

IDENTIFICATION OF AKR101 AS POTENTIAL NEW DRUG FOR CHRONIC WOUND THERAPY

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BACKGROUND

Chronic wounds are a major health issue worldwide. Despite the high medical need, no new drugs have been approved in this field over the last >20 years.

Wound exudates (WEs) can transfer the clinical phenotype of healing or non-healing wounds onto primary human dermal fibroblasts in cell culture. In contrast to healing WEs, non-healing WEs impair fibroblast proliferation. In addition, WEs induce transcription of several genes involved in inflammation and in Endoplasmic Reticulum (ER)-stress¹.

We used this assay to screen substance libraries and identify compounds which can revert the negative effect of non-healing WEs and thus offer so far unknown potential for chronic wound therapy.

Ex vivo assay for personalized assessment of chronic wounds

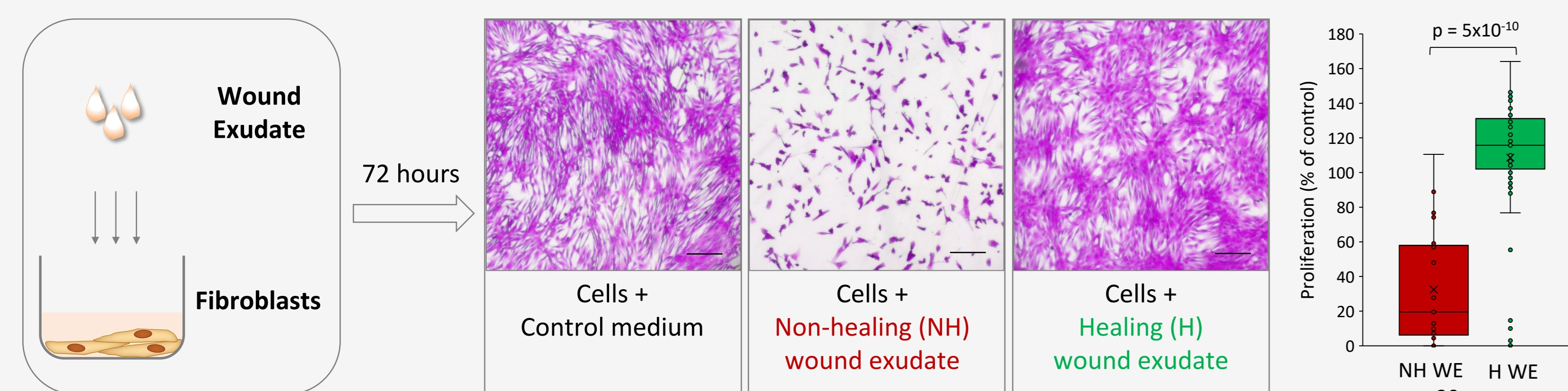


Figure 1: Fibroblast growth in the assay differentiates between exudates from non-healing and healing wounds¹.

METHODS

Patient material:

- Collection of WEs from healing or non-healing venous-, arterial-, mixed-, pressure- and diabetic ulcers, as well as trauma/surgery wounds was performed in accordance with the Declaration of Helsinki, including approval by Ethics Committees and patient consents. WEs were obtained from individual patients receiving negative pressure wound therapy (NPWT, from sponges or gel-free containers) or by using nylon-flocked swabs.

Cell culture and incubation with WEs:

- Cell proliferation: Primary human dermal fibroblasts (HDF) from healthy donors were seeded at a density of 2500 cells/well in 384 well plates and incubated with medium +/- sterile-filtered WEs. Proliferation (after 72 hours) and FDM (after 8 days) was assessed after fixation with 4% paraformaldehyde and staining with Sulforhodamine B. Production of ECM proteins and inflammatory cytokines after 72 hours was assessed by qRT-PCR. Expression of inflammatory cytokines after 6 hours was assessed by mRNA sequencing.

Abbreviations:

ECM, extracellular matrix; FDM, fibroblast-derived matrix; H, healing, NH, non-healing; WE, wound exudate; PDGF: Platelet-Derived Growth Factor.

