Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Efficacy of p62-expressing plasmid in treatment of canine ATOPIC DERMATITIS: A Pilot study IN companion dogs

Vladimir Gabai
CureLab Veterinary, Boston, USA
Evgeny Bakin
CytoReason LTD, Tel-Aviv, Israel
Maxim Langs
Miramonte High School, CA, USA
Robert Delvin
CureLab Veterinary, Boston, USA
Alexis Nahama
CureLab Veterinary, Boston, USA
Sergei Krasny
N.N. Alexandrov National Cancer Centre of Belarus, Minsk, Belarus
Yauheni Baranau
Minsk City Clinical Oncologic Centre, Belarus
Sergey Polyakov
N.N. Alexandrov National Cancer Centre of Belarus Minsk, Belarus
Maksim Patapovich
Belarusian State University, Minsk, Belarus
Sergey Gvozdev
BELVITUNIFARM, Belarus
Aliaksei Bazyleuski
Veterinary Center, Minsk, Belarus
Siarhei Isachenka
Vetetaclinic, Minsk, Belarus
Hanna Lisnenko
NAN animal clinic
Nastassia Kandratovich
Alfa-Vet Veterinary Clinic
Elena Zueva
ZOOevaVet

Alexander Shneider

ashneider@curelab.com

CureLab Veterinary, Boston, USA

Research Article

Keywords: DNA vaccine, inflammation, clinical trial, plasmid, immunotherapy, canine

Posted Date: April 7th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9223428/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations:
The authors declare potential competing interests as follows: VG, RD, AN and AS were employed by CureLab Veterinary Inc. SG was employed by BELVITUNIFARM. EB was employed by CytoReason LTD.

Ethics Statement Formal ethical approval was not required for this study under Belarusian legislation or institutional policy. National regulations specify that research involving client□owned animals undergoing routine diagnostic or therapeutic procedures does not require review by an animal ethics committee. All procedures were performed as part of standard clinical care, and written informed consent was obtained from all owners prior to enrollment. This is consistent with international guidance for veterinary clinical research in privately owned animals, including the principles outlined by the World Organisation for Animal Health and the International Association of Veterinary Editors.

EFFICACY OF P62-EXPRESSING PLASMID IN TREATMENT OF CANINE ATOPIC DERMATITIS: A PILOT STUDY IN COMPANION DOGS

Vladimir Gabai[1], Eugene Bakin[2], Maxim Langs[3], Robert Devlin[1], Alexis Nahama[1], Sergei Krasny[4], Yauheni Baranou[5], Sergei Polyakov[4], Maksim Patapovich[6], Sergey Gvozdev[7], Aliaksei Bazyleuski[8] , Siarhei Isachenka[9], Hanna Lisnenko[10], Nastassia Kandratovich[11], Elena Zueva[12], Alexander Shneider[1,13*]

[1]CureLab Veterinary Inc, Boston, MA, USA

[2]CytoReason LTD, Tel-Aviv, Israel

[3]Miramonte High School, Orinda, CA, USA

[4]N.N. Alexandrov National Cancer Centre of Belarus, Minsk, Belarus

[5]Minsk City Clinical Oncologic Centre, Minsk, Belarus

[6]Department of Microbiology, Faculty of Biology, Belarusian State University, Minsk, Belarus

[7] BELVITUNIFARM, Vitebsk district, Belarus

[8] Veterinary Center, Minsk, Belarus

[9]Vetetaclinic, Minsk, Belarus

[10]NAN animal Clinic, Minsk, Belarus

[11]Alfa-Vet Veterinary Clinic, Minsk, Belarus

[12]ZOOevaVet, Postavi, Belarus

[13] Department of Molecular Biology, Ariel University, Ariel, Israel

*Correspondence: Alexander Shneider[1]

ashneider@curelab.com

ABSTRACT

BACKGROUND: Canine Atopic Dermatitis (AD) is a genetically predisposed chronic inflammatory and pruritic allergic skin disease. Despite several drugs for treatment of AD, there are still unmet needs for therapies that have significant clinical response, yet minimal adverse effects.

OBJECTIVE: We have recently developed a plasmid DNA encoding SQSTM1/p62, which demonstrated efficacy in preclinical models of inflammatory diseases as well as in osteoarthritis pain in dogs. Here we test a hypothesis that the p62 plasmid may alleviate severity of AD symptoms associated with the chronic inflammatory nature of the disease.

