characterize its tolerability and pharmacological profile in that context.

Conclusion

Topical application of a formulation containing DHA_vD and barrier-repairing agents was associated with clinically meaningful reductions in 12-PSS scores in a multicenter cohort of 1,000 patients with chronic pruritus across diverse etiological categories. This clinical improvement was supported by significant downregulation of five pruritogenic genes in symptomatic skin, suggesting potential multi-target attenuation of type 2 inflammatory signaling, neurogenic inflammation, and peripheral sensitization. Collectively, these findings support the concept that combined modulation of NK1R-mediated neurogenic pathways and epidermal barrier function may produce convergent clinical and molecular effects.

Ethics approval

The research adhered to the Declaration of Helsinki and received approval from the institutional review board of Studio Minoretti (reference: XN20_25). Prior to participation, all individuals provided written informed consent.

Acknowledgments

We thank Bionativa SpA for providing the samples used in the study free of charge.

Competing interests

The authors declare no conflict of interest.

Funding

This study received no external funding.

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

No artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

How to cite

Ribero S, Vaccaro M, Borgia F, *et al.* Real-world effectiveness of a topical dihydroavenanthramide D formulation for chronic pruritus: A multicenter clinical study with molecular evidence of pruritogenic pathway modulation. *Narra J* 2026; 6 (2): e3116 - <http://doi.org/10.52225/narra.v6i2.3116>.

References

1. Butler DC, Berger T, Elmariah S, *et al.* Chronic pruritus: A review. *JAMA* 2024;331(24):2114-2124.

2. Criado PR, Jardim Criado RF, Ianhez M, *et al.* Chronic pruritus: A narrative review. *An Bras Dermatol* 2025;100(3):487-519.
3. Ramcke T, Kaplan DH. From neuroimmune circuits to targeted therapy of chronic pruritus. *Pharmacol Rev* 2026;78(2):100121.
4. Garibyan L, Rheingold CG, Lerner EA. Understanding the pathophysiology of itch. *Dermatol Ther* 2013;26(2):84-91.
5. Champion M, Smith L, Gatault S, *et al.* Interleukin-4 and interleukin-13 evoke scratching behaviour in mice. *Exp Dermatol* 2019;28(12):1501-1504.
6. Jha MK, Han Y, Liu Z, *et al.* Type 2 cytokines pleiotropically modulate sensory nerve architecture and neuroimmune interactions to mediate itch. *J Allergy Clin Immunol* 2025;156(4):1066-1081.e12.
7. Tatu AL, Nadasdy T, Arbune A, *et al.* Interrelationship and sequencing of interleukins 4, 13, 31, and 33 – An integrated systematic review: Dermatological and multidisciplinary perspectives. *J Inflamm Res* 2022;15:5163-5184.
8. Cevikbas F, Wang X, Akiyama T, *et al.* A sensory neuron-expressed IL-31 receptor mediates T helper cell-dependent itch: Involvement of TRPV1 and TRPA1. *J Allergy Clin Immunol* 2014;133(2):448-460.
9. Zhu Z, Bhatia M. Inflammation and organ injury – The role of substance P and its receptors. *Int J Mol Sci* 2023;24(7):6140.
10. Ständer S, Yosipovitch G. Substance P and neurokinin 1 receptor are new targets for the treatment of chronic pruritus. *Br J Dermatol* 2019;181(5):932-938.
11. Indo Y. Nerve growth factor, pain, itch and inflammation: Lessons from congenital insensitivity to pain with anhidrosis. *Expert Rev Neurother* 2010;10(11):1707-1724.
12. Yamaguchi J, Aihara M, Kobayashi Y, *et al.* Quantitative analysis of nerve growth factor (NGF) in the atopic dermatitis and psoriasis horny layer and effect of treatment on NGF in atopic dermatitis. *J Dermatol Sci* 2009;53(1):48-54.
13. Fölster-Holst R, Reimer R, Neumann C, *et al.* Comparison of epidermal barrier integrity in adults with classic atopic dermatitis, atopic prurigo and non-atopic prurigo nodularis. *Biology (Basel)* 2021;10(10):1008.
14. Huang Y, Liu J, Zhang X, *et al.* Patients with senile pruritus have a distinct skin microbiota and epidermal barrier in comparison with healthy controls. *J Dermatol* 2021;48(12):1892-1899.
15. Beck LA, Cork MJ, Amagai M, *et al.* Type 2 inflammation contributes to skin barrier dysfunction in atopic dermatitis. *JID Innov* 2022;2(5):100131.
16. Meng J, Xiao H, Xu F, *et al.* Systemic barrier dysfunction in type 2 inflammation diseases: Perspective in the skin, airways, and gastrointestinal tract. *Immunol Res* 2025;73(1):60.
17. Lotts T, Agelopoulos K, Phan NQ, *et al.* Dihydroavananthramide D inhibits mast cell degranulation and exhibits anti-inflammatory effects through the activation of neurokinin-1 receptor. *Exp Dermatol* 2017;26(8):739-742.
18. Park J, Shin JY, Kim D, *et al.* Dihydroavananthramide D enhances skin barrier function through upregulation of epidermal tight junction expression. *Curr Issues Mol Biol* 2024;46(9):9255-9268.
19. Akiska YM, Gandhi I, Adler R, *et al.* Pathogenesis and therapeutics for chronic pruritus of unknown origin: A systematic review. *Int J Dermatol* 2026;65(4):706-723.
20. Emanuele E, Bertona M, Altabas K, *et al.* Anti-inflammatory effects of a topical preparation containing nicotinamide, retinol, and 7-dehydrocholesterol in patients with acne: A gene expression study. *Clin Cosmet Investig Dermatol* 2012;5:33-37.
21. Reich A, Božek A, Janiszewska K, *et al.* 12-Item pruritus severity scale: Development and validation of new itch severity questionnaire. *Biomed Res Int* 2017;2017:3896423.
22. Alam M, Buddenkotte J, Ahmad F, *et al.* Neurokinin 1 receptor antagonists for pruritus. *Drugs* 2021;81(6):621-634.
23. Ho PY, Huang YC, Lin YT. Systemic neurokinin-1 receptor antagonists mitigate chronic pruritus: A systematic review and meta-analysis. *J Eur Acad Dermatol Venereol* 2025;39(10):1738-1749.
24. Singh B, Jegga AG, Shanmukhappa KS, *et al.* IL-31-driven skin remodeling involves epidermal cell proliferation and thickening that lead to impaired skin-barrier function. *PLoS One* 2016;11(8):e0161877.
25. Hänel KH, Cornelissen C, Lüscher B, *et al.* Cytokines and the skin barrier. *Int J Mol Sci* 2013;14(4):6720-6745.
26. Furue M, Furue M. Interleukin-31 and pruritic skin. *J Clin Med* 2021;10(9):1906.
27. Szöllősi AG, Oláh A, Lisztes E, *et al.* Pruritus: A sensory symptom generated in cutaneous immuno-neuronal crosstalk. *Front Pharmacol* 2022;13:745658.
28. García-Domínguez M. NGF in neuropathic pain: Understanding its role and therapeutic opportunities. *Curr Issues Mol Biol* 2025;47(2):93.
29. Roblin D, Yosipovitch G, Boyce B, *et al.* Topical TrkA kinase inhibitor CT327 is an effective, novel therapy for the treatment of pruritus due to psoriasis: Results from experimental studies, and efficacy and safety of CT327 in a phase 2b clinical trial in patients with psoriasis. *Acta Derm Venereol* 2015;95(5):542-548.