References:

- Doerfler P, et al. (2024) "Development of a cellular assay as a personalized model for testing chronic wound therapeutics." *J Invest Dermatol*, <https://doi.org/10.1016/j.jid.2024.05.029>.

RESULTS

AKR101 rescues fibroblasts from chronic wound exudate-induced damage

In the *ex vivo* chronic wound assay, AKR101 restored fibroblast proliferation in the presence of non-healing wound exudates. The AKR101 effect was dose-dependent with ED₅₀ values of 12-33nM.

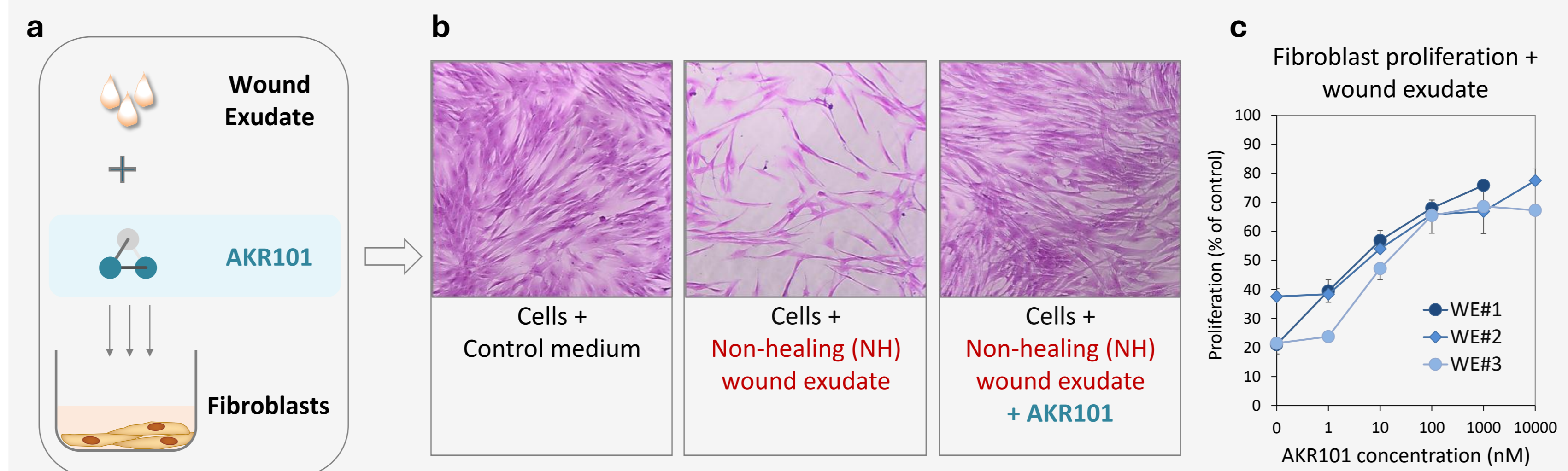


Figure 2: Rescue effect of AKR101. a) illustration of experimental set-up, b) microscopic images of fibroblast proliferation in medium control, with a non-healing WE alone and with the same WE in the presence of AKR101 (1µM), c) effect of increasing concentrations of AKR101 on proliferation of fibroblasts exposed to non-healing WEs.

AKR101 acts by promotion of tissue regeneration and reduction of inflammation

Increasing concentrations of AKR101 in the presence of non-healing wound exudates led to enhancement of tissue regeneration (cell growth, matrix deposition and expression of collagen) and inhibition of inflammation (e.g., IL-1β). This reflects the transition of a non-healing to a healing state observed with patient samples¹. AKR101 also reduced expression of inflammatory cytokines by fibroblasts in the presence of different WEs.