ANIMALS: Twenty privately owned animals with diagnosed severe chronic AD

MATERIALS AND METHODS: This was single arm open label study. All dogs were treated with p62 plasmid (1 mg) weekly either intramuscular or subcutaneously (10 dogs in each group) and AD severity by pVAS score was assessed weekly for 11 wks.

RESULTS: Treatment with p62 plasmid decreased pVAS score from baseline 8.05+/- 1.36 to 5.52+/- 1.8 (mean+/-SD) with a highly significant statistical difference ($P=10^{-5}$). The effect of the p62 plasmid treatment were developed over time, starting within 2 weeks and with a maximum effect after 7 weeks. Estimated average decrease in pVAS score was 0.23 per week with high statistical significance ($p = 3.9 \cdot 10^{-19}$). There were no side effects reported. Given the severity of AD, some owners were allowed to continue administration of the same AD medications (steroids or Apoquel®), which they used before. No potential interaction (positive or negative) with p62 plasmid was observed.

CONCLUSION AND CLINICAL RELEVANCE: The p62 plasmid demonstrated modest but statistically significant alleviation of AD in dogs without observed side effects. These results are encouraging to conduct further trials.

KEYWORDS: DNA vaccine; inflammation, clinical trial, plasmid, immunotherapy, dog, canine, atopic dermatitis, itch

INTRODUCTION

Canine Atopic Dermatitis (AD) is a genetically predisposed chronic inflammatory and pruritic allergic skin disease. It is usually associated with IgE antibodies to environmental allergens (1). AD occurs in about 10-15% of dogs, with strong breed predispositions and onset in young adulthood (2). It shares many clinical characteristics with its counterpart in humans, such as a genetic predisposition, early age of onset, predilection sites of affected skin (3). Onset of Immune response during AD is dominated by T_H2 cells and involves cytokines such as IL-4, IL-5, IL-6, IL-13, and IL-31 (1,4). Development of chronic inflammation, however, involves a mix of T_H1, T_H2, T_H17, and T_H22-cell mediators (4) (5). Given etiology of the disease, medications to treat AD include a range of anti-inflammatory drugs, focused anti-itch medications (JAK inhibitors), and monoclonal antibodies. The most common drugs include cyclosporin A (Atopica), which inhibit production of IL-2, IL-4 and INF, oclacitinib (Apoquel®) which suppresses JAK and IL-2, IL-4, IL-6, IL-13, and IL-31 in particular (5) (6) (7). Among old but still often used ant-inflammatory drugs are glucocorticoids, which are inexpensive and with rapid actions (2), but are known to potentially have short and long-term side effects. A more recent drug is anti-canine monoclonal antibody IL-31 mAb (Lokivetmab®). However, all these drugs often have limited efficiency and adverse effects. For instance, glucocorticoids, especially in long term use, have a range of side effect such as polyuria, polydipsia, polyphagia, and gastrointestinal and endocrine disorders (e.g. Cushing's Disease), whereas for most of the other drugs listed the most common side effects are vomiting, diarrhea, and anorexia (2). Therefore, there are still unmet needs for therapies that have broad patient response, good efficacy in relieving itch, yet minimal adverse effects.

Curelab has developed a plasmid DNA encoding SQSTM1/p62. This plasmid-encoding protein p62 plays multiple functions in cells, controlling, among others, autophagy, signal transduction, and inflammation (7, 8). Initially p62 plasmid was introduced as an anti-cancer vaccine, which revealed anti-tumor activity in canine mammary tumors (9) as well as in clinical trials in humans, and it demonstrated a good safety profile (10, 11) (12). During our preclinical studies, we found a quite unexpected effect of p62 plasmid which apparently was unrelated to its immune response action– it improved symptoms in diseases of chronic inflammation (see ref (7) for review). In particular, we found that the plasmid decreases generation of pro-inflammatory cytokines such as TNF, IL-1, IL-6, while increasing anti-inflammatory cytokines (e.g. IL-4 and IL-10) and decreased ovariectomy-induced osteoporosis in mice and rats (13) (14). Also, anti-inflammatory effect of human p62 plasmid showed a decrease in disease symptoms as observed in rats with metabolic syndrome/obesity (15), and age-related macular degeneration (AMD) (16). Of note, all these inflammatory and anti-inflammatory cytokines are also involved in AD as mentioned above.

