Vitiligo: Mechanistic insights lead to novel treatments

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Vitiligo is an autoimmune disease of the skin characterized by patchy depigmentation. Current treatments are moderately effective at reversing disease by suppressing autoimmune inflammation in the skin and promoting melanocyte regeneration. Recent basic and translational research studies have significantly improved our understanding of disease pathogenesis, which is now leading to emerging treatment strategies based on targeted therapy. Here we discuss important clinical characteristics of vitiligo, current therapies and their limitations, advances in understanding disease pathogenesis, emerging targeted treatments, and strategies to optimize clinical trials to efficiently and effectively test these new treatments. (J Allergy Clin Immunol 2017;140:654-62.)

Key words: *Vitiligo, treatment, T cell, IFN-γ, CXCL9, CXCL10, CXCR3, resident memory, regulation, stress, innate immunity, inflammasome, NLRP1, NLRP3, melanocyte, regeneration, clinical trials, repigmentation, hair*

Abbreviations used
JAK: Janus kinase
NB-UVB: Narrow-band UVB
Treg: Regulatory T
Trm: Resident memory T
WNT: Wingless-related integration site

Vitiligo is an autoimmune disease of the skin that results in disfiguring white spots (Fig 1, *A*), which often negatively affect patients' self-esteem and quality of life.[1,2] Like many autoimmune diseases, complex interactions among genetic, environmental, and stochastic factors contribute to disease pathogenesis in patients with vitiligo.[3] However, unlike many other autoimmune diseases, current treatments are capable of reversing vitiligo by suppressing the immune system and stimulating regrowth from a natural reservoir of melanocyte stem cells. These stem cells can survive autoimmune attack because of their location within hair follicles, which are immune privileged sites similar to other organs, such as the brain and eye. As a result, successful repigmentation of the skin of patients with vitiligo initially presents in a perifollicular pattern (Fig 1, *B* and *C*). Certain anatomic sites that lack hair follicles are difficult to treat, including the hands (particularly the knuckles and fingertips), feet, other bony prominences, lips, and genitals (Fig 1, *D*). In addition, lesions in which the hair is depigmented have a poor prognosis. Thus patients' expectations must be managed with this information in mind.

Existing treatments for vitiligo (Table I) include topical steroids and calcineurin inhibitors, which suppress immune responses in the superficial skin, where the immune infiltrate is located. These are most useful for treating disease that is localized, comprising less than 5% of the body surface area.[4,5] Phototherapy, which was administered initially as psoralen plus UVA (PUVA) but now primarily as narrow-band UVB (NB-UVB), is effective for more widespread disease or disease that is active and expanding with the appearance of new lesions. The excimer laser, which emits a single wavelength of light in the NB-UVB range, is also effective when treating localized disease that is unresponsive to topical therapies or when a more rapid response is desired.[6] When highly active, vitiligo can be stabilized through oral pulse steroid therapy, which is continued until another therapy (usually NB-UVB) proves effectively therapeutic.[7] Although moderately effective, current treatments for vitiligo are both financially and practically burdensome.

Over the past 2 decades, basic and translational research studies have identified several mechanistic pathways that promote vitiligo, which offer promising opportunities to develop new targeted treatments. The discovery that $CD8^+$ T cells are both necessary and sufficient to mediate melanocyte destruction in human subjects provided a foundation for ongoing investigation of T cells in patients with vitiligo disease.[8] Likewise, early findings that melanocytes from patients with vitiligo grew poorly *ex vivo* and were more susceptible to oxidative stress provided insight that melanocyte abnormalities contribute to vitiligo pathogenesis.[9-11] Current evidence supports involvement by both $CD8^+$ T cells and melanocyte stress, and thus understanding of both aspects of the disease presents opportunities for therapeutic development.

IFN-γ PATHWAY AND T-CELL RECRUITMENT IN PATIENTS WITH VITILIGO

$CD8^+$ T cells are key effectors that drive melanocyte destruction in patients with vitiligo, and several studies have identified

