

## New Therapeutic Opportunities in Dermatology: Low Dose Cytokines Treatment for Vitiligo

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### Abstract

Vitiligo is a skin disorder characterized by skin depigmentation caused by the loss of melanocytes. The causes of melanocyte loss are unclear, but a relevant number of observations lead researchers to ascribe cellular immunity as having an important role in vitiligo pathogenesis.

Acting at the origin of the inflammatory phenomena, rebalancing the immune response with specific low dose SKA interleukins and antibodies and stimulating melanocytes to produce melanin via up-regulation of transmembrane receptors through SKA low dose basic-Fibroblast Growth Factor, represents the proposed Low Dose Medicine approach for Vitiligo treatment.

**Keywords:** Vitiligo; Inflammation; Low dose medicine; Sequential kinetic activation; Interleukin-4; Interleukin-10; Anti-Interleukin-1 antibodies; Basic-fibroblast growth factor

### Introduction

In recent years, researchers and clinicians operating in the field of Low Dose Medicine (LDM), a new medical approach born from Molecular Biology and Psycho-Neuro-Endocrine-Immunology (P.N.E.I.) and developed thanks to the results of research in the field of pharmacology of low doses [1-6] have investigated the possibility of the treatment of Vitiligo utilizing low dose activated interleukins, antibodies, neuropeptides and growth factors. The results are extremely encouraging and drawing new scenarios for the treatment of this severe disease.

### Vitiligo Etiopathology

Vitiligo is a dermatologic disorder characterized by a progressive skin depigmentation which is caused by the reduction of melanocytes number and activity at the cutaneous level. The causes of melanocyte loss are still unclear, but a relevant number of observations lead researchers to ascribe cellular immunity and oxidative stress as having an important role in vitiligo onset and progression. At the cutaneous level in active lesions level an imbalance in cytokine expression is observed; the perilesional area is immunologically active, recording high levels of IL-1 and IL-17 (markers of increased Th1/Th17 cellular subsets activation) which demonstrate a chronic pro-inflammatory shift of the immune system response. Reduced Tregs/Th2 activity is also assessed by low IL-4 levels [7,8] and may be part of the etiology of this autoimmune disease. TNF- $\alpha$  also plays a pivotal role in oxidative stress-mediated cytotoxicity directed against melanocytes and keratinocytes [9].

High levels of reactive oxygen species (ROS) [10] are detected and contribute to skin structure damage affecting the keratinocyte-melanocytes cross-talk, which is a key factor for a correct skin pigmentation. Hyper-sensibility to ROS is recognized as pivotal for the disease onset; in healthy conditions melanocytes react against ROS over-expression producing some typical phase II enzymes such as hemeoxygenase-1 (HO-1), superoxide dismutase (SOD) and catalase. Antioxidant enzyme synthesis is regulated by the nuclear translocation of NF-E2-related factor (Nrf2) which binds AREs sequences within the enzymes' encoding genes. Nrf2/ARE/HO-1 axis is compromised in Vitiligo [11] and consequently the ROS-scavengers enzymatic pool is

reduced and/or less effective. An alteration in anti-oxidative response [primarily driven by PAR-2 (Protease-Activated Receptor-2, which enhances Nrf2-mediated response against oxidative stress)] also affects perilesional and lesional keratinocytes in vitiliginous depigmented patches. The breakdown of the PAR-2/Nrf2 pathway in keratinocytes results in reduced Nrf2 nuclear translocation and a subsequent defective anti-oxidant response [12] (Figure 1).

In summary, decreased antioxidant enzymatic activity and increased ROS levels, due to a chronic inflammatory condition, driven by Th1/Th17 subsets, may be linked in Vitiligo with a Nrf2 pathway alteration both in keratinocytes and melanocytes.

The sophisticated mechanism of cross-talk between keratinocytes and melanocytes is essential for proper skin pigmentation; the network between keratinocytes and melanocytes includes cytokine and growth factors such as SCF (stem cells factor), ETs (endothelins) and b-FGF.

Keratinocytes-melanocytes crosstalk is mainly modulated by basic-Fibroblast Growth Factor (b-FGF) which induces melanocytes growth and differentiation and melanin synthesis; it is also involved in the ROS detoxifying processes by inhibiting them (and related damages) via activation of PI3K/Akt and consequent block of NF-kB nuclear translocation.

As previously described, both keratinocytes and melanocytes are directly damaged by the altered skin microenvironment which characterizes Vitiligo in terms of the hyperexpression of the proinflammatory cytokine network [13,14].