Recently, we also observed in companion dogs that the p62 plasmid significantly reduced osteoarthritis pain associated with chronic inflammation (17). Given that AD is also considered as a disease of chronic inflammation, and canine AD has greater translational value to human AD than rodent models, we hypothesize that p62 plasmid may alleviate symptoms of AD in both companion animals and humans. This pilot study was conducted to test this hypothesis in companion dogs.

MATERIALS AND METHODS

P62 plasmid. Human p62 (SQSM1, isoform 1) was cloned in pcDNA3.1 vector, and plasmid DNA was produced by Aldevron (ND, USA) in GMP-like conditions as described before (9). Quality of plasmid preparation was tested by standard assays, which demonstrated 80% supercoiled DNA and less than 5 EU/mg of endotoxin. It was formulated in phosphate buffered saline and 1 mg in 1 ml was injected intramuscular (IM) or subcutaneously (SC).

Ethics

This one-arm open label study of 20 companion dogs with AD was conducted in veterinary clinics in the Republic of Belarus in 2024-2025. All owners provided written informed consent. Best practice of individual veterinary patient care was followed.

Enrollment criteria

Inclusion criteria

Client-owned dogs (irrespective of breed, age or sex) with visible signs of pruritic skin disease and diagnosed with chronic AD based on published guidelines (1) were included in the study. Dogs were in overall good health apart from the AD and free from external parasites at the time of the first visit; appropriate parasite control/prevention was used, if necessary, throughout the study. Dogs were initially assessed for entry into the study by the owners based on a Visual Analog Scale (pVAS) score of pruritus (18) of at least 6 cm (10 cm scale).

Exclusion criteria

Exclusion criteria included dogs with evidence of malignant neoplasia, evidence of demodicosis within the past year, evidence of immune suppression such as hyperadrenocorticism, dogs that received systemic antimicrobial therapy for bacterial or fungal skin infections, dogs with known food allergy and lactating bitches or dogs (male or female) intended for use as breeding animals.

Study protocol

Study was a single-arm, longitudinal trial in which 20 dogs with AD were assessed weekly using a standardized itch clinical severity score. On week 0 through week 10th, the dogs were treated with 1 mg of p62 plasmid, either IM as described before for osteoarthritic dogs (10 dogs) (17) or

SC (10 dogs) once a week for 10 weeks. Dogs were evaluated on the day of enrolment (day 0) and then every week for 11 weeks (week after the last treatment administered). A validated owner-assessed pruritus Visual Analog Scale (pVAS) score (0-10 scale) was used to determine severity of pruritus at each evaluation. The primary endpoint was the trajectory of the pVAS score over time. Some dogs, especially with severe AD, remained on treatment with steroids or Apoquel®, which allowed to study effect of p62 plasmid in combination with these drugs; no other drugs were employed.

Adverse effect including vomiting, diarrhea, colic, seizure were monitored according to FDA guidelines (https://www.fda.gov/animal-veterinary/report-problem/how-report-animal-drug-and-device-side-effects-and-product-problems

Statistical methods

Non-parametric trend analysis. To quantify individual longitudinal trends, we calculated, for each dog, Spearman's rank correlation between score and visit number. A one-sample t-test assessed whether the mean of these per-dog correlations differed from zero (null: $\rho = 0$), using a two-sided $\alpha = 0.05$. Between-group comparisons of trend magnitude (i.e. IM vs. SC injection, steroids vs. non-steroids, Apoquel vs non-Apoquel), were performed via pairwise tests on the per-dog correlation coefficients.

Linear mixed-effects modeling Population-level trajectories were modeled with linear mixed-effects regression. A random intercept for each dog accounted for within-animal correlation. Fixed effects included visit number, treatment subgroup (steroid yes/no). The second model substituted the administration category (IM vs. SC). Estimates of slope, group differences, and 95% confidence intervals were obtained from the fitted models.

Statistical inference All hypothesis tests were two-sided with $\alpha = 0.05$, and effect estimates are accompanied by 95% confidence intervals. Analyses were implemented in R (version 4.x) using standard libraries for non-parametric tests, mixed-effects modeling, and segmented regression

RESULTS

Study population

The study population (20 animals) was diverse consisting of several breeds (mostly pure breed, except 1 dog), both female (70%) and male (30%) were represented, with a mean age (+/-SD) of 4.8+/-1.9 years (Table 1). All dogs were treated with p62 plasmid either IM or SC weekly for 10 weeks with the dose of 1 mg (17).