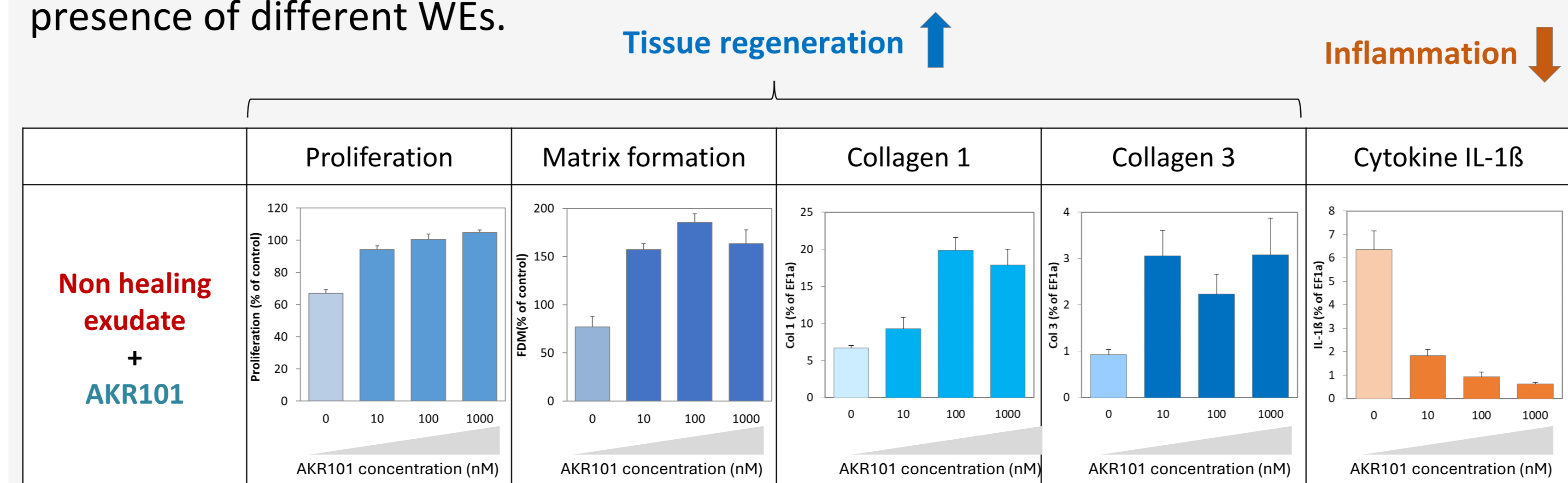


Figure 3: AKR101 mechanism of action. Effect of increasing concentrations of AKR101 in the presence of a non-healing WE was assessed for proliferation, ECM formation (FDM assay), and expression of collagen-1, collagen-3, and IL-1β mRNAs.

Analyte*	Medium		WE43		WE78	
	no cpd	AKR101	no cpd	AKR101	no cpd	AKR101
IL-1alpha	2	0,3	367	154	39	17
IL-1beta	60	4	10172	4942	918	323
TNFalpha	0,0	0,0	2,1	0,6	0,5	0,3
IL-6	140	11	13369	9412	3838	994
IL-11	324	7	439	66	441	53
IL-33	97	2	93	13	93	22
CCL2 (MCP-1)	794	111	6930	4099	3474	2857
CXCL1 (GRO-1)	438	41	13492	8795	4553	4902
CXCL2 (MIP-2)	8	1	1258	395	184	111
CXCL8 (IL-8)	2059	56	47252	23752	13369	8362
CXCL6 (GCP-2)	234	29	1397	628	717	517

*examples of the top most significant cytokines and chemokines

Figure 4: AKR101 anti-inflammatory action. Treatment with AKR101 (1µM) resulted in reduction of mRNA expression of different inflammatory cytokines and chemokines by fibroblasts exposed to two non-healing WEs. Values are given as the average of counts per million (cpm) of mapped reads from triplicates. Cell were exposed to WEs +/- AKR101 for 6 hours

RESULTS

AKR101 is superior to current standard therapies

The rescue effect of AKR101 was compared to current standard therapies (the growth factor PDGF and the active dressing ingredient silver sulfadiazine) in the same 101 non-healing WEs of different wound etiologies. AKR101 showed the best rescue effect, increasing proliferation of fibroblasts with ~90% of the WEs.