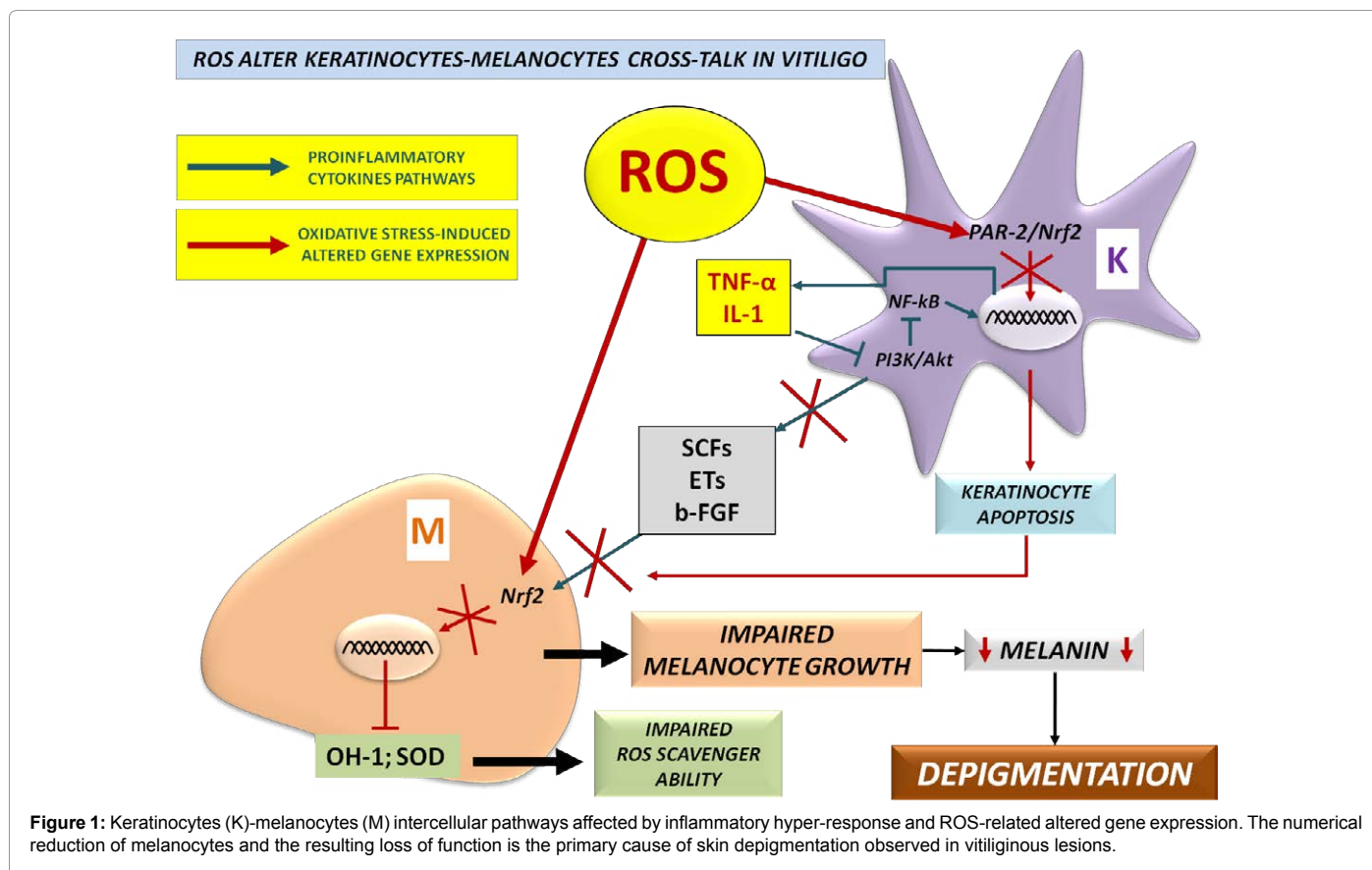
Taken together, these alterations in dermis cellular composition and function result in the depigmentation phenomena which represents the expression of the Vitiligo etiopathogenetic pathway.

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b-FGF, as noted, exerts a trophic action or it exerts regulating cellular proliferative and pro-active mechanisms.

These biological characteristics make it a potential molecule with pharmacological properties resulting from its ability to modulate both the growth phase and that of differentiation and migration of a set of target cells. Recombinant b-FGF (bovine or human) has been tested in a number of clinical trials mainly directed to the evaluation of its applicative use as enhancer of bone repair and soft tissues lesion healing by direct stimulation of fibroblasts and the dosages used are in the order of micrograms [15-17]. Some side effects, although not supported by statistical significance, are associated to the treatment with high doses of this growth factor.

Acting as a messenger mediator, b-FGF plays its biological functions in a concentration-dependent manner. In particular, the dose-response ratio is not linear but follows a typical bell-shaped curve with a peak of activity in a concentration range identified between picograms and nanograms.