AD pVAS scores were measured by owners once a week; also, the general wellbeing of dogs, blood analysis and possible side effects of the treatment were monitored once a week. Most dogs before the treatment demonstrated a severe AD disease: mean pVAS score (+/-SD) was 8.05+/-1.36, with 25% of dogs having the highest score (10 in 0-10 scale) (Table 1). By the end of the treatment, mean pVAS score decreased significantly to 5.52+/- 1.8 ($P=10^{-5}$) (Table 1). As shown in Table 1, p62 plasmid treatment decreased mean pVAS score by 32% by the end of 10-week treatment, and 5 dogs (20%) achieved 50% or greater score reduction. Effect of p62 plasmid on pVAS was apparently independent of dog weight. For instance, from 5 dogs achieving maximal effect, two had weight of 4 and 7 kg, and three - 14, 27, and 33 kg (Table 1). Such lack of dose dependence is a general characteristics of DNA plasmids, and, accordingly, we did not observed dose dependence of p62 plasmid in our previous preclinical or clinical studies (11, 19).

Table 1 Patients characteristics

#	Breed	Gender	Age, yr	Weight, kg	Injection IM or SC	Apoquel	Steroids	Initial pVAS	Final pVAS
3	Miniature Schnauzer	male	6	7	IM	NR*	NR*	6	2
4	Pekinese	male	5	4	SC	-	-	10	2
5	Labrador Retriever	female	7	30	IM	+	+	8	6
6	Bulldog	female	5.5	14	IM	+	+	10	5
7	Staffordshire Terrier	male	4	33	IM	-	-	10	2
8	Chihuahua	female	2	2	SC	+	+	8	6

9	Mixed Breed	female	5.3	30	IM	+	+	10	8
10	French Bulldog	female	2	12	IM	-	-	8	8
11	French Bulldog	male	4	13.5	SC	-	-	6	8
12	Pit Bull	female	2	27	SC	-	-	6	2
13	Staffordshire Terrier	female	4	25	SC	-	-	6	5
14	Chihuahua	female	9	2	IM	-	+	8	5
15	French Bulldog	female	2	16	IM	-	+	8	6
16	West Highland White Terrier	male	6	6	IM	+	+	8	6
17	German Boxer	female	8	43	SC	+	+	7	7
18	Beagle	female	4	7	IM	-	-	10	7
19	Chinese Crested	female	4	5	SC	+	+	8	6

20	Pit Bull	female	4	37	SC	-	-	8	7
21	Samoyed	male	5	27	SC	+	-	8	6
22	West Highland White Terrier	female	6	8	SC	+	+	8	8
	Means (SD)		**4.6 (1.8)**	**17.8 (12.5)**				**8.05(1.36)**	**5.52(1.8)**

($p=10^{-5}$)

***NR - not reported**

As in previous studies in dogs and humans, no adverse effects of the treatment including vomiting, diarrhea, colic, seizure were observed over the study period (9, 11, 17).

The pVAS score trend over the time of the treatment in total dog population

We next addressed how pVAS score changed over time. As shown in Fig.1, most of dogs demonstrated decline in score meaning that their conditions improved as treatment progressed. Using Spearman correlation (Non-Parametric Analysis) we found that average correlation between number of weekly injections and pVAS score was -0.59, which is a on scale from -1 to +1 indicated a strong negative trend with very high statistical significance ($p = 4.26 \cdot 10^{-3}$) (Table 2, Fig.2). As seen in Fig.1, pVAS scores started to slowly decline already after 1-2 injections, with a change point about 7 weeks. The score decreasing rate of before this change point was -0.311 per week, while after 7 weeks it was almost zero. (Fig.1). Thus, p62 plasmid started to work rather fast with a maximum effect at 7 weeks and then it reached plateau (Fig.1)

Table 2. **Distribution of Spearman correlation coefficients (non-parametric approach)**

Characteristic	**N = 20***	**95% CI**
Correlation	Min = -0.94, Max = 0.31, Mean = -0.59 (0.41)	-0.78, -0.39

*Min = Min, Max = Max, Mean = Mean (SD)

