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Effectiveness of OR-101 in Murine Models of Skin Disorders

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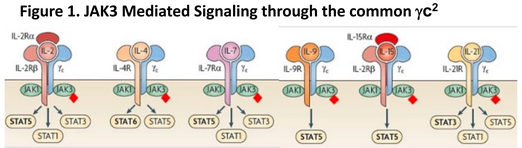
Abstract

Psoriasis (PS) is a chronic, autoimmune, inflammatory skin disorder that affects 2 to 3% of the world population. The skin of PS patients is characterized by excessive keratinocyte proliferation, as well as aberrant keratinocyte differentiation in the stratum corneum. Multiple inflammatory cell populations are observed within the lesions, including autoreactive T lymphocytes, mainly represented by Th17, Th1, and Th22 cells, that release IL-17, IFN-γ, IL-22, and TNF-α to potentiate disease pathogenesis. Other cytokines, such as IL-6 and IL-21, have additionally been shown to enhance IL-17 production from Th17 cells in a JAK-STAT–dependent manner. OR-101 is a next generation JAK3-selective compound that blocks down-stream signaling mediated by receptors that employ the common gamma chain (γc) of the type I cytokine receptor family (e.g. IL-2R, IL-4R, IL-7R, IL-9R, IL-15R, and IL-21R) and therefore has potential in a number of inflammatory conditions, including psoriasis. Here we evaluated the efficacy of OR-101 in a humanized murine model of psoriasis. Specifically, fifty immunodeficient mice were xenotransplanted with human psoriatic skin and then treated for 2 weeks with daily oral OR-101 (30 mg/kg, 60 mg/kg, or 100 mg/kg; n=10 each dose), or vehicle (n=10), or with topical 2 mg dexamethasone twice daily as a positive control (n=10). Dose-dependent improvements were seen in epidermal thickness measured by ocular micrometer (p<0.001 vs. vehicle; 249±122 µm for 100 mg, 373±206 µm for 60 mg, 437±294 µm for 30 mg, 723±91 µm for vehicle, 225±35 µm for dexamethasone) and epidermal proliferation (p<0.01 vs. vehicle; 14.6%±7.9 for 100 mg, 23.8%±21 for 60 mg, 27.9%±23 for 30 mg, 41%±11.4 for vehicle, 11%±4.2 for dexamethasone). Blinded histology mean total scores on the Baker system were significantly better (p<0.05) for the highest OR-101 dose (1.4±2.1) versus the 2 lower doses (3±4.4 for 60 mg, 4.5±4.6 for 30 mg) and placebo (9.1±0.8) and were similar to those of the positive control (0.35±0.4). Recovery of psoriatic features (e.g., hyperkeratosis, parakeratosis, acanthosis) based on histology was also dose-dependent. Similar improvements were observed for infiltrating CD4 and CD8 T cells, antimicrobial peptides (hBD4, S100A7), and inflammatory cytokines (TNF-α, IL-17, IL-22). These animal model results demonstrate the potential of OR-101 in psoriasis and other TH17-driven inflammatory skin diseases.

Developing a Next-GEN JAKinib

- Aberrant cytokine signaling has been shown to drive immune-mediated disease through type I and type II cytokine receptors. These receptors rely on Janus family of kinases (JAK) for signal transduction.
- Pharmacological targeting of the JAKs has proven to be efficacious in treating immune and inflammatory diseases; however, the lack of selectivity has been associated with significant safety concerns[1]
- Selective small-molecule inhibition of both JAK3 and ITK is challenging due to the highly conserved ATP binding pocket within the Janus kinase family (JAK1, JAK2, JAK3, TYK2) and the TEC kinase family (ITK, RLK, BMX, BTK, TEC)
- OR-101 is a selective dual inhibitor of Tyr (Y) kinases, JAK3 and ITK, interfering with the JAK-STAT6 and T Cell Receptor signaling pathways

Figure 1. JAK3 Mediated Signaling through the common γc[2]



Study Objective

- Psoriatic "humanized" mice are defined here as immunodeficient mice (SCID/beige) that are xenotransplanted with relevant functional human biological systems, i.e., human split-thickness skin (with all its skin appendages) injected with activated peripheral blood cells of psoriatic patients[3]
- This study evaluated the effectiveness of OR-101 on the histological and immunohistochemical (IHC) parameters of psoriasis in the humanized model of the disease.

