



Wydział Biologii
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**Rola białka Hsp90
w atopowym zapaleniu skóry**
The role of Hsp90 in atopic dermatitis

Praca przedstawiona
Radzie Dyscypliny Nauki biologiczne Uniwersytetu Gdańskiego
celem uzyskania stopnia doktora
w dziedzinie nauk ścisłych i przyrodniczych
w dyscyplinie nauki biologiczne

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GDAŃSK 2025

Moje najserdeczniejsze podziękowania kieruję do **prof. dr hab. Stefana Tukaja**, bez którego ta praca by nie powstała. Dziękuję też licznym współautorom, a w szczególności **Michałowi Starke**. Dziękuję mojej wspaniałej żonie **Marcie**, za wspieranie moich marzeń i bycie mi omphalosem. Dziękuję też **Rodzicom**, którzy są początkiem wszystkich naszych osiągnięć.

Pracę dedykuję mojemu synowi **Feliksowi...**

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1. WYKAZ PRAC WCHODZĄCYCH W SKŁAD ROZPRAWY DOKTORSKIEJ

1. Praca poglądowa:

Heat Shock Protein 90 (HSP90) and HSP70 as Potential Therapeutic Targets in Autoimmune Skin Diseases

Stefan Tukaj, Krzysztof Sitko

Biomolecules, 2022, doi: 10.3390/biom12081153

IF₂₀₂₂ 5,5|MNiSW 100

2. Praca oryginalna:

Circulating heat shock protein 90 (HSP90) and autoantibodies to HSP90 are increased in patients with atopic dermatitis

Krzysztof Sitko, Marta Bednarek, Jagoda Mantej, Magdalena Trzeciak, Stefan Tukaj

Cell Stress and Chaperones, 2021, doi: 10.1007/s12192-021-01238-w

IF₂₀₂₁ 3,827|MNiSW 70

3. Praca oryginalna:

Differences in the detection of circulating HSP90 alpha between patients with atopic dermatitis and dermatitis herpetiformis

Krzysztof Sitko, Sarolta Kárpáti, Grzegorz Węgrzyn, Grzegorz Mincewicz, Magdalena Trzeciak, Michael Kasperkiewicz, Stefan Tukaj

Frontiers in Medicine, 2024, doi: 10.3389/fmed.2023.1327144

IF₂₀₂₃ 3|MNiSW 70

4. Praca oryginalna:

Heat shock protein 90 (HSP90) inhibitor STA-9090 (Ganetespib) ameliorates inflammation in a mouse model of atopic dermatitis

Krzysztof Sitko, Michał Starke, Stefan Tukaj

Cell Stress and Chaperones, 2023, doi: 10.1007/s12192-023-01387-0

IF₂₀₂₃ 3,3|MNiSW 70

5. Praca oryginalna:

Topical Application of the HSP90 Inhibitor 17-AAG Reduces Skin Inflammation and Partially Restores Microbial Balance: Implications for Atopic Dermatitis Therapy

Krzysztof Sitko, Ewa Piotrowska, Magdalena Podlacha, Natalia Zagórska, Michał Starke, Magdalena Trzeciak, Stefan Tukaj

Scientific Reports, 2025, doi: 10.1038/s41598-025-05307-3

IF₂₀₂₃ 3,9|MNiSW 140

Łączna wartość wskaźnika oddziaływania (IF): 19,527

Łączna punktacja MNiSW: 450

Prace naukowe wchodzące w skład rozprawy doktorskiej były finansowane z grantu Narodowego Centrum Nauki (NCN) numer 2020/39/B/NZ6/00357 przyznanego prof. dr hab. Stefanowi Tukajowi.

2. OBJAŚNIENIE SKRÓTÓW I NAZW

17-AAG – Tanespimycyna, inhibitor HSP90, pochodna geldanamycyny (**GA**), antybiotyku z grupy makrolidów aromatycznych.

17-DMAG – Alvespimycyna, inhibitor HSP90.

ATP – Adenozynotrifosforan.

AZS – Atopowe zapalenie skóry.

BiP – Białko wiążące immunoglobuliny, należące do rodziny HSP70.

CD – Choroba Crohna.

CD4+ – Subpopulacja limfocytów T pomocniczych.

CHIP – Ligaza ubikwitynowa E3, która współdziała z białkami HSP w degradacji białek.

CTD – Domena C-końcowa, sekwencja aminokwasowa znajdująca się przy grupie karboksylowej łańcucha polipeptydowego.

DH – Opryszczkowate zapalenie skóry, inaczej choroba Duhringa - choroba autoimmunologiczna, skórna postać celiakii.

DNCB – 1-Chloro-2,4-dinitrobenzen, substancja silnie immunogenna.

DNAJP1 – Białko należące rodziny chaperonów HSP40, wspomagające funkcję HSP70.

ELISA – Test immunoenzymatyczny, służący do wykrywania białek.

EPX – peroksydaza eozynofilowa, enzym obecny w ziarnistościach eozynofili, marker ich aktywności.

FDA – Amerykańska Agencja Żywności i Leków odpowiedzialna za dopuszczanie leków i żywności do obrotu.

FLG – Filagryna, białko kluczowe dla prawidłowego funkcjonowania bariery skórnej.

Grp94/gp96 – białko należące do rodziny HSP90 zlokalizowane w siateczce śródplazmatycznej.

Hch1 – Białko modulujące aktywność foldazową HSP90.

HLA – Ludzki układ zgodności tkankowej, kluczowy czynnik odpowiedzi immunologicznej.

HSP27 – Białko z rodziny małych białek szoku termicznego.

HSP40 – Rodzina opiekuńczych białek wspomagających funkcję HSP70.

HSP60 – Rodzina mitochondrialnych białek opiekuńczych.

HSP70 – Rodzina białek szoku cieplnego wspomagająca m.in. fałdowanie nowo syntetyzowanych białek.

HSP90 – Rodzina białek szoku cieplnego pełniąca rolę opiekuńczą w regulacji aktywności wielu białek kluczowych dla przetrwania komórki.

HSP90α – Indukowana izoforma HSP90,

szczególnie ważna w odpowiedzi na stres komórkowy.

eHSP90α – zewnątrzkomórkowe białko HSP90α.

HSP90β – Konstytutywna forma HSP90, niezbędna do prawidłowego funkcjonowania komórki.

HSP100 – Rodzina białek opiekuńczych uczestnicząca w rozpadzie nieprawidłowych agregatów białkowych.

HSF-1 – Czynn timeranskrypcyjny aktywujący ekspresję genów kodujących białka szoku cieplnego.

HSR – Komórkowa odpowiedź na stres cieplny obejmująca zwiększoną ekspresję białek opiekuńczych.

IgA – Immunoglobulina typu A, dominująca w błonach śluzowych.

IgE – Immunoglobulina typu E, związana z reakcjami alergicznymi i odpowiedzią na pasożyty.

IgG – Immunoglobulina typu G, najczęściej występująca immunoglobulina w surowicy, kluczowa dla odporności wtórnej.

IgM – Immunoglobulina typu M, produkowana w odpowiedzi na antygen, uczestniczy w aktywacji układu dopełniacza.

IL-10 – Interleukina 10, odgrywa rolę w regulacji odpowiedzi immunologicznej.

IL-13 – Interleukina 13, cytokina typu

Th2, zaangażowana w m.in. w patogenezę chorób alergicznych.

IL-17 – Interleukina 17, cytokina prozapalna wytwarzana głównie przez komórki Th17, odgrywająca rolę w chorobach autoimmunologicznych.

IL-22 – Interleukina 22, odgrywająca rolę w odpowiedzi immunologicznej błon śluzowych i skóry.

IL-31 – Interleukina 31, cytokina odgrywająca kluczową rolę w świadczeniu neurogennym.

IL-33 – Interleukina 33, alarmina wydzielana przez uszkodzone komórki nabłonka, aktywująca odpowiedź typu Th2.

IL-4 – Interleukina 4, cytokina promująca różnicowanie limfocytów Th2 i produkcję IgE, ważna w odpowiedzi alergicznej.

JAK/STAT – Szlak sygnalizacji komórkowej, aktywowany przez cytokiny, kluczowy w regulacji odpowiedzi immunologicznej.

MAPK – Kinazy aktywowane mitogenami, uczestniczące w transdukcji sygnałów prowadzących do proliferacji komórek.

MD – Domena środkowa białka, sekwencja aminokwasów znajdująca się w środku łańcucha polipeptydowego.

NFκB – Czynn timeranskrypcyjny regulujący ekspresję genów związanych z odpowiedzią zapalną i odpornościową.

NGS – Sekwencjonowanie nowej generacji

NTD – N-końcowa domena białka, sekwencja aminokwasów znajdująca się przy grupie aminowej łańcucha polipeptydowego, w rodzinie HSP90 zawiera miejsce wiązania ATP.

P23 – Białko stabilizujące kompleksy HSP90 z białkami klientowymi.

PTM – Modyfikacje potranslacyjne, zmiany białek zachodzące w procesie ich dojrzewania.

RAG – Geny aktywujące rekombinację, kodują elementy kompleksu białkowego odpowiedzialnego za rekombinację genów immunoglobulin i receptorów limfocytów T.

RGRN-305 – Debio 0932, inhibitor HSP90.

RT-qPCR - Ilościowa reakcja łańcuchowa polimerazy w czasie rzeczywistym.

SARS-CoV-2 – Wirus wywołujący chorobę COVID-19.

SCORAD – Skala oceny nasilenia objawów AZS, używana w badaniach klinicznych i diagnostyce.

STA-9090 – Ganetespib, syntetyczny inhibitor HSP90.

STAT-1/3 – Czynn timeranskrypcyjne

należące do szlaku sygnalizacji komórkowej JAK/STAT.

Sti1 – Białko uczestniczące w przekazywaniu substratów między HSP70 a HSP90.

Th – Limfocyty T pomocnicze, komórki układu odpornościowego kluczowe w odpowiedzi immunologicznej.

Th1, Th2, Th17 – Subpopulacje limfocytów Th, komponenty immunologicznej odpowiedzi komórkowej.

TNF-α – Czynn timerartwicy nowotworów alfa, to cytokina prozapalna zaangażowana w inicjację stanu zapalnego.

TSLP – Limfopoetyna zrębu grasicy, cytokina pochodzenia nabłonkowego aktywująca m.in. komórki dendrytyczne i limfocyty Th2.

WAS – Gen kodujący białko, którego mutacje prowadzą do zespołu Wiskotta-Aldricha.

3. SŁOWA KLUCZOWE

Atopowe zapalenie skóry, AZS; Białka szoku termicznego, HSP; HSP90; choroby skóry; terapia; 17-AAG; STA-9090

Atopic dermatitis, AD; Heat shock proteins, HSP; HSP90; skin diseases; therapy; 17-AAG; STA-9090

4. STRESZCZENIE W JĘZYKU POLSKIM

Białka szoku termicznego (HSP, ang. *Heat Shock Proteins*) to grupa wysoce ewolucyjnie konserwowanych białek opiekuńczych (ang. *chaperones*) oraz proteaz, występujących we wszystkich poznanych organizmach prokariotycznych i eukariotycznych. Białka HSP klasyfikuje się według masy cząsteczkowej wyrażonej w kilodaltonach (kDa) do rodzin: HSP100, HSP90, HSP70, HSP60, HSP40 oraz sHSP (małych białek szoku termicznego). Ich aktywność, zarówno ATP-niezależna, jak i zależna od ATP, wspomaga procesy dojrzewania białek, ponownego fałdowania białek zdenaturowanych, stabilizacji ich natywnej struktury, degradacji nieprawidłowo sfałdowanych białek oraz transportu polipeptydów. Na początku lat dziewięćdziesiątych minionego wieku N-końcowa domena HSP90 (NTD, ang. *N-terminal domain*) została rozpoznana jako potencjalny cel molekularny w terapii nowotworów. Najnowsze badania wskazują również na udział HSP90 w rozwoju nieinfekcyjnych chorób zapalnych i autoimmunologicznych. HSP90 odpowiada za regulację aktywności ponad 300 substratów białkowych, w tym kinaz, ligaz ubikwitynowych, receptorów steroidowych, cyklin i czynników transkrypcyjnych, które uczestniczą w podstawowych procesach komórkowych, takich jak przeżycie, wzrost, różnicowanie i apoptoza. U ssaków wyróżnia się cztery główne izoformy HSP90: GRP94 (zlokalizowaną w retikulum endoplazmatycznym), TRAP-1 (obecną w mitochondriach) oraz izoformy cytoplazmatyczne – HSP90 α i HSP90 β . HSP90 β ulega konstytutywnej ekspresji, natomiast ekspresja HSP90 α może być indukowana przez różnego rodzaju stresory komórkowe, takie jak stres oksydacyjny, promieniowanie UV czy hipertermia. HSP90 α może być także wydzielana do środowiska zewnątrzkomórkowego (eHSP90 α), gdzie pełni funkcje sygnalizacyjne i immunomodulacyjne, m.in. uczestniczy w procesach gojenia ran, angiogenezy i metastazy. Co istotne, eHSP90 α może indukować odpowiedź humoralną prowadzącą do wytwarzania przeciwciał anti-HSP90, których podwyższone stężenie obserwowano w niektórych chorobach autoimmunologicznych. Inhibitory aktywności chaperonowej HSP90, takie jak 17-AAG i STA-9090, wiążą się z domeną NTD z większym powinowactwem niż ATP, co prowadzi do proteasomalnej degradacji białek zależnych od HSP90. Uważa się, że immunosupresyjny efekt tego typu inhibicji może wynikać m.in. z aktywacji czynnika transkrypcyjnego HSF-1, który reguluje ekspresję genów immunoregulacyjnych, takich jak HSP70 i IL-10; ekspansji immunosupresyjnych populacji limfocytów T i B regulatorowych; inaktywacji prozapalnych czynników

zależnych od NF- κ B; oraz blokowania szlaków sygnalizacyjnych, takich jak JAK-STAT i MAPK.

Atopowe zapalenie skóry (AZS) jest jedną z najczęstszych przewlekłych chorób zapalnych skóry, o globalnej częstości występowania do 15-30% u dzieci i 2-10% u dorosłych. Jest to schorzenie o podłożu alergicznym, charakteryzujące się intensywnym neurogennym świądem oraz nawracającymi zmianami skórnymi w postaci rumienia, przesuszenia i nadżerek. Patogeneza AZS nie jest w pełni poznana, choć kluczowe znaczenie przypisuje się zaburzeniom ciągłości bariery skórnej. AZS wiąże się z mutacjami genu kodującego filagrynę – kluczowe białko barierowe – co prowadzi do zwiększonej aktywacji układu odpornościowego. Zaburzenia immunologiczne wywołują stan zapalny, który wtórnie uszkadza barierę skórną, zaburza mikrobiom i ułatwia penetrację alergenów. W patogenezie AZS udział biorą różne populacje komórkowe, w tym subpopulacje limfocytów pomocniczych Th1, Th2 i Th17, których nadmierna aktywacja prowadzi do nasilonej produkcji cytokin prozapalnych. Aktywacja limfocytów T sprzyja również proliferacji, migracji i aktywacji eozynofili. Istotną rolę odgrywają także keratynocyty, fibroblasty i komórki dendrytyczne, które wydzielają liczne cytokiny, mediatory, chemokiny i alarminy. U około 80% pacjentów z AZS stwierdza się podwyższone stężenie krążących IgE, które mogą aktywować komórki tuczne. Leczenie AZS ma charakter indywidualny i ukierunkowane jest na odbudowę bariery naskórkowej, redukcję stanu zapalnego oraz łagodzenie świądu. Obejmuje ono stosowanie emolientów, miejscowych kortykosteroidów lub inhibitorów kalcyneuryny, a w cięższych przypadkach ogólnoustrojową immunosupresję lub leczenie biologiczne. Mimo skuteczności leczenia objawowego, żadna z obecnych terapii nie prowadzi do trwałego wyleczenia, a ich stosowanie wiąże się z ryzykiem działań niepożądanych.

Celem niniejszej rozprawy było zbadanie roli HSP90 w rozwoju AZS oraz ocena wpływu inhibicji HSP90 za pomocą STA-9090 i 17-AAG, na procesy zapalne charakterystyczne dla AZS, zarówno *in vitro*, jak i w mysim modelu choroby.

W toku badań wykazano istotnie wyższe stężenie eHSP90 α oraz przeciwciał IgE anti-HSP90 α w surowicy pacjentów z AZS w porównaniu do dopasowanej grupy kontrolnej. Zaobserwowano również dodatnią korelację pomiędzy poziomem eHSP90 α

a nasileniem objawów choroby ocenianych w skali SCORAD (ang. *Scoring Atopic Dermatitis*). Swoistość eHSP90 α jako biomarkera AZS potwierdzono poprzez analizę porównawczą z opryszczkowatym zapaleniem skóry (DH, łac. *Dermatitis herpetiformis*, choroba Duhringa), co może mieć znaczenie w diagnostyce różnicowej. W tym badaniu 83,9% (26/31) pacjentów z AZS miało podwyższone stężenie HSP90 α , w porównaniu do 0% (0/26) pacjentów z DH. Kolejnym etapem badań zmierzających do określenia roli Hsp90 w patogenezie AZS były eksperymenty prowadzone w mysim modelu AZS, indukowanym przez naskórną aplikację 1-chloro-2,4-dinitrobenzenu (DNCB). Dootrzewnowe podanie STA-9090 prowadziło do istotnego zmniejszenia aktywności choroby (w ocenie skali SCORAD), redukcji hiperplazji naskórka, mniejszej infiltracji leukocytów oraz łagodzenia świądu. Również aplikacja miejscowa STA-9090 obniżała aktywność choroby, zmniejszała poziom IgE w surowicy i zwiększała ekspresję filagryny w skórze. Obie formy terapii były dobrze tolerowane i nie wywoływały skutków ubocznych. Z uwagi na większą skuteczność oraz potencjał aplikacyjny, dalsze badania przedkliniczne skoncentrowano na miejscowej inhibicji HSP90 za pomocą 17-AAG. Terapia ta zmniejszała aktywność choroby, redukowała hiperplazję naskórka oraz obniżała ekspresję TSLP, IL-5, IL-6 oraz aktywność NF- κ B w skórze. Obserwowano również spadek liczby eozynofili we krwi oraz obniżenie aktywności peroksydazy eozynofilowej (EPX) w skórze. Immunosupresyjny efekt 17-AAG na ograniczenie produkcji alarmin, cytokin charakterystycznych dla odpowiedzi typu Th oraz reaktywnych form tlenu został dodatkowo rozwinięty w badaniach *in vitro* z wykorzystaniem ludzkich hodowli keratynocytów, limfocytów T CD4⁺ oraz eozynofili stymulowanych odpowiednimi czynnikami zapalnymi. Co istotne, sekwencjonowanie nowej generacji (NGS) wykazało, że miejscowa terapia 17-AAG częściowo przywraca równowagę mikrobiomu jelitowego u myszy z AZS. Ponadto wykazano, że 17-AAG zmniejsza zdolność *Staphylococcus aureus* do produkcji biofilmu – patogenu oportunistycznego uznawanego za istotny w patogenezie AZS ze względu na jego rolę jako źródła superantygenów oraz czynnika zaostrzającego przebieg choroby. Zaobserwowano również zwiększoną aktywność (=deacetylację) HSP90 oraz wzrost aktywności EPX w leukocytach izolowanych od pacjentów z AZS. Dodatkowo, badania *in vitro* wykazały, że inhibicja aktywności chaperonowej HSP90 prowadzi do zwiększonej acetylacji HSP90 α , co może częściowo wyjaśniać obserwowane efekty immunomodulujące i terapeutyczne u zwierząt.

Uzyskane w ramach rozprawy wyniki po raz pierwszy wskazują na istotną rolę HSP90 w patogenezie atopowego zapalenia skóry. Co szczególnie ważne, wykazano potencjał diagnostyczny eHSP90 α jako biomarkera AZS oraz potencjał terapeutyczny inhibicji HSP90 - zwłaszcza w formie terapii miejscowej - w łagodzeniu objawów choroby. Otwiera to nowe perspektywy dla opracowania bardziej skutecznych, ukierunkowanych terapii oraz narzędzi diagnostycznych w leczeniu AZS.

5. STRESZCZENIE W JĘZYKU ANGIELSKIM

Heat shock proteins (HSP) are a highly evolutionarily conserved family of molecular chaperones and proteases present in all studied prokaryotic and eukaryotic cells. Heat shock proteins are classified into families by their molecular weight expressed in kilodaltons (kDa) and include: HSP100, HSP90, HSP70, HSP60, HSP40 and sHSP (small heat shock proteins). Both their adenosine triphosphate -dependent and -independent activity promotes protein maturation, refolding of denatured proteins, stabilizing native protein structures, degradation of misfolded proteins and facilitates peptide transport. In the first half of the 90's the N-terminal domain of HSP90 was recognized as a potential molecular target in cancer. Current research implicates HSPs in the pathogenesis and progression of noncommunicable inflammatory and autoimmune disorders. HSP90 is responsible for the proper function of over 300 proteins, including kinases, ubiquitin ligases, steroid receptors, cyclins and transcription factors, most of which are crucial in the cell's survival, growth, differentiation and apoptosis. In mammals four main isoforms of HSP90 have been described: GRP94 (in the endoplasmic reticulum), TRAP-1 (in mitochondria), and two localized in the cytoplasm: HSP90 α and HSP90 β . HSP90 β expression is constant whereas HSP90 α can be induced by various stressors, such as oxidative stress, UV radiation or hyperthermia. Furthermore, HSP90 α can be released to the extracellular milieu (eHSP90 α), where it acts as a signal transducer and immunomodulating factor, its functions include wound healing, angiogenesis and metastasis. Moreover, eHSP90 α can induce humoral response leading to the production of anti-HSP90 antibodies which are elevated in some autoimmune diseases. HSP90's chaperone activity inhibitors, namely 17-AAG and STA-9090, bind to the NTD region with higher affinity than ATP, which leads to the proteasomal degradation of HSP90's protein substrates. Immunosuppressive effects of such inhibition could arise due to: activation of transcription factor HSF-1, which regulates immunoregulatory gene expression i.e. HSP70 and IL-10; expansion of immunosuppressive populations of regulatory B and T cells; inactivation of NF- κ B-dependent proinflammatory molecules; and the inhibition of signaling pathways such as JAK-STAT or MAPK.

Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases, with reported global incidence of 15-30% in children and 2-10% in adults.

AD is classified as an allergic disease characterized by intense neuropathic itch and recurring skin lesions in the form of erythema, dryness and erosion. AD pathogenesis is not clearly understood, however disruption of skin barrier integrity plays a crucial role. One of the better understood genetic factors underlying AD are filaggrin mutations. Filaggrin is a crucial skin barrier protein, whose impaired activity or lowered expression leads to overactive immune response which in turn leads to inflammation which further damages the skin, leading to the imbalance of the skin microbiome and facilitated allergen penetration. Various cell populations, including helper T cells Th1, Th2 and Th17, participate in AD pathogenesis, their excessive activation leads to the increased production of proinflammatory cytokines. T cell activation also promotes the proliferation, migration and activation of eosinophils. Keratinocytes, fibroblasts and dendritic cells also play a significant role, as they secrete numerous cytokines, mediators, chemokines and alarmins. In approximately 80% of AD patients, elevated levels of circulating IgE which can activate mast cells, are observed. Current treatment of AD is individualized and aims to restore the skin barrier function, reduce inflammation and relieve pruritus. It includes the use of emollients, topical corticosteroids or calcineurin inhibitors, with systemic immunosuppression or novel biological therapeutics reserved for more severe cases. Despite the effectiveness of symptomatic treatment, none of the current approaches constitute a cure for AD, and their use is associated with a risk of adverse effects.

The aim of this dissertation was to investigate the role of HSP90 in the development of AD and to evaluate the effect of HSP90 inhibition, using STA-9090 and 17-AAG, on inflammatory processes characteristic of AD, both *in vitro* and in a murine model of the disease.

In the course of this study, a significantly higher concentration of eHSP90 α and anti-HSP90 α IgE antibodies was discovered in the serum of patients with AD compared to a closely matched control group. Furthermore, a positive correlation was observed, between eHSP90 α concentration and the severity of the disease symptoms assessed using the SCORAD (Scoring Atopic Dermatitis) index. The specificity of eHSP90 α as a biomarker of AD was confirmed through comparative analysis with dermatitis herpetiformis (DH, Dühring's disease), which is relevant for differential diagnosis. It was noted that 83.9%

(26/31) patients with AD had elevated eHSP90 α levels, as compared to 0% (0/26) of patients with DH. Further experiments used a murine model of AD induced by topical application of 1-chloro-2,4-dinitrobenzene (DNCB). Intraperitoneal administration of STA-9090 led to a significant reduction in disease activity (as assessed by SCORAD index), a decrease in epidermal hyperplasia, reduction in leukocyte infiltration, and the amelioration of pruritus. Topical application of STA-9090 also lowered disease activity, decreased serum IgE levels, and increased filaggrin expression in the skin. Both therapies were well-tolerated and did not produce observable adverse effects. Due to greater efficacy and therapeutic potential, further preclinical studies focused on topical inhibition of HSP90 chaperone activity using 17-AAG. This approach significantly reduced disease activity, epidermal hyperplasia, lowered the expression of TSLP, IL-5, IL-6 and NF- κ B activity. A reduction in the number of circulating eosinophils and in eosinophil peroxidase (EPX) activity in the skin was also detected. This limiting effect of 17-AAG on the levels of cytokines and alarmins characteristic of the Th-type immune response, and reactive oxygen species production was confirmed *in vitro* in appropriately stimulated cultures of human keratinocytes, CD4⁺ T lymphocytes, and eosinophils. Notably, next generation sequencing approaches revealed that the 17-AAG topical therapy partially restores gut microbiome balance in AD-like mice. Furthermore, it was demonstrated that 17-AAG reduces biofilm formation capabilities of *Staphylococcus aureus* – an opportunistic pathogen studied extensively in AD due to its role as a source of superantigens and a factor exacerbating disease severity. Increased HSP90 activity (=deacetylation) and elevated EPX activity were observed in leukocytes isolated from AD patients. Additional *in vitro* investigations revealed that the inhibition of HSP90's chaperone activity leads to increased acetylation of HSP90 α , which may partially explain the observed immunomodulatory and therapeutic effects.

These results, for the first time, demonstrate a significant role of HSP90 in the pathogenesis of atopic dermatitis. The diagnostic potential of eHSP90 α as a biomarker of AD and the symptom-alleviating therapeutic potential of HSP90 inhibition, especially in the form of topical therapy, cannot be overstated. This provides new avenues for the development of more effective, molecularly targeted therapies and diagnostic tools in the treatment of AD.

6. WPROWADZENIE

Białka szoku termicznego (HSP, ang. *Heat shock proteins*) to grupa wysoce ewolucyjnie konserwowanych białek opiekuńczych występujących we wszystkich zbadanych organizmach prokariotycznych i eukariotycznych. W ujęciu tradycyjnym, ich aktywność, zarówno zależna od adenozynotrójfosforanu (ATP) – foldazy i ATP-niezależna – holdazy, wspomaga poprawne dojrzewanie białek, ponowne fałdowanie zdenaturowanych białek, stabilizację natywnej struktury białek, transport polipeptydów i degradację niepoprawnie uformowanych białek (Rutledge et al. 2022). Białka HSP podzielone zostały na rodziny według ich masy cząsteczkowej wyrażonej w kilodaltonach (kDa), do których zaliczane są HSP100, HSP90, HSP70, HSP60, HSP40 i sHSP (ang. *small Heat Shock Proteins*), czyli małe białka szoku termicznego. Białka HSP zlokalizowane są w różnych przedziałach komórkowych, a ich ekspresja może być konstytutywna lub indukowana wieloma stresorami m.in. hipertermią, stresem oksydacyjnym, etanolem i promieniowaniem UV (Tukaj & Sitko 2022).

Jedną z lepiej poznanych rodzin HSP jest rodzina ssaczych białek HSP90 (ang. *Heat Shock Protein 90*). Rodzina ta, składa się z czterech członków: dwóch izoform cytozolowych (HSP90 α i HSP90 β), zlokalizowanego w retikulum endoplazmatycznym białka Grp94/gp96 oraz w znajdujące się w mitochondrium białka TRAP-1 (Sitko et al. 2023). W budowie białek HSP90 wyróżniamy trzy domeny: domena N-końcowa (NTD, ang. *N-terminal domain*) wiążąca ATP; domena środkowa (MD, ang. *middle domain*) odpowiedzialna za wiązanie substratów, nazywanych białkami klientowymi i kochaperonów (białek wspomagających działanie białek opiekuńczych); oraz domena końca karboksylowego (CTD, ang. *carboxy-terminal domain*) odpowiedzialna za dimeryzację i oligomeryzację (Hoter et al. 2018). HSP90 reguluje aktywność biologiczną ponad 300 białek klientowych, do których należą: kinazy, ligazy ubikwitynowe, receptory steroidowe, cykliny i czynniki transkrypcyjne odpowiedzialne za wiele procesów komórkowych, między innymi - przeżycie, wzrost, różnicowanie i apoptoza (Serwetnyk & Blagg 2021).

W toku obrad 11-tej międzynarodowej konferencji na temat funkcji białka opiekuńczego HSP90 w Seon-Seebruck (2024), zaproponowano cztery analogie przedstawiające skomplikowane i ważne funkcje tego białka (Karras et al. 2025).

HSP90 jak scyzoryk szwajcarski, jest wszechstronną i wielofunkcyjną platformą fałdowania białek, jego dimer przybiera różne konformacje, odsłaniając fragmenty o różniącej się hydrofobowości i ładunku, które bezpośrednio uczestniczą w fałdowaniu substratów (Lopez et al. 2021). Izoformy HSP90 wykazują unikalne wypadkowe właściwości powierzchni na różnych etapach cyklu konformacyjnego, co umożliwia im oddziaływanie ze zróżnicowanymi substratami. Do zmiany konformacji HSP90 kluczowe jest wiązanie i hydroliza ATP, której tempo jest różne dla każdej z izoform HSP90 (Noddings et al. 2023). Otwarta konformacja HSP90 sprzyja wiązaniu białek klientowych i jest stanem wyjściowym tego dimeru. W momencie przyłączenia ATP do HSP90, indukowana jest rotacja NTD, powodująca zamknięcie dimeru (Minari et al. 2024). Wiele białek wymaga powtarzających się cykli wiązania, zamykania i uwalniania przez HSP90, aby zachować stabilność (Karras et al. 2025).

HSP90 można również porównać do silnika samochodowego, który wykorzystuje ATP jak paliwo. Niektóre kochaperony, takie jak Sti1 i Hch1, spowalniają tempo katalizy, czyli „obroty silnika” (Karras et al. 2025). Inne, np. Aha1, przyspieszają fałdowanie białek klientowych przez HSP90 (Amoah et al. 2025). Kluczową rolę na końcowych etapach fałdowania i uwalniania białek klientowych pełni p23, który można porównać do sprzęgła (Baischew et al. 2023; Karras et al. 2025). Regulacja tempa cyklu konformacyjnego HSP90, pozwala na dostosowanie czasu fałdowania do specyficznych wymagań każdego z białek klientowych. HSP90 zdolne jest także do fałdowania niektórych białek klientowych (np. białko Tau) bez hydrolizy ATP, co przypomina pracę silnika na biegu jałowym (Karras et al. 2025).

HSP90 przypomina również centralę telefoniczną, ponieważ działa jak dynamiczna platforma koordynująca i podtrzymująca liczne „połączenia” w zmieniających się warunkach środowiska wewnątrz i zewnątrzkomórkowego. Każdy przełącznik odpowiada konkretnemu zadaniu, umożliwiając HSP90 jednoczesne zarządzanie wieloma procesami związanymi z homeostazą białek (biosynteza, fałdowanie, degradacja, agregacja, transport), a każdy przełącznik aktywowany jest jedynie przy określonych warunkach (Karras et al. 2025). Specyficzność połączeń, kontrolowanych przez HSP90, jest regulowana przez jego kochaperony. Na przykład, białko CHIP (ligaza ubikwitynowa E3) współpracując z HSP90 i HSP70, „przełącza” aktualnie związany substrat, kierując go na szlak degradacji (Chakraborty & Edkins 2023).

Podobnie jak dyrygent kieruje różnymi sekcjami muzyków orkiestry, HSP90 stabilizuje liczne białka i kompleksy białkowe w komórce, zapewniając homeostazę proteomu (Karras et al. 2025). Białka klientowe HSP90 często zajmują kluczowe pozycje w szlakach sygnałowych, co daje HSP90 kontrolę nad wieloma funkcjami wyższego rzędu takimi jak, odpowiedź na stres komórkowy lub komunikacja międzykomórkowa. Co więcej, dzięki swojej aktywności opiekuńczej HSP90 może łagodzić fenotypowe skutki mutacji, powodując akumulację ukrytej zmienności genetycznej w populacjach (Karras et al. 2017). Jego pojemność buforowa jest natomiast ograniczona. Podobnie jak dyrygent może zostać zdekoncentrowany przez hałas na widowni, tak stres proteotoksyczny może przeciążyć HSP90, prowadząc do ujawnienia utajonych wcześniej wariantów genetycznych. W większości przypadków, zmienność zależna od HSP90 jest szkodliwa, istnieje jednak prawdopodobieństwo, że nowo ujawnione warianty mogą okazać się korzystne w zmieniającym się środowisku. Sugeruje to, że HSP90 może sprzyjać ewolucji adaptacyjnej (Aguilar-Rodríguez et al. 2024). Aby sprostać tym złożonym zadaniom, HSP90 występuje w komórce w nadmiarze i działa z zapasem funkcjonalnym. W ten sposób HSP90 wpływa na tempo i kierunek ewolucji, niczym dyrygent dostosowujący tempo i nastrój symfonii mimo błędów i zakłóceń (Karras et al. 2025).

Żeby poprawnie i wydajnie wypełniać te wszechstronne role, HSP90 ulega wielu modyfikacjom potranslacyjnym (PTM, ang. *post-translational modifications*) np. fosforylacji, acetylacji, monometylacji, tiokarmabylacji, S-nitrozytacji, sumoilacji, ubikwitynacji, nitracji i O-GlcNacylacji. Wpływają one m.in. na interakcję z kochaperonami, regulują aktywność ATP-azową, aktywację substratów i wiązanie inhibitorów do HSP90 (Backe et al. 2020).

Przez wzgląd na jego obszernie funkcje regulatorowe, HSP90 stało się atrakcyjnym celem w terapii nowotworów, gdy na początku lat dziewięćdziesiątych domenę NTD tego białka rozpoznano jako potencjalny cel molekularny dla terapii przeciwnowotworowych (Sanchez et al. 2020). Jednakże, większość odkrytych inhibitorów wiążących NTD, które od tamtego czasu trafiły do badań klinicznych (np. 17-DMAG, 17-AAG i STA-9090), nie zostało zatwierdzonych przez FDA (ang. *Food and Drug Administration*) ze względu na wysoką toksyczność, słabe profile farmakokinetyczne lub brak skuteczności klinicznej (Tukaj & Sitko 2022). Brak efektywności terapeutycznej inhibitorów HSP90 wynika między innymi z uruchamiania mechanizmu przetrwania w komórkach nowotworowych,

określanego jako odpowiedź na stres cieplny (HSR, ang. *Heat Shock Response*), prowadzącego do aktywacji czynnika HSF-1 (ang. *Heat Shock Factor 1*) (Wang & McAlpine 2015). Aktywacja HSF-1 jest cechą wspólną wielu typów nowotworów, a jego ekspresja koreluje ze złośliwością nowotworu i śmiertelnością wśród chorych. Co więcej, aktywacja białek opiekuńczych zależnych od HSF-1 (np. HSP70, HSP40 i HSP27) przyczynia się do wzrostu i zwiększonej przeżywalności komórek nowotworowych (Tukaj & Sitko 2022). Z drugiej strony, aktywacja mechanizmów kompensacyjnych może mieć zastosowanie w terapii chorób zapalnych i autoimmunologicznych. Inhibitory HSP90, takie jak geldanamycyna (GA) i oparte na jej strukturze półsyntetyczne pochodne (np. 17-DMAG, 17-AAG) lub nowoczesne inhibitory o strukturach projektowanych metodami inżynierii molekularnej (np. STA-9090, RGRN-305), wiążą się z N-kończową kieszenią nukleotydową HSP90 z większym powinowactwem niż ATP, co prowadzi do degradacji proteasomalnej białek klientowych HSP90. Przyjmowane jest, że immunosupresyjna aktywność tego typu inhibicji HSP90 może wynikać z aktywacji HSF-1, który reguluje ekspresję wielu genów, w tym immunoregulacyjnych *HSP70* i *IL-10*; ekspansji immunosupresyjnych populacji limfocytów (B i T-regulatorowych); inaktywacji zależnych od NF- κ B czynników zapalnych i regulacyjnych, w tym TNF- α , IL-6, IL-8 i IL-17; lub blokady cząsteczek sygnalizacyjnych komórek, takich jak MAPK (Tukaj & Sitko 2022). Terapia anti-HSP90, została z powodzeniem zastosowana w mysich modelach: zapalenia mózgu i rdzenia kręgowego, zapalenia stawów indukowanego kolagenem lub adiuwantem, toczenia rumieniowatego układowego, zapalenia błony naczyniowej oka wywołanego lipopolisacharydem oraz wrzodziejącego zapalenia jelita grubego indukowanego siarczanem dekstranu sodu (Tukaj & Sitko 2022). HSP90 odgrywa również ważną rolę w autoimmunizacyjnych chorobach pęcherzowych skóry (AIBDs, ang. *autoimmune bullous diseases*), gdzie promuje powstawanie autoprzeciwciał, prowadzi do aktywacji neutrofili i odpowiedzi zapalnej keratynocytów warstwy epidermalnej, a doniesienia przedkliniczne sugerują, że farmakologiczna inhibicja HSP90 może być skuteczną formą terapii (Tukaj 2025). Kolejną jednostką chorobową, w której z powodzeniem zastosowano terapię inhibitorami HSP90 jest łuszczyca. W badaniu klinicznym pierwszej fazy, oralnie podawany inhibitor HSP90 (RGRN-305), wywołał znaczną poprawę objawów u 6 z 11 pacjentów (redukcja 71-94% mierzona w skali PASI, ang. *Psoriasis Area and Severity Index*) prowadząc do inhibicji sygnalizacji IL-23, TNF- α i IL-17A i powodując poprawę obrazu histologicznego oraz profilu ekspresji genów,

w obrębie zmian łuszczykowych (Bregnhøj et al. 2022). W związku z powyższym, najnowsze badania przedkliniczne oraz kliniczne dostarczają dowodów popierających zastosowanie terapii anty-HSP90 w leczeniu chorób autoimmunologicznych lub zapalnych skóry.

Jedną z najczęściej diagnozowanych obecnie przewlekłych chorób zapalnych skóry, o globalnej częstości występowania do 15-30% u dzieci i 2-10% u dorosłych, jest atopowe zapalenie skóry (AZS) (Langan et al. 2020). Bardzo trudno oszacować całkowity koszt społeczno-ekonomiczny AZS (Drucker et al. 2017). W badaniu obejmującym 9 krajów europejskich, średni dodatkowy koszt życia pacjenta obciążonego AZS określony został na 927€ rocznie, z czego największą część stanowią wydatki na emolienty 332€, następnie leki 213€ i wizyty u specjalistów 104€ (Zink et al. 2019). Pośrednie koszty choroby, wynikają w dużej mierze z prezenteizmu lub nieobecności w pracy, w skali roku 57% pacjentów zgłosiło od 1 do 5 dni nieobecności w pracy, 26% od 6 do 10 dni i 13% opuściło co najmniej 11 dni pracy (Zink et al. 2019). W 2006 roku przeprowadzono badania na populacji 2000 osób z 8 krajów, z których wynika, że 27% pacjentów doświadczyło nękania z powodu AZS. W podziale wiekowym, to aż 39% grupy nieletnich 8-17 lat, oraz 11% dorosłych odczuwających dyskryminację i 14% zgłaszających negatywny wpływ choroby na ich ścieżkę kariery zawodowej (Zuberbier et al. 2006). AZS jest przewlekłą i nieuleczalną chorobą o podłożu zapalnym, alergicznym i immunologicznym. Jej główne objawy to intensywny neurogeny świąd i nawracające zmiany skórne w postaci zaczerwienienia, przesuszeń i drobnych ran. Zarówno czynniki genetyczne, jak i środowiskowe przyczyniają się do rozwoju AZS (Langan et al. 2020). Warto wyróżnić też liczne czynniki zakłócające, takie jak status społeczno-ekonomiczny, środowisko fizyczne oraz dostęp do opieki medycznej i jej jakość, które mają duży wpływ na dane epidemiologiczne. Dodatkowo, czynniki wpływające na zwiększającą się liczbę przypadków AZS na świecie to stopień zanieczyszczenia środowiska, warunki klimatyczne, styl życia oraz czynniki psychologiczne, takie jak ostry stres. Hipoteza higieniczna zakłada, że większa różnorodność mikrobiologiczna we wczesnym dzieciństwie zapobiega chorobom atopowym i alergicznym w późniejszym życiu, co za tym idzie, postępująca urbanizacja społeczeństwa może także mieć duży wpływ na wzrastającą ilość notowanych przypadków AZS (Guttman-Yassky et al. 2025). Tradycyjnie, postuluje się dwie koncepcje patogenezy AZS, czyli hipotezę

zewn trzpochodn  (ang. *extrinsic*) oraz hipotez  wewn trzpochodn  (ang. *intrinsic*) (Czarnowicki et al. 2019). U podłoża zewn trzpochodnego AZS leży zaburzenie ci głości bariery nask rkowej zwi zane z niefunkcjonalnym wariantem genu koduj cego kluczowe bia ko barierowe, filagryn  (FLG). Prowadzi to do zwi kszonego wnikania alergen w, co skutkuje lokaln  aktywacj  uk adu odpornościowego i rozwojem choroby (Guttman-Yassky et al. 2025). Wewn trzpochodne AZS, charakteryzuje si  pierwotn  wad  uk adu immunologicznego, a wywo any tym stan zapalny sk ry prowadzi do wt rnego zaburzenia bariery nask rka, zaburzenia r wnowagi mikrobiomu sk rnego i zwi kszonej penetracji alergen w. W literaturze określa si  konkretne geny koreluj ce z wy szym ryzykiem wyst pienia AZS. Opr cz najcz ściej wyst puj cych mutacji *FLG*, s  to m.in. mediatory prezentacji antygenu limfocyt w T (np. *HLA*) oraz geny reguluj ce stan zapalny (np. *IL13*, *TNFSF4*, *IL22*, *IFNG*). Patogenez  wewn trzpochodn  popiera r wnieŹ fakt, Źe liczne powaŹne choroby immunologiczne prowadz  do wyst pienia objaw w przypominaj cych wyprysk atopowy, np. zesp ł Omenn, gen *RAG*; zesp ł Wiskott-Aldrich, gen *WAS*; zesp ł Job, gen *STAT3* (Guttman-Yassky et al. 2025). Kolejnym argumentem za hipotez  wewn trzpochodn  jest kluczowa rola limfocyt w T w AZS, ktor  potwierdzaj  efekty terapeutyczne lek w takich jak cyklosporyna, oraz nowoczesnych lek w biologicznych (Hijnen et al. 2007; Simpson et al. 2016). Ponadto, aktywacja odpowiedzi Th2, kt ra prowadzi m.in. do uwalniania cytokin IL-4, IL-5 i IL-13 w sk rze, prowadzi do obniŹenia ekspresji peptyd w przeciwdrobnoustrojowych oraz bia ek barierowych sk ry (Kim et al. 2019; Kokubo et al. 2022). W zwi zku z tym, aktywacja obwodowego uk adu odpornościowego i zaburzenie bariery sk rnej s  ściśle spr Źone, a obie opisane koncepcje wydaj  si  odgrywa  waŹn  rol  w AZS. Kolejnym kluczowym w AZS czynnikiem jest świ d neurogenny, wywo uj cy u pacjent w niezwykle intensywne cykle świ du i drapania. Drapanie zmian w AZS prowadzi do zaostrzenia świ du, poniewaŹ neurony czuciowe posiadaj  receptory dla cytokin typu Th2 (Oetjen et al. 2017). Dowodem na kluczow  rol  świ du w AZS jest skuteczność terapii lekami biologicznymi na przyk ad Nemolizumabem (anty-CD31RA mAb), kt ry prowadzi do inhibicji cytokin typu Th2 (Kabashima et al. 2020). IL-4 i IL-13, b d ce elementami odpowiedzi Th2, nie tylko po rednicz  w wywo ywaniu stanu zapalnego sk ry, ale mog  teŹ wywo ywa  sensytyzacj  neuronaln , wyja nia to silny świ d w przebiegu AZS oraz dzia anie przeciwswi dowe lek w biologicznych, takich jak Dupilumab i Lebrikizumab, kt re obniŹaj  aktywność tych cytokin (Simpson et al. 2016; Oetjen et al. 2017). Niemniej

jednak, terapia celowana przeciwko odpowiedzi Th2 prowadzi do istotnej poprawy klinicznej u zaledwie jednej trzeciej pacjentów cierpiących na AZS. Sugeruje to, że dodatkowe czynniki są istotnie zaangażowane w przebieg choroby u znacznej liczby pacjentów. U niektórych pacjentów w obrębie zmian przewlekłych nasiloną jest odpowiedź komórkowa typu Th1, a w populacjach azjatyckich zaobserwowano wzmożoną aktywność Th17, jednakże terapeutyczne znaczenie tych czynników pozostaje niejasne (Guttman-Yassky et al. 2025). Znacznie lepiej poznanym mechanizmem, jest występujące u większości pacjentów z AZS podwyższone stężenie przeciwciał IgE specyficznych wobec różnego rodzaju alergenów, korelujące z zaostrzeniami objawów (Liu et al. 2011). Populacją komórek szczególnie wrażliwych na aktywację IgE są mastocyty. Ich intensywna degranulacja, przy jednoczesnym braku zwiększonej infiltracji do skóry, obserwowana jest w ostrych zmianach AZS; z drugiej strony, zmiany przewlekłe, charakteryzują się zwiększoną infiltracją mastocytów (Liu et al. 2011). Kolejną populacją komórek, które są szczególnie wrażliwe na sygnalizację IgE są komórki dendrytyczne posiadające receptory FcεRI. Ich liczba jest istotnie wyższa w skórze zmienionej chorobowo, mogą one ułatwiać pobieranie i prezentację antygenów a także wzmacniać aktywację limfocytów T w skórze (Novak 2012). Wzmocniona aktywacja limfocytów T przez komórki prezentujące antygen prowadzi do wyrzutu cytokin charakterystycznych dla odpowiedzi Th2, promuje to proliferację, infiltrację i wzmacnia miejscową aktywność kolejnej kluczowej w przebiegu AZS populacji komórek - eozynofili (Liu et al. 2011). Za wyrzut cytokin prozapalnych w obrębie zmian AZS, odpowiedzialne są także keratynocyty i fibroblasty. Wydzielają one cytokiny charakterystyczne dla odpowiedzi typu Th2, oraz mediatory, czynniki chemotaktyczne i alarminy aktywujące liczne sprzężenia zwrotne, zarówno lokalnie jak i na obwodzie organizmu. Niemniej jednak inhibicja cytokin pochodzących z komórek naskórka, takich jak IL-33, IL-17C, IL-36 i limfopoetyna zrębu grasicy (TSLP), jak na razie, nie przyniosła zadowalających efektów terapeutycznych w AZS (Guttman-Yassky et al. 2025).

Obecnie, leczenie AZS jest dostosowywane do każdego pacjenta i polega na kontrolowaniu objawów choroby przez odtwarzanie bariery naskórkowej, redukcję stanu zapalnego oraz świądu, dzięki czemu uzyskuje się poprawę jakości życia i przedłuża okres remisji. Najczęściej stosuje się terapię miejscową, dedykowaną do łagodnej postaci choroby oraz leczenie ogólnoustrojowe, wdrażane w przypadkach umiarkowanych i ciężkich (Luger et al. 2021). Podstawą profilaktyki przed zaostrzeniami AZS jest unikanie

czynników wyzwalających (m.in. alergenów, substancji drażniących, niekorzystnych warunków środowiskowych) i drapania zmian oraz regularne stosowanie emolientów, a także przestrzeganie higieny w formie krótkich ciepłych kąpieeli/pryszniców z użyciem kosmetyków niezawierających mydła i stosowania mokrych opatrunków (Martin et al. 2025). Dermatolodzy przeważnie zalecają leczenie miejscowe, z wykorzystaniem kortykosteroidów (np. Hydrokortyzon), inhibitorów kalcyneuryny (np. Takrolimus), inhibitorów fosfodiesterazy-4 (np. Kryzaborol) lub agonistów receptora węglowodorów aromatycznych (np. Ruksolitynib) (Luger et al. 2021). Kortykosteroidy miejscowe, skutecznie redukują stan zapalny, jednak ich długotrwałe stosowanie może prowadzić do niepożądanych efektów, takich jak rozstępy, trądzik różowaty, atrofia skóry i postępująca oporność na lek (Coondoo et al. 2014). Inhibitory kalcyneuryny, działają przeciwzapalnie bez ryzyka atrofii skóry, więc mogą być stosowane do leczenia miejsc gdzie skóra jest delikatna - twarz, powieki, szyja, fałdy skórne i krocze. Jednakże, mogą one powodować uczucie pieczenia lub świąd, a ulotki tych środków zawierają ostrzeżenie o podwyższonym ryzyku wystąpienia chłoniaka wśród pacjentów poniżej 2 roku życia (Luger et al. 2021). W umiarkowanych lub ciężkich postaciach AZS, gdy leczenie miejscowe okazuje się niewystarczające, mogą zostać wdrożone terapie wspomagające np. fototerapia wąskopasmowym promieniowaniem ultrafioletowym UVB, jednak jej szerokie stosowanie jest ograniczone ze względu na długość trwania zabiegów i dostępność ośrodków świadczących takie usługi (Guttman-Yassky et al. 2025). Dla pacjentów nieodpowiadających w zadowalającym stopniu na leczenie miejscowe, dostępne są długo obecne na rynku i stosunkowo tanie, ogólnoustrojowe leki immunosupresyjne, takie jak Cyklosporyna i Metotreksat, jednakże ze względu na potencjalną toksyczność narządową i brak oficjalnych wskazań w leczeniu AZS, a także szybki nawrót objawów po ustaniu terapii, ich stosowanie jest rzadkie w praktyce klinicznej (Guttman-Yassky et al. 2025). Obecnie, najszybciej rozwijającym się i najbardziej obiecującym typem terapii AZS są nowoczesne leki biologiczne. Na rynku amerykańskim dostępne są cztery takie preparaty, zatwierdzone przez FDA do leczenia umiarkowanych i ciężkich postaci AZS. Należą do nich: Dupilumab (blokujący receptor IL-4R α , prowadząc do inhibicji IL-4 i IL-13), Tralokinumab (hamujący wiązanie IL-13 do receptorów IL-13R α 1 i 2), Lebrikizumab (hamujący powstawanie kompleksu IL-4R α /IL-13R α 1) i Nemolizumab (blokujący receptor IL-31R α , odpowiedzialny za aktywację bazofili). Choć leki te charakteryzują się korzystnym profilem bezpieczeństwa,

to Dupilumab, Tralokinumab i Lebrikizumab wiążą się z ryzykiem wystąpienia zapalenia spojówek. W nowoczesnej terapii ogólnoustrojowej stosuje się także doustne inhibitory JAK (Abrocycynib i Upadacycynib), które mimo zapewniania szybkiej poprawy objawów, wiążą się z podwyższonym ryzykiem wystąpienia poważnych zakażeń, zgonów, nowotworów złośliwych, ciężkich zdarzeń sercowo-naczyniowych oraz incydentów zakrzepowo-zatorowych, co ogranicza ich zastosowanie u niektórych grup pacjentów (Martin et al. 2025).