Abbreviation: CI = Confidence Interval

Table 3. Linear mixed-models analysis (parametric approach)

Term	Estimate	Std.error	Statistic	df	p.value	conf.low	conf.high
Intercept	7.66	0.37	20.73	24.64	4.2e-17	6.90	8.43
Timepoint	-0.23	0.02	-9.85	216.12	3.9e-19	-0.28	-0.19

Effect of IM vs SC injections

In our previous preclinical studies and clinical trials, we used IM injections for plasmid administration (9, 11), which is widely used for such medications. However, for veterinarians and dog's owners SC injections are more convenient; therefore, we tested to see whether there is a difference in the results between these methods of administering the plasmid injections. For this, equal number of dogs were injected weekly IM or SC with the same dose of the plasmid (1 mg). As seen in Table 4, Spearman correlation coefficients for IM and SC injection were very close and statistically insignificant (p=0.899) indicating equal efficacy of IM or SC injections.

Table 4. Spearman correlation coefficients for IM and SC injection

Characteristic	**intramuscular** N = 10*	**95% CI**	**subcutaneous** N = 10*	**95% CI**
Correlation	Min = -0.94, Max = 0.31, Mean = -0.58 (0.45)	-0.90, -0.25	Min = -0.93, Max = 0.17, Mean = -0.60 (0.40)	-0.88, -0.32

*Min = Min, Max = Max, Mean = Mean (SD)

Abbreviation: CI = Confidence Interval

Given the severity of AD in dogs and unknown effect of p62 plasmid in the treatment of the disease, some owners, for ethical reasons, were allowed to continue administration the same AT medications (steroids or/and Apoquel), which they used prior to the trial. However, since these preceding treatments were not successful, the owners expected that these medications may

better work in combination with p62 plasmid. For instance, female bulldog (patient #6) was being treated with both steroids and Apoquel, but her initial score, despite the treatment, was 10 (Table 1). Although complicating the picture, such combination setting allowed us to additionally study whether there any interaction of these standard of care medications with p62 plasmid (see below)

Effect of steroids

Next the dogs were stratified by status of steroid therapy: half of them that received standard steroid therapy (prednisolone or methylprednisolone 2 mg daily per os) along with p62 plasmid were compared with those treated with p62 plasmid only. As seen in **Table 5**, dogs with and without steroid therapy demonstrated close Spearman correlation coefficients (0.61 vs 0.56 respectively) and absence of statistical significance (p=0.804). Thus, addition of steroids to the plasmid did not differ from the effect of the plasmid alone.

Table 5. Spearman correlation coefficients for presence or absence steroid injection

Characteristic	**steroid-** N = 10*	**95% CI**	**steroid+** N = 10*	**95% CI**
Correlation	Min = -0.93, Max = 0.31, Mean = -0.56 (0.45)	-0.89, -0.24	Min = -0.94, Max = 0.00, Mean = -0.61 (0.40)	-0.90, -0.33

*Min = Min, Max = Max, Mean = Mean (SD)

Abbreviation: CI = Confidence Interval

Effect of Apoquel

Although Apoquel is considered as standard of care for AD, it is not always effective, and has side effects. In our trial, 9 dogs received Apoquel (4 or 8 mg daily per os) along with p62 plasmid, while other 11 dogs were treated with plasmid only. As we found, similar to steroids, there was no difference in Spearman correlation coefficients between Apoquel+p62 plasmid and plasmid only (0.60 vs 0.57) (p=0.894) indicating that addition of Apoquel to the plasmid did not differ from the effect of the plasmid alone.

Table 6 Spearman correlation coefficients for presence or absence Apoquel injections

Characteristic	**apoquel-** N = 11*	**95% CI**	**apoquel+** N = 9*	**95% CI**
Correlation	Min = -0.94, Max = 0.31, Mean = -0.60 (0.44)	-0.90, -0.30	Min = -0.93, Max = 0.00, Mean = -0.57 (0.40)	-0.88, -0.26

*[1]Min = Min, Max = Max, Mean = Mean (SD)

Abbreviation: CI = Confidence Interval

Association between Apoquel and steroid administration

As seen in Table 1, some dogs were administered, besides plasmid, steroids or/and Apoquel. Thus, we addressed whether steroid therapy is associated with Apoquel therapy. We found a a strong association between the administration of the two therapies (Odds ratio (OR) = 27.33, 95%

CI [2.06, 1740], $p = 5.5 \cdot 10^{-3}$) (Table 7). This finding may be related to the fact that dogs in more severe condition tended to receive both agents in combination (Table 1).