Study Design

Animals

- 50 female C.B-17/IcrHsd-scid-bg mice (beige-SCID, Harlan Laboratories Ltd., Jerusalem, Israel) were housed under pathogen-free conditions, approved by the Institutional Committee on Animal Use in the Rappaport Faculty of Medicine, Technion – Israel Institute of Technology

Human Blood and Tissue Collection

- Clinically healthy abdominal skin with a width of 0.4 mm and surface area of 1 x 1 cm was obtained from two healthy female volunteers (aged 44±6 years) who underwent elective surgery.
- Peripheral blood mononuclear cells (PBMCs) were collected from six psoriatic female (aged 35±8 years) and four psoriatic male (41±11 years) volunteers who had not used any biologic agent or undergone either systemic treatments or psoriasis treatment within six months prior to the blood being drawn.
- All human materials used in these studies were obtained after receiving informed written consent from the participants
- The study protocol reviewed and approved by the Rambam Health Care Campus Institutional Review Board in accordance with the Declaration of Helsinki Principles.

PBMC Preparation and Activation

- PBMCs were isolated and cultured in the presence of a high dose of IL-2 (Prospec),100 U/mL of media – RPMI 1640,10% human AB serum (Sigma, St. Louis, MO), 1% glutamine, 1% antibiotics (media components; Biological Industries, Kibbutz Beit Ha'Emek, Israel), as previously described.[4] (Gilhar, 2011; Nousbeck, 2011; Schafer, 2010).

Xenotransplantation

- Xenotransplantation of healthy human split-thickness skin was performed as described (Keren, 2018). Briefly, one 1 cm^2/0.4 mm-thick skin sample was transplanted onto female SCID/beige mice.
- Four weeks following the engraftment, each mouse was injected (intradermally) with $1x10^7$ IL-2 activated allogeneic PBMCs from psoriatic patients ($1x10^7$ cells injected/mouse). Cells from different psoriasis patients were distributed equally between the treatment groups.
- Two weeks following the injection of cells, mice were treated as outlined in Table 1.

Table 1. Experimental Groups

Group	Dexamethasone (Topically Applied BID)	OR-101 (Orally QD)
1	2 mg/kg/day	-
2	-	Vehicle
3	-	30 mg/kg
4	-	60 mg/kg
5	-	100 mg/kg

Immunohistochemistry

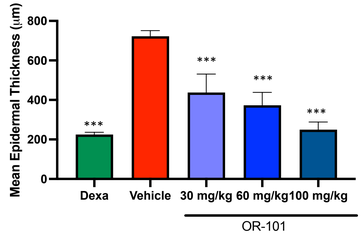
- The human skin xenotransplants were harvested and fixed in 10% saline-buffered formalin overnight followed by 70% ethanol. The sections were either stained and visualized with H&E staining or used for IHC analysis as previously described.
- Samples were examined using light microscopy and all slides were evaluated by experienced observers. The fields of immunostained sections were counted in at least three areas and scored as outlined in Table 2.

Table 2. Qualitative Staining Scoring

Epidermal Staining	Definition
Negative	scattered expression of less than 15% of the epidermis
Focal	scattered expression of less than 50% of the epidermis
Diffuse	scattered expression of greater than 50% of the epidermis
Dermal Staining	**Definition**
Mild	minor level of cell infiltrates/cytokine positive cells along the dermis
Moderate	average level of cell infiltrates/cytokine positive cells along the dermis
Dense	intense level of cell infiltrates/cytokine positive cells along the dermis

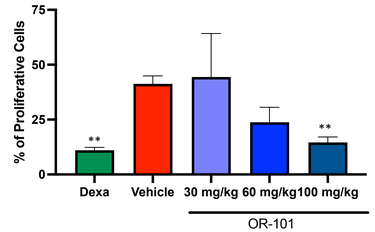
Efficacy of OR-101 in Humanized Mouse Model of Psoriasis

Epidermal Thickening



Epidermal thickness was determined using an ocular micrometer at a minimum of 50 points along the epidermis selected to represent points of maximal and minimal thickness. The thickness of the suprapapillary plate was measured similarly at 50 points per sample. *** P<0.001 compared to Vehicle