Chociaż AZS nie jest obecnie klasyfikowane jako choroba autoimmunologiczna, w jego patofizjologię zaangażowane jest wiele rodzajów komórek układu odpornościowego zlokalizowanych w skórze lub na obwodzie oraz wiele cząsteczek i szlaków sygnałowych opisywanych w chorobach autoimmunologicznych (Tukaj & Sitko 2022). AZS charakteryzuje się też specyficznym przebiegiem, nazywanym wzrocom nawracająco-ustępującym; wzorzec ten jest charakterystyczny dla wielu chorób autoimmunologicznych (Guttman-Yassky et al. 2025). Ponieważ u pacjentów z AZS zaobserwowano odpowiedzi immunologiczne skierowane przeciwko białkom autologicznym, nie można też wykluczyć, że reakcja autoimmunologiczna odgrywa istotną rolę w progresji tej choroby. Co więcej, w metaanalizach wykryto związek AZS z występowaniem chorób autoimmunologicznych takich jak łysienie plackowate, celiakia, choroba Leśniowskiego-Crohna, reumatoidalne zapalenie stawów, toczeń rumieniowaty układowy, wrzodziejące zapalenie jelita grubego czy bielactwo nabyte (Tukaj & Sitko 2022).

Białkiem, które może aktywować humoralną odpowiedź immunologiczną, prowadząc do powstawania przeciwciał autologicznych jest wysoce immunogenne, pozakomórkowe HSP90 α (eHSP90 α). Podwyższone miano przeciwciał anti-HSP90 obserwowane jest w niektórych chorobach autoimmunologicznych takich jak cukrzyca typu pierwszego, toczeń rumieniowaty układowy, reumatoidalne zapalenie stawów (RZS), opryszczkowe zapalenie skóry (choroba Duhringa) oraz celiakia. Istnieją rozległe doniesienia przedkliniczne dotyczące stosowania szczepionek opartych na HSP lub inhibicji HSP w zwierzęcych modelach chorób autoimmunologicznych, z których wiele zostało rozszerzone o badania kliniczne pierwszego stopnia, co daje nadzieję na rozwój leków nowej generacji (Tukaj & Sitko 2022). Obiecujące badania kliniczne zostały przeprowadzone na przykład u pacjentów z RZS. Wykorzystano tam terapię polegającą na doustnej aplikacji dnaJP1 (silnie konserwowanego ewolucyjnie 15-aminokwasowego

regionu białka HSP40), jak również pełnej długości białek HSP, takich jak BiP (białko wiążące immunoglobuliny, należące do rodziny HSP70) i HSP10. Co więcej, pomyślnie rozpoczęto trzecią fazę badań klinicznych w leczeniu nowo zdiagnozowanych pacjentów z cukrzycą typu pierwszego, za pomocą peptydu DiaPep277 (fragmentu HSP60) (Tukaj & Sitko 2022). Zarówno podejścia terapeutyczne oparte na modyfikacji aktywności i ekspresji HSP oraz szczepionki oparte na HSP, wydają się być zatem obiecującym sposobem terapii chorób autoimmunologicznych skóry (Tukaj & Sitko 2022). Jednakże, ocena roli HSP90 w przebiegu chorób, bazująca na ocenie jego ekspresji w zajętych chorobą tkankach lub korelowania jego stężenia z parametrami klinicznymi nie są miarodajne, gdyż mogą wynikać z procesów kompensacyjnych. Jest to szczególnie istotne przy badaniach nad HSP90, ponieważ jego szybka i wydajna ekspresja w komórkach wystawionych na działanie wielu stresorów jest kluczowym elementem pierwotnej odpowiedzi komórkowej. Z tego powodu, dogłębne analizy funkcjonalne oraz podejścia eksperymentalne, zarówno *in vitro*, jak i wykorzystujące modele zwierzęce, powinny być stosowane w celu określenia funkcji HSP90 w chorobach o podłożu immunologicznym.

7. CELE PRACY

Celem przewodnim niniejszej rozprawy doktorskiej było zbadanie roli HSP90 w rozwoju AZS oraz ocena wpływu inhibicji HSP90 za pomocą STA-9090 i 17-AAG, na procesy zapalne charakterystyczne dla AZS, zarówno *in vitro*, jak i w mysim modelu choroby.

Cele szczegółowe:

1. Pogłębienie i systematyzacja dostępnej wiedzy naukowej z badań podstawowych, przedklinicznych oraz klinicznych poprzez przegląd literatury na temat roli białek szoku termicznego, należących do rodziny HSP90 i HSP70, jako potencjalnych celów terapeutycznych w nieinfekcyjnych chorobach zapalnych skóry, ze szczególnym uwzględnieniem autoimmunologicznych chorób pęcherzowych skóry, łuszczycy i AZS.
2. Określenie roli zewnątrzkomórkowego białka HSP90 α (eHSP90 α) oraz autoprzeciwciał skierowanych przeciwko HSP90 α u pacjentów z AZS.
3. Ocena przydatności diagnostycznej eHSP90 α w diagnostyce różnicowej AZS i opryszczkowatego zapalenia skóry (choroby Duhringa).
4. Ocena wpływu inhibicji aktywności opiekuńczej HSP90 za pomocą STA-9090 (Ganetespib), na stan zapalny i obraz kliniczny w mysim modelu AZS, z uwzględnieniem porównania różnych dróg podania inhibitora.
5. Ocena bezpieczeństwa, skuteczności i mechanizmu działania naskórnej aplikacji inhibitora HSP90 17-AAG (Tanespimycin), na stan zapalny, obraz kliniczny i skład mikrobiomu w mysim modelu AZS.

8. OMÓWIENIE PUBLIKACJI WCHODZĄCYCH W SKŁAD ROZPRAWY DOKTORSKIEJ

Publikacja 1

W publikacji poglądowej pt. **„Heat Shock Protein 90 (HSP90) and HSP70 as Potential Therapeutic Targets in Autoimmune Skin Diseases”** przedstawiono aktualny stan wiedzy dotyczącej roli białek HSP90 i HSP70 w odpowiedzi immunologicznej, w kontekście terapii chorób autoimmunologicznych skóry. W pracy uwzględniono również rozdział poświęcony AZS, mimo że choroba ta klasyfikowana jest jako przewlekła, zapalna, genetycznie uwarunkowana, wypryskowa dermatоза związana z predyspozycją do atopii (World Health Organization 2019). Jej uwzględnienie w tym przeglądzie podyktowane jest najnowszą wiedzą naukową dotyczącą mechanizmu tej choroby, w który zaangażowane jest wiele rodzajów komórek układu odpornościowego zlokalizowanych zarówno w skórze, jak i na obwodzie. Ponadto, w patogenezę AZS zaangażowanych jest wiele cząsteczek i szlaków sygnałowych opisywanych w chorobach autozapalnych i autoimmunologicznych. Dodatkowo, uwzględnienie AZS w tym artykule uzasadnia obserwowane w metaanalizach współwystępowanie AZS z chorobami autoimmunologicznymi takimi jak: łysienie plackowate, celiakia, choroba Leśniowskiego-Crohna, reumatoidalne zapalenie stawów, toczeń rumieniowaty układowy, wrzodziejące zapalenie jelita grubego i bielactwo nabyte.

W treści artykułu opisano funkcje białek szoku termicznego i nomenklaturę związaną z klasyfikacją rodzin HSP. Następnie, szczegółowo scharakteryzowano białka HSP90 i HSP70 oraz przedstawiono aktualne hipotezy dotyczące ich roli w odpowiedzi immunologicznej, ze szczególnym uwzględnieniem ich wpływu na czynniki kluczowe w patofizjologii wybranych chorób autoimmunologicznych. Publikacja ta, zawiera także wykaz przedklinicznych i klinicznych interwencji terapeutycznych modulujących aktywność bądź ekspresję HSP90 lub HSP70 w: nabytym pęcherzowym oddzielaniu się naskórka, łuszczycy, bielactwie, łysieniu plackowatym, toczniu rumieniowatym układowym, twardzinie układowej i AZS. Pracę kończy rozdział poświęcony perspektywom wykorzystania modulacji aktywności HSP oraz szczepionek opartych na HSP w terapii chorób autoimmunologicznych skóry.

Publikacja 2

W publikacji pt. **„Circulating heat shock protein 90 (HSP90) and autoantibodies to HSP90 are increased in patients with atopic dermatitis”** przedstawiono wyniki analizy porównawczej występowania białka eHSP90α oraz autoprzeciwciał klasy IgG, IgE, IgA i IgM, skierowanych przeciwko eHSP90α w surowicach pacjentów z AZS (n = 29) oraz dopasowanych wiekiem i płcią osób zdrowych (n = 70).

W artykule, dokonano syntezy aktualnej wiedzy na temat białek szoku termicznego z rodziny HSP90 oraz patogenezy AZS z wyszczególnieniem roli adaptacyjnej odpowiedzi immunologicznej i zaburzeń bariery naskórka, na które mogą wpływać między innymi alergenowo swoiste i autoreaktywne przeciwciała klasy IgE. Kierunek badań opisanych w tej pracy wynika z literatury naukowej, która opisuje podwyższony poziom autoprzeciwciał anty-HSP90 obserwowany w niektórych chorobach autoimmunologicznych oraz kluczową w patogenezie AZS, zależną od IgE, swoistą odpowiedź na autoantygeny.

Przedstawione w manuskrypcie analizy porównawcze poziomu eHSP90α, wykazały 2,79-krotny wzrost poziomu eHSP90α w surowicy pacjentów z AZS w odniesieniu do zdrowych kontroli. Co więcej, poziom eHSP90α, w sposób istotny statystycznie, korelował z nasileniem objawów choroby określonym w skali SCORAD (ang. *SCORing Atopic Dermatitis*). W toku badań wykryto również istotnie wyższe miano autoprzeciwciał anty-HSP90 klasy IgE w surowicy pacjentów z AZS, w porównaniu do zdrowych kontroli; analiza kontyngencji, wykazała seropozytywność u 48.27% pacjentów z AZS i jedynie 2.85% osób zdrowych. Co ważne, nie wykryto korelacji między poziomem tych autoprzeciwciał a całkowitą pulą IgE w surowicy, a także stopniem nasilenia objawów choroby w skali SCORAD. Jednakże, brak istotnego wzrostu miana wśród pozostałych analizowanych klas autoprzeciwciał oraz brak korelacji z analizowanymi chorobami współistniejącymi: astmą, alergicznym nieżytem nosa i zapaleniem spojówek, mogą sugerować specyficzną dla AZS odpowiedź autoimmunologiczną anty-HSP90 IgE.

Zatem, eHSP90α i autoprzeciwciał anty-HSP90 IgE wydają się pełnić ważną rolę w patogenezie AZS. Artykuł ten może stanowić punkt wyjścia do opracowania nowoczesnych biomarkerów. Jednakże, zaprezentowane w nim badania wymagają pogłębienia oraz rekrutacji większej i bardziej zróżnicowanej grupy badawczej.

Publikacja 3

W publikacji pt. **„Differences in the detection of circulating HSP90 alpha between patients with atopic dermatitis and dermatitis herpetiformis”** rozszerzono wcześniejsze doniesienia dotyczące roli eHSP90 w patogenezie AZS. Nasze wcześniejsze badania wykazały podwyższony poziom eHSP90 α oraz autoprzeciwciał anty-HSP90 w klasie IgE u pacjentów z AZS, co sugeruje istotną rolę tego białka w jego patogenezie (Sitko et al. 2021). W celu potwierdzenia specyficzności tych potencjalnych biomarkerów wobec AZS, wykonano analizę porównawczą z opryszczkowatym zapaleniem skóry (DH, łac. *Dermatitis Herpetiformis*, choroba Duhringa), skórnej postaci celiakii. DH to choroba autoimmunologiczna, której ryzyko zapadalności jest istotnie wyższe wśród pacjentów cierpiących na AZS. Istnieją doniesienia o trudnościach w diagnostyce różnicowej DH i AZS, wynikających z pozornego podobieństwa objawów klinicznych. Dlatego, identyfikacja nowych i swoistych biomarkerów jest pożądana. Biorąc pod uwagę wcześniejsze obserwacje dotyczące podwyższonego poziomu autoprzeciwciał anty-HSP90 klasy IgE w surowicy pacjentów z AZS i klasy IgG w surowicy pacjentów z DH, zbadano czy poziom białka eHSP90 α może stanowić taki biomarker.

W tym celu, określono stężenie eHSP90 α w surowicach pacjentów z AZS (n = 31), celiakią (n = 15), DH (n = 26) i osób zdrowych (n = 55). Analiza kontyngencji stężenia eHSP90 α w surowicy osób zrekrutowanych, w której punktem odcięcia była dwukrotność odchylenia standardowego zdrowych kontroli wykazała, że 83.9% pacjentów z AZS charakteryzowało się istotnie podwyższonym poziomem eHSP90 α , podczas gdy nie odnotowano takiego wzrostu w surowicy u żadnego z pacjentów cierpiących na DH. Dodatkowo, w związku z wyjątkową sytuacją epidemiologiczną w chwili powstawania pracy, wykluczono wpływ przebytego zakażenia SARS-CoV-2 lub szczepień przeciwko COVID-19 na stężenie eHSP90 α w surowicy.

Na podstawie przeprowadzonych analiz można sądzić, że eHSP90 α charakteryzuje duży potencjał do zastosowania w diagnostyce różnicowej AZS i DH. Może to mieć istotne implikacje dla praktyki klinicznej, otwierając perspektywy na opracowanie nowych narzędzi diagnostycznych i terapeutycznych. Potrzeba jednak dalszych badań, na większej grupie pacjentów, w celu potwierdzenia tych wniosków.

Publikacja 4

W publikacji pt. **„Heat shock protein 90 (HSP90) inhibitor STA-9090 (Ganetespib) ameliorates inflammation in a mouse model of atopic dermatitis”** po raz pierwszy odnotowano poprawę objawów klinicznych w mysim modelu AZS, dzięki zastosowaniu eksperymentalnej terapii, z wykorzystaniem inhibitora aktywności opiekuńczej HSP90, STA-9090, podawanego zarówno miejscowo, jak i systemowo. Do badań wykorzystano myszy model AZS, indukowany za pomocą 1-chloro-2,4-dinitrobenzenu (DNCB), który jest jednym z najczęściej wykorzystywanych modeli w badaniach nad nowymi terapiami AZS, ponieważ chemiczna, kontaktowa sensytyzacja skóry za pomocą DNCB wywołuje wiele objawów charakteryzujących ostrą postać AZS u ludzi.

W artykule, STA-9090 podawany był myszom w zastrzykach dootrzewnowych lub aplikowany na pozbawioną sierści skórę pleców. Przedstawione analizy, wykazały istotną statystycznie kliniczną skuteczność systemowej inhibicji aktywności HSP90 za pomocą STA-9090, co związane było ze zmniejszoną hiperplazją naskórka, obniżoną infiltracją leukocytów do skóry właściwej oraz ograniczeniem świądu u zwierząt. Zaobserwowano również nieznaczną (5.96%) utratę masy ciała u myszy w tej grupie badawczej. Drugi typ terapii eksperymentalnej, zastosowanie miejscowe, powodował istotną statystycznie redukcję objawów klinicznych, czemu towarzyszyło obniżenie poziomu IgE w surowicy oraz zwiększona ekspresja filagryny w skórze. W obu typach terapii nie zaobserwowano wpływu na poziom histaminy w surowicy oraz infiltrację mastocytów do skóry.

Co za tym idzie, inhibitor STA-9090 został skutecznie zastosowany do terapii przedklinicznego modelu AZS. Co więcej, miejscowe stosowanie inhibitorów HSP90 wydaje się oferować większe korzyści terapeutyczne w leczeniu AZS, dzięki zmniejszonemu ryzyku ogólnoustrojowych działań niepożądanych. Jednakże, potrzebne są dalsze badania z uwzględnieniem dodatkowych modeli choroby oraz pomiary dodatkowych czynników kluczowych w patogenezie AZS, w celu określenia dokładnego mechanizmu terapeutycznego inhibicji HSP90.

Publikacja 5

W publikacji pt. „**Topical Application of the HSP90 Inhibitor 17-AAG Reduces Skin Inflammation and Partially Restores Microbial Balance: Implications for Atopic Dermatitis Therapy**” opisano mechanizm działania inhibitora aktywności opiekuńczej HSP90, 17-AAG, w terapii miejscowej przedklinicznego modelu AZS oraz rozszerzono wiedzę na temat roli HSP90 w AZS. Do badań wykorzystano myszy model AZS, indukowany za pomocą 1-chloro-2,4-dinitrobenzenu (DNCB), naskórek pacjentów z AZS, hodowle *in vitro* keratynoców, limfocytów i eozynofili.

Eksperymentalna terapia przedklinicznego modelu AZS za pomocą 17-AAG, prowadzona była zarówno w formie profilaktycznej, jak i terapeutycznej. W obu przypadkach wykazano jej istotną statystycznie kliniczną skuteczność. Dodatkowo, w terapii profilaktycznej zademonstrowano, istotną statystycznie, zależność efektu klinicznego 17-AAG od dawki podania. Miejscowej terapii 17-AAG towarzyszyły: zmniejszenie hiperplazji naskórka; redukcja ekspresji genów kluczowych w AZS, tj. *TSLP*, *IL-5*, *IL-6*; redukcja stężenia przeciwciał IgE w surowicy i obniżenie lokalnej aktywności czynnika transkrypcyjnego NFκB. Wykryto także spadek odsetku eozynofili w krwioobiegu i obniżoną aktywność peroksydazy eozynofilowej (EPX) w skórze, pomimo niezmienionej infiltracji skóry. Nie odnotowano wpływu terapii na infiltrację skóry przez komórki CD4⁺ i komórki tuczne. Jednakże, wykryto istotny spadek stężenia histaminy w surowicy. Co więcej, wykazano wpływ 17-AAG na poprawę równowagi mikrobiomu jelit u myszy. Wykonane analizy morfologiczne i biochemiczne wykazały, że dawki inhibitora zastosowane w tej pracy, tj. 0.1 μM, 0.5 μM, 1 μM były dobrze tolerowane i bezpieczne dla zwierząt, co popiera zaproponowaną wcześniej hipotezę o bezpieczeństwie miejscowej terapii inhibitorami HSP90 (Sitko et al. 2023).

Powyższe obserwacje przedkliniczne zostały rozszerzone o badania mechanistyczne w eksperymentach z wykorzystaniem komórek stymulowanych *in vitro*, w których wykazano zależny od dawki pozytywny efekt terapeutyczny 17-AAG na: proliferację komórek naskórka; ekspresję IL-33, TSLP, IL-6, FLG, IL-22, IL-17A, IL-5; poziom p-NFκB, p-STAT-1 i p-STAT-3; zahamowanie wydzielania reaktywnych form tlenu przez aktywowane eozynofile pochodzące od osób zdrowych, a także redukcję tworzenia biofilmu przez *S. aureus*. Wykazano również zwiększoną aktywność HSP90, analizując poziom acetylacji tego białka w hodowlach keratynocytów stymulowanych *in vitro*.

Co więcej, wykazano zwiększoną aktywność EPX we frakcji leukocytów pobranych od pacjentów z AZS. Warto zaznaczyć, że analizy naskórka pobranego z obszaru zmian chorobowych oraz skóry niezmienionej pacjentów z AZS, nie ujawniły istotnych różnic w ekspresji HSP90.

Z przeprowadzonych badań wynika, że celem terapii 17-AAG w AZS jest zmniejszenie aktywności białka HSP90 bez wpływu na jego ekspresję, a główne efekty terapeutyczne dotyczą warstwy epidermalnej skóry. Brak efektu na infiltrację eozynofili, komórek tucznych i limfocytów T do skóry, z jednoczesnym wpływem 17-AAG na aktywność tych populacji komórek może sugerować, że przyczyną obserwowanych efektów terapeutycznych jest modulacja aktywności komórek kluczowych w AZS zależna od sygnalizacji NF- κ B, bez bezpośredniego wpływu na infiltrację tych populacji do skóry. Co za tym idzie, miejscowa terapia anty-HSP90 jest bezpieczna i prowadzi do złagodzenia objawów charakterystycznych dla AZS w modelu przedklinicznym, a następstwem tych badań powinno być rozpoczęcie badań klinicznych z wykorzystaniem inhibitorów HSP90.

9. PODSUMOWANIE CAŁOŚCI ROZPRAWY

Celem niniejszej rozprawy doktorskiej było zbadanie roli białka HSP90 w patogenezie AZS oraz ocena wpływu jego inhibicji za pomocą dwóch inhibitorów, STA-9090 i 17-AAG, na procesy zapalne charakterystyczne dla AZS, zarówno w warunkach *in vitro*, jak i w mysim modelu tej choroby. Badania obejmowały analizy przeprowadzone na modelach komórkowych, zwierzęcych oraz próbkach pochodzących od pacjentów z AZS i osób zdrowych. Wyniki eksperymentów zostały przedstawione w formie czterech oryginalnych publikacji naukowych opublikowanych w recenzowanych czasopismach międzynarodowych. Uzupełnieniem pracy eksperymentalnej jest artykuł poglądowy poświęcony roli białek HSP90 i HSP70 w nieinfekcyjnych chorobach zapalnych skóry, stanowiący tło teoretyczne i kontekst interpretacyjny dla przedstawionych badań eksperymentalnych. Rezultaty badań zostały również zaprezentowane społeczności naukowej podczas międzynarodowych konferencji naukowych.

W toku rozprawy wykazano istotnie wyższe stężenie eHSP90 α oraz przeciwciał IgE anty-HSP90 α w surowicy pacjentów z AZS; wykryto korelację pomiędzy poziomem eHSP90 α a nasileniem objawów AZS; dowiedziono swoistości podwyższonego poziomu eHSP90 α jako potencjalnego biomarkera w kontekście diagnostyki różnicowej z opryszczkowatym zapaleniem skóry (DH, łac. *dermatitis herpetiformis*, choroba Dühringa); z użyciem mysiego modelu AZS i eksperymentów *in vitro* udowodniono skuteczność i bezpieczeństwo terapii polegającej na inhibicji aktywności opiekuńczej HSP90, a w szczególności terapii miejscowej z użyciem 17-AAG, gdzie szczegółowo opisano potencjalne mechanizmy obserwowanych efektów terapeutycznych; analizy NGS wykazały, że miejscowa terapia 17-AAG częściowo przywraca równowagę mikrobiomu jelit u myszy z AZS; udokumentowano także wzrost aktywności HSP90 α przy jednoczesnym braku podwyższonej ekspresji HSP90 α w skórze i leukocytach pacjentów. Co więcej, badania *in vitro* wykazały, że inhibicja HSP90 za pomocą 17-AAG, prowadzi do zwiększonej acetylacji HSP90 α , co może częściowo wyjaśniać obserwowane efekty immunomodulujące i terapeutyczne.

Uzyskane w ramach tej rozprawy wyniki, po raz pierwszy udowadniają istotną rolę HSP90 w patogenezie AZS. Otwiera to nowe perspektywy na opracowanie bardziej skutecznych, ukierunkowanych molekularnie, terapii oraz narzędzi diagnostycznych w leczeniu AZS i innych chorób zapalnych skóry.

10. PIŚMIENNICTWO

Aguilar-Rodríguez J, Jakobson CM, Jarosz DF (2024) The Hsp90 Molecular Chaperone as a Global Modifier of the Genotype-Phenotype-Fitness Map: An Evolutionary Perspective. *J Mol Biol* 436:168846. <https://doi.org/10.1016/j.jmb.2024.168846>

Amoah DP, Hussein SK, Johnson JL, LaPointe P (2025) Ordered ATP hydrolysis in the Hsp90 chaperone is regulated by Aha1 and a conserved post-translational modification. *Protein Science* 34:e5255. <https://doi.org/https://doi.org/10.1002/pro.5255>

Backe SJ, Sager RA, Woodford MR, Makedon AM, Mollapour M (2020) Post-translational modifications of Hsp90 and translating the chaperone code. *J Biol Chem* 295:11099-11117. <https://doi.org/10.1074/jbc.REV120.011833>

Baischew A, Engel S, Taubert MC, Geiger TM, Hausch F (2023) Large-scale, in-cell photocrosslinking at single-residue resolution reveals the molecular basis for glucocorticoid receptor regulation by immunophilins. *Nat Struct Mol Biol* 30:1857-1866. <https://doi.org/10.1038/s41594-023-01098-1>

Bregnhøj A, Thuesen KKH, Emmanuel T, Litman T, Grek CL, Ghatnekar GS, Johansen C, Iversen L (2022) HSP90 inhibitor RGRN-305 for oral treatment of plaque-type psoriasis: efficacy, safety and biomarker results in an open-label proof-of-concept study. *Br J Dermatol* 186:861-874. <https://doi.org/10.1111/bjd.20880>

Chakraborty A, Edkins AL (2023) CHIP: A Co-chaperone for Degradation by the Proteasome and Lysosome. *Subcellular Biochemistry*, vol 101. Springer, Cham. https://doi.org/10.1007/978-3-031-14740-1_12

Coondoo A, Phiske M, Verma S, Lahiri K (2014) Side-effects of topical steroids: A long overdue revisit. *Indian Dermatol Online J* 5:416-425. <https://doi.org/10.4103/2229-5178.142483>

Czarnowicki T, He H, Krueger JG, Guttman-Yassky E (2019) Atopic dermatitis endotypes and implications for targeted therapeutics. *J Allergy Clin Immunol*. 143:1-11. <https://doi.org/10.1016/j.jaci.2018.10.032>

Drucker AM, Wang AR, Li WQ, Sevetson E, Block JK, Qureshi AA (2017) The Burden of Atopic Dermatitis: Summary of a Report for the National Eczema Association. *J Invest Dermatol* 137:26-30. <https://doi.org/10.1016/j.jid.2016.07.012>

Guttman-Yassky E, Renert-Yuval Y, Brunner PM (2025) Atopic dermatitis. *The Lancet* 405:583-596. [https://doi.org/10.1016/S0140-6736\(24\)02519-4](https://doi.org/10.1016/S0140-6736(24)02519-4)

Hijnen DJ, ten Berge O, Timmer-de Mik L, Bruijnzeel-Koomen CA, de Bruin-Weller MS (2007) Efficacy and safety of long-term treatment with cyclosporin A for atopic dermatitis. *J Eur Acad Dermatol Venereol* 21:85-89. <https://doi.org/10.1111/j.1468-3083.2006.01877.x>

- Hoter A, El-Sabban ME, Naim HY (2018) The HSP90 Family: Structure, Regulation, Function, and Implications in Health and Disease. *Int J Mol Sci* 19:25650. <https://doi.org/10.3390/ijms19092560>
- Kabashima K, Matsumura T, Komazaki H, Kawashima M (2020) Trial of Nemolizumab and Topical Agents for Atopic Dermatitis with Pruritus. *N Engl J Med* 383:141-150. <https://doi.org/10.1056/NEJMoa1917006>
- Karras GI, Colombo G, Kravats AN (2025) Hsp90: Bringing it all together. *Cell Stress Chaperones* 30:69-79. <https://doi.org/10.1016/j.cstres.2025.01.002>
- Karras GI, Yi S, Sahni N, Fischer M, Xie J, Vidal M, D'Andrea AD, Whitesell L, Lindquist S (2017) HSP90 Shapes the Consequences of Human Genetic Variation. *Cell* 168:856-866.e812. <https://doi.org/10.1016/j.cell.2017.01.023>
- Kim J, Kim BE, Ahn K, Leung DYM (2019) Interactions Between Atopic Dermatitis and Staphylococcus aureus Infection: Clinical Implications. *Allergy Asthma Immunol Res* 11:593-603. <https://doi.org/10.4168/aaair.2019.11.5.593>
- Kokubo K, Onodera A, Kiuchi M, Tsuji K, Hirahara K, Nakayama T (2022) Conventional and pathogenic Th2 cells in inflammation, tissue repair, and fibrosis. *Front Immunol* 13:945063. <https://doi.org/10.3389/fimmu.2022.945063>
- Langan SM, Irvine AD, Weidinger S (2020) Atopic dermatitis. *Lancet* 396:345-360. [https://doi.org/10.1016/s0140-6736\(20\)31286-1](https://doi.org/10.1016/s0140-6736(20)31286-1)
- Liu F-T, Goodarzi H, Chen H-Y (2011) IgE, Mast Cells, and Eosinophils in Atopic Dermatitis. *Clin Rev Allergy Immunol* 41:298-310. <https://doi.org/10.1007/s12016-011-8252-4>
- Lopez A, Dahiya V, Delhommel F, Freiburger L, Stehle R, Asami S, Rutz D, Blair L, Buchner J, Sattler M (2021) Client binding shifts the populations of dynamic Hsp90 conformations through an allosteric network. *Science Advances* 7:eabl7295. <https://doi.org/doi:10.1126/sciadv.abl7295>
- Luger T, Paller AS, Irvine AD, Sidbury R, Eichenfield LF, Werfel T, Bieber T (2021) Topical therapy of atopic dermatitis with a focus on pimecrolimus. *J Eur Acad Dermatol Venerol* 35:1505-1518. <https://doi.org/https://doi.org/10.1111/jdv.17272>
- Martin G, Aldredge L, DiRuggiero D, Young M, Simpson E (2025) An Overview of Atopic Dermatitis Disease Burden, Pathogenesis, and the Current Treatment Landscape: Recommendations for Appropriate Utilization of Systemic Therapies. *J Clin Aesthet Dermatol* 18:51-66.
- Minari K, Balasco Serrão VH, Borges JC (2024) New Insights into Hsp90 Structural Plasticity Revealed by cryoEM. *BioChem* 4:62-89. <https://doi.org/10.3390/biochem4020004>

Noddings CM, Johnson JL, Agard DA (2023) Cryo-EM reveals how Hsp90 and FKBP immunophilins co-regulate the glucocorticoid receptor. *Nat Struct Mol Biol* 30:1867-1877. <https://doi.org/10.1038/s41594-023-01128-y>

Novak N (2012) An update on the role of human dendritic cells in patients with atopic dermatitis. *J Allergy Clin Immunol* 129:879-886. <https://doi.org/10.1016/j.jaci.2012.01.062>

Oetjen LK, Mack MR, Feng J, Whelan TM, Niu H, Guo CJ, Chen S, Trier AM, Xu AZ, Tripathi SV, Luo J, Gao X, Yang L, Hamilton SL, Wang PL, Brestoff JR, Council ML, Brasington R, Schaffer A, Brombacher F, Hsieh CS, Gereau RWt, Miller MJ, Chen ZF, Hu H, Davidson S, Liu Q, Kim BS (2017) Sensory Neurons Co-opt Classical Immune Signaling Pathways to Mediate Chronic Itch. *Cell* 171:217-228.e213. <https://doi.org/10.1016/j.cell.2017.08.006>

Rutledge BS, Choy WY, Duennwald ML (2022) Folding or holding?-Hsp70 and Hsp90 chaperoning of misfolded proteins in neurodegenerative disease. *J Biol Chem* 298:101905. <https://doi.org/10.1016/j.jbc.2022.101905>

Sanchez J, Carter TR, Cohen MS, Blagg BSJ (2020) Old and New Approaches to Target the Hsp90 Chaperone. *Curr Cancer Drug Targets* 20:253-270. <https://doi.org/10.2174/1568009619666191202101330>

Serwetnyk MA, Blagg BSJ (2021) The disruption of protein-protein interactions with co-chaperones and client substrates as a strategy towards Hsp90 inhibition. *Acta Pharm Sin B* 11:1446-1468. <https://doi.org/10.1016/j.apsb.2020.11.015>

Simpson EL, Bieber T, Guttman-Yassky E, Beck LA, Blauvelt A, Cork MJ, Silverberg JI, Deleuran M, Kataoka Y, Lacour JP, Kingo K, Worm M, Poulin Y, Wollenberg A, Soo Y, Graham NM, Pirozzi G, Akinlade B, Staudinger H, Mastey V, Eckert L, Gadkari A, Stahl N, Yancopoulos GD, Ardeleanu M (2016) Two Phase 3 Trials of Dupilumab versus Placebo in Atopic Dermatitis. *N Engl J Med* 375:2335-2348. <https://doi.org/10.1056/NEJMoa1610020>

Sitko K, Bednarek M, Mantej J, Trzeciak M, Tukaj S (2021) Circulating heat shock protein 90 (Hsp90) and autoantibodies to Hsp90 are increased in patients with atopic dermatitis. *Cell Stress Chaperones* 26:1001–1007. <https://doi.org/10.1007/s12192-021-01238-w>

Sitko K, Starke M, Tukaj S (2023) Heat shock protein 90 (Hsp90) inhibitor STA-9090 (Ganetespib) ameliorates inflammation in a mouse model of atopic dermatitis. *Cell Stress Chaperones* 28:935-942. <https://doi.org/10.1007/s12192-023-01387-0>

Tukaj S (2025) Hsp90 as a pathophysiological factor and emerging therapeutic target in atopic dermatitis. *Front Immunol* 16:1658399. <https://doi.org/10.3389/fimmu.2025.1658399>

Tukaj S, Sitko K (2022) Heat Shock Protein 90 (Hsp90) and Hsp70 as Potential Therapeutic Targets in Autoimmune Skin Diseases. *Biomolecules* 12:1153.
<https://doi.org/10.3390/biom12081153>

Wang Y, McAlpine SR (2015) N-terminal and C-terminal modulation of Hsp90 produce dissimilar phenotypes. *Chem Commun (Camb)* 51:1410-1413.
<https://doi.org/10.1039/c4cc07284g>

World Health Organization (2019) EA80 Atopic eczema. International statistical classification of diseases and related health problems (11th ed.)
<https://icd.who.int/browse/2025-01/mms/en#215767047>

Zink AGS, Arents B, Fink-Wagner A, Seitz IA, Mensing U, Wettemann N, de Carlo G, Ring J (2019) Out-of-pocket Costs for Individuals with Atopic Eczema: A Cross-sectional Study in Nine European Countries. *Acta Dermato-Venereologica* 99:263-267.
<https://doi.org/10.2340/00015555-3102>

Zuberbier T, Orlov SJ, Paller AS, Taïeb A, Allen R, Hernanz-Hermosa JM, Ocampo-Candiani J, Cox M, Langeraar J, Simon JC (2006) Patient perspectives on the management of atopic dermatitis. *J Allergy Clin Immunol* 118:226-232.
<https://doi.org/10.1016/j.jaci.2006.02.031>

11. PUBLIKACJE WCHODZĄCE W SKŁAD ROZPRAWY DOKTORSKIEJ

Review

Heat Shock Protein 90 (Hsp90) and Hsp70 as Potential Therapeutic Targets in Autoimmune Skin Diseases

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Abstract: Over a hundred different autoimmune diseases have been described to date, which can affect every organ in the body, including the largest one, the skin. In fact, up to one-fifth of the world's population suffers from chronic, noninfectious inflammatory skin diseases, the development of which is significantly influenced by an autoimmune response. One of the hallmarks of autoimmune diseases is the loss of immune tolerance, which leads to the formation of autoreactive lymphocytes or autoantibodies and, consequently, to chronic inflammation and tissue damage. The treatment of autoimmune skin diseases mainly focuses on immunosuppression (using, e.g., corticosteroids) but almost never leads to the development of permanent mechanisms of immune tolerance. In addition, current therapies and their long-term administration may cause serious adverse effects. Hence, safer and more effective therapies that bring sustained balance between pro- and anti-inflammatory responses are still desired. Both intra- and extracellular heat shock proteins (Hsps), specifically well-characterized inducible Hsp90 and Hsp70 chaperones, have been highlighted as therapeutic targets for autoimmune diseases. This review presents preclinical data on the involvement of Hsp90 and Hsp70 in modulating the immune response, specifically in the context of the treatment of selected autoimmune skin diseases with emphasis on autoimmune bullous skin diseases and psoriasis.

Keywords: heat shock proteins; Hsp90; Hsp70; skin diseases; autoimmunity; therapy



Citation: Tukaj, S.; Sitko, K. Heat Shock Protein 90 (Hsp90) and Hsp70 as Potential Therapeutic Targets in Autoimmune Skin Diseases.

Biomolecules **2022**, *12*, 1153. <https://doi.org/10.3390/biom12081153>

Academic Editor: Vladimir N. Uversky

Received: 27 July 2022

Accepted: 18 August 2022

Published: 20 August 2022

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1. Introduction

Present in both prokaryotic and eukaryotic cells, highly evolutionarily conserved heat shock proteins (Hsps) are grouped into six major families: Hsp100, Hsp90, Hsp70, Hsp60 (chaperonins), Hsp40, and the small Hsps, according to their approximate molecular weight expressed in kilodaltons (kDa). Their classical ATP-dependent (foldase) or ATP-independent (holdase) chaperone activity is to assist in proper protein folding during translation, re-folding of denatured proteins, native protein stabilization, polypeptide transport, or degradation of misfolded proteins. Hsps can be synthesized constitutively or induced in the cell under stress conditions and are present in various cellular compartments. Multiple stress factors, e.g., hyperthermia, oxidative stress, ethanol, or UV radiation, can increase the production of well-characterized inducible Hsp90 and Hsp70 chaperones, which, in turn, interact with protein substrates (clients) and (co-)chaperones to participate in almost every cellular process, including inflammation [1–4]. In addition to their classical (canonical) role in the cell, some of these molecular chaperones (e.g., Hsp70) can be either passively (due to necrosis) or actively (via lipid vesicles) secreted into the extracellular space to mediate cell–cell communication via binding to several cell-surface receptors, including TLR4, CD91, LRP-1, SCARF1, and LOX-1 [5,6]. Moreover, some Hsps may form complexes with intracellular antigens, which are subsequently directed toward either the MHC class I or MHC class II associated pathways, leading to the activation of T lymphocytes [7]. As a result of findings described above, both intra- and extracellular Hsps have become the subject of interest of scientists in the context of the inflammatory and autoimmune process [8–10].

2. Heat Shock Protein 90 (Hsp90)

In humans, the Hsp90 family consists of four members (i.e., Hsp90 α , Hsp90 β , Grp94/gp96, and TRAP-1) localized in different cellular compartments. Structurally, all members of the Hsp90 family comprise a common domain structure consisting of the nucleotide-binding domain (NTD, N-terminal domain), a middle domain (MD) that has high affinity for co-chaperones and client proteins, and the carboxy-terminal domain (CTD) responsible for dimerization and oligomerization [11–13]. Hsp90 α , so far the best described member of the Hsp90 family, is a stress-inducible mammalian cytosolic isoform. It is one of the key chaperones responsible for the biological activity of hundreds of its protein substrates, among which there are key signaling molecules (e.g., mitogen-activated protein kinase, MAP kinases) and transcription factors (e.g., nuclear factor-kappa B, NF- κ B) that regulate important cellular processes, such as growth, cell cycle, and differentiation. Moreover, Hsp90 is involved in the stabilization of oncogenic proteins (e.g., Bcr-Abl, HER-2, EGFR, and HIF-1 α); hence, the inhibitors of its chaperone activity are currently being tested as cancer treatment in advanced clinical trials [14,15]. In addition, it was shown that extracellular Hsp90 α mediates tumor metastasis but can also contribute to physiological processes such as wound healing [16,17].

Inhibition of Hsp90 Activity

The structure and chaperone activities of Hsp90 have already been thoroughly discussed in other review articles [18–22]. Here, we present a brief overview of this issue to provide the reader with a basic background for understanding the mechanism of action of Hsp90 inhibitors and their classification, which may be considered for use in the treatment of autoimmune skin diseases. The majority of discovered Hsp90 inhibitors that target the ATP-binding pocket in the N-terminal domain of Hsp90 have entered clinical trials (e.g., 17-DMAG, 17-AAG, and STA-9090); however, they have not been approved by the FDA due to high toxicity, poor pharmacokinetic profiles, or simply a lack of clinical efficacy [20]. The lack of effectiveness of this type of Hsp90 inhibitor is due to, *inter alia*, triggering a survival mechanism in cancer cells, referred to as heat shock response (HSR), driving heat shock factor 1 (HSF-1) activation [23]. In fact, activation of HSF-1 is a common feature of numerous cancer types, and its expression is associated with malignancy and mortality. Moreover, activation of HSF1-dependent chaperones (e.g., Hsp70, Hsp40, and Hsp27) participates in cancer cell growth and survival [10]. The above-mentioned insufficient therapeutic strategy in cancer diseases can be improved by using combined therapy. For instance, treatment with 17-AAG along with small interfering RNA (siRNA), knocking down Hsp90 α , exhibited significant anticancer activity in glioma [24]. Additionally, Hsp90 siRNA was able to inhibit the proliferation, migration, and invasion of angiosarcoma cells [25]. Nevertheless, due to the significant role of Hsp90 in neoplastic diseases, the search for effective and safer inhibitors of this chaperone is still ongoing. For instance, isoform-selective inhibitors (e.g., Gamitrinib or Radamide) as well as regulators targeting the MD (e.g., Sansalvamide A, Kongesin A) or C-terminal region of Hsp90 (e.g., Novobiocin, Deguelin, Epigallocatechin-3-gallate (EGCG)) were reported as alternative strategies for developing potent and safer Hsp90 inhibitors for clinical use [18–22,26–28]. The latter group of Hsp90 inhibitors is an especially attractive chemotherapeutic approach, as they do not trigger an HSR [23] (Figure 1).

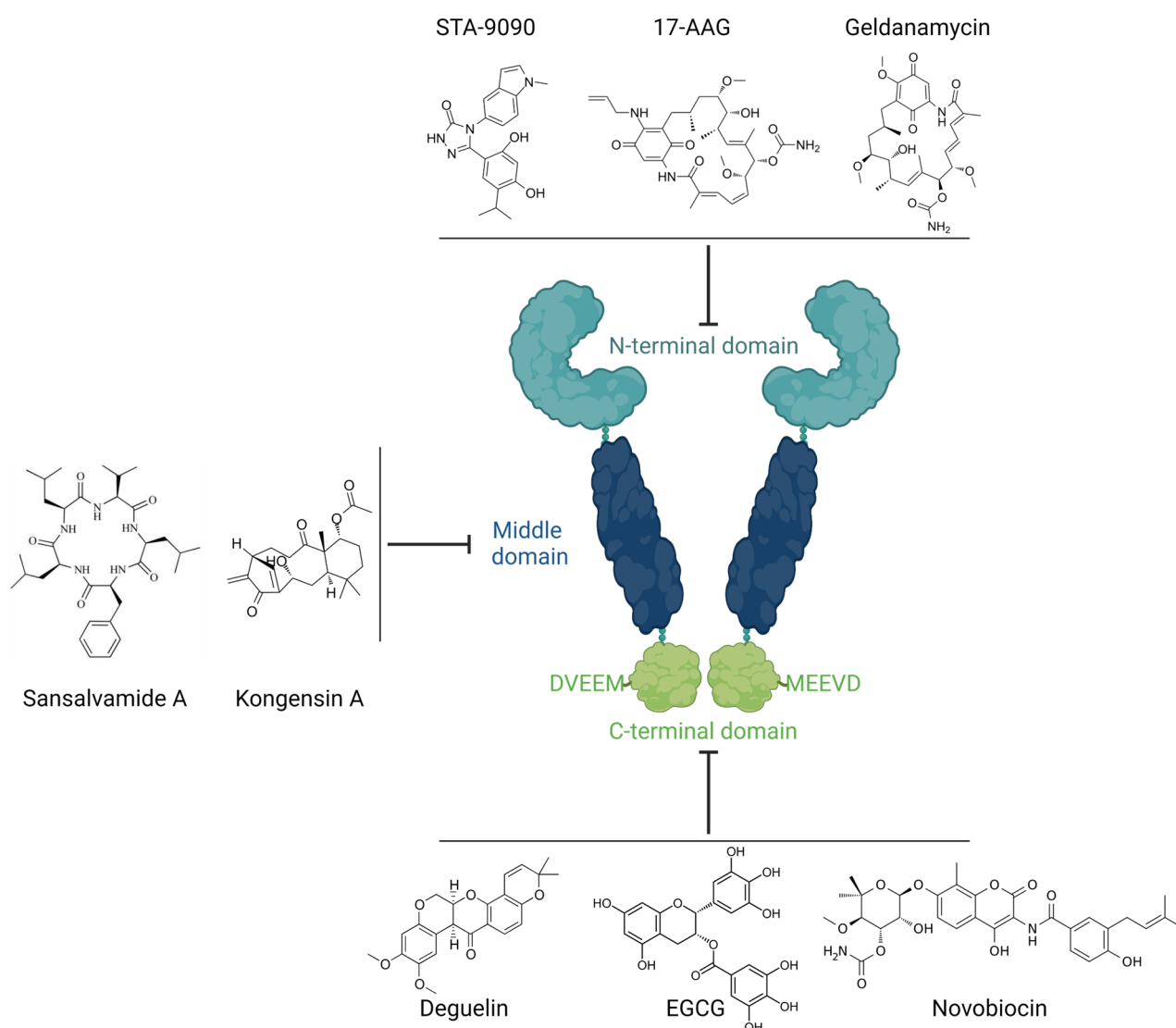


Figure 1. Schematic representation of the structural domains of Hsp90 along with their specific inhibitors, categorized by binding sites. Created with [BioRender.com](https://www.biorender.com/) (accessed on 9 August 2022).

Importantly, numerous studies have shown that intracellular Hsp90 is involved in the activation of the innate and adaptive components of the immune response, thereby promoting an inflammatory/autoimmune response. An indirect contribution of Hsp90 in the autoimmune process was confirmed in experimental preclinical studies using Hsp90 inhibitors, belonging solely to the N-terminal binding type, which seem to be more attractive for the treatment of autoimmune diseases due to the activation of an HRS [10,23]. This anti-Hsp90 therapy has been successfully applied in murine models of encephalomyelitis, collagen-induced arthritis, adjuvant-induced arthritis, systemic lupus erythematosus (SLE), lipopolysaccharide-induced uveitis (EIU), and DSS-induced ulcerative colitis [10,29]. Mechanistically, Hsp90 inhibitors such as geldanamycin (GA) and its semi-synthetic derivatives (e.g., 17-DMAG or 17-AAG) bind to the N-terminal nucleotide binding pocket of Hsp90 with higher affinity than ATP, which drives Hsp90-dependent ‘clients’ to proteasomal degradation. It is believed that immunosuppressive activity of this type of Hsp90 inhibition may result from (i) the activation of HSF-1, which regulates the expression of multiple genes, including immunoregulatory Hsp70 and IL-10; (ii) the expansion of immunosuppressive lymphocytes (both T and B regulatory cells); (iii) the inactivation of NF- κ B-dependent inflammatory/regulating factors, including TNF- α , IL-6, IL-8, and IL-17; or (iv) blockade of the cell signaling molecules, such as MAP kinase [10,14,29–31]. There is, however, an

important issue to be resolved. Namely, will Hsp90 inhibitors, which already found use in the treatment of cancer and do not activate an HSR, also be effective in the treatment of autoimmune diseases? The basic mechanism and cellular consequences concerning the inhibition of Hsp90 in regard to autoimmune diseases are schematically presented in Figure 2.