Table 7. Association of Apoquel and steroid injections

therapy	Apoquel-	Apoquel+
steroid-	9	1
steroid+	2	8

estimate	p.value	conf.low	conf.high
27.33	5.5e-03	2.06	1,740.08

DISCUSSION

Result of this pilot trial demonstrates: 1) treatment with p62 plasmid once a week might alleviate AD in dogs: 2) IM or IC injections seem to have the same effect; 3) there were no side effects observed; 4) p62 plasmid on AD required several weeks to reach its peak effect; 5) there seem to be no interactions, positive or negative, between oclacitinib (Apoquel), glucocorticoids and p62 plasmid. Although the effect of p62 plasmid is rather moderate (decrease in pVAS score by 35%), the clinical benefit and absence of side effects observed in this and in previous trials in dogs and human are very encouraging and further (controlled) studies are needed to determine if p62 plasmid could become a treatment of choice when other options fail. Of note, the owners were not financially compensated and had no incentive to manipulate the scores.

Since this was a pilot study to address only safety and efficacy of p62 plasmid, MOA of the plasmid in AT was not studied. AT is considered as inflammatory disease where, along with acute Th2-assoctiated response, development of chronic inflammation involves Th1, Th17, Th22 and responses, in particular IL-2, IL-12, TNF, and interferon-gamma (4) (5). Additionally, another pathways of chronic inflammation, inflammasome/IL-1 and NF-kB, were also involved in AT (20, 21) (22). In our previous studies in different preclinical models of chronic inflammation such as osteoporosis, obesity and Alzheimer, we observed suppression of IL-1, IL-2, IL-12, IL-17 cytokines along with NF-kB pathway (13) (14) (15) (23). Furthermore, p62 plasmid alleviated pain in companion dogs with osteoarthritis, another chronic inflammatory disease (17). Based on these data, we hypothesize that p62 may exert its anti-AT effect via suppression of chronic rather than acute phase of inflammation, and we will plan to test this hypothesis in our future studies.

Study limitations

There are several limitations of the study

1. Absence of placebo randomized control or standard of care for comparison. However, the study is a pilot study for which a controlled group was not considered necessary and we preferred assessing as is before evaluation against an active control (e.g., lokivetmab/oclacitinib.
2. • Single-arm, open label design and owner-reported outcome only (pVAS) creates a high risk of expectancy/placebo, regression-to-the-mean, seasonal fluctuation in a chronic waxing/waning disease like AD.
3. Small sample size. Although it was a pilot study with a limited dog numbers, we have managed to obtain a high statistical significance of p62 plasmid effect. However, it is clear from our data that individual variations in pVAS scores between dogs and their response to the treatment varied significantly: some of them responded well, but others almost did not respond (Fig.1). Larger sample size may allow us to analyze, among others, how dog's bread, age, diseases severity, and treatment history would affect efficacy of p62 plasmid. This allow to choose those group of dogs for whom the treatment with the p62 plasmid would be most effective.

REFERENCES

1. Hensel P, Santoro D, Favrot C, Hill P, Griffin C. Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification. BMC veterinary research. 2015;11:196.
2. Drechsler Y, Dong C, Clark DE, Kaur G. Canine Atopic Dermatitis: Prevalence, Impact, and Management Strategies. Veterinary medicine (Auckland, NZ). 2024;15:15-29.
3. Marsella R, De Benedetto A. Atopic Dermatitis in Animals and People: An Update and Comparative Review. Veterinary Sciences. 2017;4(3):37.
4. Sroka-Tomaszewska J, Trzeciak M. Molecular Mechanisms of Atopic Dermatitis Pathogenesis. International Journal of Molecular Sciences. 2021;22(8):4130.
5. Nuttall TJ, Marsella R, Rosenbaum MR, Gonzales AJ, Fadok VA. Update on pathogenesis, diagnosis, and treatment of atopic dermatitis in dogs. J Am Vet Med Assoc. 2019;254(11):1291-300.
6. Olivry T, DeBoer DJ, Favrot C, Jackson HA, Mueller RS, Nuttall T, et al. Treatment of canine atopic dermatitis: 2015 updated guidelines from the International Committee on Allergic Diseases of Animals (ICADA). BMC veterinary research. 2015;11:210.
7. Sabbieti MG, Marchegiani A, Sufianov AA, Gabai VL, Shneider A, Agas D. P62/SQSTM1 beyond Autophagy: Physiological Role and Therapeutic Applications in Laboratory and Domestic Animals. Life. 2022;12(4):539.
8. Gabai V, Shifrin V. Feasibility analysis of p62 (SQSTM1) – encoding DNA vaccine as a novel cancer immunotherapy. International Reviews of Immunology. 2014;33(5):375-82.