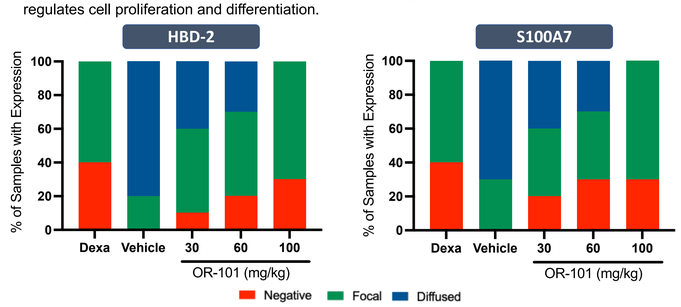
Epidermal Proliferation



Epidermal proliferation was determined by counting the % of Ki-67 positive cells in the dermal/epidermal compartment within an area of 0.66mm^2 ** P<0.01 compared to Vehicle

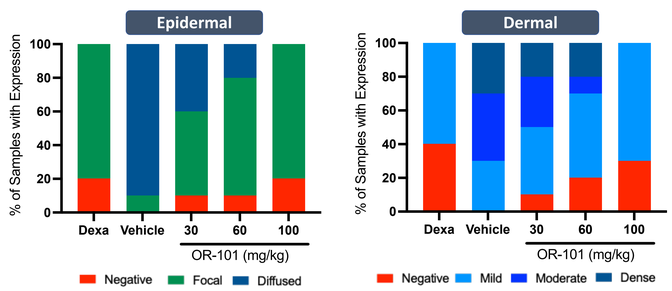
Antimicrobial Peptides

- Human beta defensin 2 (HBD-2) is increased in psoriasis in response to proinflammatory mediators and was probably responsible for the low rate of infection in psoriatic skin.
- S100A7 (psoriasin) is an EF-hand type calcium-binding protein localized in epithelial cells, regulates cell proliferation and differentiation.



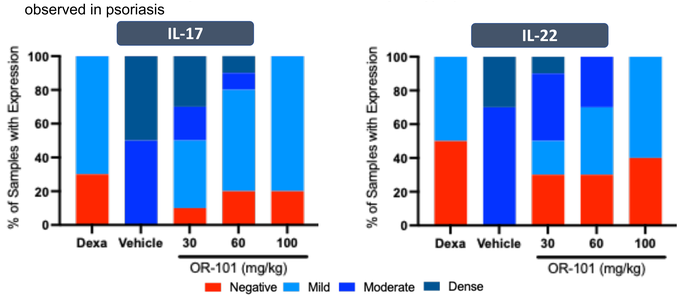
TNF-α

- TNF-α, a pro-inflammatory cytokine, is increased in the skin lesions of psoriatic patients. TNF-α staining in the dermal/epidermal compartments was assessed in an area of 0.66mm^2



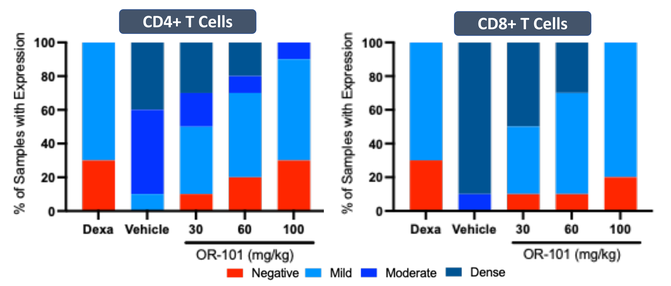
IL-17 and IL-22

- IL-17 acts directly on keratinocytes, resulting in keratinocyte proliferation and the production of psoriasis-related cytokines, chemokines, and antimicrobial peptides
- IL-22 is an epithelial cytokine that drives keratinocyte hyperproliferation and the acanthosis observed in psoriasis



T Cell Infiltration

- Psoriatic lesions are characterized by increased numbers of CD4 and CD8 positive T cells that drive disease pathogensis



Conclusions

- OR-101 dose-dependently reduced psoriasis-associated antimicrobial peptides, cytokines, and inflammatory cells
- Efficacy was similar to or greater than topically applied dexamethasone
- Overall, this supports the potential for OR-101 in psoriasis and other Th17 driven inflammatory skin diseases

References

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2. Rochman et al. 2009. *Nature Immunology.* 9: 480–490.
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Disclosures

M. Howell, P. Walker, W. Ahmad, and F. Hasan are shareholders in Ornovi