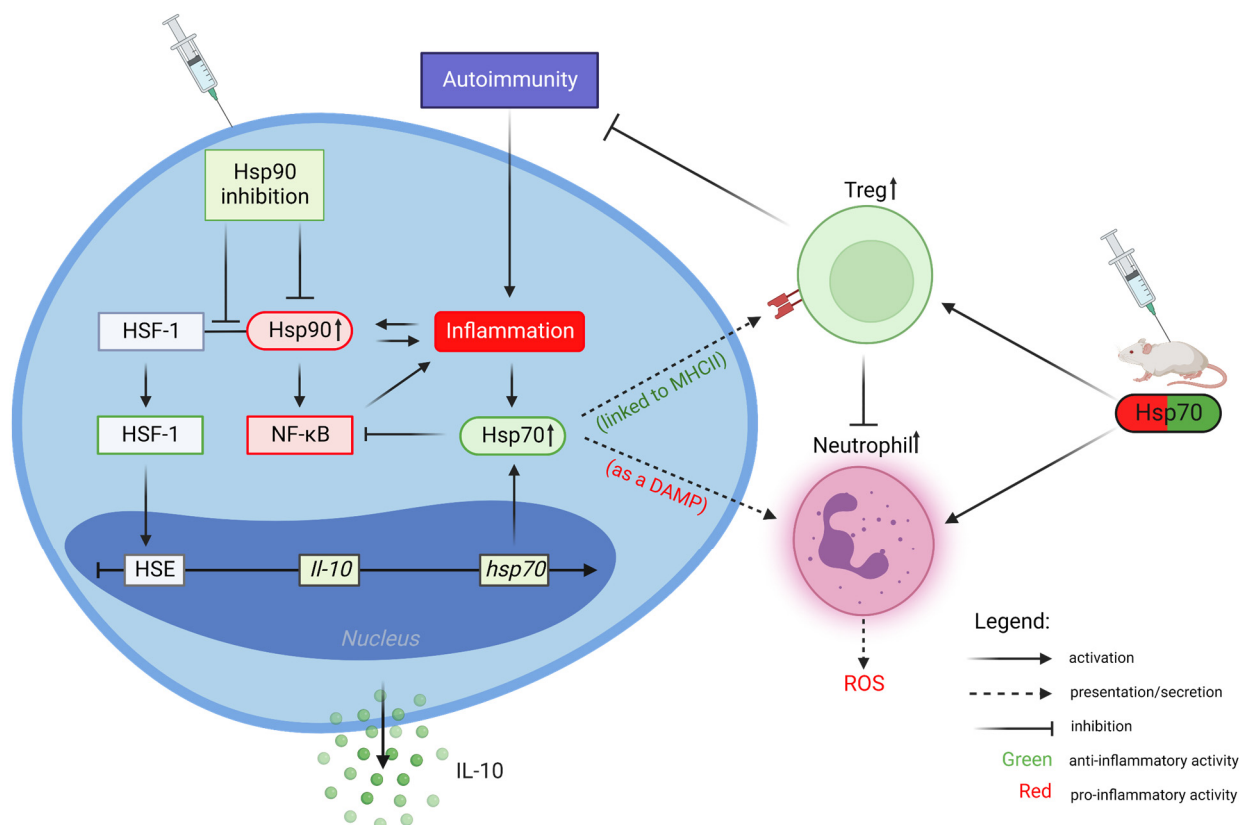


Figure 2. Contribution of Hsp90 and Hsp70 to the immune response and their significance in the therapy of autoimmune diseases. Inflammation leads to Hsp90 upregulation and, vice versa, Hsp90 promotes proinflammatory signaling. Blockade of Hsp90 activates heat shock factor 1 (HSF-1), which upregulates the expression of Hsp70 and IL-10. Stress stimuli or Hsp90 inhibition lead to the induction of Hsp70, which downregulates NF- κ B activation. In parallel, Hsp70 presented by major histocompatibility complex (MHC) class II molecules activates disease-modulating (immunosuppressive) T regulatory cells (Treg). On the other hand, acting as a damage-associated molecular pattern (DAMP), extracellular Hsp70 activates neutrophils and promotes the secretion of reactive oxygen species (ROS). Hsp70-based immunization stimulates either pro- or anti-inflammatory immune responses/cells. Created with [BioRender.com](https://www.biorender.com) (accessed on 11 August 2022).

3. Heat Shock Protein 70 (Hsp70)

The Hsp70 family of molecular chaperones (comprises 13 gene products in humans) represents one of the most ubiquitous classes of constitutively (e.g., Hsc70) or stress-induced (collectively termed Hsp70 or HSPA1) proteins [32]. Structurally, Hsp70 isoforms consist of three functional domains, including the nucleotide-binding domain (NBD), substrate-binding domain (SBD), and a C-terminal peptide-binding domain [33]. The Hsp70 is involved in a large variety of cellular processes, e.g., protein folding and remodeling, together with other molecular (co-)chaperones, including Hsp40 or Hsp90 [34]. While the inhibitors of Hsp70 chaperone activity are currently under investigation in therapy for cancerous diseases [28,35,36], a potential use of this therapeutic strategy in autoimmune and noncancerous inflammatory diseases remains unclear. This is especially intriguing as pharmacological co-inducers of Hsp70 expression (e.g., carvacrol) were able to down-

regulate the inflammation process in preclinical models of autoimmune arthritis [37–39]. Mechanistically, in contrast to Hsp90, the upregulation of intracellular Hsp70 inhibits the activity of NF- κ B, a transcription factor that plays a key role in the inflammation process and autoimmunity [8]. In addition, DNA vaccines coding for Hsps including Hsp70 are considered as potential treatment of autoimmune disorders [40].

Extracellular Hsp70 Activities

Bacterial and autologous Hsp70 may be released from normal and stressed cells and impact the host's immune components belonging to the innate and acquired arms of the immune system. While data concerning the immunosuppressive activity of intracellular Hsp70 are generally consistent, with some exceptions, the role of extracellular Hsp70 in the inflammation process and the development of autoimmune diseases is still not fully determined, as scientists working in this field present often-conflicting data. These differences may be the result of interpretation errors that stem from presenting an incomplete picture of the immune responses to Hsp70, insufficient/inadequate quality/purity of protein preparations used in cell cultures, or simply due to fundamental disparity in the way in vitro and in vivo experiments are conducted. These apparently ambiguous activities may also be related to the ability of this chaperone to interact with multiple receptors that are displayed on different types of cells in the immune system. Finally, it is suggested that this dual nature corresponds to the mechanism of secretion/presentation. While Hsp70 presented by the antigen-presenting cells (APC) may activate regulatory T helper cells (Treg), thus promoting anti-inflammatory mechanisms, Hsp70 liberated from damaged (necrotic) cells can act as a damage-associated molecular pattern (DAMP) via Toll-like receptors 2 and 4 (TLR2 and TLR4), which stimulates proinflammatory reactions [5,8–10,41,42]. It should also be kept in mind that, despite the common features of autoimmune diseases, there are some substantial differences (involving different cells of the immune system) that may ultimately elicit the dual (pleiotropic) nature of extracellular Hsp70 and other Hsps. Multiple studies presenting the immunosuppressive activities of Hsp70-derived molecules are based on preclinical models of arthritis [43]. For instance, the research team of professor van Eden W. reported, in a very elegant way, that highly conserved Hsp70-peptide (HSP70-B29) used for active immunization of animals could be regarded as a potential treatment target for rheumatoid arthritis (RA) via induction of immunosuppressive Hsp70-specific Tregs [44]. The description of the dual role of extracellular Hsp70 in the development of autoimmune skin diseases is presented in later chapters and in Figure 2.

4. Hsp90 and Hsp70 as Potential Therapeutic Targets in Autoimmune Skin Diseases

The skin, which protects us from environmental and microbial insults thanks to physical and immunological barriers, is the largest organ of the human body. This protection is ensured by the skin's unique anatomy and cellular composition, in particular, the network of immune cells including macrophages, dendritic cells, mast cells, $\gamma\delta$ T cells, and innate lymphoid cells. During microbial skin infection, different activated T cell subpopulations, monocytes, and granulocytes may be additionally recruited to the skin to support host defense [45]. When the body overcomes an infection, the primary activity status of the immune cells is restored thanks to immunoregulatory mechanisms. Colloquially, autoimmunity may occur when the balance between the effector arm and the regulatory arm of the innate and adaptive immune systems is disturbed or improperly regulated. Therefore, the primary therapies for autoimmune skin diseases involve the use of immunosuppressive medications, including corticosteroids (applied both topically and systemically), methotrexate, or azathioprine, which inhibit the proinflammatory immune response. Currently, precisely targeted therapies relying on proinflammatory cytokine blockade (e.g., TNF, IL-17, and TSLP), cell depletion (e.g., B cells), blockade of intracellular signaling (e.g., JAK-STAT), or a costimulatory blockade (e.g., CD28/CD80-86/CTLA-4) are approved by the FDA or under clinical evaluation [46,47]. Despite the huge breakthrough made in the treatment of autoimmune skin diseases, currently available therapy still fails to bring a satisfactory

effect in a number of cases. Hence, safer and more effective therapies that bring a sustained balance between pro- and anti-inflammatory responses are still desired.

There is growing evidence from preclinical studies confirming the contribution of either Hsp90 or Hsp70 to the development and therapy of autoimmune skin diseases, such as autoimmune bullous skin diseases, psoriasis, systemic lupus erythematosus, vitiligo, alopecia areata, or systemic sclerosis. Studies targeting Hsp90 or Hsp70 in autoimmune skin diseases based on preclinical and clinical studies are presented in Table 1.

Table 1. Overview of studies targeting Hsp90 or Hsp70 in autoimmune skin diseases based on preclinical and clinical observations.

Disease	Animal Model/ Clinical Observation	Target	Inhibitor	Outcome	Literature
Epidermolysis bullosa acquisita	COL7 or anti-COL7 IgG immunized mouse models	Hsp90	TCBL-145	Clinical and histological improvement	[48]
			17-DMAG		[48,49]
			17-AAG		[50]
		Hsp70	None	Hsp70- or anti-Hsp70 IgG-treated EBA mice had more intense disease activity	[51,52]
Psoriasis	Mouse xenograft transplantation model	Hsp90	Debio 0932	Clinical and histological improvement	[53]
	Imiquimod-induced mouse model	Hsp70	Myricetin Quercetin Ellagic acid	Clinical and histological improvement	[54]
		Hsp70	None	Topically applied Hsp70 ameliorated disease activity	[55]
		Hsp70	None	Hsp70-based immunization ameliorated disease activity	[56]
	Phase I–II evaluation of safety and efficacy	Hsp90	Debio 0932	Clinical improvement during unrelated (oncological diseases) clinical trial	[53]
	Phase Ib proof-of-concept study	Hsp90	RGRN-305	Clinical and serological improvement	[57]
Vitiligo	Mouse model of autoimmune vitiligo	Hsp70	None	Human- and mouse-derived inducible Hsp70-vaccinated mice displayed accelerated depigmentation	[58]
Alopecia areata	C3H/HeJ spontaneous mouse model of AA	Hsp70	Quercetin	Clinical and histological improvement	[59]
Systemic lupus erythematosus	MRL/lpr mouse model of SLE	Hsp90	17-AAG	Clinical and functional improvement	[60]
			STA-9090		[61]
			17-DMAG		[62]
	(NZB × NZW)F1 mouse model of SLE	Hsp90	None	Vaccination with DNA encoding Hsp90 protected from murine lupus	[63]
		Hsp70	None	Vaccination with DNA encoding Hsp70 led to disease suppression	[64]
Systemic sclerosis	Mouse model of Ssc	Hsp90	17-DMAG	Histological and functional improvement	[65]
Atopic dermatitis	OVA-induced mouse model	Hsp70	None	Subcutaneous administration of recombinant Hsp70 led to clinical, histological, and serological improvement	[66]

4.1. Autoimmune Bullous Diseases

Autoimmune bullous diseases (AIBDs) belong to a relatively rare and potentially life-threatening organ-specific group of inflammatory skin diseases characterized by the presence of autoantibodies against various structural proteins of the skin present in desmosomes (e.g., pemphigus vulgaris-PV) and hemidesmosomes (e.g., bullous pemphigoid-BP and epidermolysis bullosa acquisita-EBA), or against epidermal/tissue transglutaminases present in Dühring disease (also known as dermatitis herpetiformis-DH). Despite understanding of the pathophysiology of AIBDs, in which both innate and adaptive mechanisms of the immune response are undoubtedly involved, treatment for this group of diseases remains a challenge, due to frequent relapses after the discontinuation of therapy, numerous side effects associated with using, e.g., corticosteroids, or due to the lack of a fully effective drug [67–69].

4.1.1. Bullous Pemphigoid

Bullous pemphigoid (BP) is one of the most common types of AIBDs, characterized by the presence of tense blisters and tissue-bound autoantibodies directed to two hemidesmosomal structure proteins, namely, BP180 (specifically to its immunodominant region, NC16A) and BP230. In addition to the T-cell-dependent humoral autoreactivity, the importance of complement activation, neutrophils, macrophages, mast cells, and various proteases including neutrophil elastase and matrix metalloproteinases (MMPs) for blister formation are indicated [70].

It has been found that Hsp90 is accumulated/overexpressed in the perilesional skin of BP patients as compared to normal skin of healthy controls. Experimental approaches revealed that the accumulation of this chaperone in BP is mediated by the presence of circulating anti-BP180-NC16A IgG autoantibodies, since the levels of Hsp90 in circulation were significantly lower in affected patients compared to healthy controls and inversely correlated to the titer of anti-BP180-NC16A IgG in those patients. In addition, while Hsp90 expression was upregulated in activated (by BP serum) human keratinocytes (HaCaT), the presence of purified anti-BP180-NC16A IgG blocked the secretion of Hsp90 from HaCaT cells in vitro [71]. The role of the Hsp90 in BP has also been proven experimentally using an Hsp90 inhibitor—17-DMAG. The presence of this inhibitor in HaCaT cultures stimulated by anti-BP180-NC16A IgG (an in vitro approximation of BP ‘model’) led to the inhibition of NF- κ B activation and a decreased expression/secretion of IL-8, which is one of the key chemokines in BP. In addition, 17-DMAG treatment was associated with Hsp70 induction in the cells [72].

4.1.2. Epidermolysis Bullosa Acquisita

Epidermolysis bullosa acquisita (EBA) is an anti-type VII collagen (COL7) autoantibody-mediated autoimmune blistering skin disease with two major clinical subtypes, including mechanobullous or inflammatory variants, with the latter resembling BP [70]. In the BP-like experimental model of EBA, anti-COL7 IgG binding is followed by reactive oxygen species (ROS) generation and MMPs expression by neutrophils. Both directly lead to the degradation of the dermal–epidermal junction and blister formation [73].

Anti-Hsp90 therapy was examined in COL7-immunized mice using intraperitoneally applied 17-DMAG or nontoxic peptide TCBL-145. Both inhibitors ameliorated the clinical symptoms of EBA, suppressed anti-COL7 IgG, and reduced dermal neutrophilic infiltration. While total or antigen-specific plasma cells and germinal center B cells were unaffected by anti-Hsp90 treatment, human or mouse B cell as well as T cell activation were potentially suppressed by the inhibitors [48,49]. Mechanistically, 17-DMAG treatment led to the induction of HSF-1 and Hsp70 and reduced Th1 and Th17 frequencies in human activated B and T cell cultures, respectively [49,74]. In addition, anti-Hsp90 therapy led to the induction of regulatory B cells (Breg), the activation of which was proven ex vivo [49]. In a dose-dependent manner, 17-DMAG inhibited the detachment of the epidermis from the dermis in the human skin biopsy (cryosection assay) treated with anti-COL7 IgG-activated

granulocytes, as well as inhibiting the production/release of ROS by activated human neutrophils. Finally, since extracellular Hsp90 has been found to interact with MMP2 and MMP12 in the sera of EBA patients, it may suggest that these basement-membrane-degrading enzymes are substrates of this chaperone and are thus potentially dependent on its activity [75]. The effectiveness of anti-Hsp90 therapy has also been demonstrated locally. Application of a less toxic geldanamycin analog, 17-AAG, directly to skin lesions attenuated clinical disease severity (both in prophylactic and therapeutic treatment) without skin or systemic toxicity in experimental EBA models. The therapy led to the reduction in skin infiltration by neutrophils and NF- κ B activation, as well as decreases in MMP2, MMP9, MMP12, and Flii expression. In addition, topical 17-AAG application led to the induction of Hsp70 in the skin [50].

It has been frequently reported that Hsp70-derived peptides, used for active immunization of animals, could be regarded as a potential treatment target for RA via the induction of immunosuppressive mechanisms, which include IL-10 and Tregs [43]. Since blood levels of autologous inducible Hsp70 were found to be elevated in an experimental EBA mouse model [51], the role of this protein in EBA has yet to be determined. This disease is characterized by a different mechanism of development than RA. Surprisingly, Hsp70-treated EBA mice displayed more severe symptoms compared to untreated EBA mice. This effect was accompanied by increased levels of cutaneous MMP9 and circulating H₂O₂. ROS release assay using human granulocytes stimulated by EBA-specific immune complexes confirmed the proinflammatory properties of autologous Hsp70 [51]. Additionally, the humoral autoimmune response to Hsp70 has been observed to play a role in EBA. Circulating anti-Hsp70 IgG autoantibodies were significantly elevated in EBA patients as compared to healthy individuals and positively correlated with IFN- γ in patients. The importance of these newly discovered, pathologically relevant autoantibodies has been proven in vivo, since anti-Hsp70 IgG-treated EBA mice had a more intense clinical and histological disease activity [52]. The above outcomes suggest that both autologous Hsp70 and autoantibodies to Hsp70 display proinflammatory activities in the context of EBA development [51,52].

4.1.3. Dermatitis Herpetiformis

Dermatitis herpetiformis (DH) is an autoimmune blistering skin manifestation of celiac disease that develops mostly in patients with latent gluten-sensitive enteropathy. It is manifested by the presence of gluten-induced IgA autoantibodies against epidermal (eTG) and tissue (tTG) transglutaminases [76]. In fact, circulating autoantibodies to Hsp60, Hsp70, and Hsp90 were found to be significantly elevated in DH patients (but not in BP and PV patients) in the active phase of the disease. Interestingly, remitted patients were characterized by a significant decrease in the level of anti-Hsps autoantibodies, as well as autoantibodies directed to eTG and tTG. These serological observations may suggest that autoantibodies to Hsps participate in the development and maintenance of DH, which suggests a potential novel use as disease biomarkers [77].

4.2. Psoriasis

Psoriasis is one of the most common chronic autoimmune skin conditions, characterized by scaly and itchy patches of reddened skin resulting from excessive proliferation and abnormal differentiation of epidermal keratinocytes caused by an impaired local and systemic immune responses, the presence of susceptibility alleles, and environmental factors [78]. There is a growing body of research suggesting that psoriasis is a systemic incurable disease where proinflammatory T helper cells, specifically the Th17 subpopulation, play an important effector role in initiating systemic inflammation [79]. Since proving that Hsp90 was involved in IL-17-mediated skin inflammation, the chaperone was found to be significantly upregulated in keratinocytes and mast cells in the lesional skin of patients with psoriasis [80]. Therefore, it has been described as a potential novel therapeutic target in this disease. In fact, the use of an Hsp90 inhibitor in the treatment of patients with psoriasis was discovered accidentally during the first clinical trials of a new oral Hsp90

inhibitor (Debio 0932) in the treatment of oncological diseases, such as advanced solid tumors, lymphomas, and non-small cell lung cancer. Symptoms of psoriasis, which one of the oncological patients was suffering from, had gone into complete remission as a result of the experimental treatment administered during the trial. The confirmation of these unexpected results was provided in a psoriasis xenograft transplantation model. In this mouse model, oral administration of Debio 0932 to animals significantly arrested both the development of the clinical manifestation of psoriasis and histological parameters, such as epidermal thickness, as well as psoriasis pattern and vessel scores [53]. In vitro studies using another Hsp90 inhibitor (RGRN-305) on psoriasis-like inflammatory response in human keratinocytes further confirmed the contribution of this chaperone to the development of psoriasis. RGRN-305 significantly reduced the IL-17A- and TNF α -induced expression of CCL20, NFKBIZ, IL-36G, and IL-23A in human keratinocyte cultures [81]. These preclinical observations have resulted in the use of this experimental therapy in clinical trials. A phase Ib proof-of-concept study has been recently launched to evaluate the safety and efficacy of RGRN-305 in the treatment of plaque psoriasis [57]. It has been reported that six of the eleven psoriasis patients enrolled in this clinical study responded positively to RGRN-305 treatment, as a 71–94% reduction in the Psoriasis Area and Severity Index (PASI) in RGRN-305-treated patients was noted. While five out of eleven patients were considered non-responders, no serious side effects were reported. Treatment with RGRN-305 resulted in a marked inhibition of the IL-23, TNF- α , and IL-17A signaling pathways and the normalization of both histological changes and the gene expression profiles of psoriasis, further supporting that Hsp90 may serve as a novel target in the treatment of psoriasis [57].

Hsp70 has also been considered as a therapeutic target in psoriasis. This claim was tested using a well-described imiquimod (IMQ)-induced mouse model of psoriasis-like skin inflammation. Interestingly, topical application of either Hsp70 inhibitors or plant-derived Hsp70 protein contributed to a significant inhibition of clinical and histological symptoms and a modulation of the selected disease-associated cytokines, including IL-17A, IL-4, IL-5, TNF- α , IL-22, and IL-23 [54,55]. These distinct therapeutic approaches (i.e., inhibition of Hsp70 activity or topical Hsp70 application) had surprisingly convergent clinical outcomes. It must therefore be clarified whether the chaperone activity of intracellular Hsp70 or the immunogenicity of the extracellular fraction of this protein is crucial in the development of psoriasis. Given the assumption that immunization with Hsp70 expanded Treg and ameliorated autoimmune arthritis in a model [82], the role of Hsp70-based immunization therapy on psoriasis-like skin inflammation (IMQ-induced mouse) was evaluated. Immunization of naïve BALB/c mice (two weeks prior to psoriasis induction) with autologous or plant Hsp70 resulted in decreased PASI and histological severity. Mechanistically, therapy with plant-derived Hsp70 led to the expansion of two populations of Tregs, i.e., CD4⁺FoxP3⁺ and CD4⁺CD25⁺. A functional assay revealed that concomitantly induced circulating anti-Hsp70 IgG in the immunized animals could also inhibit disease progress, since passive transfer of anti-Hsp70 IgG led to the attenuation of disease activity and the inhibition of Th17 frequencies in the spleens. Antiproliferative/immunosuppressive effects of Hsp70 on keratinocytes/T cells were also directly confirmed in cell culture experiments [56].

4.3. Vitiligo

Vitiligo is an autoimmune T-cell-dependent depigmenting disorder resulting from the loss of melanocytes in the epidermis [83]. It has been initially reported that 4-tertiary butyl phenol (4-TBP)-treated vitiligo PIG3V melanocytes may mediate the activation of disease-effector dendritic cells (DCs) through the release of Hsp70 [84]. Further, it has been shown that using human- and mouse-derived inducible Hsp70 in vaccination (plasmids encoding Hsp70s) accelerated depigmentation in a mouse model of autoimmune vitiligo [58], representing a potential therapeutic target [85]. Moreover, two independent studies have found cutaneous upregulation of Hsp70, which was associated with the development of vitiligo in patients [86,87]. Additionally, using an immortalized human vitiligo melanocyte cell line, both cytoplasmic Hsp70 and Hsp90 were found to be translocated into apoptotic bodies

along with autoantigens (e.g., tyrosinase-related protein 1 or cleavage nuclear membrane antigen Lamin A/C) associated with vitiligo [88].

4.4. Alopecia Areata

Alopecia areata (AA) is a chronic inflammatory CD8⁺ T cells-mediated disease characterized by an autoimmune reaction to hair follicles, which consequently leads to the loss of hair in focal regions, the complete scalp including eyelashes and eyebrows, or even the entire body [89]. Quantitative proteomic analysis revealed that Hsp90 and Hsp70 chaperones were among 104 downregulated proteins found in lesional compared to non-lesional skin biopsies of AA patients [90]. Further studies demonstrated that Hsp90 and Lamin A/C interact with each other, and both play an essential role in the growth, migration, and self-aggregation of dermal papilla cells and can be linked to AA. It is suggested that the disruption of such interactions may contribute to the pathogenesis of AA via the dysfunction of dermal papilla cells [91].

Preclinical studies revealed not only an enhanced skin Hsp70 expression in C3H/HeJ mice strain that developed AA spontaneously [92], but also that the blockade of Hsp70 by quercetin proved an effective treatment for AA in this model [59].

4.5. Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a prototype autoimmune disease characterized by antinuclear antibodies, immune complex deposition, and heterogeneous clinical manifestations involving various organs and tissues, e.g., skin, joints, or kidneys. The disease manifests with various abnormalities in the phenotypes and functions of the innate and acquired immune cells [93]. The role of Hsp90 in SLE and its clinical relevance has been confirmed independently by several research teams. Higher expression of Hsp90 as well as enhanced circulating levels of soluble Hsp90 and anti-Hsp90 autoantibodies were associated with disease progression. Clinical improvement and multimodal regulatory effects of Hsp90 inhibition on, e.g., cell signaling, proinflammatory cytokine secretion, dsDNA antibodies, proteinuria, or lymphocytes activity (e.g., expansion of Treg or reduction in pathogenic T and B cell lineage populations) in SLE was revealed using experimental animal models. Taken together, Hsp90 contributes to inflammation and SLE progression, and therefore, targeting of its expression/activity may be a viable treatment for SLE [60–63,94–100].

Independent approaches revealed Hsp70 gene polymorphisms associated with SLE pathogenesis [101,102]. In addition, the expression of Hsp70 (HSPA1A) was significantly upregulated in patients with SLE and positively linked with the disease-specific autoantibodies in patients [103]. Preclinical studies, however, indicate a dual role for Hsp70 in the development of SLE. On the one hand, vaccination with DNA encoding Hsp70 suppressed a murine model of SLE via the induction of tolerogenic immune responses and marked suppression of anti-dsDNA antibody production, reduction in renal disease, and anti-inflammatory responses [64]. On the other hand, blockade of HSC70/Hsp73 chaperone expression via the P140 peptide displayed protective properties in MRL/lpr lupus-prone mice by decreasing autoreactive T cell priming and signaling [104].

4.6. Systemic Sclerosis

Systemic sclerosis (SSc) is a rare systemic autoimmune disease, one of the connective tissue diseases, characterized by widespread skin (scleroderma) and internal organ fibrosis, disturbances of innate and acquired immune responses, as well as vascular abnormalities [105]. Multiple observations provide evidence for the contribution of Hsp90 in the development of skin fibrosis [106]. It has been found, for instance, that increased expression of Hsp90 in the skin of patients with SSc is critical for TGF- β signaling and that a pharmacological blockade of Hsp90 inhibited the profibrotic effects of TGF- β in cultured fibroblasts and in animal models of SSc [65]. Importantly, the chaperone's extracellular presence, i.e., elevated serum Hsp90 levels, were associated with increased systemic inflammation, worse

lung functions, and skin involvement in SSc patients [107]. Likewise, increased levels of circulating Hsp70 were associated with pulmonary fibrosis, skin sclerosis, renal vascular damage, oxidative stress, and inflammation in SSc patients, suggesting that extracellular Hsp70 may be a useful serological marker for evaluating both cellular stresses and disease severity in SSc patients [108].

4.7. Atopic Dermatitis

Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases (prevalence: 15–30% in children and 2–10% in adults), characterized by intense itching and recurrent skin lesions. AD is a chronic and incurable immune-mediated disease that can be controlled by the use of topical emollients, calcineurin inhibitors, or corticosteroids [109]. Since immune responses to self-proteins have been observed in AD patients, it cannot be excluded that an autoimmune response plays an important role in the progression of this disease [110]. Even though AD is not currently classified as an autoimmune disease, recent observational studies revealed the potential association between AD and autoimmune disorders. Systematic review and meta-analysis studies showed a significant association of AD with various autoimmune diseases, including AA, celiac disease, Crohn's disease, RA, SLE, ulcerative colitis, and vitiligo [111].

Highly immunogenic extracellular Hsp90 can activate the humoral immune response driving the generation of circulating anti-Hsp90 autoantibodies that were found to be elevated in several autoimmune disorders [8,9]. In fact, one study found circulating Hsp90 to be significantly elevated in AD patients compared to healthy controls and positively correlated with the severity of AD (SCORAD; Scoring Atopic Dermatitis). In the same study, anti-Hsp90 IgE serum positivity was characterized for about 50% of AD patients and less than 3% of healthy controls. These results suggest a possible role of the extracellular Hsp90 and anti-Hsp90 IgE autoantibodies in the development of AD, as well as providing potential novel disease biomarkers [112].

It has been found that the upregulation of Hsp65 and Hsp72/73 in skin lesions of patients with AD was positively associated with the disease's severity [113]. Another study revealed that elevated levels of anti-Hsp70 antibodies were associated with metal allergy in AD patients [114]. On the other hand, subcutaneous administration of recombinant Hsp70 to mice with an OVA-induced AD-like phenotype ameliorated disease severity and cellular skin inflammation via the induction of systemic Th1- and inhibition of Th2-like immune responses, as well as the inhibition of cutaneous TSLP expression [66].

5. Prospective

Extensive preclinical studies using Hsp-based vaccines or the inhibition of Hsp expression/activity in experimental autoimmune animal models have been validated in the first clinical trials and proof-of-concept studies, giving hope for the development of next-generation drugs. Promising clinical trials have been performed in patients with rheumatoid arthritis using Hsp40-derived dnaJP1 (a highly conserved 15 aa peptide), as well as full-length Hsps, such as immuno-globulin binding protein (BiP, belonging to the Hsp70 family) and Hsp10. In parallel, the treatment of newly diagnosed type 1 diabetes patients with Hsp60-derived DiaPep277 peptide successfully entered phase III clinical trials [8,9,43,115,116]. In addition, the latest clinical trials confirm the safety and efficacy of Hsp90 inhibition by RGRN-305 in the treatment of plaque psoriasis [57]. Taken together, both therapeutic approaches, i.e., modulation of Hsp activity/expression and Hsp-based vaccines, seem to represent a new promising direction in the treatment of autoimmune skin diseases. To fully validate this approach, long-term efficacy and safety, optimal dosage, and the use of Hsp-derived proteins/chaperone inhibitors in combination with current therapy all require further research. Therefore, further and more extensive studies using animal experimental models should be performed to determine the roles of such chaperones in the pathogenesis of autoimmune skin diseases. It is also worth noting that studies suggesting the involvement of Hsp90/70 in autoimmune/inflammatory diseases based

solely on observations of changes in the level of protein expression in inflamed tissues may not be sufficient to draw a final conclusion. Upregulation of Hsp90/70 and the correlations between the levels of these chaperones both inside and outside the cell with selected clinical parameters cannot unequivocally determine their contribution in the pathological process since the presence of such associations may result from compensation mechanisms. Therefore, functional assays or animal experimental models should be implemented to determine the role of such chaperones in the pathogenesis of autoimmune diseases. This is particularly important in the study of Hsp90/70, whose rapid and efficient expression in a cell exposed to multiple stressors is a key element of first response, enabling the repair of any damage that has occurred.

Author Contributions: Conceptualization, S.T.; writing—original draft preparation, S.T.; writing—review and editing, K.S.; visualization, S.T. and K.S.; supervision, S.T.; funding acquisition, S.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Polish National Science Centre (NCN), grant number 2020/39/B/NZ6/00357.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

- De Maio, A.; Vazquez, D. Extracellular heat shock proteins: A new location, a new function. *Shock* **2013**, *40*, 239–246. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zuehlke, A.D.; Moses, M.A.; Neckers, L. Heat shock protein 90: Its inhibition and function. *Philos. Trans. R. Soc. B Biol. Sci.* **2018**, *373*, 20160527. [\[CrossRef\]](#) [\[PubMed\]](#)
- Rutledge, B.S.; Choy, W.Y.; Duennwald, M.L. Folding or holding?—Hsp70 and Hsp90 chaperoning of misfolded proteins in neurodegenerative disease. *J. Biol. Chem.* **2022**, *298*, 101905. [\[CrossRef\]](#) [\[PubMed\]](#)
- Mitra, R.; Wu, K.; Lee, C.; Bardwell, J.C.A. ATP-Independent Chaperones. *Annu. Rev. Biophys.* **2022**, *51*, 409–429. [\[CrossRef\]](#)
- Calderwood, S.K.; Gong, J.; Murshid, A. Extracellular HSPs: The Complicated Roles of Extracellular HSPs in Immunity. *Front. Immunol.* **2016**, *7*, 159. [\[CrossRef\]](#)
- Murshid, A.; Borges, T.J.; Bonorino, C.; Lang, B.J.; Calderwood, S.K. Immunological Outcomes Mediated Upon Binding of Heat Shock Proteins to Scavenger Receptors SCARF1 and LOX-1, and Endocytosis by Mononuclear Phagocytes. *Front. Immunol.* **2019**, *10*, 3035. [\[CrossRef\]](#)
- Murshid, A.; Gong, J.; Calderwood, S.K. The role of heat shock proteins in antigen cross presentation. *Front. Immunol.* **2012**, *3*, 63. [\[CrossRef\]](#)
- Tukaj, S. Heat Shock Protein 70 as a Double Agent Acting Inside and Outside the Cell: Insights into Autoimmunity. *Int. J. Mol. Sci.* **2020**, *21*, 5298. [\[CrossRef\]](#)
- Tukaj, S.; Kaminski, M. Heat shock proteins in the therapy of autoimmune diseases: Too simple to be true? *Cell Stress Chaperones* **2019**, *24*, 475–479. [\[CrossRef\]](#)
- Tukaj, S.; Węgrzyn, G. Anti-Hsp90 therapy in autoimmune and inflammatory diseases: A review of preclinical studies. *Cell Stress Chaperones* **2016**, *21*, 213–218. [\[CrossRef\]](#)
- Hoter, A.; El-Sabban, M.E.; Naim, H.Y. The HSP90 Family: Structure, Regulation, Function, and Implications in Health and Disease. *Int. J. Mol. Sci.* **2018**, *19*, 2560. [\[CrossRef\]](#) [\[PubMed\]](#)
- Li, J.; Buchner, J. Structure, function and regulation of the hsp90 machinery. *Biomed. J.* **2013**, *36*, 106–117. [\[CrossRef\]](#) [\[PubMed\]](#)
- Biebl, M.M.; Buchner, J. Structure, Function, and Regulation of the Hsp90 Machinery. *Cold Spring Harb. Perspect. Biol.* **2019**, *11*, a034017. [\[CrossRef\]](#) [\[PubMed\]](#)
- Schopf, F.H.; Biebl, M.M.; Buchner, J. The HSP90 chaperone machinery. *Nat. Rev. Mol. Cell. Biol.* **2017**, *18*, 345–360. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zuehlke, A.D.; Beebe, K.; Neckers, L.; Prince, T. Regulation and function of the human HSP90AA1 gene. *Gene* **2015**, *570*, 8–16. [\[CrossRef\]](#)
- Hance, M.W.; Nolan, K.D.; Isaacs, J.S. The double-edged sword: Conserved functions of extracellular hsp90 in wound healing and cancer. *Cancers* **2014**, *6*, 1065–1097. [\[CrossRef\]](#)
- Li, W.; Sahu, D.; Tsen, F. Secreted heat shock protein-90 (Hsp90) in wound healing and cancer. *Biochim. Biophys. Acta* **2012**, *1823*, 730–741. [\[CrossRef\]](#)

18. Donnelly, A.; Blagg, B.S. Novobiocin and additional inhibitors of the Hsp90 C-terminal nucleotide-binding pocket. *Curr. Med. Chem.* **2008**, *15*, 2702–2717. [[CrossRef](#)]
19. Der Sarkissian, S.; Aceros, H.; Williams, P.M.; Scalabrini, C.; Borie, M.; Noiseux, N. Heat shock protein 90 inhibition and multi-target approach to maximize cardioprotection in ischaemic injury. *Br. J. Pharmacol.* **2020**, *177*, 3378–3388. [[CrossRef](#)]
20. Sanchez, J.; Carter, T.R.; Cohen, M.S.; Blagg, B.S.J. Old and New Approaches to Target the Hsp90 Chaperone. *Curr. Cancer Drug Targets* **2020**, *20*, 253–270. [[CrossRef](#)]
21. Mielczarek-Lewandowska, A.; Hartman, M.L.; Czyz, M. Inhibitors of HSP90 in melanoma. *Apoptosis* **2020**, *25*, 12–28. [[CrossRef](#)] [[PubMed](#)]
22. Bickel, D.; Gohlke, H. C-terminal modulators of heat shock protein of 90 kDa (HSP90): State of development and modes of action. *Bioorg. Med. Chem.* **2019**, *27*, 115080. [[CrossRef](#)] [[PubMed](#)]
23. Wang, Y.; McAlpine, S.R. N-terminal and C-terminal modulation of Hsp90 produce dissimilar phenotypes. *Chem. Commun.* **2015**, *51*, 1410–1413. [[CrossRef](#)] [[PubMed](#)]
24. Mehta, A.; Shervington, A.; Howl, J.; Jones, S.; Shervington, L. Can RNAi-mediated hsp90 α knockdown in combination with 17-AAG be a therapy for glioma? *FEBS Open Bio* **2013**, *3*, 271–278. [[CrossRef](#)] [[PubMed](#)]
25. Yamada-Kanazawa, S.; Kajihara, I.; Fukushima, S.; Jinnin, M.; Masuzawa, M.; Masuzawa, M.; Amoh, Y.; Hoshina, D.; Abe, R.; Ihn, H. Inhibition of heat shock protein 90 exerts an antitumour effect in angiosarcoma: Involvement of the vascular endothelial growth factor signalling pathway. *Br. J. Dermatol.* **2017**, *177*, 456–469. [[CrossRef](#)]
26. Yu, J.; Zhang, C.; Song, C. Pan- and isoform-specific inhibition of Hsp90: Design strategy and recent advances. *Eur. J. Med. Chem.* **2022**, *238*, 114516. [[CrossRef](#)]
27. Li, L.; Chen, N.N.; You, Q.D.; Xu, X.L. An updated patent review of anticancer Hsp90 inhibitors (2013–present). *Expert Opin. Ther. Pat.* **2021**, *31*, 67–80. [[CrossRef](#)]
28. Shevtsov, M.; Multhoff, G.; Mikhaylova, E.; Shibata, A.; Guzhova, I.; Margulis, B. Combination of Anti-Cancer Drugs with Molecular Chaperone Inhibitors. *Int. J. Mol. Sci.* **2019**, *20*, 5284. [[CrossRef](#)]
29. Li, L.; Wang, L.; You, Q.D.; Xu, X.L. Heat Shock Protein 90 Inhibitors: An Update on Achievements, Challenges, and Future Directions. *J. Med. Chem.* **2020**, *63*, 1798–1822. [[CrossRef](#)]
30. Trepel, J.; Mollapour, M.; Giaccone, G.; Neckers, L. Targeting the dynamic HSP90 complex in cancer. *Nat. Rev. Cancer* **2010**, *10*, 537–549. [[CrossRef](#)]
31. Jackson, S.E. Hsp90: Structure and function. *Top Curr. Chem.* **2013**, *328*, 155–240. [[CrossRef](#)]
32. Radons, J. The human HSP70 family of chaperones: Where do we stand? *Cell Stress Chaperones* **2016**, *21*, 379–404. [[CrossRef](#)] [[PubMed](#)]
33. Kityk, R.; Kopp, J.; Sinning, I.; Mayer, M.P. Structure and dynamics of the ATP-bound open conformation of Hsp70 chaperones. *Mol. Cell* **2012**, *48*, 863–874. [[CrossRef](#)] [[PubMed](#)]
34. Rosenzweig, R.; Nillegoda, N.B.; Mayer, M.P.; Bukau, B. The Hsp70 chaperone network. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 665–680. [[CrossRef](#)] [[PubMed](#)]
35. Kumar, S.; Stokes, J., 3rd; Singh, U.P.; Scisum Gunn, K.; Acharya, A.; Manne, U.; Mishra, M. Targeting Hsp70: A possible therapy for cancer. *Cancer Lett.* **2016**, *374*, 156–166. [[CrossRef](#)]
36. Ambrose, A.J.; Chapman, E. Function, Therapeutic Potential, and Inhibition of Hsp70 Chaperones. *J. Med. Chem.* **2021**, *64*, 7060–7082. [[CrossRef](#)]
37. Wieten, L.; Broere, F.; van der Zee, R.; Koerkamp, E.K.; Wagenaar, J.; van Eden, W. Cell stress induced HSP are targets of regulatory T cells: A role for HSP inducing compounds as anti-inflammatory immuno-modulators? *FEBS Lett.* **2007**, *581*, 3716–3722. [[CrossRef](#)]
38. Wieten, L.; van der Zee, R.; Spiering, R.; Wagenaar-Hilbers, J.; van Kooten, P.; Broere, F.; van Eden, W. A novel heat-shock protein coinducer boosts stress protein Hsp70 to activate T cell regulation of inflammation in autoimmune arthritis. *Arthritis Rheum.* **2010**, *62*, 1026–1035. [[CrossRef](#)]
39. Wieten, L.; van der Zee, R.; Goedemans, R.; Sijtsma, J.; Serafini, M.; Lubsen, N.H.; van Eden, W.; Broere, F. Hsp70 expression and induction as a readout for detection of immune modulatory components in food. *Cell Stress Chaperones* **2010**, *15*, 25–37. [[CrossRef](#)]
40. Quintana, F.J.; Cohen, I.R. DNA vaccines coding for heat-shock proteins (HSPs): Tools for the activation of HSP-specific regulatory T cells. *Expert Opin. Biol. Ther.* **2005**, *5*, 545–554. [[CrossRef](#)]
41. De Maio, A. Extracellular Hsp70: Export and function. *Curr. Protein Pept. Sci.* **2014**, *15*, 225–231. [[CrossRef](#)] [[PubMed](#)]
42. Hulina, A.; Grdić Rajković, M.; Jakšić Despot, D.; Jelić, D.; Dojder, A.; Čepelak, I.; Rumora, L. Extracellular Hsp70 induces inflammation and modulates LPS/LTA-stimulated inflammatory response in THP-1 cells. *Cell Stress Chaperones* **2018**, *23*, 373–384. [[CrossRef](#)] [[PubMed](#)]
43. van Eden, W. Immune tolerance therapies for autoimmune diseases based on heat shock protein T-cell epitopes. *Philos. Trans. R. Soc. B Biol. Sci.* **2018**, *373*, 20160531. [[CrossRef](#)] [[PubMed](#)]
44. Van Herwijnen, M.J.; Wieten, L.; van der Zee, R.; van Kooten, P.J.; Wagenaar-Hilbers, J.P.; Hoek, A.; den Braber, I.; Anderton, S.M.; Singh, M.; Meiring, H.D.; et al. Regulatory T cells that recognize a ubiquitous stress-inducible self-antigen are long-lived suppressors of autoimmune arthritis. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 14134–14139. [[CrossRef](#)]
45. Tay, S.S.; Roediger, B.; Tong, P.L.; Tikoo, S.; Weninger, W. The Skin-Resident Immune Network. *Curr. Dermatol. Rep.* **2014**, *3*, 13–22. [[CrossRef](#)]

46. Vesely, M.D. Getting Under the Skin: Targeting Cutaneous Autoimmune Disease. *Yale J. Biol. Med.* **2020**, *93*, 197–206.
47. Ujiie, H.; Rosmarin, D.; Schön, M.P.; Ständer, S.; Boch, K.; Metz, M.; Maurer, M.; Thaci, D.; Schmidt, E.; Cole, C.; et al. Unmet Medical Needs in Chronic, Non-communicable Inflammatory Skin Diseases. *Front. Med.* **2022**, *9*, 875492. [\[CrossRef\]](#)
48. Kasperkiewicz, M.; Müller, R.; Manz, R.; Magens, M.; Hammers, C.M.; Somlai, C.; Westermann, J.; Schmidt, E.; Zillikens, D.; Ludwig, R.J.; et al. Heat-shock protein 90 inhibition in autoimmunity to type VII collagen: Evidence that nonmalignant plasma cells are not therapeutic targets. *Blood* **2011**, *117*, 6135–6142. [\[CrossRef\]](#)
49. Tukaj, S.; Tiburzy, B.; Manz, R.; de Castro Marques, A.; Orosz, A.; Ludwig, R.J.; Zillikens, D.; Kasperkiewicz, M. Immunomodulatory effects of heat shock protein 90 inhibition on humoral immune responses. *Exp. Dermatol.* **2014**, *23*, 585–590. [\[CrossRef\]](#)
50. Tukaj, S.; Bieber, K.; Kleszczyński, K.; Witte, M.; Cames, R.; Kalies, K.; Zillikens, D.; Ludwig, R.J.; Fischer, T.W.; Kasperkiewicz, M. Topically Applied Hsp90 Blocker 17AAG Inhibits Autoantibody-Mediated Blister-Inducing Cutaneous Inflammation. *J. Investig. Dermatol.* **2017**, *137*, 341–349. [\[CrossRef\]](#)
51. Tukaj, S.; Mantej, J.; Sitko, K.; Bednarek, M.; Zillikens, D.; Ludwig, R.J.; Bieber, K.; Kasperkiewicz, M. Evidence for a role of extracellular heat shock protein 70 in epidermolysis bullosa acquisita. *Exp. Dermatol.* **2022**, *31*, 528–534. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Tukaj, S.; Mantej, J.; Sitko, K.; Zillikens, D.; Ludwig, R.J.; Bieber, K.; Kasperkiewicz, M. Pathological Relevance of Anti-Hsp70 IgG Autoantibodies in Epidermolysis Bullosa Acquisita. *Front. Immunol.* **2022**, *13*, 877958. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Stenderup, K.; Rosada, C.; Gavillet, B.; Vuagniaux, G.; Dam, T.N. Debio 0932, a new oral Hsp90 inhibitor, alleviates psoriasis in a xenograft transplantation model. *Acta Derm. Venereol.* **2014**, *94*, 672–676. [\[CrossRef\]](#)
54. Raghuwanshi, N.; Yadav, T.C.; Srivastava, A.K.; Raj, U.; Varadwaj, P.; Pruthi, V. Structure-based drug designing and identification of *Woodfordia fruticosa* inhibitors targeted against heat shock protein (HSP70-1) as suppressor for Imiquimod-induced psoriasis like skin inflammation in mice model. *Mater. Sci. Eng. C* **2019**, *95*, 57–71. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Seifarth, F.G.; Lax, J.E.; Harvey, J.; DiCorleto, P.E.; Husni, M.E.; Chandrasekharan, U.M.; Tytell, M. Topical heat shock protein 70 prevents imiquimod-induced psoriasis-like inflammation in mice. *Cell Stress Chaperones* **2018**, *23*, 1129–1135. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Tukaj, S.; Mantej, J.; Sobala, M.; Potrykus, K.; Tukaj, Z.; Zillikens, D.; Ludwig, R.J.; Bieber, K.; Kasperkiewicz, M. Therapeutic Implications of Targeting Heat Shock Protein 70 by Immunization or Antibodies in Experimental Skin Inflammation. *Front. Immunol.* **2021**, *12*, 614320. [\[CrossRef\]](#)
57. Bregnhøj, A.; Thuesen, K.K.H.; Emmanuel, T.; Litman, T.; Grek, C.L.; Ghatnekar, G.S.; Johansen, C.; Iversen, L. HSP90 inhibitor RGRN-305 for oral treatment of plaque-type psoriasis: Efficacy, safety and biomarker results in an open-label proof-of-concept study. *Br. J. Dermatol.* **2022**, *186*, 861–874. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Denman, C.J.; McCracken, J.; Hariharan, V.; Klarquist, J.; Oyarbide-Valencia, K.; Guevara-Patiño, J.A.; Le Poole, I.C. HSP70i accelerates depigmentation in a mouse model of autoimmune vitiligo. *J. Investig. Dermatol.* **2008**, *128*, 2041–2048. [\[CrossRef\]](#)
59. Wikramanayake, T.C.; Villasante, A.C.; Mauro, L.M.; Perez, C.I.; Schachner, L.A.; Jimenez, J.J. Prevention and treatment of alopecia areata with quercetin in the C3H/HeJ mouse model. *Cell Stress Chaperones* **2012**, *17*, 267–274. [\[CrossRef\]](#)
60. Hong, L.J.; Chen, A.J.; Li, F.Z.; Chen, K.J.; Fang, S. The HSP90 Inhibitor, 17-AAG, Influences the Activation and Proliferation of T Lymphocytes via AKT/GSK3 β Signaling in MRL/lpr Mice. *Drug Des. Dev. Ther.* **2020**, *14*, 4605–4612. [\[CrossRef\]](#)
61. Liu, Y.; Ye, J.; Shin Ogawa, L.; Inoue, T.; Huang, Q.; Chu, J.; Bates, R.C.; Ying, W.; Sonderfan, A.J.; Rao, P.E.; et al. The HSP90 Inhibitor Ganetespib Alleviates Disease Progression and Augments Intermittent Cyclophosphamide Therapy in the MRL/lpr Mouse Model of Systemic Lupus Erythematosus. *PLoS ONE* **2015**, *10*, e0127361. [\[CrossRef\]](#)
62. Shimp, S.K., 3rd; Chafin, C.B.; Regna, N.L.; Hammond, S.E.; Read, M.A.; Caudell, D.L.; Rylander, M.; Reilly, C.M. Heat shock protein 90 inhibition by 17-DMAG lessens disease in the MRL/lpr mouse model of systemic lupus erythematosus. *Cell. Mol. Immunol.* **2012**, *9*, 255–266. [\[CrossRef\]](#)
63. Liu, A.; Shi, F.D.; Cohen, I.R.; Castaldo, G.; Matarese, G.; Quintana, F.J.; La Cava, A. DNA vaccine encoding heat shock protein 90 protects from murine lupus. *Arthritis Res. Ther.* **2020**, *22*, 152. [\[CrossRef\]](#)
64. Liu, A.; Ferretti, C.; Shi, F.D.; Cohen, I.R.; Quintana, F.J.; La Cava, A. DNA Vaccination With Hsp70 Protects Against Systemic Lupus Erythematosus in (NZB $\times$ NZW)F1 Mice. *Arthritis Rheumatol.* **2020**, *72*, 997–1002. [\[CrossRef\]](#)
65. Tomcik, M.; Zerr, P.; Pitkowski, J.; Palumbo-Zerr, K.; Avouac, J.; Distler, O.; Becvar, R.; Senolt, L.; Schett, G.; Distler, J.H. Heat shock protein 90 (Hsp90) inhibition targets canonical TGF- β signalling to prevent fibrosis. *Ann. Rheum. Dis.* **2014**, *73*, 1215–1222. [\[CrossRef\]](#)
66. Kao, J.K.; Hsu, T.F.; Lee, M.S.; Su, T.C.; Lee, C.H.; Hsu, C.S.; Shieh, J.J.; Wang, J.Y.; Yang, R.C. Subcutaneous injection of recombinant heat shock protein 70 ameliorates atopic dermatitis skin lesions in a mouse model. *Kaohsiung J. Med. Sci.* **2020**, *36*, 186–195. [\[CrossRef\]](#)
67. Schmidt, E.; Kasperkiewicz, M.; Joly, P. Pemphigus. *Lancet* **2019**, *394*, 882–894. [\[CrossRef\]](#)
68. Bieber, K.; Ludwig, R.J. Drug Development in Pemphigoid Diseases. *Acta Derm. Venereol.* **2020**, *100*, adv00055. [\[CrossRef\]](#)
69. Izumi, K.; Bieber, K.; Ludwig, R.J. Current Clinical Trials in Pemphigus and Pemphigoid. *Front. Immunol.* **2019**, *10*, 978. [\[CrossRef\]](#)
70. Schmidt, E.; Zillikens, D. Pemphigoid diseases. *Lancet* **2013**, *381*, 320–332. [\[CrossRef\]](#)
71. Tukaj, S.; Kleszczyński, K.; Vafia, K.; Groth, S.; Meyersburg, D.; Trzonkowski, P.; Ludwig, R.J.; Zillikens, D.; Schmidt, E.; Fischer, T.W.; et al. Aberrant expression and secretion of heat shock protein 90 in patients with bullous pemphigoid. *PLoS ONE* **2013**, *8*, e70496. [\[CrossRef\]](#)
72. Tukaj, S.; Grüner, D.; Zillikens, D.; Kasperkiewicz, M. Hsp90 blockade modulates bullous pemphigoid IgG-induced IL-8 production by keratinocytes. *Cell Stress Chaperones* **2014**, *19*, 887–894. [\[CrossRef\]](#)