9. Gabai V, Venanzi FM, Bagashova E, Rud O, Mariotti F, Vullo C, et al. Pilot study of p62 DNA vaccine in dogs with mammary tumors. Oncotarget. 2014;5(24):12803-10.
10. Ponomarenko DM, Gabai VL, Sufianov AA, Kolesnikov SI, Shneider AM. Response of a chemo-resistant triple-negative breast cancer patient to a combination of p62-encoding plasmid, Elenagen, and CMF chemotherapy. Oncotarget. 2019;11(3):294-9.
11. Ponomarenko DM, Klimova ID, Chapygina YA, Dvornichenko VV, Zhukova NV, Orlova RV, et al. Safety and efficacy of p62 DNA vaccine ELENAGEN in a first-in-human trial in patients with advanced solid tumors. Oncotarget. 2017;8(34):56030-40.
12. Krasny S, Baranau Y, Polyakov S, Zharkova E, Streltsova O, Filimonava A, et al. Clinical efficacy of plasmid encoding p62/SQSTM1 (Elenagen) in combination with gemcitabine in patients with platinum-resistant ovarian cancer: a randomized controlled trial. Frontiers in Oncology. 2024;14.
13. Sabbieti MG, Agas D, Capitani M, Marchetti L, Concetti A, Vullo C, et al. Plasmid DNA-coding p62 as a bone effective anti-inflammatory/anabolic agent. Oncotarget. 2015;6(6):3590-9.
14. Agas D, Marchegiani A, Laus F, Gabai V, Sufianov AA, Shneider A, et al. p62/SQSTM1 indirectly mediates remote multipotent mesenchymal cells and rescues bone loss and bone marrow integrity in ovariectomized rats. Journal of Cellular Physiology. 2023;238(2):407-19.
15. Halenova T, Savchuk O, Ostapchenko L, Chursov A, Fridlyand N, Komissarov AB, et al. P62 plasmid can alleviate diet-induced obesity and metabolic dysfunctions. Oncotarget. 2017;8(34):56030-40.
16. Kolosova NG, Kozhevnikova OS, Telegina DV, Fursova AZ, Stefanova NA, Muraleva NA, et al. p62 /SQSTM1 coding plasmid prevents age related macular degeneration in a rat model. Aging. 2018;10(8):2136-47.
17. Gabai V, Bakin E, Langs M, Devlin R, Krasny S, Baranou Y, et al. Efficacy of P62-expressing plasmid in treatment of canine osteoarthritis pain: a pilot study. Front Vet Sci. 2025;12:1519881.
18. Hill PB, Lau P, Rybnicek J. Development of an owner-assessed scale to measure the severity of pruritus in dogs. Veterinary dermatology. 2007;18(5):301-8.
19. Venanzi F, Shifrin V, Sherman MY, Gabai V, Kisilev O, Komissarov A, et al. Broad-spectrum anti-tumor and anti-metastatic DNA vaccine based on p62-encoding vector. Oncotarget. 2013;4(10):1829-35.
20. Martin P, Goldstein JD, Mermoud L, Diaz-Barreiro A, Palmer G. IL-1 Family Antagonists in Mouse and Human Skin Inflammation. Front Immunol. 2021;12:652846.
21. Calabrese L, Fiocco Z, Mellett M, Aoki R, Rubegni P, French LE, et al. Role of the NLRP1 inflammasome in skin cancer and inflammatory skin diseases. British Journal of Dermatology. 2023;190(3):305-15.
22. Mahjoubin-Tehran M, Rezaei S, Butler AE, Sahebkar A. Decoy oligonucleotides targeting NF-κB: a promising therapeutic approach for inflammatory diseases. Inflammation Research. 2025;74(1):47.
23. Cecarini V, Bonfili L, Gogoi O, Lawrence S, Venanzi FM, Azevedo V, et al. Neuroprotective effects of p62(SQSTM1)-engineered lactic acid bacteria in Alzheimer's disease: a pre-clinical study. Aging. 2020;12(16):15995-6020.