### LDM for Vitiligo Management: The Possible Therapeutic Role of Low Dose SKA b-FGF, Cytokines and Neuropeptides

Vitiligo is a systemic chronic autoimmune inflammatory disease and its onset is due to an etiologic loop between oxidative stress and chronic inflammatory phenomena, secondary to up-regulation of Th1/Th17 cytokines (IL-1, IL-17, IFN- $\gamma$  and TNF- $\alpha$ ); inflammation results in keratinocytes-melanocytes cross-talk disruption with consequent skin depigmentation.

For a correct epidermal pigmentation it is essential that the

keratinocyte produces the signaling molecules which control physiological melanocytes proliferation, survival rates and proper melanin production; an original and innovative treatment for Vitiligo must be centered on modulation of chronic inflammation and intercellular cross-talk enhancement simultaneously.

LDM gives the opportunity to use low doses of orally administered SKA [sequential kinetic activation [18,19] b-FGF in order to restore one of the most important melanocytes stimulating mediator pathways, but the single therapeutic use of b-FGF allows to act only on the last step of the etiopathogenetic cascade of Vitiligo. Acting at the origin of the inflammatory phenomena, counteracting pro-inflammatory cytokines with specific low dose SKA interleukins and antibodies (IL-10, Anti-IL-1 and IL-4) and, in the meantime, stimulating melanocytes to produce melanin via up-regulation of transmembrane receptors through SKA low dose b-FGF, represents the hypothesized new LDM approach for Vitiligo treatment.

### LDM for Vitiligo Management: Preclinical and Clinical Efficacy Assessment

Starting from this preliminary theoretical approach, Barygina et al. [20] designed and conducted a basic research *ex vivo* study on human primary keratinocytes obtained from perilesional skin biopsies in order to evaluate the effects of low dose SKA IL-4, IL-10, b-FGF, and  $\beta$ -endorphin in the modulation of intra- and extra-cellular oxidative stress and on the cellular proliferation rate.

Published results highlighted a substantial reduction of intracellular oxidative stress in samples treated with low dose SKA IL-4, IL-10 and b-FGF with mean values of  $18.1 \pm 0.5\%$ ,  $19.2 \pm 15\%$  and  $21 \pm$

6% respectively; extra-cellular oxidative stress was also reduced in cell groups treated with low dose SKA IL-4 and b-FGF (mean values:  $26 \pm 5.6\%$  and  $36.2 \pm 11.5\%$  respectively).

Cell viability assays revealed that the cell treatment with low dose SKA IL-10, b-FGF and  $\beta$ -endorphin, induced an increase of the proliferation rates of  $9.2 \pm 1\%$ ,  $15.7 \pm 3.26\%$  and  $13.5 \pm 2.7\%$  respectively (compared to untreated perilesional keratinocytes).

The evidence of the *ex vivo* effectiveness of low dose SKA molecules against oxidative stress induced Lotti et al. [21] to perform a retrospective spontaneous clinical study in order to quantify and compare the results obtained with a LDM therapeutic approach versus a conventional one. The authors evaluated (in terms of re-pigmented skin surface and disease spread reduction) some groups of patients who received different topical and systemic therapeutic treatments. All evaluated subjects had a depigmented skin surface that did not exceed 15% of the total. Two groups were treated respectively with orally administered low dose SKA IL-4; IL-10; Anti-IL-1 antibodies (Guna S.p.a – Italy) together and low dose SKA b-FGF (Guna S.p.a – Italy) alone were evaluated and compared with other groups of patients topically treated with cortisone (dexamethasone) cream (alone and in association with both groups of low dose SKA molecules) and narrow-band UVB radiation (alone and in association with both groups of low dose SKA molecules). Two groups of subjects who received topical sunlight exposure and systemic oral intake of Ginkgo biloba extract treatments were evaluated as control groups.

The patients/group with a moderate (reduction of depigmentation in 25-50% of the affected area) to excellent (reduction of depigmentation in >75% of the affected area) response was globally considered a positive result: low dose SKA b-FGF induced an improvement in 74% of patients and the association of low dose SKA IL-4, IL-10 and anti-IL-1 antibodies was effective in 77% of the evaluated cases.

The association of low dose SKA treatments with the topical UVB-based treatment provides 92/93% of the positive results, allowing the authors to evaluate the opportunity of an integrated use of the two therapeutic tools.

In summary, the preclinical study demonstrated the efficacy of low doses of SKA signaling molecules in order to reduce oxidative damages, one of the most important inflammatory triggers in Vitiligo. Furthermore, clinical results demonstrated the ability of all low dose SKA administered molecules to significantly reduce the depigmented skin surfaces and to block the spread of the lesions.

The efficacy and safety of low dose SKA signaling molecules in the treatment of Vitiligo were also assessed, and no adverse effects were reported.

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