73. Kasperkiewicz, M.; Sadik, C.D.; Bieber, K.; Ibrahim, S.M.; Manz, R.A.; Schmidt, E.; Zillikens, D.; Ludwig, R.J. Epidermolysis Bullosa Acquisita: From Pathophysiology to Novel Therapeutic Options. *J. Investig. Dermatol.* **2016**, *136*, 24–33. [\[CrossRef\]](#)
74. Tukaj, S.; Zillikens, D.; Kasperkiewicz, M. Inhibitory effects of heat shock protein 90 blockade on proinflammatory human Th1 and Th17 cell subpopulations. *J. Inflamm.* **2014**, *11*, 10. [\[CrossRef\]](#)
75. Tukaj, S.; Hellberg, L.; Ueck, C.; Hänsel, M.; Samavedam, U.; Zillikens, D.; Ludwig, R.J.; Laskay, T.; Kasperkiewicz, M. Heat shock protein 90 is required for ex vivo neutrophil-driven autoantibody-induced tissue damage in experimental epidermolysis bullosa acquisita. *Exp. Dermatol.* **2015**, *24*, 471–473. [\[CrossRef\]](#)
76. Kárpáti, S. Dermatitis herpetiformis. *Clin. Dermatol.* **2012**, *30*, 56–59. [\[CrossRef\]](#)
77. Kasperkiewicz, M.; Tukaj, S.; Gembicki, A.J.; Silló, P.; Görög, A.; Zillikens, D.; Kárpáti, S. Evidence for a role of autoantibodies to heat shock protein 60, 70, and 90 in patients with dermatitis herpetiformis. *Cell Stress Chaperones* **2014**, *19*, 837–843. [\[CrossRef\]](#)
78. Schön, M.P. Adaptive and Innate Immunity in Psoriasis and Other Inflammatory Disorders. *Front. Immunol.* **2019**, *10*, 1764. [\[CrossRef\]](#)
79. Boehncke, W.H.; Schön, M.P. Psoriasis. *Lancet* **2015**, *386*, 983–994. [\[CrossRef\]](#)
80. Kakeda, M.; Arock, M.; Schlapbach, C.; Yawalkar, N. Increased expression of heat shock protein 90 in keratinocytes and mast cells in patients with psoriasis. *J. Am. Acad. Dermatol.* **2014**, *70*, 683–690.e681. [\[CrossRef\]](#)
81. Hansen, R.S.; Thuesen, K.K.H.; Bregnhøj, A.; Moldovan, L.I.; Kristensen, L.S.; Grek, C.L.; Ghatnekar, G.S.; Iversen, L.; Johansen, C. The HSP90 inhibitor RGRN-305 exhibits strong immunomodulatory effects in human keratinocytes. *Exp. Dermatol.* **2021**, *30*, 773–781. [\[CrossRef\]](#) [\[PubMed\]](#)
82. van Eden, W. Vaccination against autoimmune diseases moves closer to the clinic. *Hum. Vaccines Immunother.* **2020**, *16*, 228–232. [\[CrossRef\]](#) [\[PubMed\]](#)
83. Karagaiah, P.; Valle, Y.; Sigova, J.; Zerbinati, N.; Vojvodic, P.; Parsad, D.; Schwartz, R.A.; Grabbe, S.; Goldust, M.; Lotti, T.; et al. Emerging drugs for the treatment of vitiligo. *Expert Opin. Emerg. Drugs* **2020**, *25*, 7–24. [\[CrossRef\]](#) [\[PubMed\]](#)
84. Kroll, T.M.; Bommiasamy, H.; Boissy, R.E.; Hernandez, C.; Nickoloff, B.J.; Mestrl, R.; Caroline Le Poole, I. 4-Tertiary butyl phenol exposure sensitizes human melanocytes to dendritic cell-mediated killing: Relevance to vitiligo. *J. Investig. Dermatol.* **2005**, *124*, 798–806. [\[CrossRef\]](#) [\[PubMed\]](#)
85. Mosenson, J.A.; Zloza, A.; Klarquist, J.; Barfuss, A.J.; Guevara-Patino, J.A.; Poole, I.C. HSP70i is a critical component of the immune response leading to vitiligo. *Pigment Cell Melanoma Res.* **2012**, *25*, 88–98. [\[CrossRef\]](#)
86. Abdou, A.G.; Maraee, A.H.; Reyad, W. Immunohistochemical expression of heat shock protein 70 in vitiligo. *Ann. Diagn. Pathol.* **2013**, *17*, 245–249. [\[CrossRef\]](#)
87. Doss, R.W.; El-Rifaie, A.A.; Abdel-Wahab, A.M.; Gohary, Y.M.; Rashed, L.A. Heat Shock Protein-70 Expression in Vitiligo and its Relation to the Disease Activity. *Indian J. Dermatol.* **2016**, *61*, 408–412. [\[CrossRef\]](#)
88. Tian, J.; Wang, Y.; Ding, M.; Zhang, Y.; Chi, J.; Wang, T.; Jiao, B.; Jian, Z.; Yi, X.; Huang, Y.; et al. The Formation of Melanocyte Apoptotic Bodies in Vitiligo and the Relocation of Vitiligo Autoantigens under Oxidative Stress. *Oxidative Med. Cell. Longev.* **2021**, *2021*, 7617839. [\[CrossRef\]](#)
89. Gilhar, A.; Laufer-Britva, R.; Keren, A.; Paus, R. Frontiers in alopecia areata pathobiology research. *J. Allergy Clin. Immunol.* **2019**, *144*, 1478–1489. [\[CrossRef\]](#)
90. Thanomkitti, K.; Kanlaya, R.; Fong-Ngern, K.; Kapincharanon, C.; Sueksakit, K.; Chanchaem, P.; Thuangtong, R.; Thongboonkerd, V. Differential proteomics of lesional vs. non-lesional biopsies revealed non-immune mechanisms of alopecia areata. *Sci. Rep.* **2018**, *8*, 521. [\[CrossRef\]](#)
91. Thanomkitti, K.; Fong-Ngern, K.; Sueksakit, K.; Thuangtong, R.; Thongboonkerd, V. Molecular functional analyses revealed essential roles of HSP90 and lamin A/C in growth, migration, and self-aggregation of dermal papilla cells. *Cell Death Discov.* **2018**, *4*, 53. [\[CrossRef\]](#) [\[PubMed\]](#)
92. Wikramanayake, T.C.; Alvarez-Connelly, E.; Simon, J.; Mauro, L.M.; Guzman, J.; Elgart, G.; Schachner, L.A.; Chen, J.; Plano, L.R.; Jimenez, J.J. Heat treatment increases the incidence of alopecia areata in the C3H/HeJ mouse model. *Cell Stress Chaperones* **2010**, *15*, 985–991. [\[CrossRef\]](#) [\[PubMed\]](#)
93. Fanouriakis, A.; Tziolos, N.; Bertsias, G.; Boumpas, D.T. Update on the diagnosis and management of systemic lupus erythematosus. *Ann. Rheum. Dis.* **2021**, *80*, 14–25. [\[CrossRef\]](#) [\[PubMed\]](#)
94. Latchman, D.S.; Isenberg, D.A. The role of hsp90 in SLE. *Autoimmunity* **1994**, *19*, 211–218. [\[CrossRef\]](#) [\[PubMed\]](#)
95. Minota, S.; Koyasu, S.; Yahara, I.; Winfield, J. Autoantibodies to the heat-shock protein hsp90 in systemic lupus erythematosus. *J. Clin. Invest.* **1988**, *81*, 106–109. [\[CrossRef\]](#)
96. Erkeller-Yüksel, F.M.; Isenberg, D.A.; Dhillon, V.B.; Latchman, D.S.; Lydyard, P.M. Surface expression of heat shock protein 90 by blood mononuclear cells from patients with systemic lupus erythematosus. *J. Autoimmun.* **1992**, *5*, 803–814. [\[CrossRef\]](#)
97. Ripley, B.J.; Isenberg, D.A.; Latchman, D.S. Elevated levels of the 90 kDa heat shock protein (hsp90) in SLE correlate with levels of IL-6 and autoantibodies to hsp90. *J. Autoimmun.* **2001**, *17*, 341–346. [\[CrossRef\]](#)
98. Zhang, M.; Gu, Y.; Huang, S.; Lou, Q.; Xie, Q.; Xu, Z.; Chen, Y.; Pan, F.; Xu, S.; Liu, S.; et al. Copy number variations and polymorphisms in HSP90AB1 and risk of systemic lupus erythematosus and efficacy of glucocorticoids. *J. Cell. Mol. Med.* **2019**, *23*, 5340–5348. [\[CrossRef\]](#)

99. Saito, K.; Kukita, K.; Kutomi, G.; Okuya, K.; Asanuma, H.; Tabeya, T.; Naishiro, Y.; Yamamoto, M.; Takahashi, H.; Torigoe, T.; et al. Heat shock protein 90 associates with Toll-like receptors 7/9 and mediates self-nucleic acid recognition in SLE. *Eur. J. Immunol.* **2015**, *45*, 2028–2041. [\[CrossRef\]](#)
100. Shukla, H.D.; Pitha, P.M. Role of hsp90 in systemic lupus erythematosus and its clinical relevance. *Autoimmune Dis.* **2012**, *2012*, 728605. [\[CrossRef\]](#)
101. Xie, Q.M.; Lou, Q.Y.; Huang, S.W.; Hu, H.Q.; Li, S.S.; Zhang, M.; Sun, X.X.; Xu, J.H.; Jiang, S.Q.; Liu, S.X.; et al. Hsp70 Gene Polymorphisms Are Associated With Disease Susceptibility and HRQOL Improvement in Chinese Han Population With Systemic Lupus Erythematosus. *J. Clin. Rheumatol.* **2020**, *26*, 134–141. [\[CrossRef\]](#) [\[PubMed\]](#)
102. Fürnrohr, B.G.; Wach, S.; Kelly, J.A.; Haslbeck, M.; Weber, C.K.; Stach, C.M.; Hueber, A.J.; Graef, D.; Spriewald, B.M.; Manger, K.; et al. Polymorphisms in the Hsp70 gene locus are genetically associated with systemic lupus erythematosus. *Ann. Rheum. Dis.* **2010**, *69*, 1983–1989. [\[CrossRef\]](#) [\[PubMed\]](#)
103. Mišunová, M.; Svitáková, T.; Pleštilová, L.; Kryštufková, O.; Tegzová, D.; Svobodová, R.; Hušáková, M.; Tomčík, M.; Bečvář, R.; Závada, J.; et al. Molecular markers of systemic autoimmune disorders: The expression of MHC-located HSP70 genes is significantly associated with autoimmunity development. *Clin. Exp. Rheumatol.* **2017**, *35*, 33–42.
104. Page, N.; Gros, F.; Schall, N.; Décossas, M.; Bagnard, D.; Briand, J.P.; Muller, S. HSC70 blockade by the therapeutic peptide P140 affects autophagic processes and endogenous MHCII presentation in murine lupus. *Ann. Rheum. Dis.* **2011**, *70*, 837–843. [\[CrossRef\]](#) [\[PubMed\]](#)
105. Denton, C.P.; Khanna, D. Systemic sclerosis. *Lancet* **2017**, *390*, 1685–1699. [\[CrossRef\]](#)
106. RuizdelRio, J.; Muñoz, P.; Carreira, P.; Maestro, D.; Pablos, J.L.; Palanca, A.; Merino, J.; Serrano-Mollar, A.; Merino, R.; Tamayo, E.; et al. Profibrotic Role of Inducible Heat Shock Protein 90 α Isoform in Systemic Sclerosis. *J. Immunol.* **2022**, *209*, 38–48. [\[CrossRef\]](#)
107. Štorkánová, H.; Oreská, S.; Špiritović, M.; Heřmánková, B.; Bubová, K.; Komarc, M.; Pavelka, K.; Vencovský, J.; Distler, J.H.W.; Šenolt, L.; et al. Plasma Hsp90 levels in patients with systemic sclerosis and relation to lung and skin involvement: A cross-sectional and longitudinal study. *Sci. Rep.* **2021**, *11*, 1. [\[CrossRef\]](#)
108. Ogawa, F.; Shimizu, K.; Hara, T.; Muroi, E.; Hasegawa, M.; Takehara, K.; Sato, S. Serum levels of heat shock protein 70, a biomarker of cellular stress, are elevated in patients with systemic sclerosis: Association with fibrosis and vascular damage. *Clin. Exp. Rheumatol.* **2008**, *26*, 659–662.
109. Langan, S.M.; Irvine, A.D.; Weidinger, S. Atopic dermatitis. *Lancet* **2020**, *396*, 345–360. [\[CrossRef\]](#)
110. Roesner, L.M.; Werfel, T. Autoimmunity (or Not) in Atopic Dermatitis. *Front. Immunol.* **2019**, *10*, 2128. [\[CrossRef\]](#)
111. Lu, Z.; Zeng, N.; Cheng, Y.; Chen, Y.; Li, Y.; Lu, Q.; Xia, Q.; Luo, D. Atopic dermatitis and risk of autoimmune diseases: A systematic review and meta-analysis. *Allergy Asthma Clin. Immunol.* **2021**, *17*, 96. [\[CrossRef\]](#) [\[PubMed\]](#)
112. Sitko, K.; Bednarek, M.; Mantej, J.; Trzeciak, M.; Tukaj, S. Circulating heat shock protein 90 (Hsp90) and autoantibodies to Hsp90 are increased in patients with atopic dermatitis. *Cell Stress Chaperones* **2021**, *26*, 1001–1007. [\[CrossRef\]](#) [\[PubMed\]](#)
113. Ghoreishi, M.; Yokozeki, H.; Hua, W.M.; Nishioka, K. Expression of 27 KD, 65 KD and 72/73 KD heat shock protein in atopic dermatitis: Comparison with those in normal skin and contact dermatitis. *J. Dermatol.* **2000**, *27*, 370–379. [\[CrossRef\]](#) [\[PubMed\]](#)
114. Jin, G.B.; Nakayama, H.; Shmyhlo, M.; Inoue, S.; Kondo, M.; Ikezawa, Z.; Ouchi, Y.; Cyong, J.C. High positive frequency of antibodies to metallothionein and heat shock protein 70 in sera of patients with metal allergy. *Clin. Exp. Immunol.* **2003**, *131*, 275–279. [\[CrossRef\]](#)
115. van Eden, W.; Jansen, M.A.A.; Ludwig, I.S.; Leufkens, P.; van der Goes, M.C.; van Laar, J.M.; Broere, F. Heat Shock Proteins Can Be Surrogate Autoantigens for Induction of Antigen Specific Therapeutic Tolerance in Rheumatoid Arthritis. *Front. Immunol.* **2019**, *10*, 279. [\[CrossRef\]](#)
116. van Eden, W.; Jansen, M.A.A.; Ludwig, I.; van Kooten, P.; van der Zee, R.; Broere, F. The Enigma of Heat Shock Proteins in Immune Tolerance. *Front. Immunol.* **2017**, *8*, 1599. [\[CrossRef\]](#) [\[PubMed\]](#)



Circulating heat shock protein 90 (Hsp90) and autoantibodies to Hsp90 are increased in patients with atopic dermatitis

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Received: 20 July 2021 / Revised: 5 September 2021 / Accepted: 7 September 2021 / Published online: 16 September 2021
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Abstract

Atopic dermatitis (AD) is one of the most common chronic inflammatory dermatoses characterized by persistent itching and recurrent eczematous lesions. While the primary events and key drivers of AD are topics of ongoing debate, cutaneous inflammation due to inappropriate IgE (auto)antibody-related immune reactions is frequently considered. Highly conserved and immunogenic heat shock protein 90 (Hsp90), a key intra- and extracellular chaperone, can activate the immune response driving the generation of circulating anti-Hsp90 autoantibodies that are found to be elevated in several autoimmune disorders. Here, for the first time, we observed that serum levels of Hsp90 and anti-Hsp90 IgE autoantibodies are significantly elevated ($p < 0.0001$) in AD patients ($n = 29$) when compared to age- and gender-matched healthy controls ($n = 70$). We revealed a positive correlation (0.378 , $p = 0.042$) between serum levels of Hsp90 and the severity of AD assessed by Scoring Atopic Dermatitis (SCORAD). In addition, seropositivity for anti-Hsp90 IgE has been found in 48.27% of AD patients and in 2.85% of healthy controls. Although further studies on a larger group of patients are needed to confirm presented data, our results suggest that extracellular Hsp90 and autoantibodies to Hsp90 deserve attention in the study of the mechanisms that promote the development and/or maintenance of atopic dermatitis.

Keywords Atopic dermatitis, AD · Allergy · IgE · Autoimmunity · Autoantibodies · Heat shock proteins, Hsps · Hsp90

Introduction

Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases, with an incidence of 15–30% in children and 2–10% in adults that is characterized by intense itching and recurrent eczematous lesions. Current therapy of AD consists of topical emollients for cutaneous barrier dysfunction and topical corticosteroids or calcineurin inhibitors for skin inflammation. In severely affected cases, phototherapy, systemic immunosuppressants, and biologic agents are indicated. In addition, immunoglobulin E (IgE)-selective immunoadsorption is proposed (Kasperkiewicz et al. 2018; Langan et al. 2020; Weidinger and Novak 2016).

While the underlying events and key drivers of AD are subject to ongoing debate, there are at least two major and converging pathophysiological abnormalities of epidermal structure due to decreased filaggrin (FLG) expression and allergen-specific IgE- and/or autoreactive IgE-mediated dermatitis (Langan et al. 2020). Defects in the epidermal barrier lead to the penetration of the skin by allergens, activation of T helper 2 (Th2)-like cell polarization via Langerhans and dendritic cells, and IgE class switching (Guttman-Yassky et al. 2013; Varricchi et al. 2018). In addition, the activity of various other immune cells belonging to both innate and adaptive arm of immune responses including eosinophils and Th1, Th17, or Th22 cell populations appears to be critically involved in the initiation and the progression of AD, especially in European, American, and Asian populations (Czarnowicki et al. 2019; Weidinger and Novak 2016).

Highly conserved and intracellularly expressed Hsp90 (constituting 2–3% of the total cellular proteins) is a key molecular chaperone that plays an essential role in protein folding and the activation of its protein substrates ('clients') both inside and outside the cell. Hsp90 is responsible for biological activity of key signalling molecules, such as

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kinases, steroid receptors, cell cycle regulators, and transcription factors regulating various cellular processes. Extracellular Hsp90 with its chaperone activity is able to induce extracellular matrix remodelling via assisting in matrix metalloproteinase (MMP) activation. Hsp90 can also activate humoral immune responses driving to the generation of self-reactive antibodies to Hsp90 (Seclì et al. 2021; Tukaj and Węgrzyn 2016). In fact, these autoantibodies are found to be elevated in several autoimmune diseases (Tukaj 2020; Tukaj and Kaminski 2019).

Hsp90 became the focus of interest of scientists in the context of the development of autoimmune/inflammatory conditions, since it is upregulated in inflamed tissues and its presence in the extracellular space had been associated with the autoimmune process (Tukaj 2020; Tukaj and Kaminski 2019; Tukaj and Węgrzyn 2016). Moreover, since IgE-dependent immune reactions to self-proteins have been associated with AD (Roesner and Werfel 2019; Tang et al. 2012),

searching for the autoantigens that may play an important role in the pathogenesis of AD is desired. This study aimed to determine serum levels of Hsp90 and anti-Hsp90 autoantibodies of the IgE, IgG, IgM, and IgA isotype in a cohort of patients with AD and in age- and gender-matched healthy controls.

Materials and methods

Patients and controls

Twenty-nine patients with atopic dermatitis (mean age: 25.86 ± 6.30 ; gender: 15 males and 14 females) (Table 1) and 70 age- and gender-matched healthy controls (mean age: 28.44 ± 8.81 ; gender: 31 males and 39 females) have been included in this study. Healthy volunteers who suffered from autoimmune, allergic, or any other skin disorder have been

Table 1 Characteristics of atopic dermatitis (AD) patients

No	Age	Gender	AD duration (years)	SCORAD	IgE (IU/ml)	Asthma	Allergic rhinitis	Allergic conjunctivitis
1	21	M	<2	51.2	1323.1		+	
2	18	M	<2	67	2794.3	+		+
3	10	F	≥2	84	1539.0	+		+
4	10	F	≥2	82	1264.6	+		+
5	53	F	≥2	80	2692.8			
6	41	F	≥2	19	84.5			
7	23	F	<2	32.6	52.8		+	+
8	16	F	<2	45	1120.3		+	
9	20	M	≥2	19	3283.0			
10	23	F	<2	48.3	1674.3	+	+	
11	20	M	≥2	38	158.4			
12	17	F	<2	32.4	1326.1	+	+	+
13	21	M	<2	39.6	150.0		+	
14	13	F	<2	42.2	395.8		+	+
15	20	F	≥2	86	1105.0	+	+	
16	15	M	<2	34.8	1369.0			
17	25	F	≥2	70.4	1349.0	+		
18	59	M	≥2	70.4	1122.2	+		
19	18	M	<2	48.3	1032.7		+	+
20	25	M	<2	71.5	1296.9		+	
21	43	M	≥2	45.5	414.9			
22	31	F	≥2	68.5	1022.1	+	+	
23	27	M	<2	43.3	1678.7		+	+
24	37	M	<2	62	781.2		+	+
25	32	M	<2	24	1221.7	+		
26	22	F	<2	58.5	1935.1			
27	37	M	<2	55.5	17.4			
28	28	M	<2	40	1039.5			
29	25	F	<2	43	834.5			

excluded from the study. The use of human biological material was approved by a bioethics committee at the regional medical chamber in Gdańsk (Poland), and written informed consents were performed in accordance with the Declaration of Helsinki.

Detection of circulating Hsp90

Hsp90 was evaluated in serum by commercially available HSP90 α (human) ELISA kit (Enzo Life Science), following the manufacturer's instructions.

Detection of anti-Hsp90 antibodies

Levels of IgE, IgG, IgM, and IgA against human Hsp90 were evaluated in the serum samples by a home-made enzyme-linked immunosorbent assay (ELISA), as described previously (Mantej et al. 2019). Briefly, medium-binding 96-well plates were coated with commercially available full-length native human Hsp90 (Enzo Life Sciences) protein at a concentration of 0.5 μ g/ml in 0.1 M bicarbonate buffer at 4 °C overnight. The wells were blocked with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) at room temperature (RT) for 1.5 h. After a washing step (three times with PBS containing 0.05% Tween 20), the sera were diluted (1:50–1:200) in PBS containing 0.1% BSA, added to the wells and were incubated at RT for 1.5 h. Plates were then incubated with horseradish peroxidase (HRP)–conjugated anti-human IgE (Abcam), anti-human IgG (Sigma), anti-human IgM (Abcam), or anti-human IgA (BioLegend) secondary antibodies diluted in PBS containing 0.1% BSA at RT for 1 h. The TMB substrate solution (Sigma) was used to visualize HRP enzymatic reaction and the reaction was stopped by adding 0.5 M H₂SO₄. Optical density measurements were performed at 450 nm with an ELISA plate reader (VICTOR Multilabel Plate Reader, PerkinElmer).

Total IgE levels

Serum IgE levels were detected by commercially available ELISA kit performed according to the manufacturer's instructions (Invitrogen).

Statistical analysis

Statistical analyses were performed using the GraphPad Prism 5 software (San Diego, CA). Data was analysed by the Mann–Whitney *U* test or Spearman's rank correlation test. *p* values less than 0.05 were considered significant.

Results

Serum levels of Hsp90 are significantly elevated in patients with AD

In the analysis, 29 AD patients and 70 healthy controls were included. Clinical characteristics of the patients are stated in Table 1. Here, we found for the first time that serum levels of autologous Hsp90 were significantly elevated (2.79-fold increase; $p < 0.0001$) in AD patients when compared to age- and gender-matched healthy controls (mean $\pm$ SD; 215.41 $\pm$ 146.14 pg/ml vs. 84.64 $\pm$ 79.96 pg/ml), as measured quantitatively by a commercially available ELISA kit (Fig. 1).

Serum levels of anti-Hsp90 IgE autoantibodies are increased in patients with AD

Using home-made indirect ELISA assay, we found for the first time that serum levels of anti-Hsp90 IgE were significantly elevated ($p < 0.0001$) in AD patients ($n = 29$) as compared to age- and gender-matched healthy controls ($n = 70$), whereas levels of anti-Hsp90 IgG, IgM, or IgA were similar between both groups (Fig. 2a). Values of sera reactivity with Hsp90 measured above the mean values for bovine serum albumin (BSA) reactivity (negative control) were regarded as positive. In addition, taking into account the cut-off value calculated as 3 $\times$ standard deviation above the mean of the control, 14 out of 29 (48.27%)

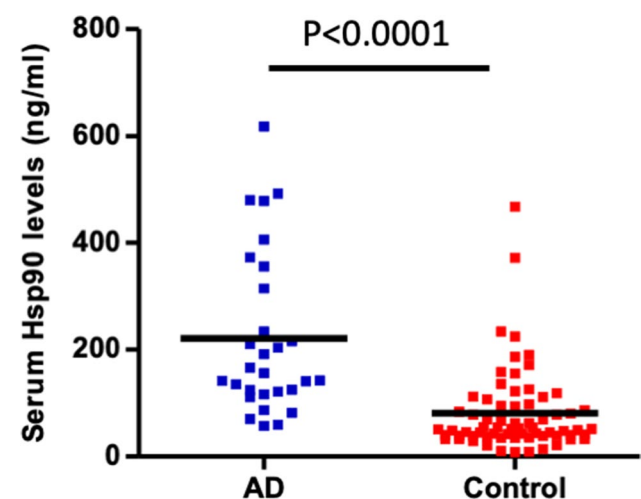
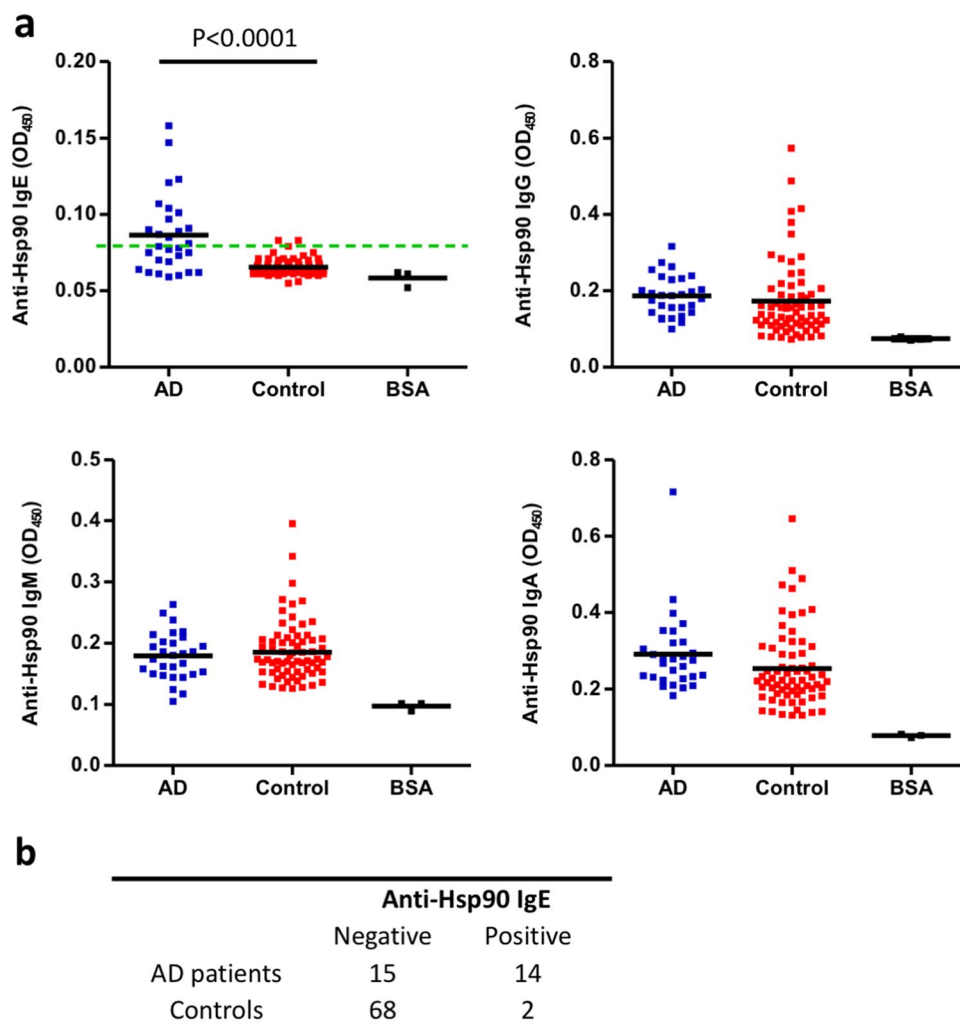


Fig. 1 Levels of Hsp90 are increased in the sera of patients with atopic dermatitis (AD). Serum levels of Hsp90 in AD ($n = 29$) and age- and gender-matched healthy controls ($n = 70$) were assessed by an enzyme-linked immunosorbent assay. The dots and horizontal bars indicate individual and mean values in each group, respectively

Fig. 2 Circulating anti-Hsp90 IgE autoantibodies are increased in patients with AD. **a** Levels of anti-Hsp90 of the IgE, IgG, IgM, and IgA autoantibody isotype in the sera of AD patients ($n=29$) and age- and gender-matched healthy controls ($n=70$), measured by enzyme-linked immunosorbent assay. The optical density was measured at 450 nm (OD_{450}). Green horizontal dashed line represents the cut-off value calculated as $3 \times$ standard deviation above the mean of the control. The dots and horizontal bars indicate individual and mean values in each group, respectively. p values less than 0.05 were considered significant. **b** Contingency analysis showing anti-Hsp90 IgE positivity in AD patients and in healthy controls



AD patients were anti-Hsp90 IgE positive, while only 2 out of 70 (2.85%) healthy controls matched such criteria (Fig. 2b).

Serum levels of Hsp90 are associated with the clinical severity of AD

Using Spearman's rank correlation coefficient test, relationships between higher levels of circulating Hsp90 or anti-Hsp90 IgE autoantibodies and selected parameters of AD patients (Table 1) including disease activity, serum levels of IgE, or comorbidities were analysed. While a significant positive correlation ($r=0.378$, $p=0.042$) between serum levels of Hsp90 and the clinical severity of AD (SCORAD) was observed (Fig. 3), there were no significant associations between Hsp90 and serum levels of total IgE (0.317, $p=0.093$) or anti-Hsp90 IgE (-0.055 , $p=0.776$) and the presence of comorbidities such as asthma (0.043, $p=0.823$), allergic rhinitis (0.091, $p=0.638$), or allergic conjunctivitis (-0.142 , $p=0.461$). Likewise, no significant correlations

between anti-Hsp90 IgE serum levels and SCORAD (-0.020 , $p=0.914$) as well as serum levels of total IgE (0.216, $p=0.258$) and the presence of comorbidities such as asthma (-0.086 , $p=0.654$), allergic rhinitis (-0.074 , $p=0.700$), or allergic conjunctivitis (0.057, $p=0.765$) could be recorded. Levels of either Hsp90 or anti-Hsp90 IgE were not dependent on the duration of AD (data not shown).

Discussion

Heat shock proteins (Hsps) are a diverse group of molecules, either expressed constitutively or stress-induced, that are classified into several families based on their molecular weight and the characteristic structural domains. They mediate a range of essential cellular functions, including folding of newly synthesized polypeptides and renaturation or stabilization of biologically active proteins (Tukaj 2020; Tukaj and Kaminski 2019). Highly expressed Hsp90 is a key molecular chaperone that can be

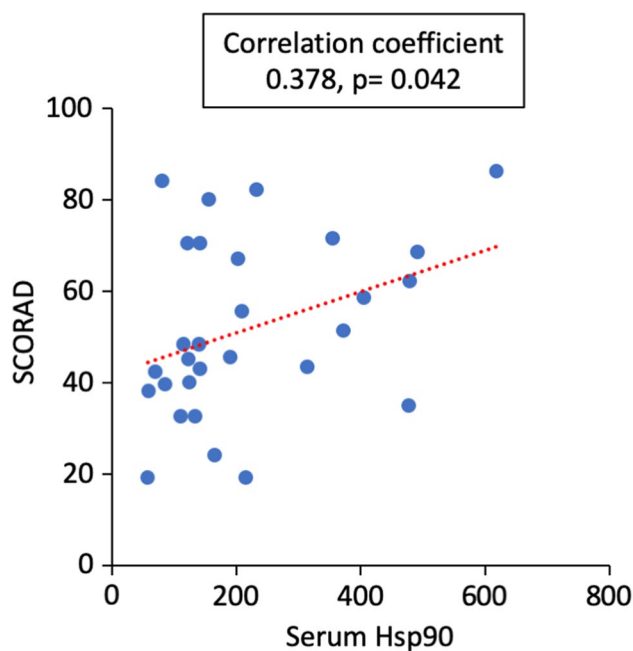


Fig. 3 Higher serum levels of Hsp90 are associated with the clinical severity of AD. Correlation analysis between Hsp90 serum levels and the clinical severity (SCORAD)

released to the extracellular milieu. Hsp90 facilitates the maturation of various ‘client’ proteins such as kinases, transcription factors, steroid hormone receptors, and E3 ubiquitin ligases that are involved in many different cellular pathways, including inflammation, growth, differentiation, and apoptosis (Schopf et al. 2017; Trepel et al. 2010). Since aberrant expression and secretion of the Hsp90 has been observed in cancer cells and chronically inflamed tissues, this chaperone has attracted scientists’ attention particularly in terms of development, progression, and treatment of cancer and autoimmune/inflammatory diseases (Tukaj 2020; Tukaj and Kaminski 2019; Tukaj et al. 2013, 2015b; Tukaj and Węgrzyn 2016). Here, for the first time, we found that serum levels of Hsp90 were significantly elevated in AD patients when compared to age- and gender-matched healthy controls and positively correlated with the clinical severity (SCORAD) of patients. While the pathological role of Hsp90 in AD needs to be further elucidated, we here propose non-exclusive explanations to the potential contribution of this chaperone in AD. It has been previously reported that MMPs are ‘client’ proteins of Hsp90 and dependent on its chaperone function (Seclì et al. 2021). As the interactions between MMPs and extracellular Hsp90 have been previously described in the context of tumour cell invasion and metastasis (Eustace et al. 2004; Garcia-Carbonero et al. 2013), we may speculate that elevated serum levels of Hsp90 could be involved in AD progression via promotion of MMP activation, the

latter being known as an important pathophysiological factor in AD (Devillers et al. 2007; Harper et al. 2010).

The role of highly immunogenic, extracellular Hsp90 in AD may also be associated with its ability to activate the humoral (auto)immune response. Under stress conditions, Hsp90 can be released to the extracellular milieu and activate both the innate and adaptive immune responses driving the generation of circulating anti-Hsp90 autoantibodies that are found to be elevated in several autoimmune diseases, e.g., diabetes type 1 (Qin et al. 2003), systemic lupus erythematosus (Ripley et al. 2001), rheumatoid arthritis (Mantej et al. 2019), dermatitis herpetiformis (Kasperkiewicz et al. 2014), and coeliac disease (Tukaj et al. 2017b). Here, for the first time, we found that serum levels of anti-Hsp90 IgE were significantly elevated in AD patients as compared to healthy controls, whereas levels of anti-Hsp90 IgG, IgM, or IgA were similar between both groups. It is well established that elevated serum levels of total IgE are found in about 80% of AD patients (Weidinger and Novak 2016). Here, seropositivity for anti-Hsp90 IgE has been found in about 50% of AD patients.

In the past, molecular mimicry hypothesis suggested that the immune response originally directed to the bacterial Hsps may be re-directed towards their human counterparts and in this way promote development of autoimmune reactions (Albani et al. 1995). While immune cross-reactions between foreign and self-antigens were experimentally confirmed in the case of Hsp40, Hsp60, or Hsp70 chaperones (Kotlarz et al. 2013; van Eden et al. 2017), clinical consequences of such immune cross-reactions have brought ambiguous outcomes (Tukaj and Kaminski 2019; Tukaj et al. 2021; van Eden et al. 2017). Information on a potential contribution of the bacterial Hsp90 (HtpG) in autoimmunity and the immune cross-reactions between HtpG and human Hsp90, however, is generally lacking. Since human Hsp90 shares about 40% sequence identity and 55% similarity with bacterial (*E. coli*) Hsp90 (Huai et al. 2005), immune cross-reactions between these molecules are theoretically possible. On the other hand, Kawano et al. (2004) found that set of antibodies raised against bacterial HtpG did not cross-react with all four human Hsp90 analogues, and vice versa. Therefore, we postulate that significantly higher levels of self-Hsp90 in the blood of AD patients led to the activation of the humoral autoimmune response and the production of anti-Hsp90 IgE autoantibodies. Since IgE-dependent immune reactions to self-proteins have been already associated with AD by many authorities on the matter (Roesner and Werfel 2019; Tang et al. 2012; Zeller et al. 2009), it is tempting to speculate that autoimmune reactions towards self-Hsp90 play an important role in the pathogenesis of AD. In fact, autoreactive IgE can also be found in other inflammatory skin diseases, such as autoimmune bullous diseases (AIBD). For example, patients suffering from the

bullous pemphigoid, the most common form of AIBD, are IgG-, IgA-, or IgE-sensitized to the hemidesmosomal BP180 NC16A protein of the dermal–epidermal junction (Liu et al. 2017). The relevance of anti-Hsp90 IgE in AD, however, needs to be further examined since no significant associations between the levels of anti-Hsp90 IgE and total IgE or the clinical severity of AD were found in this study. On the other hand, lack of significant associations between anti-Hsp90 IgE and comorbidities (i.e., asthma, allergic rhinitis, or conjunctivitis) in AD patients may suggest a disease-specific IgE-dependent immune response to Hsp90 in AD.

Finally, numerous studies have shown that pharmacological inhibition of Hsp90 is successfully used in murine models of (auto)inflammatory diseases including AIBD via modulation of humoral and cellular immune responses (Kasperkiewicz et al. 2011; Tukaj et al. 2017a, 2015a, 2014a; Tukaj and Węgrzyn 2016; Tukaj et al. 2014b; 2015b), further supporting a potential role of this chaperone in the inflammatory process.

Conclusions

Although further studies on a larger group of patients and additional experimental analysis are needed to confirm the present data, our results suggest that extracellular Hsp90 and autoantibodies to the Hsp90 deserve attention in the study of the mechanisms that promote the development and maintenance of atopic dermatitis.

Funding This research was funded by the Polish National Science Centre (Narodowe Centrum Nauki), grant number 2017/25/B/NZ6/00305 and 2020/39/B/NZ6/00357 to S.T.

Declarations

Conflict of interest The authors declare competing interests.

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References

- Albani S, Keystone EC, Nelson JL, Ollier WER, La Cava A, Montemayor AC, Weber DA, Montecucco C, Martini A, Carson DA (1995) Positive selection in autoimmunity: abnormal immune responses to a bacterial dnaJ antigenic determinant in patients with early rheumatoid arthritis. *Nat Med* 1:448–452. <https://doi.org/10.1038/nm0595-448>
- Czarnowicki T, He H, Krueger JG, Guttman-Yassky E (2019) Atopic dermatitis endotypes and implications for targeted therapeutics. *J Allergy Clin Immunol* 143:1–11. <https://doi.org/10.1016/j.jaci.2018.10.032>
- Devillers AC, van Toorenenbergen AW, Klein Heerenbrink GJ, Muldert PG, Oranje AP (2007) Elevated levels of plasma matrix metalloproteinase-9 in patients with atopic dermatitis: a pilot study. *Clin Exp Dermatol* 32:311–313. <https://doi.org/10.1111/j.1365-2230.2007.02378.x>
- Eustace BK, Sakurai T, Stewart JK, Yimlamai D, Unger C, Zehetmeier C, Lain B, Torella C, Henning SW, Beste G et al (2004) Functional proteomic screens reveal an essential extracellular role for hsp90 α in cancer cell invasiveness. *Nat Cell Biol* 6:507–514. <https://doi.org/10.1038/ncb1131>
- Garcia-Carbonero R, Carnero A, Paz-Ares L (2013) Inhibition of HSP90 molecular chaperones: moving into the clinic. *Lancet Oncol* 14:e358–369. [https://doi.org/10.1016/s1470-2045\(13\)70169-4](https://doi.org/10.1016/s1470-2045(13)70169-4)
- Guttman-Yassky E, Dhingra N, Leung DY (2013) New era of biologic therapeutics in atopic dermatitis. *Expert Opin Biol Ther* 13:549–561. <https://doi.org/10.1517/14712598.2013.758708>
- Harper JI, Godwin H, Green A, Wilkes LE, Holden NJ, Moffatt M, Cookson WO, Layton G, Chandler S (2010) A study of matrix metalloproteinase expression and activity in atopic dermatitis using a novel skin wash sampling assay for functional biomarker analysis. *Br J Dermatol* 162:397–403. <https://doi.org/10.1111/j.1365-2133.2009.09467.x>
- Huai Q, Wang H, Liu Y, Kim HY, Toft D, Ke H (2005) Structures of the N-terminal and middle domains of E. coli Hsp90 and conformation changes upon ADP binding. *Structure* 13:579–590. <https://doi.org/10.1016/j.str.2004.12.018>
- Kasperkiewicz M, Müller R, Manz R, Magens M, Hammers CM, Somlai C, Westermann J, Schmidt E, Zillikens D, Ludwig RJ et al (2011) Heat-shock protein 90 inhibition in autoimmunity to type VII collagen: evidence that nonmalignant plasma cells are not therapeutic targets. *Blood* 117:6135–6142. <https://doi.org/10.1182/blood-2010-10-314609>
- Kasperkiewicz M, Schmidt E, Ludwig RJ, Zillikens D (2018) Targeting IgE antibodies by Immunoabsorption in atopic dermatitis. *Front Immunol* 9:254. <https://doi.org/10.3389/fimmu.2018.00254>
- Kasperkiewicz M, Tukaj S, Gembicki A-J, Silló P, Görög A, Zillikens D, Kárpáti S (2014) Evidence for a role of autoantibodies to heat shock protein 60, 70, and 90 in patients with dermatitis herpetiformis. *Cell Stress Chaperones* 19:837–843. <https://doi.org/10.1007/s12192-014-0507-6>
- Kawano T, Kobayakawa T, Fukuma Y, Yukitake H, Kikuchi Y, Shoji M, Nakayama K, Mizuno A, Takagi T, Nemoto TK (2004) A comprehensive study on the immunological reactivity of the Hsp90 molecular chaperone. *J Biochem* 136:711–722. <https://doi.org/10.1093/jb/mvh178>
- Kotlarz A, Tukaj S, Krzewski K, Brycka E, Lipinska B (2013) Human Hsp40 proteins, DNAJA1 and DNAJA2, as potential targets of the immune response triggered by bacterial DnaJ in rheumatoid arthritis. *Cell Stress Chaperones* 18:653–659. <https://doi.org/10.1007/s12192-013-0407-1>

- Langan SM, Irvine AD, Weidinger S (2020) Atopic dermatitis. *Lancet* 396:345–360. [https://doi.org/10.1016/s0140-6736\(20\)31286-1](https://doi.org/10.1016/s0140-6736(20)31286-1)
- Liu Y, Li L, Xia Y (2017) BP180 is critical in the autoimmunity of bullous pemphigoid. *Front Immunol* 8:1752. <https://doi.org/10.3389/fimmu.2017.01752>
- Mantej J, Polasik K, Piotrowska E, Tukaj S (2019) Autoantibodies to heat shock proteins 60, 70, and 90 in patients with rheumatoid arthritis. *Cell Stress Chaperones* 24:283–287. <https://doi.org/10.1007/s12192-018-0951-9>
- Qin H-Y, Mahon JL, Atkinson MA, Chaturvedi P, Lee-Chan E, Singh B (2003) Type 1 diabetes alters anti-hsp90 autoantibody isotype. *J Autoimmun* 20:237–245. [https://doi.org/10.1016/S0896-8411\(03\)00035-0](https://doi.org/10.1016/S0896-8411(03)00035-0)
- Ripley BJ, Isenberg DA, Latchman DS (2001) Elevated levels of the 90 kDa heat shock protein (hsp90) in SLE correlate with levels of IL-6 and autoantibodies to hsp90. *J Autoimmun* 17:341–346. <https://doi.org/10.1006/jaut.2001.0549>
- Roesner LM, Werfel T (2019) Autoimmunity (or not) in atopic dermatitis. *Front Immunol* 10:2128. <https://doi.org/10.3389/fimmu.2019.02128>
- Schopf FH, Biehl MM, Buchner J (2017) The HSP90 chaperone machinery. *Nat Rev Mol Cell Biol* 18:345–360. <https://doi.org/10.1038/nrm.2017.20>
- Secli L, Fusella F, Avalle L, Brancaccio M (2021) The dark-side of the outside: how extracellular heat shock proteins promote cancer. *Cell Mol Life Sci* 78:4069–4083. <https://doi.org/10.1007/s00018-021-03764-3>
- Tang TS, Bieber T, Williams HC (2012) Does “autoreactivity” play a role in atopic dermatitis? *J Allergy Clin Immunol* 129:1209–1215. e1202. <https://doi.org/10.1016/j.jaci.2012.02.002>
- Trepel J, Mollapour M, Giaccone G, Neckers L (2010) Targeting the dynamic HSP90 complex in cancer. *Nat Rev Cancer* 10:537–549. <https://doi.org/10.1038/nrc2887>
- Tukaj S (2020) Heat shock protein 70 as a double agent acting inside and outside the cell: insights into autoimmunity. *Int J Mol Sci* 21:5298. <https://doi.org/10.3390/ijms21155298>
- Tukaj S, Bieber K, Kleszczyński K, Witte M, Cames R, Kalies K, Zillikens D, Ludwig RJ, Fischer TW, Kasperkiewicz M (2017a) Topically applied Hsp90 Blocker 17AAG inhibits autoantibody-mediated blister-inducing cutaneous inflammation. *J Invest Dermatol* 137:341–349. <https://doi.org/10.1016/j.jid.2016.08.032>
- Tukaj S, Görög A, Kleszczyński K, Zillikens D, Kárpáti S, Kasperkiewicz M (2017b) Autoimmunity to heat shock proteins and vitamin D status in patients with celiac disease without associated dermatitis herpetiformis. *The J Steroid Biochem Mol Biol* 173:23–27. <https://doi.org/10.1016/j.jsbmb.2016.10.002>
- Tukaj S, Hellberg L, Ueck C, Hänsel M, Samavedam U, Zillikens D, Ludwig RJ, Laskay T, Kasperkiewicz M (2015a) Heat shock protein 90 is required for ex vivo neutrophil-driven autoantibody-induced tissue damage in experimental epidermolysis bullosa acquisita. *Exp Dermatol* 24:471–473. <https://doi.org/10.1111/exd.12680>
- Tukaj S, Kaminski M (2019) Heat shock proteins in the therapy of autoimmune diseases: too simple to be true? *Cell Stress Chaperones* 24:475–479. <https://doi.org/10.1007/s12192-019-01000-3>
- Tukaj S, Kleszczyński K, Vafia K, Groth S, Meyersburg D, Trzonkowski P, Ludwig RJ, Zillikens D, Schmidt E, Fischer TW et al (2013) Aberrant expression and secretion of heat shock protein 90 in patients with bullous pemphigoid. *PLoS ONE* 8:e70496. <https://doi.org/10.1371/journal.pone.0070496>
- Tukaj S, Mantej J, Sobala M, Potrykus K, Tukaj Z, Zillikens D, Ludwig RJ, Bieber K, Kasperkiewicz M (2021) Therapeutic implications of targeting heat shock protein 70 by immunization or antibodies in experimental skin inflammation. *Front Immunol* 12:614320. <https://doi.org/10.3389/fimmu.2021.614320>
- Tukaj S, Tiburzy B, Manz R, de Castro Marques A, Orosz A, Ludwig RJ, Zillikens D, Kasperkiewicz M (2014) Immunomodulatory effects of heat shock protein 90 inhibition on humoral immune responses. *Exp Dermatol* 23:585–590. <https://doi.org/10.1111/exd.12476>
- Tukaj S, Węgrzyn G (2016) Anti-Hsp90 therapy in autoimmune and inflammatory diseases: a review of preclinical studies. *Cell Stress Chaperones* 21:213–218. <https://doi.org/10.1007/s12192-016-0670-z>
- Tukaj S, Zillikens D, Kasperkiewicz M (2014b) Inhibitory effects of heat shock protein 90 blockade on proinflammatory human Th1 and Th17 cell subpopulations. *J Inflamm (Lond)* 11:10. <https://doi.org/10.1186/1476-9255-11-10>
- Tukaj S, Zillikens D, Kasperkiewicz M (2015b) Heat shock protein 90: a pathophysiological factor and novel treatment target in autoimmune bullous skin diseases. *Exp Dermatol* 24:567–571. <https://doi.org/10.1111/exd.12760>
- van Eden W, Jansen MAA, Ludwig I, van Kooten P, van der Zee R, Broere F (2017) The enigma of heat shock proteins in immune tolerance. *Front Immunol* 8:1599. <https://doi.org/10.3389/fimmu.2017.01599>
- Varricchi G, Pecoraro A, Marone G, Criscuolo G, Spadaro G, Genovese A, Marone G (2018) Thymic stromal lymphopoietin isoforms, inflammatory disorders, and cancer. *Front Immunol* 9:1595. <https://doi.org/10.3389/fimmu.2018.01595>
- Weidinger S, Novak N (2016) Atopic dermatitis. *Lancet* 387:1109–1122. [https://doi.org/10.1016/s0140-6736\(15\)00149-x](https://doi.org/10.1016/s0140-6736(15)00149-x)
- Zeller S, Rhyner C, Meyer N, Schmid-Grendelmeier P, Akdis CA, Crameri R (2009) Exploring the repertoire of IgE-binding self-antigens associated with atopic eczema. *J Allergy Clin Immunol* 124(278–285):285.e271–277. <https://doi.org/10.1016/j.jaci.2009.05.015>

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RECEIVED 24 October 2023

ACCEPTED 11 December 2023

PUBLISHED 05 January 2024

CITATION

Sitko K, Kárpáti S, Węgrzyn G, Mincewicz G,
Trzeciak M, Kasperkiewicz M and Tukaj S (2024)
Differences in the detection of circulating
Hsp90 alpha between patients with atopic
dermatitis and dermatitis herpetiformis.
Front. Med. 10:1327144.
doi: 10.3389/fmed.2023.1327144

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Differences in the detection of circulating Hsp90 alpha between patients with atopic dermatitis and dermatitis herpetiformis

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Heat shock protein 90 alpha (Hsp90α) is one of the key intra- and extracellular chaperones responsible for the biological activity of various signaling molecules that are involved in (auto)immune-mediated inflammatory diseases. Recent epidemiologic data suggest that patients with atopic dermatitis (AD) are at risk for several autoimmune diseases, including dermatitis herpetiformis (DH), an extraintestinal manifestation of celiac disease (CD). In addition, pruritic diseases such as AD may be confused clinically with DH. In this study, we aimed to determine the role of circulating Hsp90α in patients with AD in relation to patients with DH, CD, and healthy controls. Using an enzyme-linked immunosorbent assay, levels of circulating Hsp90α were determined in serum samples derived from patients with AD ($n = 31$), DH ($n = 26$), CD ($n = 15$), and healthy controls ($n = 55$). Although serum concentrations of Hsp90α were similar between patients with DH, CD, and healthy controls, we found that serum levels of Hsp90α were significantly higher (mean value of 5.08-fold; $p < 0.0001$) in patients with AD when compared to patients with DH. A cutoff value calculated as $2 \times$ standard deviation above the mean concentration of Hsp90α in DH patients revealed that 83.9% (26/31) of AD patients were Hsp90α positive, whereas none of the DH patients (0/26) displayed such a positivity. This preliminary study suggests a distinct role for extracellular Hsp90α in the pathogenesis of AD compared to DH and its potential use in distinguishing AD from DH. Nevertheless, the potential role of the evaluation of extracellular Hsp90α for distinguishing between AD and DH is at present speculative and requires further and careful observations.