FIGURES

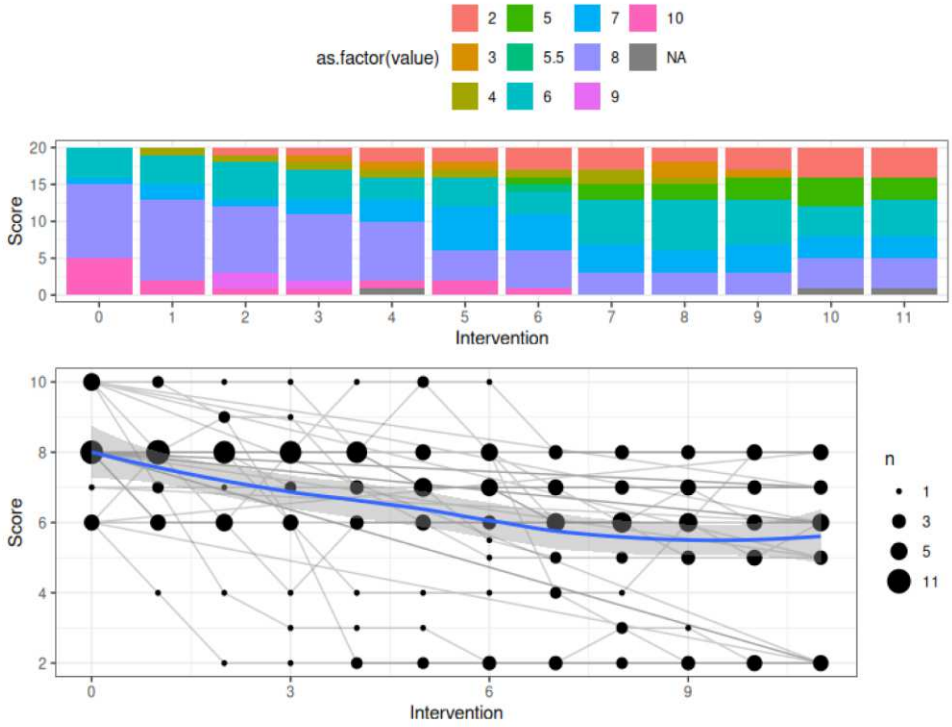


Fig.1 Effect of treatment with p62 plasmid on pVAS scores. Upper panel presents a stacked bar chart illustrating the distribution of pVAS scores across intervention time (0–11 wks.). Each bar is segmented by score values, with color-coded strata representing individual score categories. This visualization highlights the shift in score composition over time. Early intervention levels are characterized by a broader distribution and higher scores, while later stages show increasing dominance of lower score categories. This pattern supports the observed trend of clinical improvement and reduced severity over time.

Lower panel represents change in pVAS scores (from 10 to 2) of individual dogs with dot size represents a number of dogs with the certain score at a specific time point. At earlier time points prevailed dogs with score 8, but at later points some dogs demonstrated decrease in the score to normal (2).

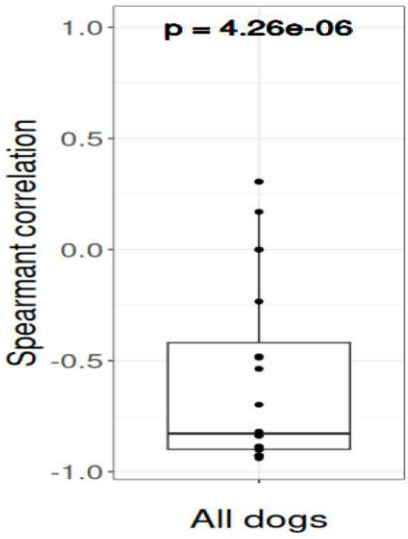


Fig. 2. Graphic presentation of distribution of Spearman correlation between the dogs.

To estimate the average weekly change in pVAS scores, we next employed Linear Mixed Model (Parametric Analysis), which allow us accounting for repeated measures. Analysis by this model estimated average decrease in pVAS score by 0.23 per week with high statistical significance ($p = 3.9 \cdot 10^{-19}$) (Table 3)