AKR101 was active with exudates of different types of wounds, including trauma/surgery, diabetic foot ulcer (DFU), venous leg ulcer (VLU), arterial leg ulcer (ALU), mixed ulcer (VLU/ALU), pressure ulcer (PU), and pyoderma gangrenosum (PG)

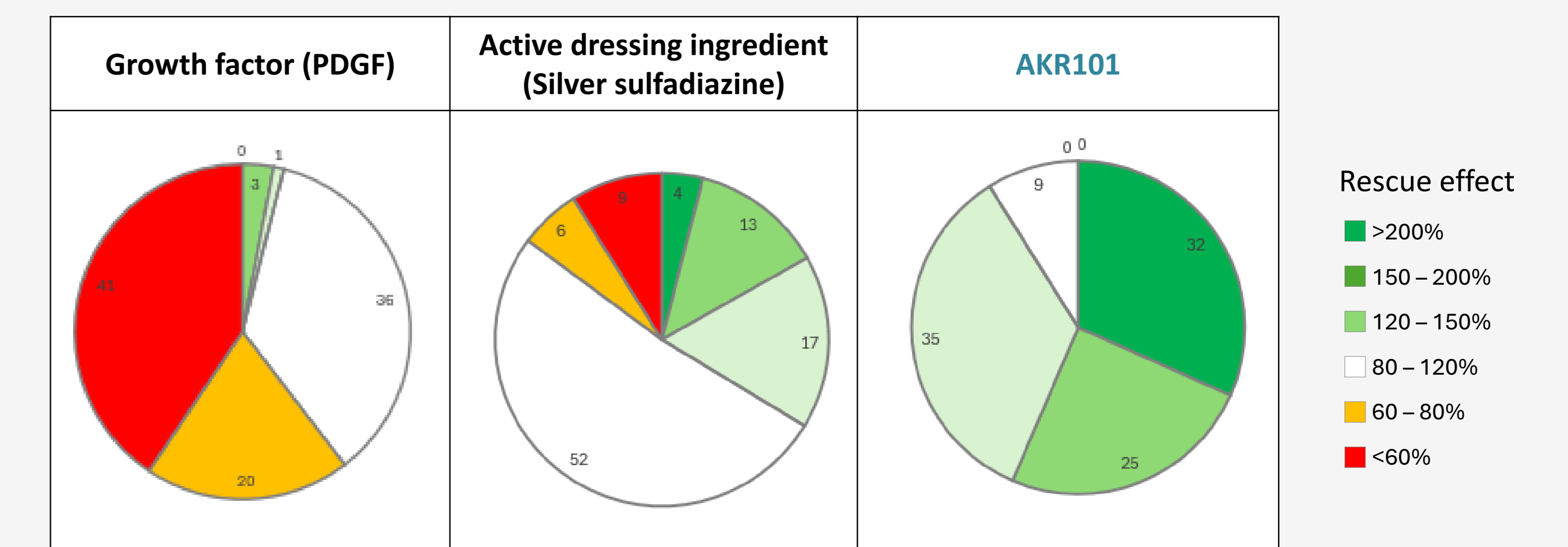


Figure 5: Rescue effect of AKR101 compared to current therapies PDGF and silver sulfadiazine. The three treatments were tested with 101 identical wound exudates. The rescue effect was calculated compared to fibroblast proliferation in the presence of WE (=100%) and color-coded. An effect above 120% was considered as rescue. AKR101 was tested at 1µM, PDGF at 20ng/ml, and silver sulfadiazine at 10µM.

CONCLUSIONS

- Non-healing wound exudates, regardless of their etiology, inhibit proliferation of normal human fibroblasts *ex vivo*.
 - ✓ Fibroblasts become "chronic wound fibroblasts" after short contact with wound exudates. → "Clinically relevant model of chronic wounds"
- AKR101 is a compound with previously unknown wound healing potential, restoring hallmarks of a healing phenotype *ex vivo*.
 - ✓ AKR101 shows excellent activity in *ex vivo* human wound assays at low nanomolar concentrations.
 - ✓ AKR101 acts *via* stimulation of tissue regeneration and reduction of inflammation.
 - ✓ AKR101 is active with exudates of all major types of wounds tested, e.g., DFU, VLU, ALU, PU, and trauma/surgery wounds.
 - ✓ AKR101 is superior to standard therapies, i.e., growth factors and silver sulfadiazine, with > 100 patient samples *ex vivo*.
- A clinical Ph2a study with AKR101 in patients with chronic venous leg ulcers is currently in preparation (projected start Q3/2026).