KEYWORDS

autoimmune diseases, atopic dermatitis (AD), dermatitis herpetiformis (DH), heat shock protein 90 (Hsp90), COVID-19

1 Introduction

The heat shock protein 90 (Hsp90) family consists of four members, i.e., the two cytosolic isoforms Hsp90α and Hsp90β, and grp94/gp96 and TRAP-1 isoforms, localized in the endoplasmic reticulum and mitochondrion, respectively. Hsp90α is an essential molecular chaperone responsible for the biological activity of hundreds of signaling molecules (e.g.,

mitogen-activated protein kinase (MAPK) or Janus kinase/signal transducer and activator of transcription (JAK/STAT)) and transcription factors (e.g., nuclear factor-kappa B (NF- κ B)) that are involved in the pathophysiology of immune-mediated diseases, including non-communicable inflammatory skin diseases (1). In fact, Hsp90 α can be passively or actively secreted into the extracellular space to promote cell motility and mediate wound healing and tumor metastasis (2). We have previously found that the sera of patients with dermatitis herpetiformis (DH) or atopic dermatitis (AD) contained significantly elevated titers of anti-Hsp90 IgG or anti-Hsp90 IgE, respectively (3, 4). In addition, circulating levels of Hsp90 α were found to be significantly elevated in AD patients when compared to healthy controls and positively associated with the severity of AD assessed by Scoring Atopic Dermatitis (SCORAD) (4). The role of circulating Hsp90 α in DH, however, has not been studied so far.

AD is one of the most common chronic inflammatory skin diseases, characterized by intense itching and relapsing skin lesions (5). In contrast, DH, an extraintestinal manifestation of celiac disease (CD) presenting similarly with intensely pruritic skin lesions, is a relatively rare autoimmune blistering skin disease (6). Due to the wide range of heterogeneity, there is no specific biomarker for AD diagnosis, and it is made based mainly on clinical features (7). On the other hand, determination of levels of anti-epidermal transglutaminase (eTG/TG3) IgA autoantibodies was shown to be a primary diagnostic strategy, with estimating levels of anti-tissue transglutaminase (tTG/TG2) and other CD-associated autoantibodies used to support the diagnosis or activity monitoring of DH (8).

Recent epidemiologic data suggest that patients with AD are at risk for several autoimmune diseases, including DH (9). In addition, DH can be misdiagnosed as AD due to a similar clinical phenotype or the general higher prevalence of AD (8). Therefore, there are still unmet needs to find more convenient, non-invasive, and reliable biomarkers that would both allow for a more efficient differentiation of these skin diseases and facilitate the proposal of appropriate or personalized therapy.

In this study, we aimed to determine the role of circulating Hsp90 α in patients with AD in relation to patients with DH, CD, and healthy controls by analyzing its serum levels and associations with disease serological markers.

2 Materials and methods

2.1 Patients

Sera from 31 patients with active atopic dermatitis (mean age: 27.77 ± 13.59 years; gender: 15 males and 16 females) with an average SCORAD index of 51.79 ± 18.99 and elevated total circulating IgE antibodies (mean 1159.06 ± 772.50 U/mL) without concomitant DH were included in this study. According to the SCORAD index, the mild form of AD was recorded in 3 patients, the moderate form in 13 patients, and the severe form in 13 patients. In two cases, the SCORAD index was not defined. Basic therapy including emollients and topical anti-inflammatory first-line drugs such as glucocorticoids or calcineurin inhibitors were used by most of AD patients prior to recruitment. AD patients using systemic treatment (e.g., phototherapy, systemic corticosteroids, cyclosporine, methotrexate, azathioprine, JAK inhibitors, and biologics) were excluded from the study. Sera

from 26 patients with active and untreated DH (mean age 35.42 ± 14.93 years; gender: 12 females and 14 males) and elevated circulating anti-eTG autoantibodies (mean 81.81 ± 87.19 U/mL) and anti-tTG autoantibodies (mean 108.80 ± 103.54 U/mL) were included in this study. Sera from 15 patients with active and untreated CD (mean age: 35.27 ± 13.84 years; gender: 12 females and 3 males) and elevated circulating anti-tTG autoantibodies (mean 129.27 ± 120.48 U/mL) and anti-eTG autoantibodies (80.94 ± 86.79) without concomitant DH were included in this study. In this study, three types of controls without a history of immunological, allergic, or skin disorders were used. Sera from (i) 26 anti-SARS-CoV-2 IgG-positive volunteers (mean age: 44.92 ± 13.82 ; gender: 4 males and 22 females) without a history of COVID-19 who received two doses of the mRNA anti-COVID-19 vaccine encoding the viral spike protein (Pfizer-BioNTech COVID-19 vaccine), (ii) 15 unvaccinated COVID-19 convalescents (anti-SARS-CoV-2 IgG-positive; mean age: 30.40 ± 13.00 ; gender: 10 males and 5 females), and (iii) 55 healthy controls (anti-SARS-CoV-2 IgG-negative; mean age: 27.91 ± 3.21 ; gender: 23 males and 32 females) have been included in this study. The collection of sera from patients with AD, DH, and CD was conducted before the outbreak of the COVID-19 pandemic. None of the patients with AD, DH, or CD were COVID-19 vaccinated. All patients and healthy volunteers that were recruited in this study were Caucasian.

2.2 Detection of circulating Hsp90 α

Hsp90 α levels were evaluated in the serum using a commercially available HSP90 α (human) ELISA kit (Enzo Life Science), following the manufacturer's instructions.

2.3 Detection of circulating anti-SARS-CoV-2 antibodies

The presence of circulating anti-SARS-CoV-2 antibodies directed to the S1 subunit of the spike protein or nucleocapsid protein (NCP) was evaluated using a commercially available anti-SARS-CoV-2 ELISA (IgG) kit (EUROIMMUN) or anti-SARS-CoV-2 NCP ELISA (IgG) kit (EUROIMMUN), respectively, following the manufacturer's instructions.

2.4 Statistical analysis

Statistical analyses were performed using the GraphPad Prism software (San Diego, CA). Data were analyzed by one-way ANOVA test with Tukey's multiple comparison test. For correlation analysis, the Spearman rank correlation coefficient test was used. Fisher's exact test was used to determine the significance of associations between groups. The *p*-values below 0.05 were considered significant.

3 Results

Although serum concentrations of Hsp90 α in patients with DH ($n=26$) or CD ($n=15$) and healthy controls ($n=55$) remained unchanged, we found that serum levels of Hsp90 α were significantly

higher (5.08-fold; $p < 0.0001$) in patients with AD ($n = 31$) when compared to patients with DH (Figure 1). The cutoff value calculated as 2 x standard deviation (SD) above the mean concentration of Hsp90 α in DH patients revealed that 83.9% (26/31) of AD patients were Hsp90 α positive, whereas none of the DH patients (0/26) displayed such positivity. As analyzed by the Fisher's exact test, these differences were found to be statistically significant ($p < 0.0001$). Neither anti-eTG IgA nor anti-tTG IgA autoantibodies present in the serum of both DH and CD patients correlated with circulating Hsp90 α levels (data not shown). Seropositivity to SARS-CoV-2, due to infection or immunization, did not impact Hsp90 α concentration measurements, as both groups of seropositive patients had comparable serum Hsp90 α levels when compared to seronegative donors (Figure 1).

4 Discussion

Epidemiologic data suggest that patients with AD are at risk for several autoimmune diseases, including DH (9). Indeed, approximately 20% of Hungarian and Finnish children with DH suffer from AD (10). Furthermore, due to the similar clinical phenotype of a severely itchy skin rash or the higher prevalence of AD in the general population, DH may mimic AD and

be misdiagnosed (8). Detecting circulating IgA autoantibodies to eTG or tTG, however, may help in diagnosing DH. Ultimately, DH can be distinguished from AD by the detection of IgA deposits in the dermal papillae or at the dermal-epidermal junction (6, 11). Nevertheless, the mean delay in the diagnosis of DH has been reported to be as long as 3.2 years (12), suggesting a still imperfect diagnostic scheme that should certainly be significantly shortened.

Our previous observations showed that sera from patients with DH, CD, and AD contained significantly increased titers of anti-Hsp90 IgG, anti-Hsp90 IgA, and anti-Hsp90 IgE, respectively (3, 4, 13). Interestingly, circulating levels of Hsp90 α were significantly increased in AD patients compared with healthy controls and positively correlated with AD severity (4). In this study, we found that serum levels of Hsp90 α were significantly higher in patients with AD when compared to patients with DH and CD or healthy controls. This preliminary outcome suggests a distinct role for extracellular Hsp90 α in the pathogenesis of AD compared to DH and its potential use as a biomarker in distinguishing AD from DH. It should also be considered that several studies on infectious, oncological, and (auto)immune diseases have already demonstrated the potential of Hsp90 as a biomarker of human diseases (14). Considering the recent COVID-19 pandemic, it is worth noting that seropositivity to SARS-CoV-2, due to infection or immunization, did not impact Hsp90 α concentration measurements, as both groups of seropositive patients had comparable serum Hsp90 α levels when compared to seronegative donors.

Distinct pathophysiological roles of extracellular Hsp90 α in the development of AD, DH, and CD are indicated by different statistical associations found between circulating levels of Hsp90 α or autoantibodies to Hsp90 and disease activity or conventional serological biomarkers. For instance, we have previously found that higher titers of anti-Hsp90 IgG are positively correlated with serum levels of anti-eTG or anti-tTG IgA autoantibodies, as well as disease activity in DH patients (3). Likewise, higher levels of anti-Hsp90 IgA were found to be positively associated with anti-tTG IgA autoantibody levels in patients with CD (13). In contrast, an association between circulating anti-Hsp90 IgE and disease activity (SCORAD) was not recorded in patients with AD (4). In this study, neither anti-eTG IgA nor anti-tTG IgA autoantibodies present in the serum of both DH and CD patients correlated with circulating Hsp90 levels.

Our study has some limitations. For instance, basic therapy, including emollients and topical anti-inflammatory first-line drugs such as glucocorticoids or calcineurin inhibitors, was used by most AD patients prior to recruitment. In contrast, only patients with untreated DH were included. On the other hand, AD patients using systemic treatment (e.g., phototherapy, systemic corticosteroids, cyclosporine, methotrexate, azathioprine, JAK inhibitors, and biologics) that could potentially influence the Hsp90 content in the blood were excluded from the study.

5 Conclusion

Our results suggest a distinct role for extracellular Hsp90 α in the pathogenesis of AD and DH and its potential use in distinguishing AD from DH. While the determination of anti-eTG and anti-tTG autoantibodies was shown to be a primary strategy used to support the diagnosis or activity monitoring of DH (specificity >90–95%) (8), we believe that the assessment of Hsp90 α levels may help the physician

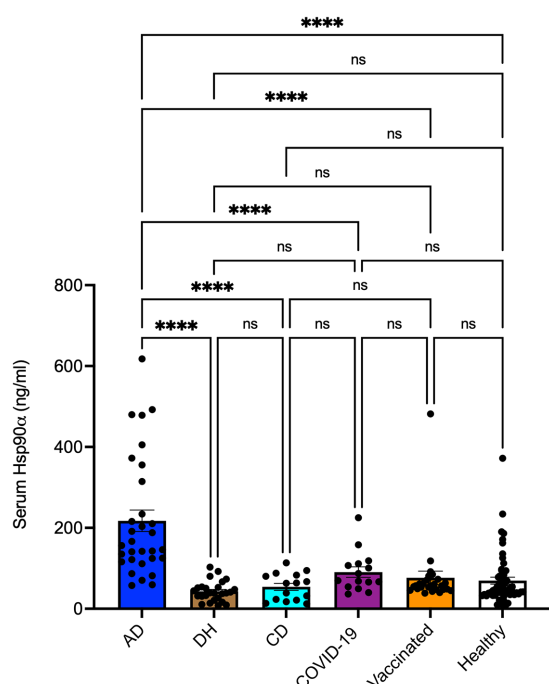


FIGURE 1

Levels of Hsp90 α in sera of patients with atopic dermatitis (AD; $n = 31$), dermatitis herpetiformis (DH; $n = 26$), celiac diseases (CD; $n = 15$), COVID-19 convalescence status (COVID-19; $n = 15$), anti-COVID-19 vaccination status (Vaccinated; $n = 26$), and healthy controls (Healthy; $n = 55$). Serum levels of Hsp90 α were assessed by an enzyme-linked immunosorbent assay. The dots and bars indicate individual and mean values in each group, respectively. Data were analyzed by a one-way ANOVA test with Tukey's multiple comparison test. **** $p < 0.0001$. The p -values below 0.05 were considered significant. ns, not significant.

distinguish DH from AD in those cases where serological results regarding the level of anti-eTG and anti-tTG autoantibodies are equivocal with a simultaneous low level of circulating Hsp90 α . Nevertheless, further research using larger cohorts is necessary to standardize the Hsp90 α detection method and to determine its accuracy, sensitivity, and specificity. Additionally, to better understand the role of Hsp90 α in the development of AD and DH, future analysis of the expression of this protein in skin biopsies from patients should be considered.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by ethics committees of the Semmelweis University, Medical University of Gdańsk, and a bioethics committee at the regional medical chamber in Gdańsk, Poland. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

KS: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. SK: Writing – review & editing. GW: Writing – review & editing. GM:

Writing – review & editing. MT: Writing – review & editing. MK: Writing – review & editing. ST: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by the Polish National Science Centre (NCN), grant no. 2020/39/B/NZ6/00357 to ST.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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References

1. Tukaj S, Sitko K. Heat shock protein 90 (Hsp90) and Hsp70 as potential therapeutic targets in autoimmune skin diseases. *Biomol Ther.* (2022) 12:1153. doi: 10.3390/biom12081153
2. Jay D, Luo Y, Li W. Extracellular heat shock Protein-90 (eHsp90): everything you need to know. *Biomol Ther.* (2022) 12:911. doi: 10.3390/biom12070911
3. Kasperkiewicz M, Tukaj S, Gembicki AJ, Silló P, Görög A, Zillikens D, et al. Evidence for a role of autoantibodies to heat shock protein 60, 70, and 90 in patients with dermatitis herpetiformis. *Cell Stress Chaperones.* (2014) 19:837–43. doi: 10.1007/s12192-014-0507-6
4. Sitko K, Bednarek M, Mantey J, Trzeciak M, Tukaj S. Circulating heat shock protein 90 (Hsp90) and autoantibodies to Hsp90 are increased in patients with atopic dermatitis. *Cell Stress Chaperones.* (2021) 26:1001–7. doi: 10.1007/s12192-021-01238-w
5. Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. *Lancet.* (2020) 396:345–60. doi: 10.1016/S0140-6736(20)31286-1
6. Reunala T, Hervonen K, Salmi T. Dermatitis herpetiformis: an update on diagnosis and management. *Am J Clin Dermatol.* (2021) 22:329–38. doi: 10.1007/s40257-020-00584-2
7. Yu L, Li L. Potential biomarkers of atopic dermatitis. *Front Med.* (2022) 9:1028694. doi: 10.3389/fmed.2022.1028694
8. Nguyen CN, Kim SJ. Dermatitis herpetiformis: an update on diagnosis, disease monitoring, and management. *Medicina.* (2021) 57:843. doi: 10.3390/medicina57080843
9. Kauppi S, Jokelainen J, Timonen M, Tasanen K, Huilaja L. Atopic dermatitis is associated with dermatitis herpetiformis and celiac disease in children. *J Invest Dermatol.* (2021) 141:191–193.e2. doi: 10.1016/j.jid.2020.05.091
10. Kárpáti S, Kósnai I, Verkasalo M, Kuitunen P, Simon Z, Koskimies S, et al. HLA antigens, jejunal morphology and associated diseases in children with dermatitis herpetiformis. *Acta Paediatr Scand.* (1986) 75:297–301. doi: 10.1111/j.1651-2227.1986.tb10202.x
11. Görög A, Antiga E, Caproni M, Cianchini G, De D, Dmochowski M, et al. S2k guidelines (consensus statement) for diagnosis and therapy of dermatitis herpetiformis initiated by the European academy of dermatology and venereology (EADV). *J Eur Acad Dermatol Venereol.* (2021) 35:1251–77. doi: 10.1111/jdv.17183
12. Rose C, Bröcker EB, Zillikens D. Clinical, histological and immunopathological findings in 32 patients with dermatitis herpetiformis Duhring. *J Dtsch Dermatol Ges.* (2010) 8:265–70. doi: 10.1111/j.1610-0387.2009.07292.x
13. Tukaj S, Görög A, Kleszczynski K, Zillikens D, Kárpáti S, Kasperkiewicz M. Autoimmunity to heat shock proteins and vitamin D status in patients with celiac disease without associated dermatitis herpetiformis. *J Steroid Biochem Mol Biol.* (2017) 173:23–7. doi: 10.1016/j.jsbmb.2016.10.002
14. Rivas-Macho A, Romeo MV, Rackles E, Olabarria G, Falcon-Perez JM, Berganza-Granda J, et al. Potential use of heat shock protein 90 as a biomarker for the diagnosis of human diseases. *Expert Rev Mol Diagn.* (2023) 23:875–84. doi: 10.1080/14737159.2023.2246883



Heat shock protein 90 (Hsp90) inhibitor STA-9090 (Ganetespib) ameliorates inflammation in a mouse model of atopic dermatitis

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Received: 11 August 2023 / Revised: 21 September 2023 / Accepted: 9 October 2023 / Published online: 18 October 2023
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Abstract

Molecular chaperones belonging to the heat shock protein 90 (Hsp90) family are implicated in inflammatory processes and described as potential novel therapeutic targets in autoimmune/inflammatory skin diseases. While the pathological role of circulating Hsp90 has been recently proposed in patients with atopic dermatitis (AD), a chronic inflammatory skin disease characterized by intense itching and recurrent skin lesions, studies aimed at investigating the role of Hsp90 as a potential target of AD therapy have not yet been conducted. Here, the effects of the Hsp90 blocker STA-9090 (Ganetespib) applied systemically or topically were determined in an experimental mouse model of dinitrochlorobenzene (DNCB)-induced AD. Intraperitoneal administration of STA-9090 ameliorated clinical disease severity, histological epidermal thickness, and dermal leukocyte infiltration in AD mice which was associated with reducing the scratching behavior in DNCB-treated animals. Additionally, topically applied STA-9090 led to lowered disease activity in AD mice, reduced serum levels of IgE, and up-regulated *filaggrin* expression in lesional skin samples. Our observations suggest that Hsp90 may be a promising therapeutic target in atopic dermatitis and potentially other inflammatory or autoimmune dermatoses.

Keywords Atopic dermatitis, AD · Heat shock proteins 90, Hsp90 · STA-9090 · Ganetespib

Introduction

The classical role of the heat shock proteins (Hsps) relies on e.g., proper protein folding during translation, re-folding of denatured proteins, and stabilization of native proteins. Widely studied mammalian Hsp90 family consists of at least four members i.e., two cytosolic isoforms, such as Hsp90 α and Hsp90 β , as well as Grp94/gp96 and TRAP-1 isoforms localized in the endoplasmic reticulum and mitochondrion, respectively. All members of the Hsp90 family comprise a common domain structure consisting of the nucleotide (ATP)-binding domain localized in N-terminal domain, a middle domain, and the C-terminal domain (Tukaj and Węgrzyn 2016). Stress-induced Hsp90 α isoform, is one of the key intra- and extracellular molecular chaperones

responsible for the biological activity of numerous protein substrates (“clients”) belonging to the key signaling molecules implicated in tumorigenesis and inflammation (Seclì et al. 2021; Tukaj 2022). Recently, due to its pleiotropic activity, Hsp90 has become the subject of interest for many researchers in the context of autoimmune/inflammatory disease development (Tukaj and Węgrzyn 2016; Li et al. 2020; Bregnhøj et al. 2022; Tukaj and Sitko 2022; Ben Abdallah et al. 2023). In fact, preclinical or clinical studies revealed that using Hsp90 inhibitors with N-terminal affinity seems to be promising for the treatment of non-infectious inflammatory skin diseases (Ben Abdallah et al. 2023) including autoimmune bullous skin diseases or psoriasis due to attenuation of numerous inflammatory immune cells and signaling pathways (Kasperkiewicz et al. 2011; Tukaj et al. 2013, 2014a, 2017; Li et al. 2020; Bregnhøj et al. 2022; Rittig et al. 2023).

Atopic dermatitis (AD) is one of the most common, non-communicable, immune-mediated inflammatory skin diseases, characterized by intense itching and recurrent skin lesions. While the underlying events and key factors involved in the development and progression of AD are the subject of ongoing debate, there are at least two major

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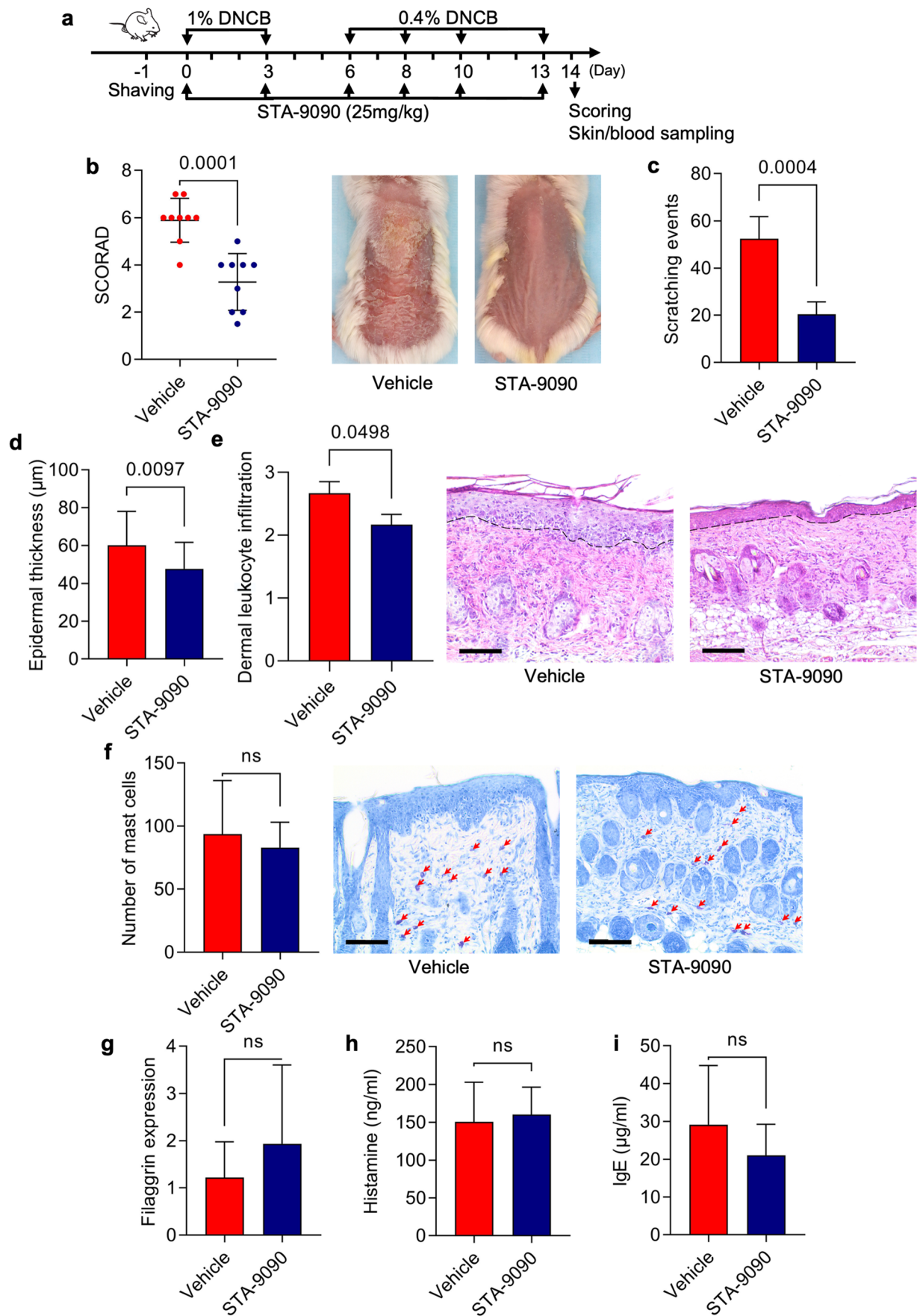


Fig. 1 Intraperitoneal administration of Hsp90 inhibitor leads to clinical and histological disease amelioration in mice with experimental AD. **(a)** Schematic illustration of the experimental setup. AD-like skin inflammation was induced in female BALB/c mice (8-weeks old) by topical dinitrochlorobenzene (DNCB) application on shaved backs starting from day 0. Mice were treated with intraperitoneal injections of STA-9090 (25 mg/kg) in volume of 50 μ L dissolved in DMSO or the equivalent volume of DMSO (Vehicle) three times a week over a 2-week treatment period starting from day 0. **(b)** Clinical disease severity of Vehicle and STA-9090-treated mice was calculated as cumulative SCORAD index at the final day (day 14) of the experiment; corresponding representative clinical presentations of vehicle- and STA-9090-treated mice at the end of the 14-day treatment period are shown on the right. **(c)** Scratching behaviour in vehicle- and STA-9090-treated animals was counted for 10 min at day 13. Histological disease severity shown by scores for **(d)** epidermal thickness, **(e)** dermal leukocyte infiltration, and **(f)** mast cells infiltration at the end of the experiment. Representative H&E and toluidine blue (red arrows indicate mast cells) staining of skin biopsies are shown next to the respective graphs. **(g)** Filaggrin (*FLG*) expression was examined in skin biopsies of vehicle- or STA-9090-treated mice at the end of the experiment by RT-qPCR. **(h)** Histamine and **(i)** IgE levels were measured in sera of vehicle- or STA-9090-treated mice at the end of the experiment by ELISA. Data are representative of three independent experiments and expressed as mean $\pm$ SEM (with individual values) of a total of 8–9 mice per group. ns, not significant. Bars = 100 μ m

and converging abnormalities in the epidermis structure due to i.e., reduced expression of filaggrin (FLG) and IgE-mediated sensitization to food and environmental allergens (Roesner and Werfel 2019; Langan et al. 2020). The abnormal structure of the epidermis leads to intensive penetration of the skin by allergens and thus to the activation of the Th2-type immune response (Guttman-Yassky et al. 2013; Varricchi et al. 2018). The involvement of innate immune cells, including mast cells and eosinophils, as well other Th subpopulations in AD, is also proven (Weidinger and Novak 2016; Czarnewicki et al. 2019).

While the pathological role of circulating Hsp90 has been recently proposed in patients with AD (Sitko et al. 2021), studies aimed at investigating the role of Hsp90 as a potential target of AD therapy have not been performed to date. Here, systemic, or local effects of the Hsp90 inhibitor STA-9090 (Ganetespib), targeting specifically ATP-binding domain of Hsp90, on development of the experimental mouse model of dinitrochlorobenzene (DNCB)-induced AD were studied.

Material and methods

Ethics statement

The animal study was reviewed and approved by local authorities of the Animal Care and Use Committee (Bydgoszcz, Poland).

Disease induction

Female BALB/c mice were purchased from the Tri-City University Animal Facility—Research Service Center (Poland). Induction of AD-like clinical symptoms in 8 weeks-old mice followed a published protocol with minor modifications (Kim et al. 2018; Jang et al. 2020). Briefly, a 6 cm² area on the mouse back was shaved and depilated on day – 1 of the experiment. On day 0 and 3, 200 μ L 1% DNCB followed by 200 μ L 0.4% DNCB dissolved in an acetone:olive oil mixture (3:1 vol/vol) was topically applied to the shaved back skin every second day starting from day 6 until day 10 and once more on day 13 as was additionally illustrated in Fig. 1 and Fig. 2.

Skin inflammation was evaluated using a modified version of the Scoring Atopic Dermatitis (SCORAD) at day 14, as described previously with some modifications (Kim et al. 2018; Jang et al. 2020). Briefly, erythema, edema, excoriation, and scaling/dryness were each scored independently by two blinded experienced investigators on a scale from 0 to 3: 0, none; 1, mild; 2, moderate; and 3, severe. The scores of these individual aspects of dermatitis were summed up to calculate the cumulative score from 0 to 12. In addition, to quantify the pruritus symptom, incidence of scratching of the body with hind paws of the mice during 10 min of observation at day 13 was recorded.

Treatment

In this study mice were treated systemically (intraperitoneally) or topically by the Hsp90 inhibitor, as described previously with minor modifications (Tukaj et al. 2014a, 2017). Briefly, 25 mg/kg of STA-9090 (Selleck Chemicals) dissolved in DMSO (Sigma) or the equivalent volume of DMSO (vehicle) was intraperitoneally injected to mice three times a week over a 2-week treatment period starting from day 0. The amount of DMSO used in this study was well tolerated by mice, with no observed toxicity such as weight loss or mortality. In the case of local treatment, STA-9090 was freshly reconstituted in a DMSO:acetone mixture (1:40 vol/vol) to a concentration of 0.5 μ mol and applied topically to back skin, 5 times a week over a two week period. Each treatment dose (administrated either systemically or topically) was applied 2 h prior to DNCB application. Blood and skin samples were taken for further experiments at day 14.

Histopathology

For the analysis of epidermal hyperplasia or dermal leukocyte infiltration, skin samples of the back obtained on the final day of the experiments (day 14) were fixed in 4% (w/v) buffered formalin and embedded in paraffin. 6- μ m tissue sections were stained with hematoxylin and eosin (H&E)

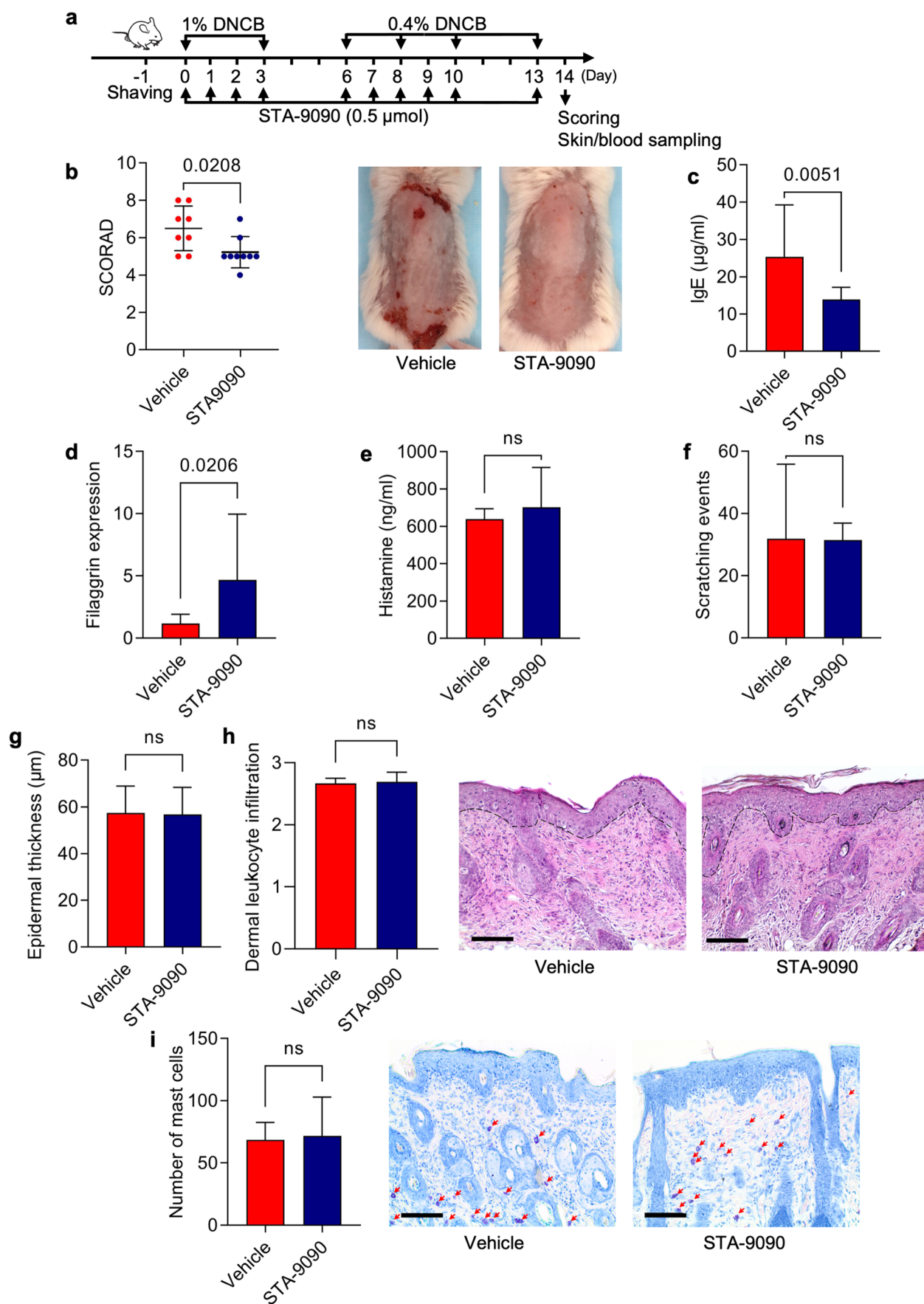


Fig. 2 Topical administration of Hsp90 inhibitor leads to clinical and molecular disease improvement in mice with experimental AD. **(a)** Schematic illustration of the experimental setup. AD-like skin inflammation was induced in female BALB/c mice (8-weeks old) by topical dinitrochlorobenzene (DNCB) application on shaved backs starting from day 0. In parallel, mice were treated with topical application of STA-9090 (0.5 μ mol) in volume of 200 μ L dissolved in DMSO:acetone mixture (1:40 vol/vol) or the equivalent volume of DMSO:acetone mixture (vehicle) 5 times a week over a 2-week treatment period starting from day 0. STA-9090 and DNCB were topically administered to mice with 2-h interval. **(b)** Clinical disease severity of vehicle and STA-9090-treated mice was calculated as cumulative SCORAD index at the final day (day 14) of the experiment; corresponding representative clinical presentations of vehicle- and STA-9090-treated mice at the end of the 14-day treatment period are shown on the right. **(c)** IgE levels were measured in sera of vehicle- or STA-9090-treated mice at the end of the experiment by ELISA. **(d)** Filaggrin (*FLG*) expression was examined in skin biopsies of vehicle- or STA-9090-treated mice at the end of the experiment by RT-qPCR. **(e)** Histamine levels were measured in sera of vehicle- or STA-9090-treated mice at the end of the experiment by ELISA. **(f)** Scratching behaviour in vehicle- and STA-9090-treated animals was counted for 10 min at day 13. Histological disease severity shown by scores for **(g)** epidermal thickness, **(h)** dermal leukocyte infiltration and **(i)** mast cells infiltration at the end of the experiment. Representative H&E and toluidine blue (red arrows indicate mast cells) staining of skin biopsies are shown next to the respective graphs. Data are representative of three independent experiments and expressed as mean $\pm$ SEM (with individual values) of a total of 8–9 mice per group. ns, not significant. Bars = 100 μ m

(Tukaj et al. 2022). To evaluate mast cell infiltration, 0.01% toluidine blue staining was used as described previously (Kim et al. 2018; Jang et al. 2020). Epidermal thickness or number of mast cells in skin sections was calculated under the light microscope at 100 $\times$ magnification in 3–6 randomly selected skin areas of each slide. Semiquantitative evaluation of dermal leukocyte infiltration in H&E skin sections was performed by two experienced researchers in a blinded manner, following a scoring scheme (1 = no infiltration, 2 = mild infiltration, 3 = moderate infiltration, 4 = severe infiltration) as described earlier (Ludwig et al. 2005).

Enzyme-linked immunosorbent assays

Serum levels of IgE and histamine were measured by commercially available ELISA kits (Invitrogen and Abcam, respectively) according to the manufacturer's protocol.

RT-qPCR analysis

Total RNA was extracted from frozen mouse dorsal skin by using RNeasy Mini Kit (QIAGEN), according to the manufacturer's protocol. RNA concentration and purity was evaluated by the ratio of absorbance at 260 nm to 280 nm. cDNA was generated from total RNA using QuantiTect Reverse Transcription Kit (QIAGEN). The mRNA levels were assessed using QuantiNova SYBR Green PCR Kit

(QIAGEN) in LightCycler® 480 Instrument II (Roche). Relative expression of *filaggrin* (*FLG*) was monitored. Here, using BestKeeper software, three reference genes (*GAPDH*, *BACT*, and *RLP13A*) were selected. The following primers were used:

FLG: forward, 5'-ATGTCCGCTCTCCTGGAAAG-3' and reverse, 5'-TGGATTCTTCAAGACTGCCTGTA-3';

GAPDH: forward, 5'-CATCACTGCCACCCAGAA GACTG-3' and reverse, 5'-ATGCCAGTGAGCTTCCCC TTCAG-3';

BACT Forward, 5'- CTCTTCCAGCCTTCCTTCCT-3' and reverse, 5'-AGCACTGTGTTGGCGTACAG-3', and.

RLP13A: forward, 5'-CTGCTCTCAAGGTTGTTC GGCT-3' and reverse, 5'-CCTTCCGTTTCTCCTCCA GAGT-3'.

The cycling conditions were as follows: 95 °C for 2 min followed by 50 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 10 s, with subsequent melting analysis. Each PCR assay was run in duplicate. The relative gene expression levels were analyzed using the $2^{-\Delta\Delta C_q}$ method.

Statistical analysis

Statistical calculations were performed using GraphPad Prism software (GraphPad, San Diego, CA, USA). Non-normal and normal distributed data were analyzed by Mann–Whitney U test and Student t-test, respectively. P values less than 0.05 were considered as significant.

Results

Intraperitoneal STA-9090 treatment reduces disease activity in a DNCB-induced atopic dermatitis mouse model

AD was induced in mice by repetitive topical administration of DNCB as described previously with minor modifications (Kim et al. 2018; Jang et al. 2020) (Fig. 1a). Intraperitoneally administration of STA-9090 (25 mg/kg) significantly reduced the development of characteristic clinical symptoms of AD i.e., erythema, edema, excoriation, and scaling/dryness (modified SCORAD index) (Fig. 1b) and scratching behavior compared with vehicle-treated animals (Fig. 1c). Histologically, epidermal hyperplasia or dermal leukocyte infiltration were significantly milder in STA-9090-treated mice compared with vehicle-treated control animals (Fig. 1d, e), whereas dermal mast cells infiltration (Fig. 1f), skin expression of *FLG* (Fig. 1g), as well as serum levels of histamine (Fig. 1h), or IgE (Fig. 1i) were not altered by the anti-Hsp90 treatment. Intraperitoneally administered STA-9090 was generally well tolerated, except for an observed average weight loss of approximately 5.96%.

Topical STA-9090 treatment reduces disease activity in a DNCB-induced atopic dermatitis mouse model

We next investigated the effect of topically applied STA-9090 on DNCB-induced AD inflammation (Fig. 2a). Topically applied STA-9090 (0.5 μ mol) significantly reduced the development of characteristic clinical symptoms of AD as expressed as modified SCORAD index compared with vehicle-treated animals (Fig. 2b). Interestingly, topical STA-9090 treatment significantly reduced serum levels of IgE (Fig. 2c) and up-regulated *FLG* expression in skin samples (Fig. 2d). Serum levels of histamine, scratching behavior, epidermal hyperplasia, dermal leukocyte infiltration, and mast cells infiltration to the skin, however, were not altered by the inhibitor (Fig. 2e-i). Topical STA-9090 treatment elicited neither skin irritation nor signs of systemic toxicity such as weight loss or death.

Discussion

We have previously found that Hsps, including Hsp70 and Hsp90 represent important pathophysiological factors and potential treatment targets in autoimmune blistering skin diseases (AIBDs), such as bullous pemphigoid, dermatitis herpetiformis, and epidermolysis bullosa acquisita (EBA), as well as psoriasis (Tukaj et al. 2013, 2021, 2022; Kasperkiewicz et al. 2014). In addition, we have recently found that significantly elevated levels of circulating Hsp90 in AD patients positively correlated with disease activity (SCORAD) (Sitko et al. 2021). The latter observations deserve attention in the study of the role Hsp90 in AD.

AD is a chronic and presently incurable immune-mediated disease. Intense itching, decreased *FLG* expression in epidermis, and increased levels of circulating IgE are common pathophysiological abnormalities in both AD patients and DNCB-induced mouse model of AD (Jang et al. 2020; Langan et al. 2020). Although, there is no specific cure for AD, it can be effectively controlled. Topical tacrolimus (calcineurin inhibitor) and topical corticosteroids belong to the first line drugs offering to the moderate-to-severe AD patients. In some severe cases, phototherapy, systemic immunosuppressors (e.g., cyclosporine A), JAK Inhibitors, and targeted biologics (e.g., dupilumab) are used. Since one of the hallmarks of AD is the elevated level of circulating IgE (typical for approximately 80% of the patients), this molecule has become AD's potential therapeutic target. For instance, the blockade of free IgE by omalizumab (anti-IgE monoclonal antibody) showed some clinical benefit in AD patients with poor response to traditional therapy (Wang et al. 2016). In addition, clinical evidence suggests that targeting IgE antibodies by immunoadsorption seems to be an effective treatment option for patients severely affected by

AD with highly elevated IgE serum levels (Kasperkiewicz et al. 2018). Despite the effectiveness of mentioned-above treatment in some AD patients, the exact role of IgE in the pathophysiology and the severity of AD is not fully understood (Wollenberg et al. 2021). All these drugs and therapies, however, may cause various side effects, therefore, the development of new treatments for AD without or with limited side effects are needed (Ujii et al. 2022).

Although most of the designed Hsp90 inhibitors targeting the ATP-binding pocket have entered oncological clinical trials, they have not been finally approved by the *Food and Drug Administration* due to toxicity, weak pharmacokinetic profiles, and insufficient clinical effectiveness. A particular obstacle to the use of these classical inhibitors may be the fact that they trigger a survival mechanism in cancer cells related to heat-shock response (HSR) which involves activation of the heat shock factor 1 (Sanchez et al. 2020). On the other hand, numerous preclinical studies using Hsp90 inhibitors, with N-terminal affinity, seem to be promising for the treatment of inflammatory/autoimmune diseases due to the activation of an HRS and attenuation of various pro-inflammatory molecules (Tukaj and Węgrzyn 2016; Tukaj and Sitko 2022). Recently, a proof-of-concept clinical trial study suggested that Hsp90 inhibition by RGRN-305 may represent a new treatment option for treating psoriasis. RGRN-305 demonstrated acceptable safety, although mild to moderate drug-induced skin rash was reported in some patients (Bregnhøj et al. 2022).

In this study, we demonstrated that ganetespib (STA-9090), a non-benzoquinone ansamycin Hsp90 inhibitor specifically targeting ATP-binding domain, significantly attenuated the development of the disease in mice with AD induced by topical application of DNCB. Although both routes of administration of this inhibitor (i.e., intraperitoneal, or topical) to AD mice resulted in suppression of disease activity as measured by the modified SCORAD index, the effect of this therapy on AD-specific cells and molecules is somewhat different. For instance, while intraperitoneal application of the STA-9090 reduced epidermal hyperplasia and incidence of scratching behavior, topically applied inhibitor had no such effects. On the other hand, only topically applied inhibitor reduced serum levels of IgE and upregulated the expression of *FLG*. Mentioned-above discrepancies, despite similar clinical effectiveness, resulted from the route of administration of the Hsp90 inhibitor to mice with AD. Several issues concerning e.g., absorption, distribution, metabolism, and elimination of the drug delivered either locally or systemically ultimately affect the target cells involved in the development of the disease. It is also noteworthy that despite the local induction of the disease, there are many pathological changes found in the periphery. Therefore, due to the heterogeneous character of AD (van der Schaft et al. 2019), potential use of anti-Hsp90

therapy (locally or peripherally) along with other drugs in AD should be personalized in the future.

Our results are in line with previously published observations concerning amelioration of other inflammatory skin condition such as EBA. We have proven that either systemically or topically applied Hsp90 inhibitors belonging to the geldanamycin derivatives (i.e., 17-DMAG or 17AAG) or short peptide derivative TCBL-145 ameliorated disease activity in experimental mouse models of EBA. The mentioned-above therapy was associated with e.g., reduced neutrophilic infiltrates, key effector cells in the pathogenesis of EBA (Kasperkiewicz et al. 2011; Tukaj et al. 2014a, 2017).

It is generally assumed that mast cells contribute to skin inflammation in AD and are mainly responsible for i.e., histamine secretion (Kawakami et al. 2009). In this study, we found no effects of the treatment on mast cell infiltration and histamine liberation. Since lesioned skin of patients with AD shows infiltration of various immune cells, including T lymphocytes, mainly belonging to the Th2 subtypes, but also Th1 and Th17 (Weidinger and Novak 2016; Langan et al. 2020), we can assume that the reduced skin infiltration observed in STA-9090-treated mice was due to the sensitivity of immune cells other than mast cells in response to the therapy. Based on our previous in vitro and pre-clinical observations, we can speculate that T lymphocytes belonging to Th1 or Th17 subpopulations (Kasperkiewicz et al. 2011; Tukaj et al. 2014b), might represent such candidates. It should also be considered that the inflammatory profile in the skin of AD patients is complex and diverse, with activation of skin-resident inflammatory cells such as e.g., dendritic cells, innate lymphoid cells, or Langerhans cells (Weidinger and Novak 2016; Langan et al. 2020). Therefore, the immunosuppressive mechanism of action of Hsp90 inhibition in alleviating AD needs to be further elucidated.

In this study, we showed that systemic or local inhibition of Hsp90 activity significantly attenuated clinical features in AD mice. Our results suggest that topical delivery of STA-9090 offers the benefit of a reduced risk of systemic adverse effects of Hsp90 inhibition. It is especially important as toxicity of these drugs has been frequently observed after systemic administration in oncological clinical trials (Sanchez et al. 2020). Although topical STA-9090 treatment elicited neither skin irritation nor signs of systemic toxicity such as weight loss or death in mice with AD, long-term safety studies of topical Hsp90 inhibitors are still needed.

Conclusion

In conclusion, systemic blockade of Hsp90 activity by STA-9090 significantly alleviated skin inflammation in mice with AD, as evidenced by lower disease activity, reduced epidermal hyperplasia or dermal leukocyte infiltration, and limited

pruritus in animals. In addition, topically applied STA-9090 was also effective in ameliorating disease symptoms that was paralleled with reduced circulating IgE secretion and upregulated *FLG* expression in AD mice. Taken together, our results suggest that the topical application of Hsp90 inhibitors offers greater therapeutic benefits due to a reduced risk of systemic side effects and therefore may be useful for controlling AD and possibly other related inflammatory skin diseases.

Acknowledgements This study was supported by the Polish National Science Centre (NCN), grant no. 2020/39/B/NZ6/00357.

Declarations

Conflict of interest There are no competing conflicts of interest.

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References

- Ben Abdallah H, Seeler S, Bregnhøj A, Ghatnekar G, Kristensen LS, Iversen L, Johansen C (2023) Heat shock protein 90 inhibitor RGRN-305 potentially attenuates skin inflammation. *Front Immunol* 14:1128897. <https://doi.org/10.3389/fimmu.2023.1128897>
- Bregnhøj A, Thuesen KKH, Emmanuel T, Litman T, Grek CL, Ghatnekar GS, Johansen C, Iversen L (2022) HSP90 inhibitor RGRN-305 for oral treatment of plaque-type psoriasis: efficacy, safety and biomarker results in an open-label proof-of-concept study. *Br J Dermatol* 186:861–874. <https://doi.org/10.1111/bjd.20880>
- Czarnowicki T, He H, Krueger JG, Guttman-Yassky E (2019) Atopic dermatitis endotypes and implications for targeted therapeutics. *J Allergy Clin Immunol* 143:1–11. <https://doi.org/10.1016/j.jaci.2018.10.032>
- Guttman-Yassky E, Dhingra N, Leung DYM (2013) New era of biologic therapeutics in atopic dermatitis. *Expert Opin Biol Ther* 13:549–561. <https://doi.org/10.1517/14712598.2013.758708>
- Jang S, Ohn J, Kim JW, Kang SM, Jeon D, Heo CY, Lee YS, Kwon O, Kim KH (2020) Caffeoil-Pro-His amide relieve DNCB-Induced Atopic Dermatitis-Like phenotypes in BALB/c mice. *Sci Rep* 10:8417. <https://doi.org/10.1038/s41598-020-65502-2>
- Kasperkiewicz M, Müller R, Manz R, Magens M, Hammers CM, Somlai C, Westermann J, Schmidt E, Zillikens D, Ludwig RJ, Orosz A (2011) Heat-shock protein 90 inhibition in autoimmunity to type VII collagen: evidence that nonmalignant plasma cells are not therapeutic targets. *Blood* 117:6135–6142. <https://doi.org/10.1182/blood-2010-10-314609>
- Kasperkiewicz M, Tukaj S, Gembicki AJ, Silló P, Görög A, Zillikens D, Kárpáti S (2014) Evidence for a role of autoantibodies to heat shock protein 60, 70, and 90 in patients with dermatitis

- herpetiformis. *Cell Stress Chaperones* 19:837–843. <https://doi.org/10.1007/s12192-014-0507-6>
- Kasperkiewicz M, Schmidt E, Ludwig RJ, Zillikens D (2018) Targeting IgE Antibodies by Immunoadsorption in Atopic Dermatitis. *Front Immunol* 9:254. <https://doi.org/10.3389/fimmu.2018.00254>
- Kawakami T, Ando T, Kimura M, Wilson BS, Kawakami Y (2009) Mast cells in atopic dermatitis. *Curr Opin Immunol* 21:666–678. <https://doi.org/10.1016/j.coi.2009.09.006>
- Kim YJ, Choi MJ, Bak DH, Lee BC, Ko EJ, Ahn GR, Ahn SW, Kim MJ, Na J, Kim BJ (2018) Topical administration of EGF suppresses immune response and protects skin barrier in DNCB-induced atopic dermatitis in NC/Nga mice. *Sci Rep* 8:11895. <https://doi.org/10.1038/s41598-018-30404-x>
- Langan SM, Irvine AD, Weidinger S (2020) Atopic dermatitis. *Lancet* 396:345–360. [https://doi.org/10.1016/s0140-6736\(20\)31286-1](https://doi.org/10.1016/s0140-6736(20)31286-1)
- Li L, Wang L, You QD, Xu XL (2020) Heat Shock Protein 90 Inhibitors: An Update on Achievements, Challenges, and Future Directions. *J Med Chem* 63:1798–1822. <https://doi.org/10.1021/acs.jmedchem.9b00940>
- Ludwig RJ, Zollner TM, Santoso S, Hardt K, Gille J, Baatz H, Johann PS, Pfeffer J, Radeke HH, Schön MP, Kaufmann R, Boehncke WH, Podda M (2005) Junctional adhesion molecules (JAM)-B and -C contribute to leukocyte extravasation to the skin and mediate cutaneous inflammation. *J Invest Dermatol* 125:969–976. <https://doi.org/10.1111/j.0022-202X.2005.23912.x>
- Rittig AH, Bregnhøj A, Johansen C, Iversen L (2023) HSP90: A potential drug target in inflammatory skin diseases. *JEADV Clin Pract* 2:450–455. <https://doi.org/10.1002/jvc2.191>
- Roesner LM, Werfel T (2019) Autoimmunity (or Not) in Atopic Dermatitis. *Front Immunol* 10:2128. <https://doi.org/10.3389/fimmu.2019.02128>
- Sanchez J, Carter TR, Cohen MS, Blagg BSJ (2020) Old and New Approaches to Target the Hsp90 Chaperone. *Curr Cancer Drug Targets* 20:253–270. <https://doi.org/10.2174/1568009619666191202101330>
- Seclì L, Fusella F, Avallè L, Brancaccio M (2021) The dark-side of the outside: how extracellular heat shock proteins promote cancer. *Cell Mol Life Sci* 78:4069–4083. <https://doi.org/10.1007/s00018-021-03764-3>
- Sitko K, Bednarek M, Mantej J, Trzeciak M, Tukaj S (2021) Circulating heat shock protein 90 (Hsp90) and autoantibodies to Hsp90 are increased in patients with atopic dermatitis. *Cell Stress Chaperones* 26:1001–1007. <https://doi.org/10.1007/s12192-021-01238-w>
- Tukaj S (2022) Circulating heat shock protein 90 (Hsp90) in atopic dermatitis and bullous pemphigoid: is there a link? *Cell Stress Chaperones* 27:601–602. <https://doi.org/10.1007/s12192-022-01298-6>
- Tukaj S, Węgrzyn G (2016) Anti-Hsp90 therapy in autoimmune and inflammatory diseases: a review of preclinical studies. *Cell Stress Chaperones* 21:213–218. <https://doi.org/10.1007/s12192-016-0670-z>
- Tukaj S, Kleszczyński K, Vafia K, Groth S, Meyersburg D, Trzonkowski P, Ludwig RJ, Zillikens D, Schmidt E, Fischer TW, Kasperkiewicz M (2013) Aberrant expression and secretion of heat shock protein 90 in patients with bullous pemphigoid. *PLoS One* 8:e70496. <https://doi.org/10.1371/journal.pone.0070496>
- Tukaj S, Tiburzy B, Manz R, de Castro MA, Orosz A, Ludwig RJ, Zillikens D, Kasperkiewicz M (2014a) Immunomodulatory effects of heat shock protein 90 inhibition on humoral immune responses. *Exp Dermatol* 23:585–590. <https://doi.org/10.1111/exd.12476>
- Tukaj S, Zillikens D, Kasperkiewicz M (2014b) Inhibitory effects of heat shock protein 90 blockade on proinflammatory human Th1 and Th17 cell subpopulations. *J Inflamm (lond)* 11:10. <https://doi.org/10.1186/1476-9255-11-10>
- Tukaj S, Bieber K, Kleszczyński K, Witte M, Cames R, Kalies K, Zillikens D, Ludwig RJ, Fischer TW, Kasperkiewicz M (2017) Topically Applied Hsp90 Blocker 17AAG Inhibits Autoantibody-Mediated Blister-Inducing Cutaneous Inflammation. *J Invest Dermatol* 137:341–349. <https://doi.org/10.1016/j.jid.2016.08.032>
- Tukaj S, Mantej J, Sitko K, Bednarek M, Zillikens D, Ludwig RJ, Bieber K, Kasperkiewicz M (2022) Evidence for a role of extracellular heat shock protein 70 in epidermolysis bullosa acquisita. *Exp Dermatol* 31:528–534. <https://doi.org/10.1111/exd.14495>
- Tukaj S, Sitko K (2022) Heat Shock Protein 90 (Hsp90) and Hsp70 as Potential Therapeutic Targets in Autoimmune Skin Diseases. *Biomolecules* 12. <https://doi.org/10.3390/biom12081153>
- Tukaj S, Mantej J, Sobala M, Potrykus K, Tukaj Z, Zillikens D, Ludwig RJ, Bieber K, Kasperkiewicz M (2021) Therapeutic Implications of Targeting Heat Shock Protein 70 by Immunization or Antibodies in Experimental Skin Inflammation. *Front Immunol* 12. <https://doi.org/10.3389/fimmu.2021.614320>
- Ujii H, Rosmarin D, Schön MP, Ständer S, Boch K, Metz M, Maurer M, Thaci D, Schmidt E, Cole C, Amber KT, Didona D, Hertl M, Recke A, Graßhoff H, Hackel A, Schumann A, Riemekasten G, Bieber K, Sprow G, Dan J, Zillikens D, Sezin T, Christiano AM, Wolk K, Sabat R, Kridin K, Werth VP, Ludwig RJ (2022) Unmet Medical Needs in Chronic. Non-Commun Inflamm Skin Dis *Front Med (lausanne)* 9:875492. <https://doi.org/10.3389/fmed.2022.875492>
- van der Schaft J, Thijs JL, Garritsen FM, Balak D, de Bruin-Weller MS (2019) Towards personalized treatment in atopic dermatitis. *Expert Opin Biol Ther* 19:469–476. <https://doi.org/10.1080/14712598.2019.1583204>
- Varricchi G, Pecoraro A, Marone G, Criscuolo G, Spadaro G, Genovese A, Marone G (2018) Thymic Stromal Lymphopoietin Isoforms, Inflammatory Disorders, and Cancer. *Front Immunol* 9:1595. <https://doi.org/10.3389/fimmu.2018.01595>
- Wang HH, Li YC, Huang YC (2016) Efficacy of omalizumab in patients with atopic dermatitis: A systematic review and meta-analysis. *J Allergy Clin Immunol* 138:1719–1722.e1711. <https://doi.org/10.1016/j.jaci.2016.05.038>
- Weidinger S, Novak N (2016) Atopic dermatitis. *Lancet* 387:1109–1122. [https://doi.org/10.1016/s0140-6736\(15\)00149-x](https://doi.org/10.1016/s0140-6736(15)00149-x)
- Wollenberg A, Thomsen SF, Lacour JP, Jaumont X, Lazarewicz S (2021) Targeting immunoglobulin E in atopic dermatitis: A review of the existing evidence. *World Allergy Organ J* 14:100519. <https://doi.org/10.1016/j.waojou.2021.100519>

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OPEN Topical application of the HSP90 inhibitor 17-AAG reduces skin inflammation and partially restores microbial balance: implications for atopic dermatitis therapy

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Heat shock proteins belonging to the HSP90 family promote inflammation and are potential therapeutic targets in inflammatory and autoimmune diseases. Here the effects of the HSP90 inhibitor 17-AAG applied topically were evaluated in a DNCB-induced murine model of atopic dermatitis (AD). The use of 17-AAG improved clinical disease activity without causing toxicity in the animals. Topical application of 17-AAG resulted in reduced epidermal hyperplasia, decreased expression of TSLP, IL-5, and IL-6, as well as reduced activation of NF- κ B in the skin. In addition, the eosinophil proportion in the blood and eosinophil peroxidase (EPX) activity in the skin were significantly reduced in 17-AAG-treated AD mice. The inhibitory effects of 17-AAG on the production of epidermal alarmins, T-helper cell-associated cytokines, and ROS release were demonstrated in cultures of activated human keratinocytes, CD4⁺ T lymphocytes, and eosinophils, respectively. Finally, next-generation sequencing metagenomic approaches revealed that topical application of 17-AAG partially restored the normal gut microbiome in AD mice. Moreover, 17-AAG inhibited *Staphylococcus aureus* biofilm formation in vitro. The findings of this study, combined with the observed increase in HSP90 and EPX activity in the leukocytes of the analyzed cohort of AD patients, support the potential therapeutic use of HSP90 inhibitors in individuals with AD.

Keywords Heat shock proteins, Mouse model, Microbiota, *Staphylococcus aureus*, Eosinophiles, Keratinocytes.

Heat shock protein 90 (HSP90) is an evolutionarily conserved family of molecular chaperones involved in the stability and activation of over 300 client proteins under normal cellular conditions¹. This family includes cytoplasmic isoforms of HSP90, comprising a constitutive form (HSP90AB) and an inducible form (HSP90AA), as well as the endoplasmic reticulum (Grp94/gp96) and the mitochondrial (TRAP1) isoforms. HSP90 functions as a crucial molecular chaperone that stabilizes oncogenic proteins, facilitating tumor growth and survival. Both intracellular and extracellular forms of HSP90 contribute to this stabilization, raising interest in HSP90 inhibitors as potential cancer therapies. Advanced clinical studies are currently assessing the efficacy of these inhibitors across various cancer types². HSP90's chaperone function is linked to its ability to bind and hydrolyze ATP, a process finely regulated by co-chaperones and post-translational modifications, including acetylation³. The N-terminal domain of HSP90 contains a nucleotide-binding pocket that serves as the primary binding site for most HSP90 inhibitors. HSP90 inhibition can disrupt pathways such as JAK-STAT and suppress the activation of NF- κ B, which are implicated in various cellular processes, including proliferation, differentiation, and inflammation⁴, making this therapy particularly attractive for inflammatory and autoimmune diseases^{5,6}. The effectiveness of this therapy has been thoroughly demonstrated in preclinical models of epidermolysis bullosa

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acquisita (EBA), an autoimmune blistering skin disorder^{7–9}. We were also the first to highlight the potential role of both extracellular and intracellular HSP90 in the development of atopic dermatitis (AD)^{10,11}, a common non-communicable inflammatory skin disease characterized by recurring eczematous lesions and intense itching¹². Additionally, recent preliminary evidence from proof-of-concept studies suggests that the orally administered HSP90 inhibitor RGRN-305 leads to significant improvements in psoriasis and shows promise in randomized trials for hidradenitis suppurativa^{13,14}. To date, there have been no studies on the use of HSP90 inhibitors in the treatment of patients with AD.

Our study examined the effects of the topically applied HSP90 inhibitor, 17-AAG, on clinical parameters, immune response, microbiome composition, and toxicity in a mouse model of 2,4-dinitrochlorobenzene (DNCB)-induced human-like AD which is widely used to assess novel treatment strategies and compounds¹⁵. Additionally, we assessed the significance of HSP90 concerning its expression and activity in tape strip skin samples and blood leukocytes obtained from AD patients, respectively. Finally, we utilized *Staphylococcus aureus* strains and human cell cultures to confirm the effects observed in mice and further elucidate the mechanisms underlying anti-HSP90 therapy. Through this research, we aimed to contribute to the development of innovative, safe, and effective therapeutic strategies for managing atopic dermatitis and related conditions.

Results

Topical 17-AAG treatment reduces disease activity in an experimental mouse model of 2,4-dinitrochlorobenzene (DNCB)-induced human-like atopic dermatitis

Topically applied 17-AAG (0.5 μ M) (Fig. 1a) significantly reduced the development of characteristic clinical symptoms of AD (i.e., erythema, edema, excoriation, and scaling/dryness) expressed as modified SCORAD index compared with vehicle-treated animals (Fig. 1b). Clinical efficacy of 17-AAG was observed in a dose-dependent manner in an additional independent experiment (Fig. S1). Of note, therapy with topically administered 17-AAG on shaved back skin (6 cm²) at a dose of 0.5 μ M per day for two weeks was safe, as it did not result in mortality, skin irritation or a decrease in body weight of the animals. Furthermore, the therapy did not induce systemic toxicity, as the parameters of peripheral blood morphology (Table S1) and serum biochemical markers (Table S2) were not significantly altered, except for the inhibitory effect of 17-AAG on the percentage of circulating eosinophils.

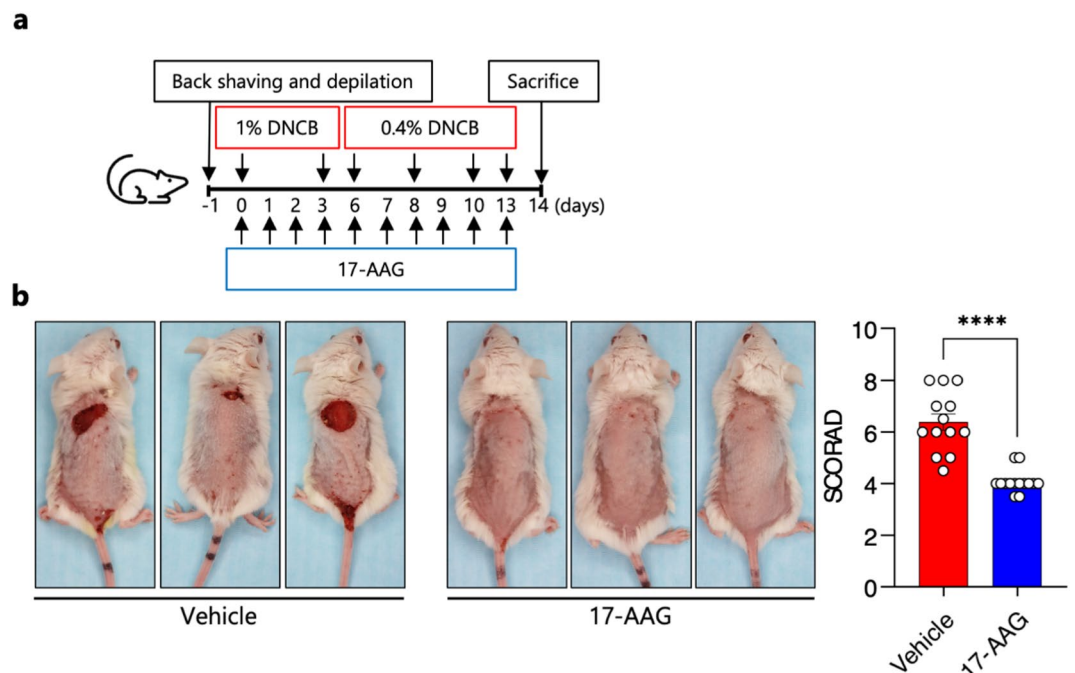


Fig. 1. Topical 17-AAG treatment reduces disease activity in an experimental mouse model of DNCB-induced human-like AD. **(a)** Schematic representation of experimental design. AD-like skin inflammation was induced in female BALB/c mice by dinitrochlorobenzene (DNCB) application to the shaved and depilated back skin (day – 1) as follows: 1% DNCB on days 0 and 3, followed by 0.4% DNCB on days 6, 8, 10 and 13. 17-AAG (0.5 μ M) or vehicle (DMSO: acetone, 1:40 vol/vol) was applied each day with a 2-h interval to DNCB. **(b)** Representative images of vehicle- and 17-AAG-treated mice at the end of the 14-day treatment period and clinical disease severity of vehicle and 17-AAG-treated mice represented as cumulative SCORAD index. Data are expressed as mean $\pm$ SEM (with individual values) of three independent experiments. **** $P < 0.0001$.

Topical application of 17-AAG influences the type 2 immune response in mice with atopic dermatitis

The topical application of 17-AAG resulted in the reduction of epidermal hyperplasia (Fig. 2a) and a decreased expression of TSLP, IL-5, and IL-6 in skin biopsies (Fig. 2b). The expression of other analyzed cytokines, including IL-4, IL-31RA, TNF- α , IL-33, IL-1 β , IL-10, IL-13, and IFN- γ , was not significantly altered by the treatment; however, some inhibitory trends were observed (Fig. S2). Statistically insignificant tendency toward a decrease in circulating IgE levels was also noted (Fig. 2c). Our findings indicate that the topical application of 17-AAG effectively reduced NF- κ B activation in the skin (Fig. 2d). Although the number of infiltrating leukocytes (Fig. 2e, f) including eosinophils (Fig. 2g), CD4⁺ T cells (Fig. 2h), and mast cells (Fig. 2i) in the skin was not significantly affected by the topical application of 17-AAG, circulating eosinophil percentages (Fig. 2j), serum levels of histamine (Fig. 2k), and eosinophil peroxidase (EPX) activity (Fig. 2l) in the skin were significantly reduced in 17-AAG-treated AD mice compared to vehicle-treated AD mice.

17-AAG inhibits epidermal IL-33 alarmin, T-helper cell-associated cytokines, and reactive oxygen species in cultures of activated human keratinocytes, CD4⁺ T lymphocytes, and eosinophils, respectively

Using a TNF- α /IFN- γ -stimulated immortalized human keratinocyte line (HaCaT cells), we found that HSP90 inhibition with non-toxic concentrations of 17-AAG significantly reduced cell proliferation (Fig. 3a), decreased IL-33 expression (but not TSLP) (Fig. 3b), and inhibited the secretion of pro-inflammatory IL-6 (Fig. 3c). These effects were associated with inhibition of STAT-1 and STAT-6 signaling (Fig. 3d), attenuation of NF- κ B activity (Fig. 3e), and upregulation of filament aggregating protein filaggrin (FLG) expression (Fig. 3f).

Additionally, the effect of 17-AAG on the cytokines produced by subpopulations of T helper cells was examined. Using anti-CD3/28 antibody-stimulated human CD4⁺ T cell cultures, we observed that 17-AAG significantly blocked the secretion of IL-5, IL-17 A, and IL-22 (Fig. 3g), cytokines characteristic of Th2, Th17, and Th22 cell populations, respectively. Finally, using IL-33- or fMLP-activated human eosinophils, we found that 17-AAG dose-dependently inhibited reactive oxygen species (ROS) production (Fig. 3h), one of the indicators of eosinophil activation status¹⁶.

Topical application of 17-AAG impacts both skin and gut microbiota in a mouse model of atopic dermatitis

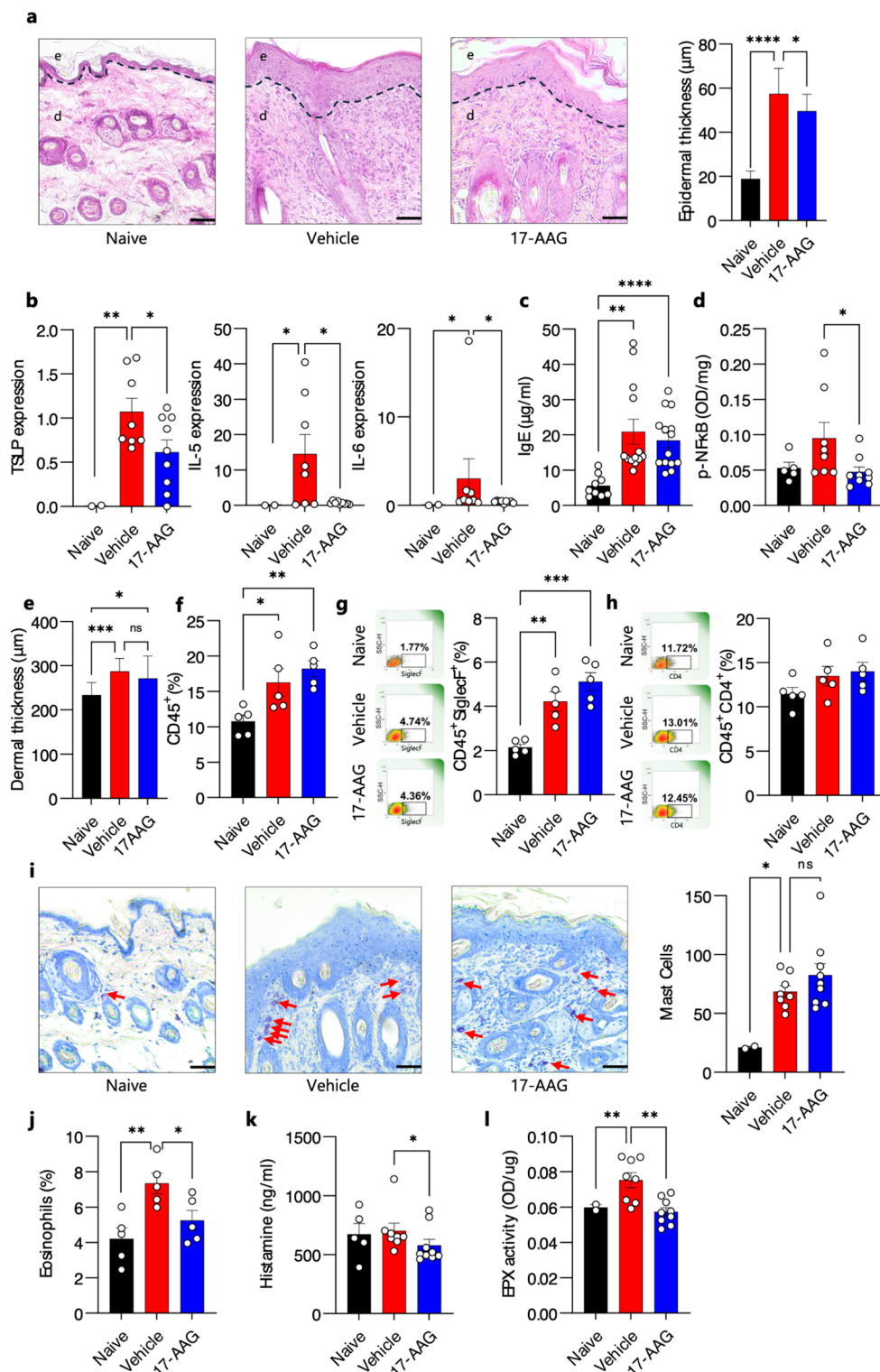
We profiled the microbiome of either back skin or fecal samples from co-housed littermates naïve, as well as vehicle- or 17-AAG-treated AD mice by next-generation sequencing (NGS) of the V3–V4 region of the 16 S rRNA gene. Our study revealed that in all analyzed groups, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* were the most abundant phyla in the skin microbiome (Fig. S3). While the relative abundances of *Proteobacteria* and *Actinobacteria* were unaffected by the topical application of DNCB or DNCB along with 17-AAG compared to naïve mice, both vehicle- and 17-AAG-treated AD mice showed significantly increased levels of *Firmicutes* and decreased levels of *Bacteroidetes* compared to naïve mice (Fig. 4a). Additionally, the microbiota diversity analysis in the samples revealed that both vehicle- and 17-AAG-treated AD mice exhibited significantly reduced alpha diversity compared to naïve mice, as indicated by the Chao1, Simpson, and Shannon indices, however no significant effect of 17-AAG therapy was observed (Fig. 4b). Similarly, DNCB sensitization significantly affected skin beta diversity, with no impact of 17-AAG treatment observed, as shown by weighted UniFrac PCoA (Fig. 4c). At the genus level, both vehicle- and 17-AAG-treated AD mice showed a significant increase in *Corynebacterium* and *Streptococcus* abundances, along with a decrease in *Duncaniella*, *Ligilactobacillus*, *Parabacteroides*, *Prevotella*, and *Turicibacter* compared to naïve mice (Fig. 4a). Additionally, 17-AAG treatment amplified *Corynebacterium* and *Mammaliicoccus* levels relative to vehicle-treated AD mice. The elevated abundance of *Streptococcus* induced by DNCB sensitization was significantly reversed in AD mice treated with 17-AAG. Likewise, the reduction in *Turicibacter* levels in AD mice was partially restored following 17-AAG treatment (Fig. 4a). We observed a significant increase in the abundance of *Staphylococcus* in AD mice. However, 17-AAG therapy showed no significant effect on *Staphylococcus* counts, including *S. aureus*. Consequently, an in vitro analysis was conducted. Our findings indicated that 17-AAG inhibits biofilm formation of two strains of *S. aureus*, i.e., methicillin-sensitive ATCC25923 and methicillin-resistant RA532, in a dose-dependent manner (Fig. 4d), without affecting their growth rate (Fig. 4e).

In the analysis of the gut microbiome, the same animals were included. Our findings revealed that *Bacteroidetes* and *Firmicutes* were the most abundant phyla across all groups (Fig. S4). We observed a notable increase in *Actinobacteria* abundance in the vehicle-treated AD mice compared to naïve mice, while 17-AAG treatment tended to restore its levels ($P=0.06$) (Fig. 4f). Vehicle-treated AD mice had on average, lower alpha diversity than the naïve group; however, the effect did not reach significance ($P=0.2$). Conversely, 17-AAG treatment significantly improved alpha diversity, as indicated by Chao1, Simpson, and Shannon indices (Fig. 4g). In addition, weighted UniFrac PCoA analysis showed significant differences between naïve and 17-AAG-treated AD mice with 30% of the variation explained (Fig. 4h).

At the genus level, we observed that DNCB sensitization significantly reduced the abundances of *Coprobacter*, *Parabacteroides*, and *Prevotella*, along with an increase in *Ligilactobacillus* compared to naïve mice. 17-AAG increased *Sodaliophilus* abundances compared to the vehicle-treated AD mice; however, its role in the skin disorders is currently unknown. Of note, topical application of 17-AAG significantly restored the levels of *Ligilactobacillus* and *Parabacteroides* to levels observed in the naïve group (Fig. 4f).

Therapeutic treatment with 17-AAG reduces the severity of the experimental AD-like model

We evaluated the therapeutic potential of 17-AAG in mice with already established clinical AD, induced by repetitive application of DNCB. Starting on day 6, mice were treated topically with either 17-AAG or vehicle, and



their condition was monitored until day 20 (Fig. 5a). Compared to vehicle-treated controls, mice receiving 17-AAG showed a clinically significant reduction in the severity of AD-like symptoms, as indicated by the modified SCORAD index (Fig. 5b), further supporting the important role of Hsp90 in the progression of experimental AD and highlighting the therapeutic potential of topically applied 17-AAG.

The activity of HSP90 and eosinophil peroxidase is elevated in the leukocytes of individuals with AD

We analyzed the gene expression of four HSP90 homologs (i.e., HSP90AA, HSP90AB, Grp94/gp96, and TRAP1) using tape-strip samples collected from 19 patients with AD, from both non-lesional and lesional skin areas. The characteristics of patients with AD are presented in the Table S3. We did not observe significant changes in the expression of the analyzed HSP90 homologs between lesional and non-lesional skin samples (Fig. 6a). Similarly,

Fig. 2. Topical Application of 17-AAG Influences the Type 2 Immune Response in Mice with AD. (a) H&E-stained skin sections of naïve, vehicle- and 17-AAG-treated mice. Bars = 50 μ m. (b) Relative expression of TSLP, IL-5, and IL-6 in the biopsies of mouse back skin, by qPCR. ELISA test results of (c) IgE level in the serum of mice and (d) NF- κ B phosphorylation in the biopsies of mouse back skin. (e) Histological skin infiltration, as well as (f) the number of infiltrating leukocytes (CD45⁺), including (g) eosinophils (CD45⁺Siglec-F⁺) and (h) T helper cells (CD45⁺CD4⁺), were assessed in dissociated skin biopsies using flow cytometry. (i) Toluidine blue- stained skin sections of naïve, vehicle- and 17-AAG-treated mice. Bars = 50 μ m. (j) Eosinophil proportion (% of WBC) in blood via hematology analyzer. (k) Histamine level in mouse serum, by ELISA. (l) Eosinophil peroxidase (EPX) activity, as measured by OPD assay in the back skin biopsies. Data are expressed as mean $\pm$ SEM (with individual values) or mean $\pm$ SD (in the case of epidermal thickness) of three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; ns, no significance; H&E, Hematoxylin and Eosin; OPD, o-phenylenediamine dihydrochloride; OD, optical density; WBC, white blood cells; e, epidermis; d, dermis.

there were no significant differences in the level of HSP90 protein isoforms in polymorphonuclear leukocytes (PMNs) (Fig. 6b) and peripheral blood mononuclear cells (PBMCs) (Fig. 6c) between patients with AD ($n = 8$) and age- and gender-matched healthy individuals ($n = 9$).

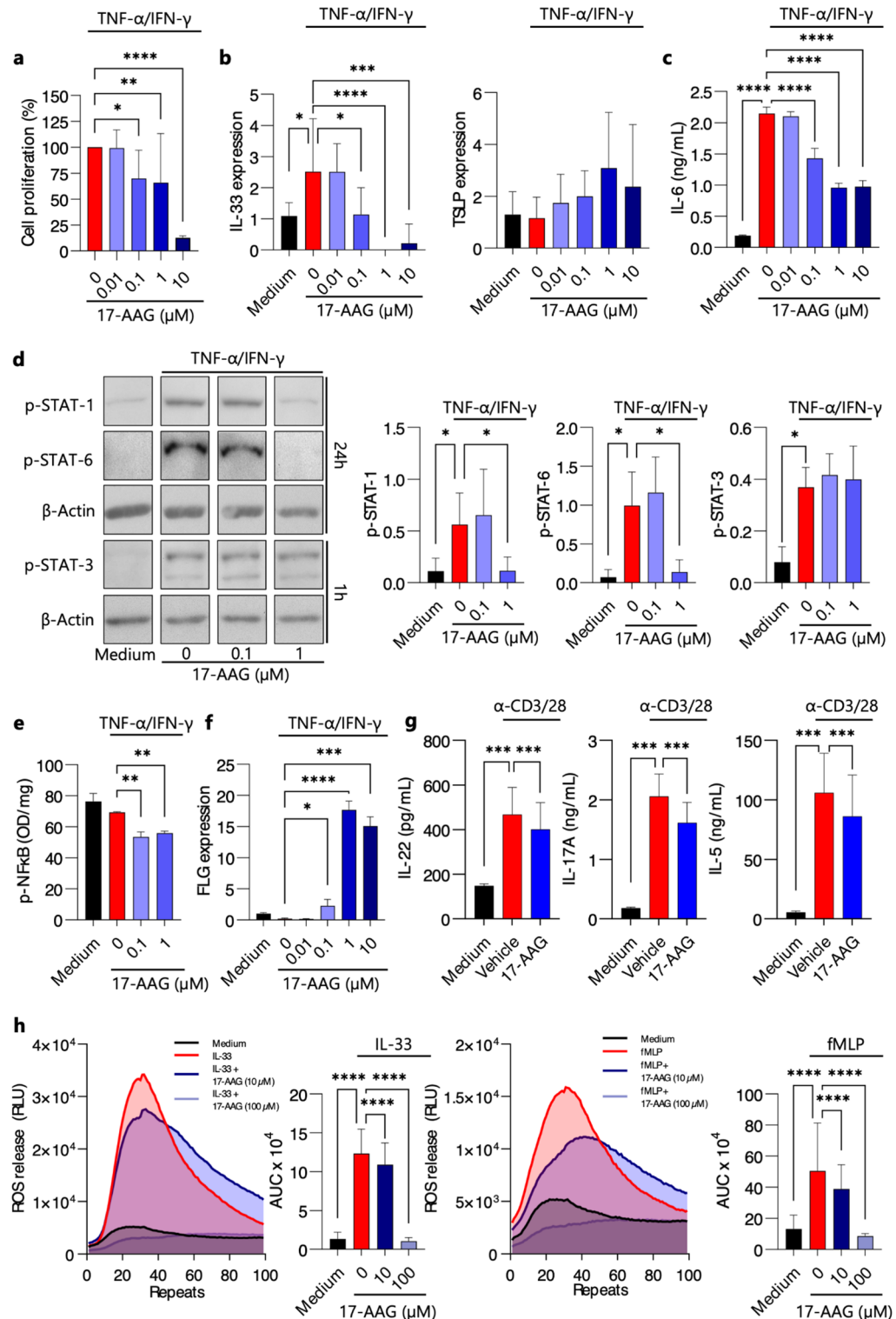
Further, we determined the level of HSP90 acetylation (acetyl-HSP90) in leukocytes from patients with AD ($n = 8$) and age- and gender-matched healthy controls ($n = 9$). Notably, we observed a significantly lower level of acetyl-HSP90 in the PBMCs of individuals with AD compared to healthy controls (Fig. 6d) and that the presence of 17-AAG in cultures of human keratinocytes (HaCaT) led to increased levels of acetyl-HSP90 (Fig. 6e). Finally, we found elevated eosinophil peroxidase (EPX) activity in the PMNs of individuals with AD compared to healthy controls (Fig. 6f).

Discussion

Our study examined the effects of the topically applied HSP90 inhibitor, 17-AAG, on clinical parameters, immune response, microbiome composition, and toxicity in a mouse model of 2,4-dinitrochlorobenzene (DNCB)-induced human-like AD which is widely used to assess novel treatment strategies and compounds¹⁵. Topically applied 17-AAG significantly reduced the development of characteristic clinical symptoms of AD in both prophylactic and therapeutic settings. The prophylactic treatment did not induce toxicity, as the parameters of peripheral blood morphology and serum biochemical markers were not significantly altered, except for the inhibitory effect of 17-AAG on the percentage of circulating eosinophils. The latter effect was desirable given the frequent occurrence of eosinophilia in patients with AD¹⁷ and the elevated eosinophil levels in mice with AD. The safety analysis of such therapy is particularly important because, although the majority of discovered HSP90 inhibitors targeting the ATP-binding pocket in the N-terminal domain of HSP90 have entered oncological clinical trials (e.g., 17-AAG) as systemic interventions, none of them was approved by the FDA due to their toxicity¹⁸. The results of our study suggest that the topical delivery of HSP90 inhibitors provides the advantage of reducing the risk of systemic adverse effects associated with orally or intravenously administered HSP90 inhibitors.

The pathophysiology of AD is characterized by a dysregulated immune response, predominantly skewed towards a type-2 phenotype. This response is driven by the release of epidermal alarmins, such as TSLP and IL-33, in reaction to allergens and various external stressors. The activation of T-helper 2 (Th2) cells subsequently stimulates the production of cytokines, including IL-4, IL-5, and IL-13, promoting B cell activation, specific IgE production, and the recruitment/activation of eosinophils and mast cells^{12,19}. In this study, the topical application of 17-AAG resulted in the reduction of epidermal hyperplasia and a decreased expression of TSLP, IL-5, and IL-6 in skin biopsies. Previous studies have established that NF- κ B mediates the induction of pro-inflammatory cytokines, such as TSLP and IL-6²⁰. Given the significant role of NF- κ B in the pathogenesis of AD²¹, there has been an effort to develop topical therapies targeting the NF- κ B pathway^{22,23}. Our findings indicate that the topical application of 17-AAG effectively reduced NF- κ B activation in the skin. Furthermore, although the number of infiltrating eosinophils, CD4⁺ T cells and mast cells in the skin was not significantly affected by the topical application of 17-AAG, circulating eosinophil percentages, serum levels of histamine, and eosinophil peroxidase (EPX) activity in the skin were significantly reduced in 17-AAG-treated AD mice compared to vehicle-treated AD mice. There is an apparent discrepancy between IL-5 expression and the number of tissue-infiltrating eosinophils. Possible explanations for this include the involvement of IL-5-independent chemotactic factors, the persistence of pre-existing eosinophils in the tissue, or the functional inactivation of eosinophils despite their presence in the skin. Therefore, it seems that the reduction in cell activity through an NF- κ B-dependent mechanism, rather than cell recruitment, may underlie the therapeutic effect of 17-AAG in reducing inflammatory responses in AD.

To further elucidate the potential molecular and cellular pathways related to AD that are targeted by HSP90 inhibition in vivo, we utilized activated human keratinocytes, CD4⁺ T lymphocytes, and eosinophil cell cultures. Using a TNF- α /IFN- γ -stimulated immortalized human keratinocyte line (HaCaT cells), we found that HSP90 inhibition with non-toxic concentrations of 17-AAG significantly reduced cell proliferation, decreased IL-33 expression, and inhibited the secretion of pro-inflammatory IL-6. These effects were associated with inhibition of STAT-1 and STAT-6 signaling, attenuation of NF- κ B activity, and upregulation of FLG expression. The results concerning the effects of HSP90 inhibition on signaling pathways involving STAT and NF- κ B are consistent with previous reports that emphasize their roles in various cellular processes, including proliferation and



inflammation⁴. The positive effect of the therapy on FLG expression is also beneficial in the context of the pathophysiology of AD, as some patients with AD have an acquired defect in its expression²⁴.

Because the pathogenesis of AD also involves an imbalanced adaptive immune system characterized by enhanced activation of Th2, Th17, and Th22 cell population²⁵, the impact of 17-AAG on the cytokines produced by these cell subpopulations was investigated. Using anti-CD3/28 antibody-stimulated human CD4⁺ T cell cultures, we observed that 17-AAG significantly blocked the secretion of IL-5, IL-17 A, and IL-22, cytokines characteristic of Th2, Th17, and Th22 cell populations, respectively. Finally, using IL-33- or fMLP-activated human eosinophils, we found that 17-AAG dose-dependently inhibited ROS production, one of the indicators of eosinophil activation status¹⁶.

The microbiome plays a crucial role in the development and maintenance of immune system homeostasis. Recent research has linked changes in microbial composition (known as dysbiosis) in the skin and gut to altered

◀ **Fig. 3.** 17-AAG inhibits the expression of IL-33, the secretion of IL-6, T-helper cell-associated cytokines, and reactive oxygen species in cultures of activated human keratinocytes, CD4⁺ T lymphocytes, and eosinophils, respectively. TNF- α /IFN- γ -stimulated human keratinocytes (HaCaT) cells were cultured in presence of DMSO 0.1% (Vehicle) or various doses of 17-AAG. **(a)** Cell proliferation ELISA results as BrdU incorporation (%) after 6 h of incubation. **(b)** Relative expression of IL-33 and TSLP, by qPCR. **(c)** IL-6 levels in culture supernatant, by ELISA. **(d)** Phosphorylated STAT-1, STAT-3, and STAT-6 protein levels, corrected for β -Actin, were analyzed in HaCaT cell lysates following 1-hour or 24-hour activation using Western blot. Representative bands are presented. **(e)** NF- κ B phosphorylation measured by ELISA. **(f)** FLG relative expression analyzed by qPCR. **(g)** IL-5, IL-17 A, and IL-22 secretion in the culture supernatant of anti-CD3/CD28 antibody-stimulated (1 μ g/mL) CD4⁺ T cells derived from healthy donors, assessed by ELISA. **(h)** Luminol-enhanced ROS release in eosinophils derived from healthy donors, activated with fMLP (2 μ M) or IL-33 (100 ng/mL). Data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; ns, no significance; BrdU, BromodeoxyUridine; RLU, Relative Light Unit; AUC, Area Under Curve; ROS, Reactive Oxygen Species; OD, optical density.

immune responses and the development of AD²⁶. Here, we profiled the microbiome of either back skin or fecal samples from co-housed littermates naïve, as well as vehicle- or 17-AAG-treated AD mice by NGS of the V3–V4 region of the 16 S rRNA gene. Our study revealed that in all analyzed groups, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* were the most abundant phyla in the skin microbiome. While the relative abundances of *Proteobacteria* and *Actinobacteria* were unaffected by the topical application of DNCB or DNCB along with 17-AAG compared to naïve mice, both vehicle- and 17-AAG-treated AD mice showed significantly increased levels of *Firmicutes* and decreased levels of *Bacteroidetes* compared to naïve mice. Additionally, the microbiota diversity analysis in the samples revealed that both vehicle- and 17-AAG-treated AD mice exhibited significantly reduced alpha diversity compared to naïve mice, as indicated by the Chao1, Simpson, and Shannon indices, however no significant effect of 17-AAG therapy was observed. Similarly, DNCB sensitization significantly affected skin beta diversity, with no impact of 17-AAG treatment observed, as shown by weighted UniFrac PCoA. At the genus level, both vehicle- and 17-AAG-treated AD mice showed a significant increase in *Corynebacterium* and *Streptococcus* abundances, along with a decrease in *Duncaniella*, *Ligilactobacillus*, *Parabacteroides*, *Prevotella*, and *Turicibacter* compared to naïve mice. Additionally, 17-AAG treatment amplified *Corynebacterium* and *Mammaliococcus* levels relative to vehicle-treated AD mice. The elevated abundance of *Streptococcus*, a recognized risk factor for AD development²⁷, induced by DNCB sensitization, was significantly reversed in AD mice treated with 17-AAG. Likewise, the reduction in *Turicibacter* levels in AD mice was partially restored following 17-AAG treatment. The increase in *Corynebacterium* abundance following topical 17-AAG treatment appears beneficial in the context of AD development and aligns with previous studies on standard AD therapies. Namely, topical applications of corticosteroids, antibiotics, and calcineurin inhibitors have been associated with enhanced microbial diversity, including colonization by *Corynebacterium*²⁸. Furthermore, the expansion of *Corynebacterium* may contribute to the inhibition of *Staphylococcus aureus* (*S. aureus*) colonization on the skin²⁸. We observed a significant increase in the abundance of *Staphylococcus* in AD mice. However, 17-AAG therapy showed no significant effect on *Staphylococcus* counts, including *S. aureus*, the predominant colonizer and pathogen in human AD²⁹. Consequently, an in vitro analysis was conducted. Our findings indicated that 17-AAG inhibits biofilm formation of two strains of *S. aureus*, i.e., methicillin-sensitive ATCC25923 and methicillin-resistant RA532, in a dose-dependent manner, without affecting their growth rate.

In the analysis of the gut microbiome, the same animals were included. Our findings revealed that *Bacteroidetes* and *Firmicutes* were the most abundant phyla across all groups. We observed a notable increase in *Actinobacteria* abundance in the vehicle-treated AD mice compared to naïve mice, while 17-AAG treatment tended to restore its levels. Vehicle-treated AD mice had on average, lower alpha diversity than the naïve group; however, the effect did not reach significance. Conversely, 17-AAG treatment significantly improved alpha diversity, as indicated by Chao1, Simpson, and Shannon indices. In addition, weighted UniFrac PCoA analysis showed significant differences between naïve and 17-AAG-treated AD mice with 30% of the variation explained. At the genus level, we observed that DNCB sensitization significantly reduced the abundances of *Coprobacter*, *Parabacteroides*, and *Prevotella*, along with an increase in *Ligilactobacillus* compared to naïve mice. 17-AAG increased *Sodaliophilus* abundances compared to the vehicle-treated AD mice; however, its role in the skin disorders is currently unknown. Of note, topical application of 17-AAG significantly restored the levels of *Ligilactobacillus* and *Parabacteroides* to levels observed in the naïve group.

In interpreting the results of microbiome analysis in animal models, two key issues must be considered. The first concerns the natural interspecies differences that exist between the microbiota of humans and animals. The second issue is the variation in microbiome composition between different mouse models of AD and the microbiome of AD patients³⁰. On the other hand, it should be emphasized that, based on our observations, the dysbiosis observed in mice treated with DNCB was partially restored by therapy with topically applied 17-AAG in the gut to levels observed in naïve mice. This comparative approach seems justified in this case. Although the exact mechanism by which topically applied 17-AAG affects the microbiome is not yet fully understood, we hypothesize that the beneficial effects of 17-AAG therapy on the microbiome may result from its direct inhibitory action on pro-inflammatory cytokine expression, *S. aureus* biofilm formation, and eosinophil count or activity. Although this is the first study on the impact of HSP90 inhibition on microbiome composition, previous research has demonstrated a link between microbiome composition and eosinophil activity^{31–33}, as well as associations between *S. aureus* colonization and AD activity²⁹. In addition, there are at least two non-exclusive hypotheses regarding the mechanism by which 17-AAG directly inhibits biofilm formation. First, 17-AAG

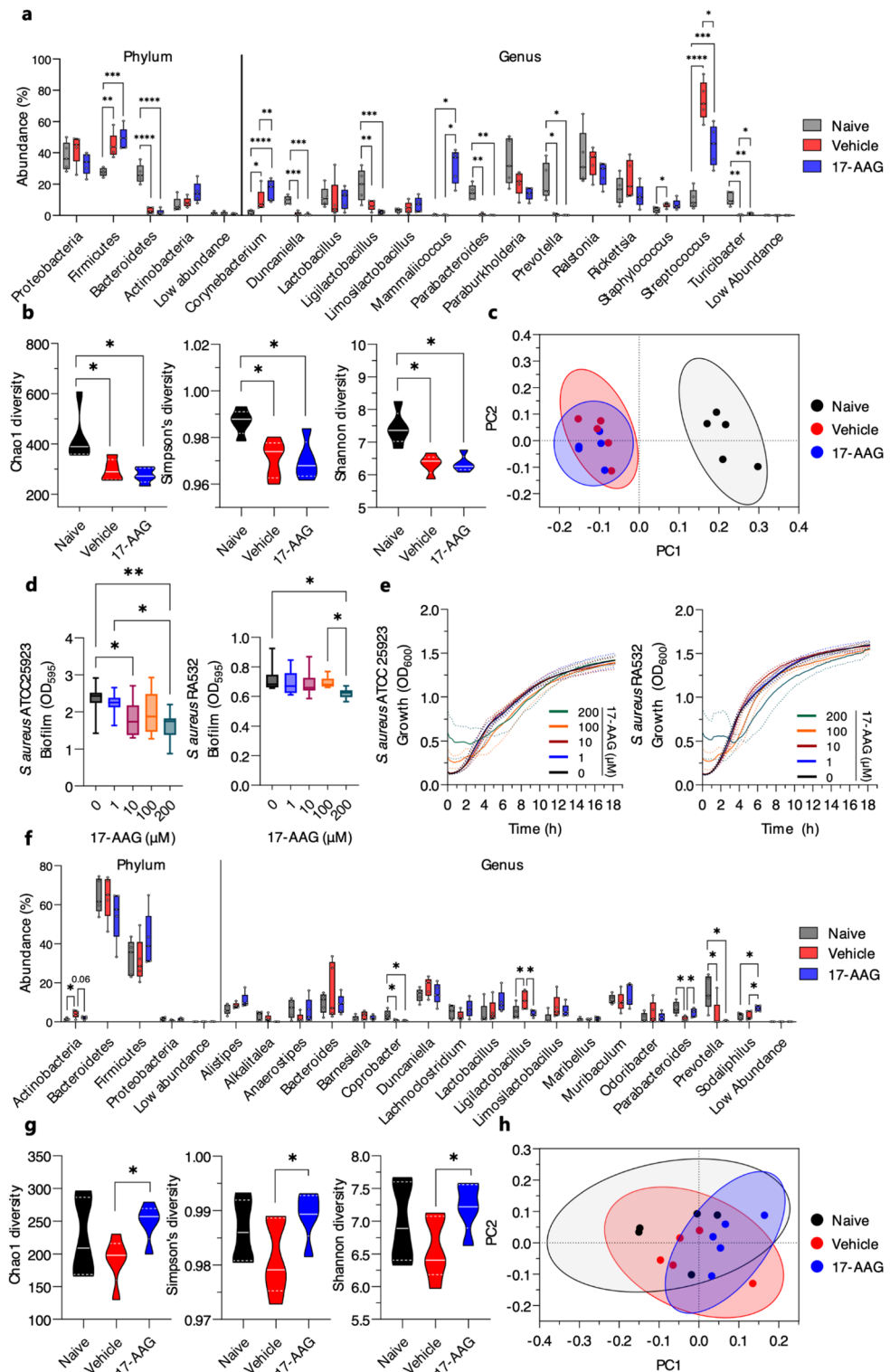


Fig. 4. Topical Application of 17-AAG Restores Both Skin and Gut Microbiota in a Mouse Model of AD. (a) Relative abundances of identified phyla and genera in back skin microbiome samples from naïve, vehicle-, and 17-AAG-treated AD mice, with (b) alpha diversity indices (Shannon, Chao1, and Simpson's) calculated and (c) weighted UniFrac PCoA analysis presented. (d) Effects of 17-AAG on biofilm formation and (e) growth rate of *S. aureus* ATCC 25,923 and RA532, expressed as min-max range and mean $\pm$ SD, respectively. (f) Relative abundances of identified phyla and genera in gut microbiome samples from naïve, vehicle-treated, and 17-AAG-treated AD mice, with (g) alpha diversity indices (Shannon, Chao1, and Simpson's) calculated and (h) weighted UniFrac PCoA analysis presented. Data expressed as mean (min-max range) or mean $\pm$ SD, respectively. Taxa with mean abundances < 1% across all groups are aggregated as "Low abundance." * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; OD, optical density.

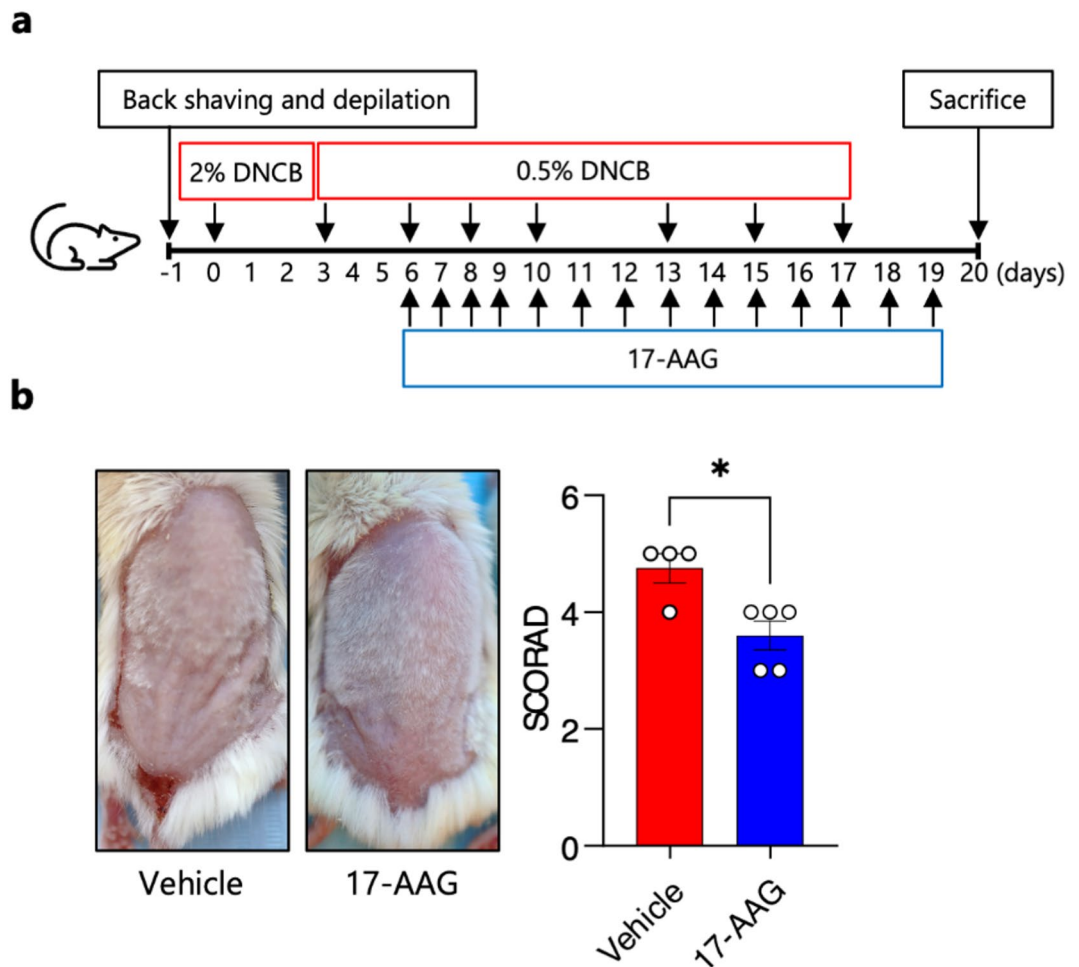
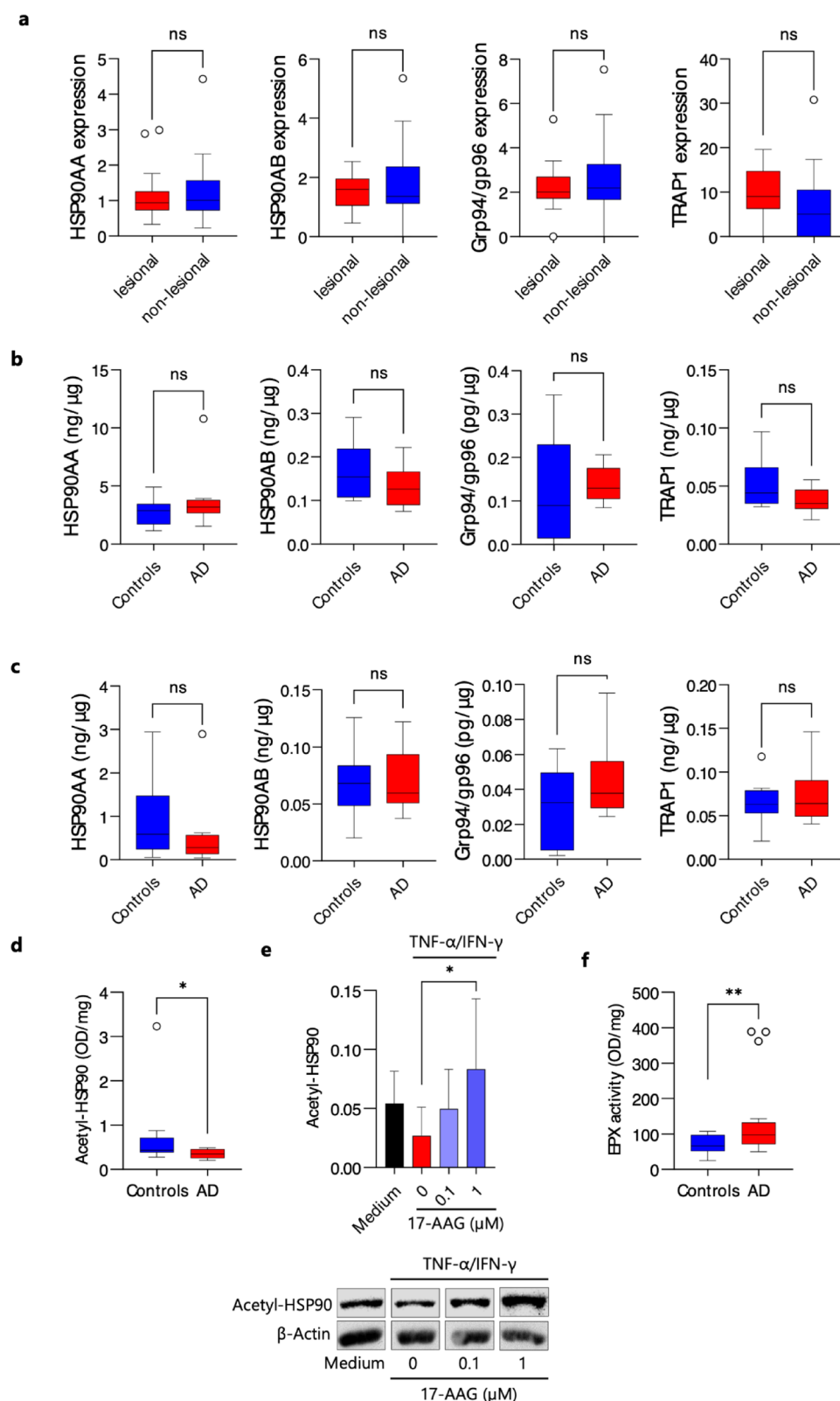


Fig. 5. Therapeutic treatment with topically applied 17-AAG reduces disease activity in an experimental mouse model of DNCB-induced human-like AD. **(a)** Schematic representation of the experimental design. AD-like skin inflammation was induced in female BALB/c mice by applying dinitrochlorobenzene (DNCB) to the shaved and depilated back skin (day – 1) as follows: 2% DNCB on day 0, followed by 0.5% DNCB on days 3, 6, 8, 10, 13, 15 and 17. 17-AAG (0.1 μ M) or vehicle (DMSO: acetone, 1:40 vol/vol) was applied daily, with a 2-hour interval following DNCB application. **(b)** Representative images of vehicle- and 17-AAG-treated mice, and clinical disease severity, represented by the cumulative SCORAD index at day 20. Data are expressed as mean $\pm$ SEM (with individual values) from one experiment. * $P < 0.05$.

inhibits the canonical function of an important molecular chaperone. Therefore, we hypothesize that targeting the bacterial HSP90 homolog, HtpG, may disrupt chaperone functions that are necessary for the production or stability of proteins involved in biofilm formation. There is evidence that 17-DMAG, another HSP90 inhibitor, disrupts *Candida albicans* biofilm formation to a degree comparable to the tetO-Hsp90/hsp90 mutant strain³⁴. Additionally, blocking HSP60 with anti-HSP60 mAb effectively reduced biofilm formation in another fungus, *Histoplasma capsulatum*³⁵. While evidence from bacteria is scarce, *Pseudomonas aeruginosa* DnaJ (HSP40) deficient mutant showed significantly reduced initial adhesion and biofilm formation, likely due to the impaired pyocyanin synthesis and decreased production of extracellular DNA³⁶. Moreover, HSP inhibitors (apoptozole and nonactin) in combination with chlorin-e6 associated photodynamic therapy, inhibited both mono- and multi-species biofilm formation and suppressed the vitality of *Streptococci* synergistically³⁷. Recent studies also demonstrated the secretion of DnaK (HSP70) and GroEL (HSP60) proteins into the extracellular space during biofilm growth in *Acinetobacter baumannii*³⁸. Secondly, one study showed that 17-AAG, in combination with azoles, could inhibit the formation of azole-resistant fungal biofilms³⁹. The same study theorised that by inhibiting ATPase activity in fungal cells, 17-AAG may also block drug efflux pumps on the fungal cell membrane³⁹. Perhaps in a similar fashion, impairing energy-dependent efflux mechanisms could sensitise *S. aureus* cells and biofilms. One such target could be the NorA efflux pump, which actively expels antibiotics and exports quorum-sensing molecules, and was shown to be successfully targeted by efflux pump inhibitors, which led to biofilm inhibition⁴⁰.

Tape strips are widely used in dermatological research as a minimally invasive method for sampling the epidermis, eliminating the need for skin biopsies⁴¹. In this study, we analyzed the gene expression of four HSP90 homologs using tape-strip samples collected from patients with AD, from both non-lesional and lesional skin



areas. We did not observe significant changes in the expression of the analyzed HSP90 homologs between lesional and non-lesional skin samples. Similarly, there were no significant differences in the level of HSP90 protein isoforms in PMNs and PBMCs between patients with AD and age- and gender-matched healthy individuals.

The primary goal of therapies using HSP90 inhibitors, such as 17-AAG, is to inhibit the chaperone activity of HSP90, thereby disrupting the function of its client proteins⁵. In fact, the activity of HSP90 is regulated by multiple mechanisms, including post-translational modifications such as acetylation³. Therefore, we determined the level of HSP90 acetylation (acetyl-HSP90) in leukocytes from patients with AD and age- and gender-matched healthy controls. Notably, we observed a significantly lower level of acetyl-HSP90 in the PBMCs of individuals with AD

◀ **Fig. 6.** Elevated Activity of HSP90 and EPX in Leukocytes of Individuals with AD. (a) Relative HSP90 homolog expression measured in duplicate from 10 consecutive tape strips sampled from lesional and non-lesional skin of patients with AD. Data are expressed as mean $\pm$ SEM (with individual values). (b) HSP90 isoform level relative to total protein content of peripheral blood mononuclear cells. (c) HSP90 isoform level relative to total protein content of polymorphonuclear leukocytes. Both cell populations were derived from patients with AD and age- and gender-matched healthy individuals. Data are expressed as box-and-whisker plots (Tukey). (d) ELISA results showing acetyl-HSP90AA levels in PBMCs from AD patients compared to age- and gender-matched healthy controls. (e) β -Actin-corrected levels of acetyl-HSP90 in activated (TNF- α /IFN- γ , 10 ng/mL) human keratinocyte (HaCaT) cells treated with 0.1% DMSO or non-toxic concentrations of 17-AAG, analyzed by Western blot with representative bands presented. (f) Eosinophil peroxidase (EPX) activity in PMNs from AD patients and age- and gender-matched healthy controls. Data are expressed as box-and-whisker plots (Tukey) or mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$. ns, no significance.

compared to healthy controls. This reduced level of acetylation suggests a higher chaperone activity of HSP90 in AD⁴². The evidence for this relationship is the observation that the presence of 17-AAG in cultures of human keratinocytes led to increased levels of acetyl-HSP90. Finally, we found elevated eosinophil peroxidase (EPX) activity in the PMNs of individuals with AD compared to healthy controls. The latter observation underscores the role of EPX in AD⁴³ and simultaneously suggests that it may be a potential therapeutic target for treating AD with 17-AAG, as demonstrated in the murine model of AD in this study.

Conclusions

In conclusion, based on our comprehensive preclinical observations regarding the efficacy of topical HSP90 inhibitor therapy in alleviating AD symptoms, as well as its impact on the immune system and microbiome, we anticipate that clinical trials involving AD patients or related diseases will soon follow. It is important to note that therapy utilizing HSP90 inhibitors is not limited solely to diseases characterized by increased expression of HSP90 family proteins. Our research suggests that the activity of these chaperones plays a crucial role, aligning with the broader concept of anti-HSP90 therapy, which aims to reduce HSP90 activity without altering its expression levels. Our findings are highly promising for future clinical studies, as the effective dose of the 17-AAG inhibitor was proven to be completely safe. Finally, based on the results of this study, we suggest that the primary cellular target of therapy with 17-AAG are epidermal cells, as indicated by both cell culture and animal model studies. Our conclusions are supported by observations such as the reduction in epidermal thickness in response to treatment, without significant changes in skin infiltration by leukocytes, including helper T cells, mast cells, and eosinophils. However, the activity of these leukocytes was significantly inhibited, therefore it appears that topical application of 17-AAG requires further investigation to evaluate its potential as a monotherapy for managing AD.

Limitations

Although our findings from both in vitro studies and the DNCB-induced AD model, along with the observed increase in HSP90 and EPX activity in the leukocytes of the analyzed cohort of AD patients, support the potential use of HSP90 inhibitors in treating individuals with AD, several issues need to be considered when planning future studies. Given the heterogeneous nature of AD, other mouse models (e.g., MC903-induced, house dust mite, and genetic models) that replicate the cellular and molecular aspects of the disease in different ways should be explored. The use of alternative HSP90 inhibitors and combination therapies should also be considered. Finally, based on next-generation sequencing and bioinformatic microbiome analyses, it appears that the topical application of 17-AAG partially restores the normal gut microbiome, while its effect on the skin microbiome seems limited to a few bacterial genera, rather than impact broad compositional metrics. We speculate that this intriguing result may be due to the therapy's specific impact on the formation of *S. aureus* biofilm, which is one of the key factors contributing to the exacerbation of atopic dermatitis symptoms, or possibly due to the inhibition of peripheral inflammatory processes mediated by eosinophils, which positively influence the gut microbiome. However, further studies are needed to confirm this hypothesis.

Materials and methods

Patients

This study included 19 patients with active AD, consisting of 13 males and 6 females, with a mean age of 26.8 years (range: 8–54 years). Patients were recruited from the outpatient Dermatology, Venerology, and Allergy clinics at the Medical University of Gdańsk, based on the AD diagnostic criteria proposed by Hanifin and Rajka (1980). All participants were of European descent. Patients receiving systemic immunosuppressive treatment, immunotherapies, or phototherapy, as well as those with skin infections, were excluded from the study. Written informed consent was obtained from all participants or their legal guardian for underage subjects. This research was conducted in accordance with the Declaration of Helsinki and was approved by the Independent Bioethics Committee for Scientific Research at the Medical University of Gdańsk, Poland. For patient details, see **Table S3**.

Animals

Female Balb/c mice (6–8 weeks old) were obtained from the Tri-City University Animal Facility – Research Service Center (Poland). The mice went through a handling period and were housed in a ventilated animal room with *ad libitum* access to food and tap water.

Ethical statement for animal use

The study protocol was approved by the Animal Care and Use Committee in Bydgoszcz, Poland. All experiments were performed by qualified personnel in accordance with National Animal Care and Use Committee guidelines as specified in Polish law by The Act on the Protection of Animals (Dz.U. 1997 nr 111 poz. 724) and European law by the Act on the Protection of Animals Used for Scientific or Educational Purposes (2010/63/EU). The research facility animals were housed in complied with the requirements of the The European Act on the Protection of Animals Used for Scientific or Educational Purposes and adhered to the European Commission's recommendations on animal welfare in scientific research.

AD induction

The induction of AD in mice using topical application of 2,4-dinitrochlorobenzene (DNCB) followed a previously published protocol¹¹. Briefly, approximately 6 cm² of back skin was shaved and depilated one day before initiating disease induction. DNCB (237329, Merck) was dissolved in an acetone: olive oil mixture (3:1 v/v), and 200 µl of a 1% DNCB solution was applied topically to the back skin on days 0 and 3. This was followed by applications of 200 µl of a 0.4% DNCB solution on days 6, 7, 10, and 13. The experiment was terminated on day 14 (Fig. 1a). Mice were euthanized according to American Veterinary Medical Association guidelines, by cervical dislocation under deep anesthesia (Ketamine-Xylazine).

Prophylactic treatment and clinical outcome analysis

A stock solution of 17-AAG (S1141, Selleckchem) was prepared in DMSO (D2650, Sigma-Aldrich) and freshly diluted in a DMSO: acetone solvent (1:40 v/v) to a concentration of 0.5 µM. A volume of 200 µl of this prepared solution was applied to the shaved back skin of the animals (Fig. 1a). The severity of clinical symptoms was assessed on days 7 and 14 using a modified Scoring Atopic Dermatitis (SCORAD) system, which evaluates four skin parameters (i.e., erythema, edema, excoriation, and scaling/dryness), each rated on a four-point scale, yielding a maximum score of 12, as previously described¹¹.

Therapeutic treatment and clinical outcome analysis

Starting on day 6, mice were treated topically with either 17-AAG (0.1 µM) or vehicle, and their condition was monitored until day 20 (Fig. 5a). The severity of clinical symptoms was assessed on day 20 using a modified Scoring Atopic Dermatitis (SCORAD) system, as previously described⁴⁴.

Histopathology

Lesional skin samples were prepared for histopathological examination as previously described⁹. Briefly, biopsy specimens from the experimental animals were collected on day 14 of the experiment, fixed in 4% buffered formalin, and embedded in paraffin. Tissue Sect. (6 µm thick) were stained with hematoxylin and eosin (H&E) to evaluate general morphology, or with 0.01% toluidine blue for mast cell detection. Epidermal thickness, and mast cell count were measured using a light microscope at 100× magnification in six randomly selected areas per slide.

Blood morphology

Blood samples were collected on day 14, prior to euthanasia of the animals. Fresh, EDTA-anticoagulated blood from naïve, vehicle- (DMSO: acetone, 1:40 v/v) or 17-AAG-treated AD mice, was analyzed using a 5160VET analyzer (URIT, China). The analysis included the number and characteristics of selected morphotic elements, as well as the percentages (%) or numbers (#) of specific cell subgroups. Table S1 provides more details.

Biochemical safety analysis

Blood samples were collected on day 14, prior to euthanasia of the animals. Fresh, EDTA-anticoagulated blood from naïve, vehicle (DMSO: acetone, 1:40 v/v)-treated AD, and 17-AAG-treated mice, was analyzed using the Exigo C-200 analyzer (Boule Diagnostics AB, Sweden) using C200 comprehensive panel (1430002, Alpha Diagnostics). Table S2 provides more details.

Microbiome analysis

Stool and skin swab samples were collected on day 14, prior to euthanasia, and processed by an external specialized company (Genomed S.A., Poland). Sequence analysis of the bacteria and archaea present in the samples was performed on the V3-V4 region of the 16 S rRNA gene using the 341f/785r primer pair. PCR amplification was conducted with Q5 Hot Start High-Fidelity 2X Master Mix (NEB, USA) following the manufacturer's instructions. Sequencing was carried out on the MiSeq platform using paired-end technology (2 × 300 nt) with the v3 Illumina reagent kit (Illumina Inc., USA). Demultiplexing and FASTQ generation were performed using MiSeq Reporter (MSR v2.6, USA). Samples were analyzed using Qiime 2⁴⁵ and Kraken 2⁴⁶, with additional filtering to remove OTUs corresponding to mitochondria, cyanobacteria, or chloroplasts, as previously described⁴⁷. Taxa with mean abundances < 1% across all groups were aggregated as "Low abundance".

Eosinophil peroxidase assay

Eosinophil peroxidase (EPX) activity was measured in mouse skin tissue extracts and human PMN extracts using a colorimetric assay on microplates, as previously described with minor modifications⁴⁸. The assay was performed in a final volume of 125 µL per well, consisting of 75 µL of OPD substrate solution containing 50 mM Tris-HCl, pH 8, 0.1% Triton X-100, 8.8 mM H₂O₂, 6 mM KBr, and 10 mM, o-phenylenediamine dihydrochloride (P1526, Merck) combined with 50 µL of the sample diluted in PBS (14190136, Thermo Fisher Scientific). The

reaction mixtures were incubated at 37 °C for 30 min, followed by the addition of 50 μ L of 2 N H₂SO₄ to stop the reaction. The absorbance was measured at 490 nm using a Synergy H1 Plate Reader (BioTek, USA).

Enzyme-linked immunosorbent and colorimetric assays

Detection of the acetylated form of HSP90 (acetyl-HSP90) was performed using an in-house ELISA. Briefly, a high-binding 96-well plate (NEST, China) was coated overnight at 4 °C with 50 μ L of 2 μ g/mL anti-Hsp90 antibodies (MABC1106, Merck) in 0.1 M Na-carbonate buffer (pH 9.6). All subsequent incubations were performed on an orbital shaker at 700 rpm. After blocking with Smartblock (113050, Candor) for 1 h at room temperature (RT), the plate was washed three times with PBS containing 0.05% Tween-20 (P1379, Merck). Cell extracts, diluted in blocking buffer, were then added to the plate and incubated for 2 h at RT. Following a washing step, anti-acetyl-Hsp90 antibodies (ABIN6285474, antibodies-online.com), diluted in blocking buffer, were added and incubated for 2 h at RT. After another washing step, HRP-conjugated secondary antibodies (ab205718, Abcam) were added and incubated for 1 h at RT. After washing, the substrate solution TMB (ab171522, Abcam) was added, and the reaction was stopped after 20 min with 0.5 M H₂SO₄. The absorbance was measured at 490 nm using a BioTek Synergy H1 Plate Reader (USA).

Commercially available ELISA-based kits were used following the manufacturer's protocols. These included the Human IgE Uncoated ELISA Kit (88-50610-88, Invitrogen), Human NFkB p65 (Phospho) [pS536] InstantOne™ ELISA Kit (85-86082, Invitrogen), Cell Proliferation ELISA, BrdU (11647229001, Roche), ELISA MAX™ Deluxe Set Human IL-6 (430504, BioLegend), ELISA MAX™ Deluxe Set Human IL-17 A (433914, BioLegend), ELISA MAX™ Deluxe Set Human IL-5 (430404, BioLegend), ELISA MAX™ Deluxe Set Human IL-22 (434504, BioLegend), as well as assays for Histamine (ab285333, abcam), HSP90AA (ADI-EKS-895, Enzo Life Sciences), HSP90AB (A314298, Antibodies.com), Grp94/gp96 (A74824, Antibodies.com), TRAP1 (A313169, Antibodies.com) and Pierce BCA Protein Assay Kit (23225, Thermo Fisher Scientific).

Tape strip analysis

Ten consecutive tape strips were collected from the same anatomical region of both lesional and non-lesional skin of AD patients and stored at −80 °C, as previously described⁴⁹.

Real-time qPCR analysis

The relative expression of selected genes in tape strips from AD patients and mouse skin biopsies was analyzed as described previously, with minor modifications¹¹. Total RNA was isolated from the samples using the RNeasy Mini Kit (74104, QIAGEN) and reverse-transcribed with the QuantiTect Reverse Transcription Kit (205311, QIAGEN). The resulting products were analyzed using the QuantiNova SYBR Green PCR Kit (208054, QIAGEN) on a LightCycler® 480 Instrument II (Roche, Switzerland). The relative expression of selected genes was analyzed as previously described¹¹. For mouse skin biopsy and tape strip analyses, three reference genes (*Gapdh*, *Actb*, and *Rpl13a*) and two reference genes (*Gapdh* and *Actb*) were chosen, respectively, using BestKeeper® software version 1.0⁵⁰. The cycling conditions were as follows: an initial denaturation step at 95 °C for 2 min, followed by 50 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 10 s, and a subsequent melting analysis. Each reaction was performed in duplicate, and the relative gene expression levels were analyzed using the 2^{−ΔΔCq} method. The primer sequences used for real-time qPCR are listed in Table S4.

Lymphocyte isolation

Peripheral blood mononuclear cells (PBMCs) and polymorphonuclear cells (PMNs) were isolated from the venous blood of AD patients (*n*=8) and healthy volunteers (*n*=9) using Histopaque-1077 (H8889, Sigma-Aldrich) and Histopaque-1119 (11191, Sigma-Aldrich) gradient centrifugation, respectively, according to the manufacturer's instructions. Human eosinophils and CD4⁺ T cells were isolated using the Eosinophil Isolation Kit (130-092-010, Miltenyi Biotec) and CD4⁺ T Cell Isolation Kit (130-096-533, Miltenyi Biotec), respectively, following the manufacturers' protocols. The purity of the isolated eosinophils and CD4⁺ T cells was assessed by staining with anti-CD16-FITC (302006, Biolegend) and anti-CD4-FITC (300538, Biolegend) antibodies, respectively, followed by flow cytometry analysis (CyFlow Cube 6 flow cytometer, Sysmex, Germany). The purity of the isolates was estimated to be approximately 95%.

Immune cell infiltrate analysis

Skin biopsies for flow cytometry analysis were performed as previously described with minor modifications⁵¹. Mice were euthanized on day 14, and lesional skin samples were collected and flash frozen in liquid nitrogen before storing in −80 °C. The frozen skin was cut into small pieces, resuspended in 2.5 mL of RPMI 1640 without phenol red (11835030, Thermo Fisher Scientific) containing 2 mg/mL type IV collagenase (C4-28-100MG, Merck) and 1 mg/mL DNase I (101041590001, Roche), and digested for 1 h at 37 °C on a magnetic stirrer. After digestion, the cell suspension was filtered through a 70 μ m strainer, diluted in FACS buffer: 0.5% BSA (05482, Sigma-Aldrich), 2 mM EDTA (115932805, Chempur), and centrifuged at 2000 rpm for 10 min at 4 °C. The skin cells were then resuspended in 1 mL of FACS buffer and stained with anti-CD45 clone I3/2.3 (147712, BioLegend), anti-CD4 clone GK1.5 (100406, BioLegend), and anti-Siglec-F clone S17007L (155504, BioLegend) antibodies for 30 min at room temperature. Viable single cells were analyzed based on forward and side light scatter properties using a CyFlow Cube 6 flow cytometer (Sysmex, Germany).

Reactive oxygen species release assay

ROS release assay was performed as described previously with minor modifications⁵². Purified eosinophils were resuspended in RPMI 1640 without phenol red (11835-030, Thermo Fisher Scientific), supplemented with 10% FBS (A3160801, Thermo Fisher Scientific), and plated onto a 96-well plate (Greiner Bio-One, Germany). The

cells were pre-incubated for 1 h, without or with varying concentrations of 17-AAG. Following pre-incubation, the cells were stimulated with either 2 μ M fMLP (F3506, Merck) or 100 ng/mL IL-33 (581802, Biolegend). Reactive oxygen species (ROS) release was immediately measured using luminol-enhanced chemiluminescence (123072, Merck) on an EnSpire Multimode Plate Reader (PerkinElmer, USA).

Cell cultures

Immortalized keratinocytes (HaCaT) were cultured in high-glucose DMEM (41966-029, ThermoFisher Scientific), supplemented with 10% heat-inactivated fetal bovine serum (A3160801, ThermoFisher Scientific) and 1% penicillin-streptomycin solution (15140122, ThermoFisher Scientific) in 75 cm² cell flasks or 96-well plates. The cultures were maintained in a humidified atmosphere containing 5% CO₂ at 37 °C. HaCaT cells were stimulated with 10 μ g/mL of IFN- γ and 10 μ g/mL of TNF- α once they reached 70–80% confluency, and subsequently cultured in the presence or absence of 17-AAG for either 1–24 h. Purified CD4⁺ T cells were resuspended at 1×10^6 cells per ml in RPMI 1640 (11835030, ThermoFisher Scientific) supplemented with 10% fetal bovine serum (A3160801, ThermoFisher Scientific) and 1% penicillin/streptomycin solution (15140122, ThermoFisher Scientific). The cells were stimulated with 1 μ g/mL immobilized anti-CD3 ϵ mAb (300414, BioLegend) and 1 μ g/mL soluble anti-CD28 mAb (302914, BioLegend), and cultured in the presence or absence of 17-AAG in 24-well culture plates at 5% CO₂ and 37 °C for 72 h.

Biofilm formation and growth of *Staphylococcus aureus*

Biofilm formation of two *Staphylococcus aureus* strains, methicillin-resistant RA532 and methicillin-sensitive ATCC25923, was assessed following a previously established protocol with minor modifications⁵³. Growth curve analysis was performed as described previously with minor modifications⁵⁴. To investigate whether 17-AAG affects biofilm formation, two strains of *S. aureus* were grown overnight at 37 °C with rotation, then diluted 1:100 in the media that in preliminary testing provided best biofilm formation for each strain i.e., LB (L3022, Merck) supplemented with 2 mg/mL glucose (G7021, Merck) and TSB (P-0120, BTL) with 10 mg/mL glucose (G7021, Merck), for ATCC25923 and RA532, respectively. Bacterial suspensions were added to a 96-well plate (701001, NEST), followed by the addition of 17-AAG. The plates were briefly mixed at 800 rpm and incubated for 72 h under stationary conditions at 25 °C. After incubation, the wells were washed with distilled water to remove any remaining planktonic cells or drug, then 0.1% crystal violet aqueous solution was added, and the plates were incubated at room temperature for 10 min, followed by another wash. Once the wells were completely dried, 33% acetic acid was added to each well, and the plate was shaken at 800 rpm for 10 min. Absorbance was measured at 595 nm using a Synergy H1 Plate Reader (BioTek, USA).

To determine whether 17-AAG affects bacterial growth, growth curve analysis was performed. Briefly, bacteria were grown overnight at 37 °C with rotation, then diluted 1:100 in LB media. Bacterial suspensions were added to a 96-well plate, and 17-AAG was introduced. The plates were placed in a Synergy H1 Plate Reader (BioTek, USA) for automated OD₆₀₀ measurements, taken every 0.5 h over a 9-hour period. During incubation, the temperature was maintained at 37 °C, with intermittent shaking between measurements.

Immunoblotting

The phosphorylation status of signaling proteins, i.e., STAT-1, STAT-3 and STAT-6, as well as HSP90 acetylation (acLys284/292), was analyzed in activated HaCaT cells cultured in the presence or absence of 17-AAG by immunoblotting. Equal amounts of cell lysates were separated on a polyacrylamide gel under denaturing conditions (SDS-PAGE) and transferred onto a nitrocellulose membrane (Bio-Rad). The membrane was incubated in blocking buffer (TBS) containing 3% non-fat milk, followed by incubation with primary antibodies targeting acetyl-HSP90 acLys284/292 (ABIN6285474, antibodies-online.com), p-STAT-1 (686401, BioLegend), p-STAT-3 (651001, BioLegend), p-STAT-6 (690101, BioLegend), and β -Actin (4970, Cell Signaling Technology) at a 1:1000 dilution. HRP-conjugated secondary antibodies, goat anti-mouse (405306, BioLegend) or anti-rabbit (A0545, Merck), were applied at a 1:2000 dilution. The Westar Supernova ECL substrate (XLS3,0100, Cyanagen) was used to visualize the protein bands. Protein levels, normalized to β -Actin, were quantified by densitometry using Image Lab 6.1.0 software (Bio-Rad, USA). Full-size scans of the exposure images corresponding to the blotting experiments presented in this study can be found in the supplementary materials (Fig. S5).

Statistical analysis

Microbiome data processing and statistical analysis were performed using RStudio (PBC, Boston, MA) and GraphPad Prism software (GraphPad, San Diego, CA, USA). Data with non-normal distribution were analyzed using the Mann-Whitney U test, while normally distributed data were analyzed using Student's t-test. For comparisons involving three or more groups, either the Kruskal-Wallis test with Dunn's multiple comparisons or one-way ANOVA with Tukey's multiple comparisons test was applied. P-values less than 0.05 were considered statistically significant. Animal research data in the study was reported in accordance with ARRIVE 2.0 guidelines.

Data availability

The datasets generated and analyzed during the current study are available in the Zenodo repository, <https://doi.org/10.5281/zenodo.14524757>.

Received: 10 December 2024; Accepted: 2 June 2025

Published online: 01 July 2025

References

- Backe, S. J., Sager, R. A., Woodford, M. R., Makedon, A. M. & Mollapour, M. Post-translational modifications of Hsp90 and translating the chaperone code. *J. Biol. Chem.* **295**, 11099–11117. <https://doi.org/10.1074/jbc.REV120.011833> (2020).
- Li, Z. N. & Luo, Y. HSP90 inhibitors and cancer: prospects for use in targeted therapies (Review). *Oncol. Rep.* **49** <https://doi.org/10.3892/or.2022.8443> (2023).
- Stetz, G., Tse, A. & Verkhivker, G. M. Dissecting Structure-Encoded determinants of allosteric Cross-Talk between Post-Translational modification sites in the Hsp90 chaperones. *Sci. Rep.* **8**, 6899. <https://doi.org/10.1038/s41598-018-25329-4> (2018).
- Schopf, F. H., Biebl, M. M. & Buchner, J. The HSP90 chaperone machinery. *Nat. Rev. Mol. Cell. Biol.* **18**, 345–360. <https://doi.org/10.1038/nrm.2017.20> (2017).
- Tukaj, S. & Węgrzyn, G. Anti-Hsp90 therapy in autoimmune and inflammatory diseases: a review of preclinical studies. *Cell. Stress Chaperones*. **21**, 213–218. <https://doi.org/10.1007/s12192-016-0670-z> (2016).
- Tukaj, S. & Sitko, K. Heat shock protein 90 (Hsp90) and Hsp70 as potential therapeutic targets in autoimmune skin diseases. *Biomolecules* **12** <https://doi.org/10.3390/biom12081153> (2022).
- Kasperkiewicz, M. et al. Heat-shock protein 90 Inhibition in autoimmunity to type VII collagen: evidence that nonmalignant plasma cells are not therapeutic targets. *Blood* **117**, 6135–6142. <https://doi.org/10.1182/blood-2010-10-314609> (2011).
- Tukaj, S., Grüner, D., Zillikens, D. & Kasperkiewicz, M. Hsp90 Blockade modulates bullous pemphigoid IgG-induced IL-8 production by keratinocytes. *Cell. Stress Chaperones*. **19**, 887–894. <https://doi.org/10.1007/s12192-014-0513-8> (2014).
- Tukaj, S. et al. Topically applied Hsp90 blocker 17AAG inhibits Autoantibody-Mediated Blister-Inducing cutaneous inflammation. *J. Invest. Dermatol.* **137**, 341–349. <https://doi.org/10.1016/j.jid.2016.08.032> (2017).
- Sitko, K., Bednarek, M., Mantej, J., Trzeciak, M. & Tukaj, S. Circulating heat shock protein 90 (Hsp90) and autoantibodies to Hsp90 are increased in patients with atopic dermatitis. *Cell. Stress Chaperones*. **26**, 1001–1007. <https://doi.org/10.1007/s12192-021-01238-w> (2021).
- Sitko, K., Starke, M. & Tukaj, S. Heat shock protein 90 (Hsp90) inhibitor STA-9090 (Ganetespib) ameliorates inflammation in a mouse model of atopic dermatitis. *Cell. Stress Chaperones*. **28**, 935–942. <https://doi.org/10.1007/s12192-023-01387-0> (2023).
- Langan, S. M., Irvine, A. D. & Weidinger, S. Atopic dermatitis. *Lancet* **396**, 345–360. [https://doi.org/10.1016/s0140-6736\(20\)31286-1](https://doi.org/10.1016/s0140-6736(20)31286-1) (2020).
- Bregnhøj, A. et al. HSP90 inhibitor RGRN-305 for oral treatment of plaque-type psoriasis: efficacy, safety and biomarker results in an open-label proof-of-concept study. *Br. J. Dermatol.* **186**, 861–874. <https://doi.org/10.1111/bjd.20880> (2022).
- Ben Abdallah, H. et al. Efficacy and safety of the heat shock protein 90 inhibitor RGRN-305 in hidradenitis suppurativa: A Parallel-Design Double-Blind trial. *JAMA Dermatol.* **160**, 63–70. <https://doi.org/10.1001/jamadermatol.2023.4800> (2024).
- Riedl, R. et al. Establishment and characterization of mild atopic dermatitis in the DNCB-Induced mouse model. *Int. J. Mol. Sci.* **24** <https://doi.org/10.3390/ijms241512325> (2023).
- Lacy, P. et al. Divergence of mechanisms regulating respiratory burst in blood and sputum eosinophils and neutrophils from atopic subjects. *J. Immunol.* **170**, 2670–2679. <https://doi.org/10.4049/jimmunol.170.5.2670> (2003).
- Uehara, M., Izukura, R. & Sawai, T. Blood eosinophilia in atopic dermatitis. *Clin. Exp. Dermatol.* **15**, 264–266. <https://doi.org/10.1111/j.1365-2230.1990.tb02086.x> (1990).
- Rastogi, S. et al. An update on the status of HSP90 inhibitors in cancer clinical trials. *Cell. Stress Chaperones*. **29**, 519–539. <https://doi.org/10.1016/j.cstres.2024.05.005> (2024).
- Çetinarslan, T., Kümper, L. & Fölster-Holst, R. The immunological and structural epidermal barrier dysfunction and skin Microbiome in atopic dermatitis-an update. *Front. Mol. Biosci.* **10**, 1159404. <https://doi.org/10.3389/fmolb.2023.1159404> (2023).
- Liu, T., Zhang, L., Joo, D. & Sun, S. C. NF-κB signaling in inflammation. *Signal. Transduct. Target. Ther.* **2**, 17023. <https://doi.org/10.1038/sigtrans.2017.23> (2017).
- Han, J. et al. TMEM232 promotes the inflammatory response in atopic dermatitis via the nuclear factor-κB and signal transducer and activator of transcription 3 signalling pathways. *Br. J. Dermatol.* **189**, 195–209. <https://doi.org/10.1093/bjd/ljad078> (2023).
- Dajee, M. et al. Blockade of experimental atopic dermatitis via topical NF-κB decoy oligonucleotide. *J. Invest. Dermatol.* **126**, 1792–1803. <https://doi.org/10.1038/sj.jid.5700307> (2006).
- Tanaka, A., Muto, S., Jung, K., Itai, A. & Matsuda, H. Topical application with a new NF-κB inhibitor improves atopic dermatitis in nc/ngatnd mice. *J. Invest. Dermatol.* **127**, 855–863. <https://doi.org/10.1038/sj.jid.5700603> (2007).
- Howell, M. D. et al. Cytokine modulation of atopic dermatitis Filaggrin skin expression. *J. Allergy Clin. Immunol.* **120**, 150–155. <https://doi.org/10.1016/j.jaci.2007.04.031> (2007).
- Alvarenga, J. M., Bieber, T. & Torres, T. Emerging biologic therapies for the treatment of atopic dermatitis. *Drugs* <https://doi.org/10.1007/s40265-024-02095-4> (2024).
- Augustine, T., Kumar, M., Al Khodor, S. & van Panhuys, N. Microbial dysbiosis Tunes the immune response towards allergic disease outcomes. *Clin. Rev. Allergy Immunol.* **65**, 43–71. <https://doi.org/10.1007/s12016-022-08939-9> (2023).
- Ito, T. & Nakamura, Y. The skin barrier and Microbiome in infantile atopic dermatitis development: can skincare prevent onset? *Int. Immunol.* **36**, 579–584. <https://doi.org/10.1093/intimm/dxae038> (2024).
- McAleer, J. P. Obesity and the Microbiome in atopic dermatitis: therapeutic implications for PPAR-γ agonists. *Front. Allergy*. **4**, 1167800. <https://doi.org/10.3389/falgy.2023.1167800> (2023).
- Schachner, L. A. et al. A consensus on Staphylococcus aureus exacerbated atopic dermatitis and the need for a novel treatment. *J. Drugs Dermatol.* **23**, 825–832. <https://doi.org/10.36849/jdd.2024.8240> (2024).
- Amar, Y. et al. Microbial dysbiosis in a mouse model of atopic dermatitis mimics shifts in human Microbiome and correlates with the key pro-inflammatory cytokines IL-4, IL-33 and TSLP. *J. Eur. Acad. Dermatol. Venereol.* **36**, 705–716. <https://doi.org/10.1111/jdv.17911> (2022).
- Ignacio, A. et al. Small intestinal resident eosinophils maintain gut homeostasis following microbial colonization. *Immunity* **55**, 1250–1267e1212. <https://doi.org/10.1016/j.immuni.2022.05.014> (2022).
- Lee, S. Y., Lee, E., Park, Y. M. & Hong, S. J. Microbiome in the Gut-Skin Axis in atopic dermatitis. *Allergy Asthma Immunol. Res.* **10**, 354–362. <https://doi.org/10.4168/air.2018.10.4.354> (2018).
- Rosenberg, H. F., Masterson, J. C. & Furuta, G. T. Eosinophils, probiotics, and the Microbiome. *J. Leukoc. Biol.* **100**, 881–888. <https://doi.org/10.1189/jlb.3RI0416> (2016).
- Becherelli, M., Tao, J. & Ryder, N. S. Involvement of heat shock proteins in Candida albicans biofilm formation. *J. Mol. Microbiol. Biotechnol.* **23**, 396–400. <https://doi.org/10.1159/000351619> (2013).
- Fregonezi, N. F. et al. Heat shock protein 60, insights to its importance in Histoplasma capsulatum: from biofilm formation to Host-Interaction. *Front. Cell. Infect. Microbiol.* **10** <https://doi.org/10.3389/fcimb.2020.591950> (2021).
- Zeng, B. et al. Heat shock protein DnaJ in Pseudomonas aeruginosa affects biofilm formation via pyocyanin production. *Microorganisms* **8**, 395. <https://doi.org/10.3390/microorganisms8030395> (2020).
- Zhang, Z. et al. Heat Shock Protein Inhibitors Show Synergistic Antibacterial Effects with Photodynamic Therapy on Caries-Related Streptococci In Vitro and In Vivo. *mSphere* **8**, e00679-00622, (2023). <https://doi.org/10.1128/msphere.00679-22>
- Massier, S. et al. Phosphorylation of extracellular proteins in Acinetobacter baumannii in sessile mode of growth. *Front. Microbiol.* **12** <https://doi.org/10.3389/fmicb.2021.738780> (2021).
- Liu, L. et al. A preliminary in vitro and in vivo evaluation of the effect and action mechanism of 17-AAG combined with Azoles against Azole-Resistant Candida spp. *Front. Microbiol.* **13** <https://doi.org/10.3389/fmicb.2022.825745> (2022).

40. Lu, X. et al. Efflux pump inhibitor combined with Ofloxacin decreases MRSA biofilm formation by regulating the gene expression of NorA and quorum sensing. *RSC Adv.* **13**, 2707–2717. <https://doi.org/10.1039/D2RA06696C> (2023).
41. Hughes, A. J., Tawfik, S. S., Baruah, K. P., O'Toole, E. A. & O'Shaughnessy, R. F. L. Tape strips in dermatology research. *Br. J. Dermatol.* **185**, 26–35. <https://doi.org/10.1111/bjd.19760> (2021).
42. Liu, P. et al. Posttranslational modification and beyond: interplay between histone deacetylase 6 and heat-shock protein 90. *Mol. Med.* **27**, 110. <https://doi.org/10.1186/s10020-021-00375-3> (2021).
43. Sánchez, J. et al. Presence of IgE autoantibodies against eosinophil peroxidase and eosinophil cationic protein in severe chronic spontaneous urticaria and atopic dermatitis. *Allergy Asthma Immunol. Res.* **13**, 746–761. <https://doi.org/10.4168/air.2021.13.5.746> (2021).
44. Wu, C. S. et al. Phloretin alleviates dinitrochlorobenzene-induced dermatitis in balb/c mice. *Int. J. ImmunoPathol Pharmacol.* **34**, <https://doi.org/10.1177/2058738420929442> (2020).
45. Bolyen, E. et al. Reproducible, interactive, scalable and extensible Microbiome data science using QIIME 2. *Nat. Biotechnol.* **37**, 852–857. <https://doi.org/10.1038/s41587-019-0209-9> (2019).
46. Wood, D. E., Lu, J. & Langmead, B. Improved metagenomic analysis with kraken 2. *Genome Biol.* **20**, 257. <https://doi.org/10.1186/s13059-019-1891-0> (2019).
47. Benítez-Cabello, A., Romero-Gil, V., Medina-Pradas, E. & Garrido-Fernández, A. Arroyo-López, F. N. Exploring bacteria diversity in commercialized table Olive biofilms by metatranscriptomic and compositional data analysis. *Sci. Rep.* **10**, 11381. <https://doi.org/10.1038/s41598-020-68305-7> (2020).
48. Ochur, S. I. et al. The development of a sensitive and specific ELISA for mouse eosinophil peroxidase: assessment of eosinophil degranulation ex vivo and in models of human disease. *J. Immunol. Methods.* **375**, 138–147. <https://doi.org/10.1016/j.jim.2011.10.002> (2012).
49. Zysk, W., Sitko, K., Tukaj, S., Zaryczńska, A. & Trzeciak, M. Altered gene expression of IL-35 and IL-36a in the skin of patients with atopic dermatitis. *Int. J. Mol. Sci.* **25** <https://doi.org/10.3390/ijms25010404> (2023).
50. Pfaffl, M. W., Tichopad, A., Prgomet, C. & Neuvians, T. P. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper–Excel-based tool using pair-wise correlations. *Biotechnol. Lett.* **26**, 509–515. <https://doi.org/10.1023/b:bile.0000019559.84305.47> (2004).
51. Polakova, A. et al. Isolation of lymphocytes from human skin and murine tissues: A rapid and Epitope-Preserving approach. *JID Innovations.* **3** <https://doi.org/10.1016/j.xjidi.2022.100155> (2023).
52. Tukaj, S. et al. Evidence for a role of extracellular heat shock protein 70 in epidermolysis Bullosa acquisita. *Exp. Dermatol.* **31**, 528–534. <https://doi.org/10.1111/exd.14495> (2022).
53. Coffey, B. M. & Anderson, G. G. Biofilm formation in the 96-well microtiter plate. *Methods Mol. Biol.* **1149**, 631–641. https://doi.org/10.1007/978-1-4939-0473-0_48 (2014).
54. Stevenson, K., McVey, A. F., Clark, I. B. N., Swain, P. S. & Pilizota, T. General calibration of microbial growth in microplate readers. *Sci. Rep.* **6**, 38828. <https://doi.org/10.1038/srep38828> (2016).

Acknowledgements

We are grateful to Anna Zaryczńska (Department of Dermatology, Venereology and Allergology, Medical University of Gdansk) for her help in collecting tape strips from patients.

Author contributions

Conceptualization: ST; Formal Analysis: KS, EP, MP, NZ, MDS, ST; Funding Acquisition: ST; Methodology: KS, MP, ST; Investigation: KS, EP, MDS, MT, ST; Project Administration: ST; Resources: MP, MT, ST; Visualization: KS, ST; Validation: KS, ST; Writing - Original Draft Preparation: ST; Writing - Review and Editing: KS, EP, MP, MT, ST.

Funding

This study was supported by the Polish National Science Centre (NCN), grant no. 2020/39/B/NZ6/00357.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Independent Bioethics Committee for Scientific Research at the Medical University of Gdańsk, Poland (approval number NKBBN/209/2021). The animal study was reviewed and approved by the local authorities of the Animal Care and Use Committee in Bydgoszcz, Poland (approval number 53/2021).

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-025-05307-3>.

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Table S1.

Analyte	Naïve N = 5 ¹	Vehicle N = 5 ¹	0.5 µM 17-AAG N = 5 ¹
WBC	4.34 (4.02, 4.45)	3.82 (3.45, 4.60)	3.89 (3.45, 4.38)
LYM%	73 (69, 73)	↓52 (46, 53)	↓55 (54, 55)
MON%	6.08 (5.81, 6.78)	7.99 (7.16, 8.71)	8.00 (6.76, 9.18)
NEU%	17 (17, 18)	↓33 (33, 35)	↓30 (30, 32)
EOS%	4.48 (3.25, 4.81)	↓7.14 (6.43, 7.85)	*4.87 (4.22, 6.35)
BASO%	0.29 (0.21, 0.43)	0.39 (0.35, 0.39)	0.50 (0.48, 0.51)
LYM#	2.72 (2.59, 2.83)	↓1.55 (1.43, 1.67)	↓1.92 (1.54, 2.02)
MON#	0.23 (0.20, 0.24)	0.26 (0.25, 0.30)	0.25 (0.22, 0.34)
NEU#	0.71 (0.62, 0.79)	↓1.10 (1.03, 1.48)	1.06 (0.99, 1.18)
EOS#	0.17 (0.10, 0.18)	0.24 (0.20, 0.27)	0.17 (0.11, 0.24)
BASO#	0.011 (0.009, 0.013)	0.012 (0.010, 0.012)	0.018 (0.011, 0.022)
RBC	7.38 (7.26, 7.55)	↓7.09 (6.85, 7.15)	↓6.81 (6.52, 7.08)
HGB	14.15 (14.10, 14.25)	↓13.05 (13.00, 13.20)	↓13.35 (12.65, 13.45)
HCT	29.55 (28.80, 30.05)	↓27.85 (26.85, 28.00)	↓26.70 (25.30, 27.50)
MCV	40.10 (39.90, 40.20)	↓39.25 (38.80, 39.30)	↓38.95 (38.95, 39.30)
MCH	19.00 (18.95, 19.40)	18.40 (18.40, 18.90)	19.35 (19.15, 19.35)
MCHC	47.35 (47.05, 49.00)	47.10 (46.85, 48.95)	49.45 (49.00, 50.00)
RDW_CV	14.30 (13.85, 14.45)	15.05 (14.85, 15.30)	15.00 (14.50, 15.00)
PLT	951 (884, 985)	↓1,144 (1,134, 1,321)	↓1,295 (1,189, 1,296)
MPV	5 (4.9, 5.05)	4.9 (4.75, 4.9)	4.95 (4.8, 4.95)
NRBC%	1.56 (1.38, 1.62)	1.83 (1.71, 2.15)	2.48 (2.32, 2.73)
NRBC#	0.05 (0.05, 0.05)	0.08 (0.05, 0.09)	0.07 (0.06, 0.10)
ALY%	1.00 (0.90, 1.28)	↓0.59 (0.48, 0.65)	↓0.53 (0.43, 0.72)
ALY#	0.035 (0.030, 0.046)	↓0.020 (0.019, 0.023)	↓0.018 (0.014, 0.024)
LIC%	0.00 (0.00, 0.00)	↓0.33 (0.26, 0.37)	↓0.37 (0.16, 0.44)
LIC#	0.000 (0.000, 0.000)	↓0.009 (0.008, 0.016)	↓0.009 (0.006, 0.016)

¹ Median (Q1, Q3); P < 0.05 vs Naïve, * P < 0.05 vs Vehicle (ANOVA)

White Blood Cells (WBC), Lymphocytes (LYM%/#), Monocytes (MON%/#), Neutrophils (NEU%/#), Eosinophils (EOS%/#), Basophils (BASO%/#), Red Blood Cells (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW_CV), Platelets (PLT), Mean Platelet Volume (MPV), Nucleated Red Blood Cells (NRBC%/#), Large Immature Cells (LIC%/#), and Atypical Lymphocytes (ALY). Data represents one experiment, n = 5 per group. Values are expressed as the median, with the first and third quartiles (Q1, Q3) shown in brackets. Ordinary one-way ANOVA results with p-value > 0.05 are indicated where applicable: ↓, vs Naïve; *, vs Vehicle.

Table S2.

Analyte	Naïve N = 5 ¹	Vehicle N = 5 ¹	0.5 µM 17-AAG N = 5 ¹
ALB	28.20 (28.20, 28.70)	↓24.50 (23.90, 25.10)	↓23.80 (22.70, 24.50)
TP	50.40 (50.40, 50.70)	50.00 (49.90, 50.20)	47.90 (47.80, 50.90)
GLOB	22.30 (22.20, 23.40)	↓25.40 (25.00, 26.40)	↓25.20 (24.00, 26.40)
A/G	1.27 (1.23, 1.27)	0.97 (0.96, 0.97)	0.94 (0.93, 0.99)
TB	1.6 (1.1, 3.3)	↓0.1 (0.1, 0.4)	↓0.1 (0.1, 0.1)
GGT	2	2	2
AST	85 (84, 94)	87 (80, 107)	84 (81, 85)
ALT	23 (20, 26)	36 (23, 40)	27 (25, 35)
ALP	5	5	5
AMY	571 (525, 576)	↓474 (448, 476)	*549 (513, 558)
CREA	18.1 (12.0, 21.4)	11.3 (10.1, 14.7)	10.2 (10.0, 10.8)
UA	10 (10, 10)	15 (10, 34)	32 (19, 39)
UREA	8.37 (8.26, 8.80)	9.85 (9.55, 10.29)	7.95 (7.91, 9.53)
U/C	439 (375, 619)	↓790 (675, 911)	↓731 (575, 1,059)
GLU	10.82 (10.22, 14.16)	8.83 (7.68, 8.88)	7.87 (6.81, 11.03)
TC	2.47 (2.34, 2.48)	2.13 (2.09, 2.36)	2.35 (2.15, 2.40)
TG	0.88 (0.38, 1.10)	↓1.58 (1.48, 1.96)	1.42 (1.08, 1.74)

¹ Median (Q1, Q3); P < 0.05 vs Naïve, * P < 0.05 vs Vehicle (ANOVA)

Albumin (ALB), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Amylase (AMY), Creatinine (CREA), Glucose (GLU), Gamma-Glutamyl Transferase (GGT), Total Bilirubin (TB), Cholesterol (TC), Triglycerides (TG), Total Protein (TP), Uric Acid (UA), Urea (UREA), Globulin (GLOB), Albumin/Globulin Ratio (A/G), and Urea/Creatinine Ratio (U/C). Data represents one experiment, n = 5 per group. Values are expressed as the median, with the first and third quartiles (Q1, Q3) shown in brackets. Ordinary one-way ANOVA results with p-value > 0.05 are indicated where applicable: ↓, vs Naïve; *, vs Vehicle.

Table S3.

Patient	Age	Sex	Onset	SCORAD	EASI	Eosino- philia	Comor-bidities	VAS	Sleep Score	Analyte
1	14	M	1 yo	Severe	Severe	No	Ast.	6	6	TS, PMNs, PBMCS
2	34	M	Early childhood	Moderate	Moderate	No	AR., Ast.	7	7	TS, PMNs, PBMCS
3	11	F	2 yo	Severe	Severe	Yes	AR., Ast.	5	10	TS, PMNs, PBMCS
4	15	F	Early childhood	Severe	Severe	No	AR., Ast., AC.	10	7	TS, PMNs, PBMCS
5	16	F	Early childhood	Moderate	Moderate	Yes	Ast.	6	4	TS
6	13	F	1 yo	Severe	Severe	Yes	AR.	5	0	TS, PMNs, PBMCS
7	54	M	Early childhood	Severe	Very severe	No		7	6	TS, PMNs, PBMCS
8	34	M	1 yo	Moderate	Moderate	Yes	AR.	3	3	TS, PMNs, PBMCS
9	35	M	Early childhood	NA	Mild	Yes		3	3	TS
10	34	M	1 yo	Severe	Severe	NA		8	8	TS, PMNs, PBMCS
11	54	F	6 mo	Severe	Severe	No	Ast.	7	7	TS
12	8	F	Early childhood	Severe	Severe	Yes	AR., AC., CA., OAS.	7	3	TS
13	32	M	Early childhood	Mild	Moderate	Yes	Ast.	2	1	TS
14	48	M	Early childhood	Severe	Severe	Yes	AR.	4	1	TS
15	14	M	3 mo	Severe	Severe	No	AR., Ast.	6	1	TS
16	34	M	> 1 yo	Severe	Severe	No	AR., Ast.	8	8	TS
17	21	M	> 1 yo	Severe	Severe	No	AR.	7	4	TS
18	22	M	7 mo	Severe	Very severe	Yes	Ast.	7	7	TS
19	16	M	> 1 yo	Severe	Severe	Yes	AR., Ast.	8	8	TS

SCORAD cutoff values: Mild (< 25); Moderate (25–50); Severe (> 50), EASI cutoff values: Clear (0); Almost clear (0.1–1.0); Mild (1.1–7.0); Moderate (7.1–21.0); Severe (21.1–50.0); Very severe (50.1–72.0), Ast., Asthma; AR, Allergic rhinitis; AC., Allergic conjunctivitis; CA., Contact allergy; OAS, Oral allergy syndrome; TS, Tape Strip; PMNs, Polymorphonuclear Cells; PBMCs, Peripheral Blood Mononuclear Cells; yo, years old; mo, months old; NA, missing data.

Table S4.

Target	Primer	Sequence (5'-3')
Mouse β -Actin (NCBI gene ID: 11461)	Forward	CTCTTCCAGCCTTCCTTCCT
	Reverse	AGCACTGTGTTGGCGTACAG
Mouse GAPDH (NCBI gene ID: 14433)	Forward	CATCACTGCCACCCAGAAGACTG
	Reverse	ATGCCAGTGAGCTTCCCGTTCAG
Mouse RPL13A (NCBI gene ID: 22121)	Forward	CTGCTCTCAAGGTTGTTCCGGCT
	Reverse	CCTTCCGTTTCTCCTCCAGAGT
Mouse TSLP (NCBI gene ID: 53603)	Forward	GCAAATCGAGGACTGTGAGAGC
	Reverse	TGAGGGCTTCTCTTGTTCCTCCG
Mouse IL-31RA (NCBI gene ID: 218624)	Forward	ACACCGAGTTGGAGAGCCGTAT
	Reverse	CTGTCCTCAGACCGATGTTCTC
Mouse IL-13 (NCBI gene ID: 16163)	Forward	GCCAGCCACAGTTCTAC
	Reverse	AGACCACCAAGGCAAGC
Mouse IL-4 (NCBI gene ID: 16189)	Forward	CGAAGAACACCACAGAGAGTGAGCT
	Reverse	GACTCATTTCATGGTGCAGCTTATCG
Mouse IL-6 (NCBI gene ID: 16193)	Forward	TGGAGTCACAGAAGGAGTGGCTAAG
	Reverse	TCTGACCACAGTGAGGAATGTCCAC
Mouse IL-10 (NCBI gene ID: 16153)	Forward	AGAAGCATGGCCCTGAAATCAAGG
	Reverse	CTTGTAGACACCTTGGTCTTGAG
Mouse IL-1 β (NCBI gene ID: 16176)	Forward	CAACCAACAAGTGATATTCTCCATG
	Reverse	GATCCACACTCTCCAGCTGCA
Mouse IFN γ (NCBI gene ID: 15978)	Forward	GGCCATCAGCAACAACATAAGCGT
	Reverse	TGGGTTGTTGACCTCAAAGTGGC
Mouse IL-33 (NCBI gene ID: 77125)	Forward	CTACTGCATGAGACTCCGTTCTG
	Reverse	AGAATCCCGTGGATAGGCAGAG
Mouse TNF α (NCBI gene ID: 21926)	Forward	CTGTAGCCACGTCGTAGC
	Reverse	TTGAGATCCATGCCGTTG
Human β -Actin (NCBI gene ID: 60)	Forward	CACCATTGGCAATGAGCGGTTTC
	Reverse	AGGTCTTTGCGGATGTCCACGT
Human GAPDH (NCBI gene ID: 2597)	Forward	GTCTCCTCTGACTTCAACAGCG
	Reverse	ACCACCCTGTTGCTGTAGCCAA
Human TRAP1 (NCBI gene ID: 10131)	Forward	CTCTGTGGAGACGGACATAGTC
	Reverse	CTCCTTCTCTGATAGGCACTCG
Human HSP90AB1 (NCBI gene ID: 3326)	Forward	CTCTGTCAGAGTATGTTTCTCGC
	Reverse	GTTTCCGCACTCGCTCCACAAA
Human HSP90B1 (NCBI gene ID: 7184)	Forward	GGAGAGTCGTGAAGCAGTTGAG
	Reverse	CCACCAAAGCACACGGAGATTC
Human Hsp90AA1 (NCBI gene ID: 3320)	Forward	TCTGCCTCTGGTGATGAGATGG
	Reverse	CGTTCCACAAAGGCTGAGTTAGC
Human TSLP (NCBI gene ID: 85480)	Forward	TATCTGGTGCCAGGCTATTTCG
	Reverse	TGAAGCGACGCCACAATCCTTG
Human IL-33 (NCBI gene ID: 90865)	Forward	GCCTGTCAACAGCAGTCTACTG
	Reverse	TGTGCTTAGAGAAGCAAGATACTC
Human FLG (NCBI gene ID: 2312)	Forward	GCTGAAGGAACTTCTGGAAAAGG
	Reverse	GTTGTGGTCTATATCCAAGTGATC

NCBI, National Center for Biotechnology Technology.

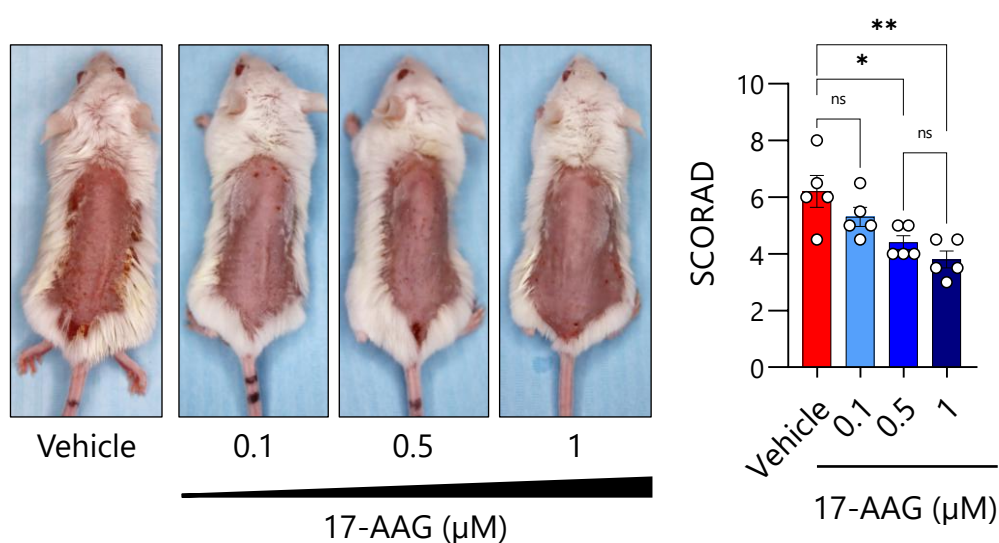


Figure S1. Topical 17-AAG treatment reduces disease activity in an experimental mouse model of DNCB-induced human-like AD in a dose-dependent manner. Representative images of AD mice treated with vehicle, 0.1 μM, 0.5 μM or 1 μM of 17-AAG, at the end of the 14-day treatment period and the clinical disease severity represented as cumulative SCORAD index. Data are expressed as mean ± SEM (with individual values, n = 5 per group). * P < .05, ** P < .01; ns, no significance.

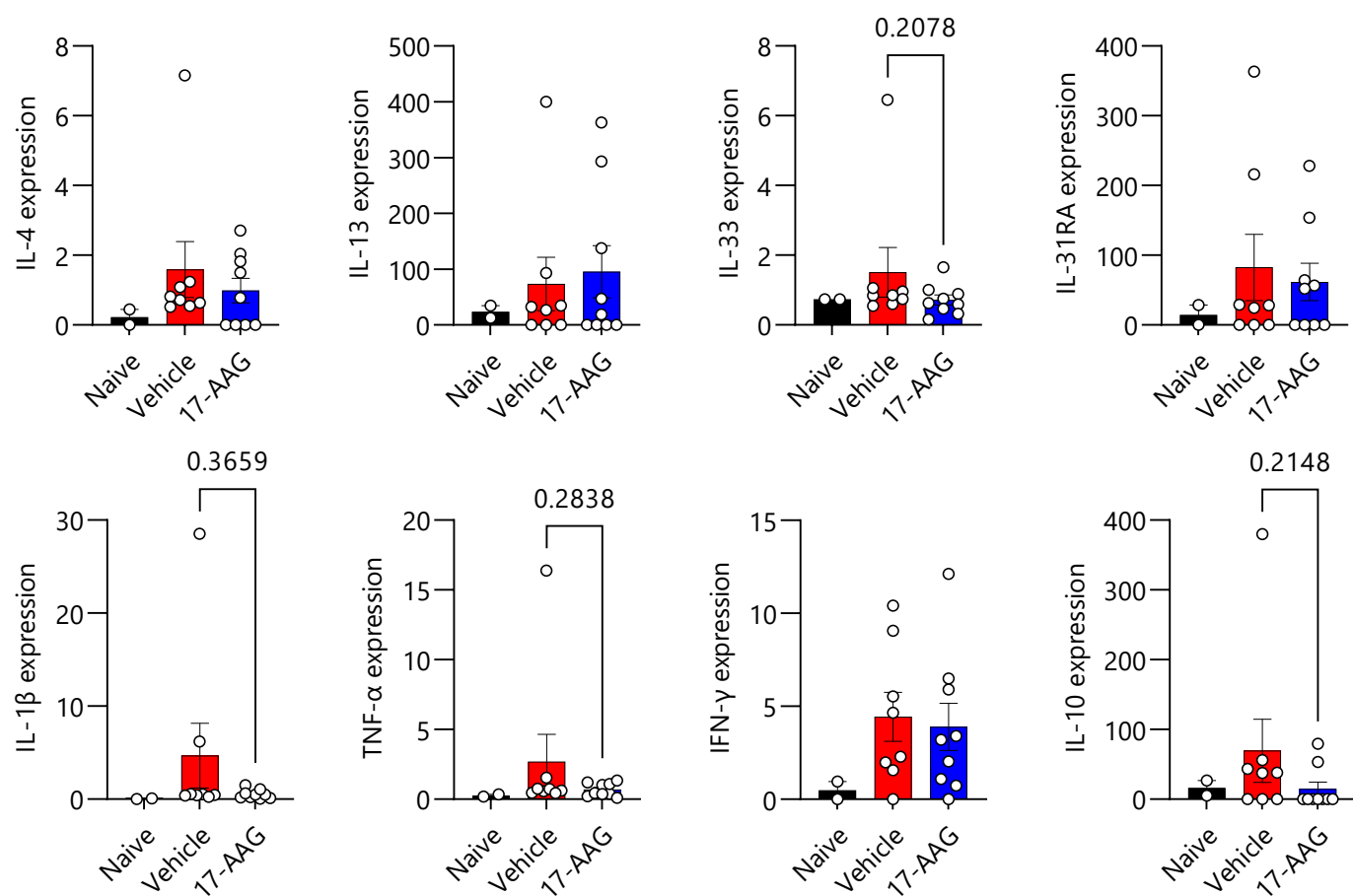


Figure S2. The relative expression of cytokines in the skin of naive, as well as vehicle- or 17-AAG-treated AD mice. Data represent three independent experiments and are expressed as mean $\pm$ SEM (with individual values). P-values from the trending Kruskal-Wallis test are indicated where applicable.

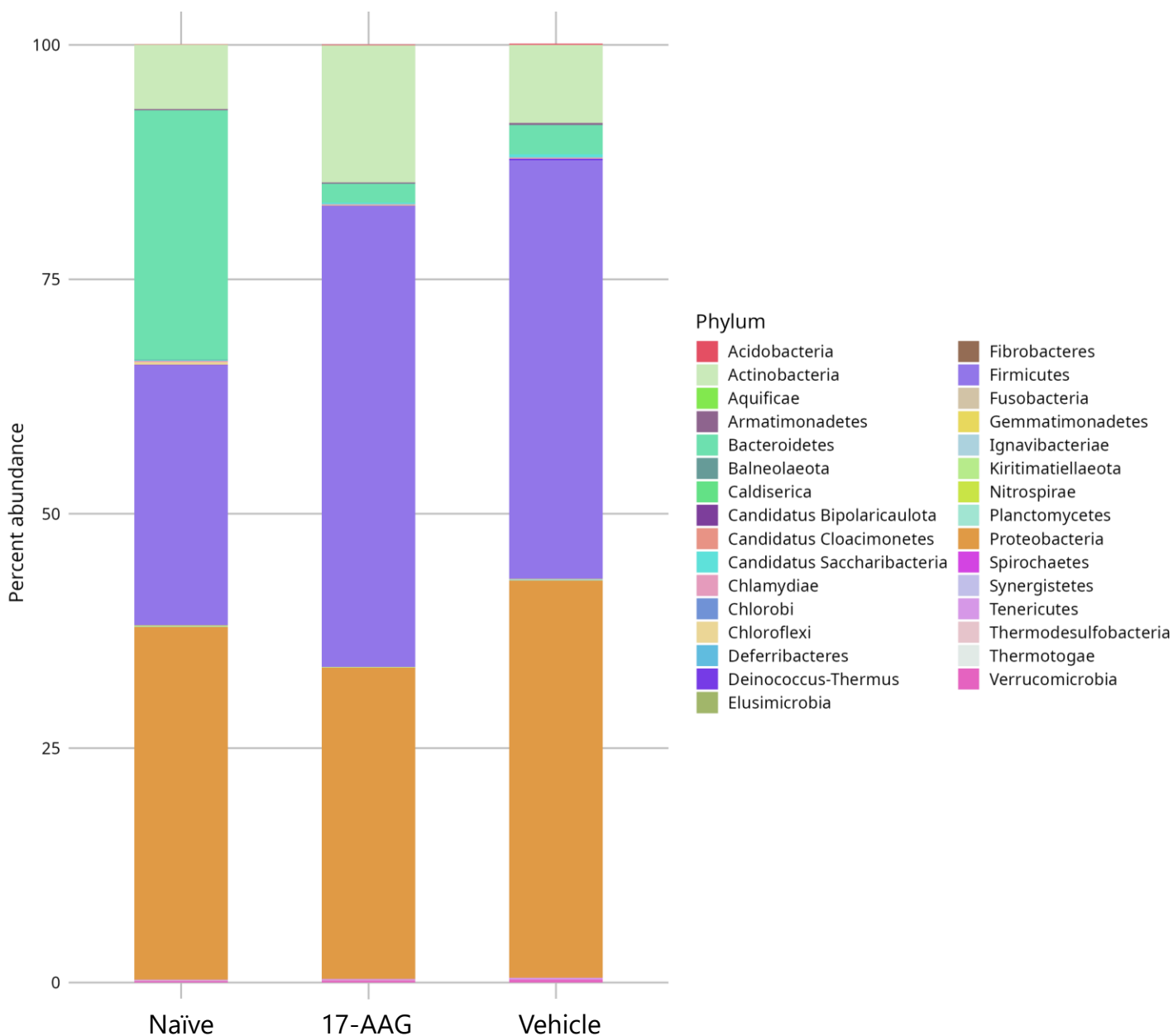


Figure S3. Phyla present in the skin microbiome of mice. Relative abundance (%) of phyla identified in the back skin microbiome samples from naïve, vehicle- and 17-AAG-treated AD mice. Data are representative of one experiment, n = 5 per group.

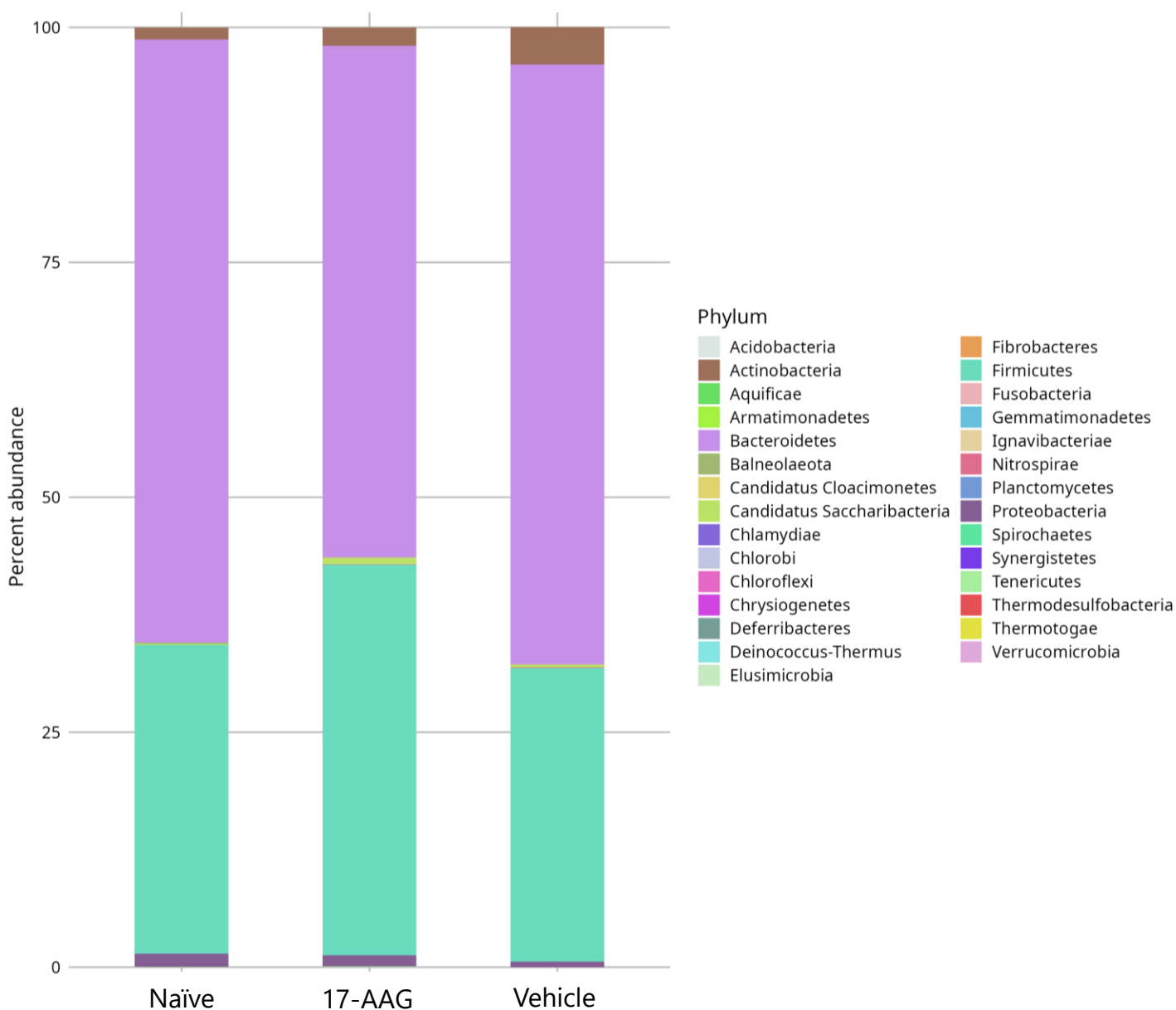


Figure S4. Phyla present in the gut microbiome of mice. Relative abundance (%) of phyla identified in the gut microbiome samples from naïve, vehicle- and 17-AAG-treated AD mice. Data are representative of one experiment, n = 5 per group.

Acetyl-Hsp90

p-STAT-1

p-STAT-6

β -Actin

p-STAT-3

β -Actin

TNF- α /IFN- γ

ctrl TNF/IFN 0.1 μ M 1 μ M

6

-100

-70

Hsp90 ac 24h

p-STAT-1 5.01.24

55.
Back
52.00
35.

ctrl Stim 0.1 μ M 1 μ M

ctrl TNF/IFN 0.1 μ M 1 μ M

p-STAT-3 21.81

ctrl TNF/IFN 0.1 1

20.

26.

56.04.54 β -actin

0 0 0 0.1 0.1 0.1 1 1 1

Medium

17-AAG (μ M)

24h

1h

Figure S5.

12. OŚWIADCZENIA WSPÓŁAUTORÓW

1. Praca pogładowa:

Heat Shock Protein 90 (HSP90) and HSP70 as Potential Therapeutic Targets in Autoimmune Skin Diseases

Stefan Tukaj, Krzysztof Sitko

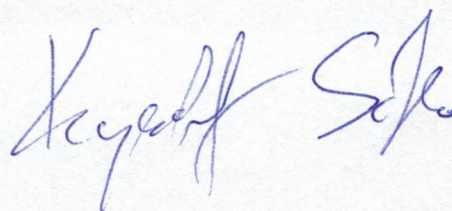
Biomolecules, 2022, doi: 10.3390/biom12081153

Gdańsk, 11.07.2025 r.

Mgr Krzysztof Sitko

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Heat Shock Protein 90 (HSP90) and HSP70 as Potential Therapeutic Targets in Autoimmune Skin Diseases", polegał na weryfikacji i redakcji oryginalnej wersji manuskryptu, przygotowaniu wizualizacji, odpowiedzi na pytania oraz uwagi recenzentów, a także przygotowaniu ostatecznej wersji manuskryptu.

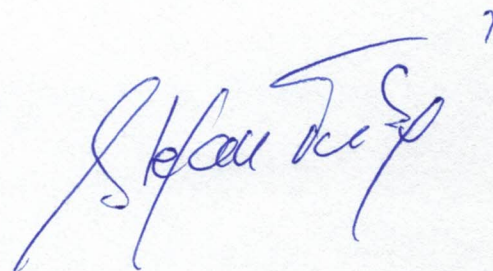
A handwritten signature in blue ink, reading "Krzysztof Sitko". The signature is written in a cursive, flowing style.

Gdańsk, 11.07.2025 r.

Prof. dr hab. Stefan Tukaj

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Heat Shock Protein 90 (HSP90) and HSP70 as Potential Therapeutic Targets in Autoimmune Skin Diseases", polegał na kierowaniu projektem i pozyskaniu funduszy, tworzeniu koncepcji publikacji, nadzorowaniu procesu tworzenia publikacji, tworzeniu wizualizacji, odpowiedzi na pytania oraz uwagi recenzentów oraz przygotowaniu pierwotnej i ostatecznej wersji manuskryptu.

A handwritten signature in blue ink, reading "Stefan Tukaj", with a small superscript "7" to its upper right.

2. Praca oryginalna:

Circulating heat shock protein 90 (HSP90) and autoantibodies to HSP90 are increased in patients with atopic dermatitis

Krzysztof Sitko, Marta Bednarek, Jagoda Mantej, Magdalena Trzeciak, Stefan Tukaj

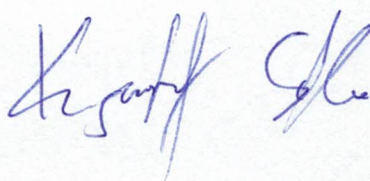
Cell Stress and Chaperones, 2021, doi: 10.1007/s12192-021-01238-w

Gdańsk, 11.07.2025 r.

Mgr Krzysztof Sitko

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Circulating heat shock protein 90 (HSP90) and autoantibodies to HSP90 are increased in patients with atopic dermatitis", polegał na opracowaniu metodologii, w tym, stworzeniu bazy danych i banku surowic, wykonaniu oznaczeń poziomu białka HSP90, przeciwciał anty-HSP90 i przeciwciał IgE, analizie statystycznej wyników, przygotowaniu wizualizacji, edycji i weryfikacji pierwotnej wersji manuskryptu, a także przygotowaniu ostatecznej wersji manuskryptu, odpowiedzi na pytania oraz uwagi recenzentów i redaktora czasopisma.

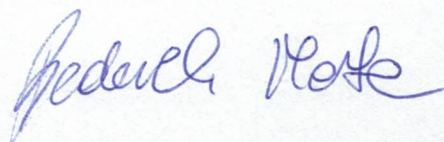


Gdańsk, 11.07.2025 r.

Dr Marta Bednarek

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Circulating heat shock protein 90 (HSP90) and autoantibodies to HSP90 are increased in patients with atopic dermatitis", polegał na przygotowaniu dokumentacji pacjentów i oznaczeniu poziomu przeciwciał anty-HSP90.



Gdańsk, 11.07.2025 r.

Dr Jagoda Mantej

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Circulating heat shock protein 90 (HSP90) and autoantibodies to HSP90 are increased in patients with atopic dermatitis", polegał na wykonaniu oznaczeń przeciwciał anty-HSP90.

Jagoda Mantej

Gdańsk, 11.07.2025 r.

Prof. dr hab. Magdalena Trzeciak

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Circulating heat shock protein 90 (HSP90) and autoantibodies to HSP90 are increased in patients with atopic dermatitis", polegał na udostępnieniu surowic i parametrów klinicznych pacjentów z AZS.

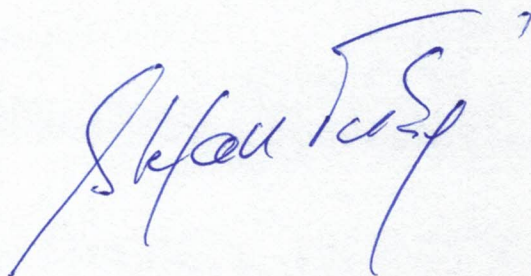
prof. dr hab. n. med. Magdalena Trzeciak
specjalista dermatolog - wenerolog
specjalista alergolog
324/606

Gdańsk, 11.07.2025 r.

Prof. dr hab. Stefan Tukaj

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Circulating heat shock protein 90 (HSP90) and autoantibodies to HSP90 are increased in patients with atopic dermatitis", polegał na kierowaniu projektem i pozyskaniu funduszy, tworzeniu koncepcji publikacji, nadzorowaniu procesu tworzenia publikacji, przygotowaniu wizualizacji, analizie statystycznej, odpowiedzi na pytania oraz uwagi recenzentów i redaktora czasopisma oraz przygotowaniu pierwotnej i ostatecznej wersji manuskryptu.



3. Praca oryginalna:

Differences in the detection of circulating HSP90 alpha between patients with atopic dermatitis and dermatitis herpetiformis

Krzysztof Sitko, Sarolta Kárpáti, Grzegorz Węgrzyn, Grzegorz Mincewicz, Magdalena Trzeciak, Michael Kasperkiewicz, Stefan Tukaj

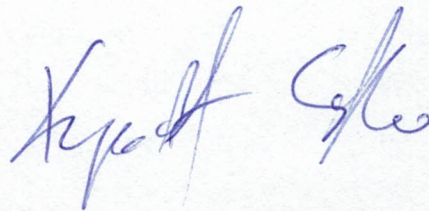
Frontiers in Medicine, 2024, doi: 10.3389/fmed.2023.1327144

Gdańsk, 11.07.2025 r.

Mgr Krzysztof Sitko

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Differences in the detection of circulating HSP90 alpha between patients with atopic dermatitis and dermatitis herpetiformis", polegał na opracowaniu metodologii, w tym, oznaczeniu poziomu przeciwciał anty-SARS-CoV-2, oznaczeniu poziomu białka HSP90, zarządzaniu danymi, wdrażaniu oprogramowania, przygotowaniu i walidacji wyników analizy statystycznej, przygotowaniu wizualizacji, edycji i redakcji pierwotnej wersji manuskryptu, a także przygotowaniu ostatecznej wersji manuskryptu, odpowiedzi na pytania oraz uwagi recenzentów i redaktora czasopisma.

A handwritten signature in blue ink, appearing to read 'Krzysztof Sitko', is positioned in the lower right area of the document.

Gdańsk, 11.07.2025 r.

Prof. dr hab. Grzegorz Węgrzyn

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Differences in the detection of circulating HSP90 alpha between patients with atopic dermatitis and dermatitis herpetiformis", polegał na edycji i redakcji pierwotnej wersji manuskryptu.

KIEROWNIK
KATEDRY BIOLOGII MOLEKULARNEJ

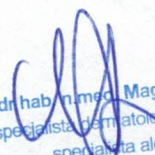
prof. dr hab. Grzegorz Węgrzyn

Gdańsk, 11.07.2025 r.

Prof. dr hab. Magdalena Trzeciak

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Differences in the detection of circulating HSP90 alpha between patients with atopic dermatitis and dermatitis herpetiformis", polegał na udostępnieniu surowic i parametrów klinicznych pacjentów z AZS.

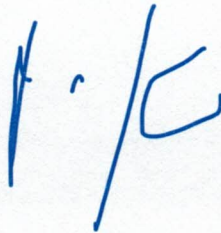

prof. dr hab. n. med. Magdalena Trzeciak
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specjalista alergolog
3561606

California, United States, 11.07.2025 r.

Michael Kasperkiewicz, MD

Author contributions

I declare that my contribution to the publication titled "Differences in the detection of circulating HSP90 alpha between patients with atopic dermatitis and dermatitis herpetiformis", included data interpretation and the review and editing of the final version of the manuscript.

A handwritten signature in blue ink, appearing to be 'M. K.', written in a stylized, cursive-like font.

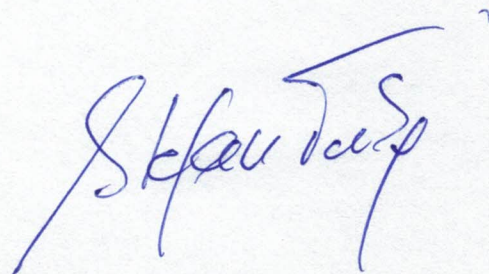
Gdańsk, 11.07.2025 r.

Prof. dr hab. Stefan Tukaj

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Differences in the detection of circulating HSP90 alpha between patients with atopic dermatitis and dermatitis herpetiformis", polegał na kierowaniu projektem i pozyskaniu funduszy, tworzeniu koncepcji publikacji, nadzorowaniu procesu tworzenia publikacji, prowadzeniu badań, opracowaniu metodologii, wdrożeniu oprogramowania, przygotowaniu i walidacji wyników analizy statystycznej, przygotowaniu wizualizacji, odpowiedzi na pytania oraz uwagi recenzentów oraz przygotowaniu pierwotnej i ostatecznej wersji manuskryptu.

Jednocześnie jako autor korespondencyjny oświadczam, że pozostali współautorzy Prof. Sarolta Kárpáti i dr hab. Grzegorz Mincewicz zaangażowani byli w udostępnieniu surowic pacjentów a także edycję i redakcję pierwotnej wersji manuskryptu. Ich oświadczenia nie zostały ujęte zgodnie z limitem pięciu oświadczeń współautorów dla prac wieloautorskich.



4. Praca oryginalna:

Heat shock protein 90 (HSP90) inhibitor STA-9090 (Ganetespib) ameliorates inflammation in a mouse model of atopic dermatitis

Krzysztof Sitko, Michał Starke, Stefan Tukaj

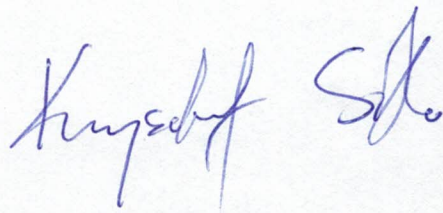
Cell Stress and Chaperones, 2023, doi: 10.1007/s12192-023-01387-0

Gdańsk, 11.07.2025 r.

Mgr Krzysztof Sitko

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Heat shock protein 90 (HSP90) inhibitor STA-9090 (Ganetespib) ameliorates inflammation in a mouse model of atopic dermatitis", polegał na opracowaniu metodologii, w tym, na wykonywaniu eksperymentów na modelu zwierzęcym, analizie parametrów klinicznych i histologicznych, oznaczeniu poziomu przeciwciał IgE i histaminy, analizie RT-qPCR, analizie statystycznej wyników, przygotowaniu wizualizacji, edycji i redakcji pierwotnej wersji manuskryptu, a także przygotowaniu ostatecznej wersji manuskryptu, odpowiedzi na pytania oraz uwagi recenzentów.

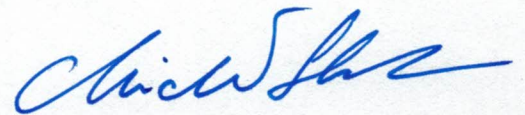
Handwritten signature of Krzysztof Sitko in blue ink.

Gdańsk, 11.07.2025 r.

mgr Michał Daniel Starke

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Topical Application of the HSP90 Inhibitor 17-AAG Reduces Skin Inflammation and Partially Restores Microbial Balance: Implications for Atopic Dermatitis Therapy", polegał na pomocy w przygotowaniu preparatów histologicznych.

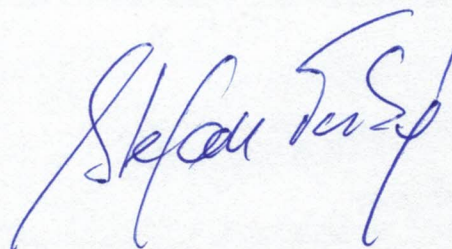


Gdańsk, 11.07.2025 r.

Prof. dr hab. Stefan Tukaj

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Heat shock protein 90 (HSP90) inhibitor STA-9090 (Ganetespib) ameliorates inflammation in a mouse model of atopic dermatitis", polegał na kierowaniu projektem i pozyskaniu funduszy, kierowaniu eksperymentami na modelu zwierzęcym, analizie parametrów klinicznych i histologicznych zwierząt, tworzeniu koncepcji publikacji, nadzorowaniu procesu tworzenia publikacji, przygotowaniu wizualizacji, analizie statystycznej wyników, odpowiedzi na pytania oraz uwagi recenzentów oraz przygotowaniu pierwotnej i ostatecznej wersji manuskryptu.

A handwritten signature in blue ink, reading "Stefan Tukaj". The signature is stylized and cursive.

5. Praca oryginalna:

Topical Application of the HSP90 Inhibitor 17-AAG Reduces Skin Inflammation and Partially Restores Microbial Balance: Implications for Atopic Dermatitis Therapy

Krzysztof Sitko, Ewa Piotrowska, Magdalena Podlacha, Natalia Zagórska, Michał Starke, Magdalena Trzeciak, Stefan Tukaj

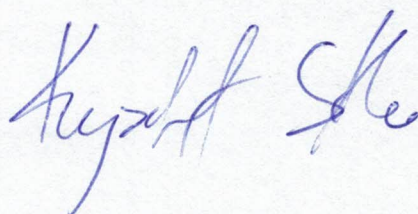
Scientific Reports, 2025, doi: 10.1038/s41598-025-05307-3

Gdańsk, 11.07.2025 r.

Mgr Krzysztof Sitko

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Topical Application of the HSP90 Inhibitor 17-AAG Reduces Skin Inflammation and Partially Restores Microbial Balance: Implications for Atopic Dermatitis Therapy", polegał na opracowaniu metodologii, w tym, wykonywaniu eksperymentów z wykorzystaniem zwierząt, analizie parametrów klinicznych i histologicznych, wykonaniu pomiarów morfologicznych i biochemicznych, oznaczeniu poziomu izoform i modyfikacji postranlacyjnych białek, wykonaniu testów ilościowych dla IgE, NFkB, BrdU, Histaminy, EPO, interleukin oraz białek szlaków sygnałowych, analizie RT-qPCR, analizie danych sekwencjonowania rRNA, analizie testów Tape-Strip, izolacji i hodowli komórek krwi obwodowej, pomiaru rekadywnych form tlenu, prowadzeniu hodowli komórkowych bakteryjnych oraz ludzkich, analizie biofilmu, tworzeniu i wdrażaniu oprogramowania, przygotowaniu i walidacji wyników analizy statystycznej, zarządzaniu danymi i zdeponowaniu ich w repozytorium, przygotowaniu wizualizacji, edycji i redakcji pierwotnej wersji manuskryptu, a także przygotowaniu ostatecznej wersji manuskryptu, odpowiedzi na pytania oraz uwagi recenzentów.



Gdańsk, 11.07.2025 r.

Dr Ewa Piotrowska

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Topical Application of the HSP90 Inhibitor 17-AAG Reduces Skin Inflammation and Partially Restores Microbial Balance: Implications for Atopic Dermatitis Therapy", polegał na pomocy w prowadzeniu hodowli keratynocytów.

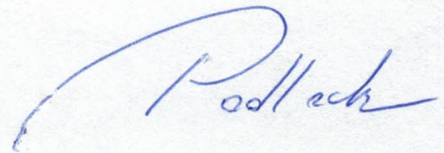
UNIwersytet Gdański
Katedra Biologii Molekularnej
dr Ewa Piotrowska

Gdańsk, 11.07.2025 r.

Dr hab. Magdalena Podlacha, Prof. UG.

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Topical Application of the HSP90 Inhibitor 17-AAG Reduces Skin Inflammation and Partially Restores Microbial Balance: Implications for Atopic Dermatitis Therapy", polegał na technicznej pomocy przy analizie morfologicznej i biochemicznej prób odzwierzęcych.

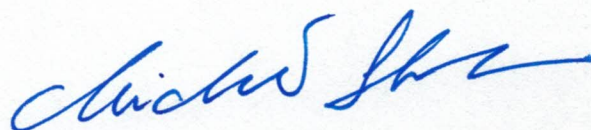


Gdańsk, 11.07.2025 r.

Mgr Michał Daniel Starke

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Heat shock protein 90 (HSP90) inhibitor STA-9090 (Ganetespib) ameliorates inflammation in a mouse model of atopic dermatitis", polegał na przygotowaniu preparatów histologicznych.



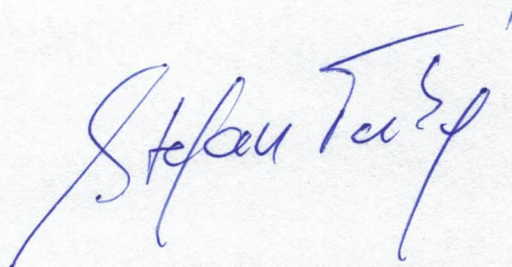
Gdańsk, 11.07.2025 r.

Prof. dr hab. Stefan Tukaj

Oświadczenie

Oświadczam, że mój wkład w powstanie pracy, pt. "Topical Application of the HSP90 Inhibitor 17-AAG Reduces Skin Inflammation and Partially Restores Microbial Balance: Implications for Atopic Dermatitis Therapy", polegał na kierowaniu projektem i pozyskaniu funduszy, tworzeniu koncepcji publikacji, nadzorowaniu procesu tworzenia publikacji, prowadzeniu badań, opracowaniu i nadzorowaniu wdrażania metodologii, przygotowaniu i walidacji wyników analizy statystycznej, przygotowaniu wizualizacji, odpowiedzi na pytania oraz uwagi recenzentów i redaktora czasopisma oraz przygotowaniu pierwotnej i ostatecznej wersji manuskryptu.

Jednocześnie jako autor korespondencyjny poświadczam, że wkład współautorki prof. dr hab. Magdalena Trzeciak dotyczył udostępnienia surowic i parametrów klinicznych pacjentów. Współautorka mgr Natalia Zagórska, zaangażowana była w oznaczenia ilości białek należących do szlaków sygnałowych. Ich oświadczenia nie zostały ujęte zgodnie z limitem pięciu oświadczeń współautorów dla prac wieloautorskich.



13. POZOSTAŁA AKTYWNOŚĆ NAUKOWA

Udzielone patenty:

W ramach rozprawy uzyskano patent nr RP.445930 (2023) autorstwa Stefana Tukaja i Krzysztofa Sitko o tytule „Inhibitory białka Hsp90 - tanespimycyna (17-AAG) lub ganetespib (STA-9090) do zastosowania w leczeniu atopowego zapalenia skóry u ludzi”.

Artykuły naukowe niewchodzące w skład rozprawy:

Tukaj, S., Sitna, M., Sitko, K. (2025) The impact of the mRNA COVID-19 vaccine on the Th-like cytokine profile in individuals with no history of COVID-19: insights into autoimmunity targeting heat shock proteins. *Frontiers in Immunology*, 16, 1549737. <https://doi.org/10.3389/fimmu.2025.1549739>

Sitko, K., Mantej, J., Bednarek, M., Tukaj, S. (2024). Detection of autoantibodies to heat shock protein 70 in the saliva and urine of normal individuals. *Frontiers in Immunology*, 15, 1454018. <https://doi.org/10.3389/fimmu.2024.1454018>

Zysk, W., Sitko, K., Tukaj, S., Zaryczańska, A., & Trzeciak, M. (2023). Altered Gene Expression of IL-35 and IL-36α in the Skin of Patients with Atopic Dermatitis. *International Journal of Molecular Sciences*, 25(1), 404. <https://doi.org/10.3390/ijms25010404>

Tukaj, S., Mantej, J., Sitko, K., Zillikens, D., Ludwig, R. J., Bieber, K., & Kasperkiewicz, M. (2022). Pathological Relevance of Anti-HSP70 IgG Autoantibodies in Epidermolysis Bullosa Acquisita. *Frontiers in Immunology*, 13, 877958. <https://doi.org/10.3389/fimmu.2022.877958>

Kosznik-Kwaśnicka, K., Podlacha, M., Łukasz Grabowski, Stasiłojć, M., Nowak-Zaleska, A., Ciemińska, K., Cyske, Z., Dydecka, A., Gaffke, L., Mantej, J., Myślińska, D., Necel, A., Pierzynowska, K., Piotrowska, E., Radzanowska-Alenowicz, E., Rintz, E., Sitko, K., Topka-Bielecka, G., Węgrzyn, G., & Węgrzyn, A. (2022). Biological aspects of phage therapy versus antibiotics against *Salmonella enterica* serovar Typhimurium infection of chickens. *Frontiers in Cellular and Infection Microbiology*, 12, 941867. <https://doi.org/10.3389/fcimb.2022.941867>

Mantej, J., Bednarek, M., Sitko, K., Świętoń, M., & Tukaj, S. (2021). Autoantibodies to heat shock protein 60, 70, and 90 are not altered in the anti-SARS-CoV-2 IgG-seropositive humans without or with mild symptoms. *Cell Stress and Chaperones*, 26(4), 735-740. <https://doi.org/10.1007/s12192-021-01215-3>

Tukaj, S., Mantej, J., Sitko, K., Bednarek, M., Zillikens, D., Ludwig, R. J., Bieber, K. & Kasperkiewicz, M. (2021). Evidence for a role of extracellular heat shock protein 70 in epidermolysis bullosa acquisita. *Experimental Dermatology*, 31(4), 528–534. <https://doi.org/10.1111/exd.14495>

Tukaj, S., Mantej, J., Sobala, M., Potrykus, K., & Sitko, K. (2020). Autologous extracellular HSP70 exerts a dual role in rheumatoid arthritis. *Cell Stress and Chaperones*, 25(6), 1105–1110. <https://doi.org/10.1007/s12192-020-01114-z>

Doniesienia konferencyjne o tematyce rozprawy:

Sitko, K., Starke M., & Tukaj, S. (2024). Topically applied HSP90 inhibitor 17-AAG ameliorates skin inflammation in a mouse model of atopic dermatitis. *European Society for Dermatological Research*. <https://doi.org/10.1016/j.jid.2024.10.298>

Sitko, K., Starke M., & Tukaj, S. (2024). Naskórnio aplikowany inhibitor białka HSP90 łagodzi stan zapalny w mysim modelu atopowego zapalenia skóry. *IV Kongres Młodej Nauki*.

Sitko, K., Trzeciak, M., & Tukaj, S. (2023). The role of heat shock protein 90 in atopic dermatitis. *5th Congress of Polish Biosciences BIO2023 "Different Faces of Biosciences"*. ISSN 1734-154X

Sitko, K., Bednarek, M., Mantej, J., Trzeciak, M., & Tukaj, S. (2022). Clinical significance of circulating heat shock protein 90 (HSP90) and autoantibodies to HSP90 in patients with atopic dermatitis. *International Sopot Youth Conference "Where the World Is Heading?"* ISBN 978-83-946541-6-0

Pozostałe doniesienia konferencyjne:

Zaryczańska, A., Sitko, K., Tukaj, S., & Trzeciak, M. (2023). The expression of IL-19 and IL-37 in the skin of atopic dermatitis patients. *13th Georg Rajka International Symposium on Atopic Dermatitis*. <https://doi.org/10.2340/actadv.v103.18470>

Sitko, K., Tukaj, S., Mantej, J., Sobala, M. & Potrykus, K. (2021). Autologous extracellular HSP70 exerts a dual role in rheumatoid arthritis. *XVII Congress of the Polish Society of Experimental and Clinical Immunology. Medical University of Białystok, Poland*.

Sitko, K., Tukaj, S., Mantej, J., MichałSobala, & Potrykus, K. (2021). Autologous extracellular HSP70 exerts a dual role in rheumatoid arthritis. *International Sopot Youth Conference "Where the World Is Heading?"* ISBN 978-83-946541-5-3

Starke, M., Sitko, K. & Stodolna, A. (2018). Kultury roślin *in vitro* w ochronie zagrożonych gatunków roślin. *IV Ogólnopolska Konferencja Dyplomatów Kierunków Przyrodniczych i Techniczno-Przyrodniczych BIOPOTENCJAŁ 2018*.