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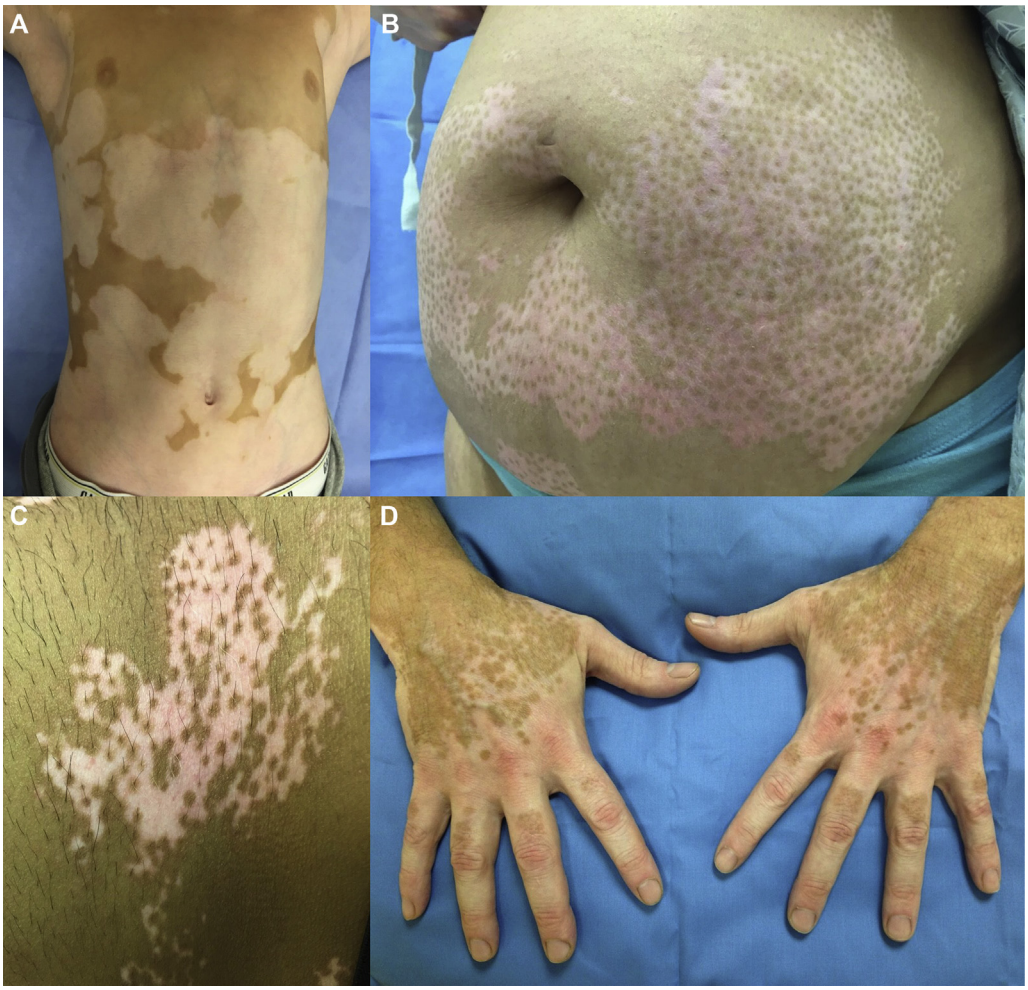

FIG 1. White patches of depigmentation characteristic of vitiligo **(A)**, perifollicular repigmentation after successful treatment of disease (**B** and **C**), and difficulty repigmenting bony prominences of the knuckles relative to the dorsum of the hands **(D)**.

TABLE I. Current and emerging vitiligo treatments

Current
Topical immunosuppression
Topical steroids
Topical calcineurin inhibitors
Phototherapy
NB-UVB
Excimer laser
Oral immunosuppression
Oral pulse steroids
Surgical
Melanocyte transplantation procedures
Emerging
Targeted immunosuppression
IFN-γ pathway inhibitors
Melanocyte growth promoters
Afamelanotide
WNT-β–catenin signaling enhancers

critical pathways involved in the recruitment of these pathogenic cells to skin tissue. Gene expression analysis of human lesional skin revealed upregulation of IFN-γ and IFN-γ–dependent genes, including the T-cell chemokine receptor CXCR3 and its multiple ligands: CXCL9, CXCL10, and CXCL11.[12] $CXCR3^+$ cells are present within skin biopsy specimens of patients with vitiligo, and antigen-specific T cells in blood of patients with vitiligo are enriched for the CXCR3 receptor.[12-15] Functional studies in a mouse model of vitiligo were consistent with human observations and then revealed that vitiligo functionally requires the IFN-γ–chemokine signaling axis. Blockade of this pathway disrupts T-cell recruitment to the skin, as well as subsequent melanocyte destruction and pigment loss. Furthermore, this approach not only prevented vitiligo in mice but was able to reverse disease, as evident by perifollicular repigmentation, which is similar to the response seen in human subjetcs.[12,16,17]

A more recent study found that keratinocytes are the major source of chemokine production during vitiligo and that disrupting IFN-γ signaling only in keratinocytes mitigated disease in mice.[18] This suggests that targeting IFN-γ signaling in keratinocytes, which can easily be done topically, can also be an effective treatment strategy.

Similar to the mouse model, blockade of the IFN-γ pathway appears to successfully treat vitiligo in human subjects. IFN-γ activates its associated receptor (IFNGR) and a downstream

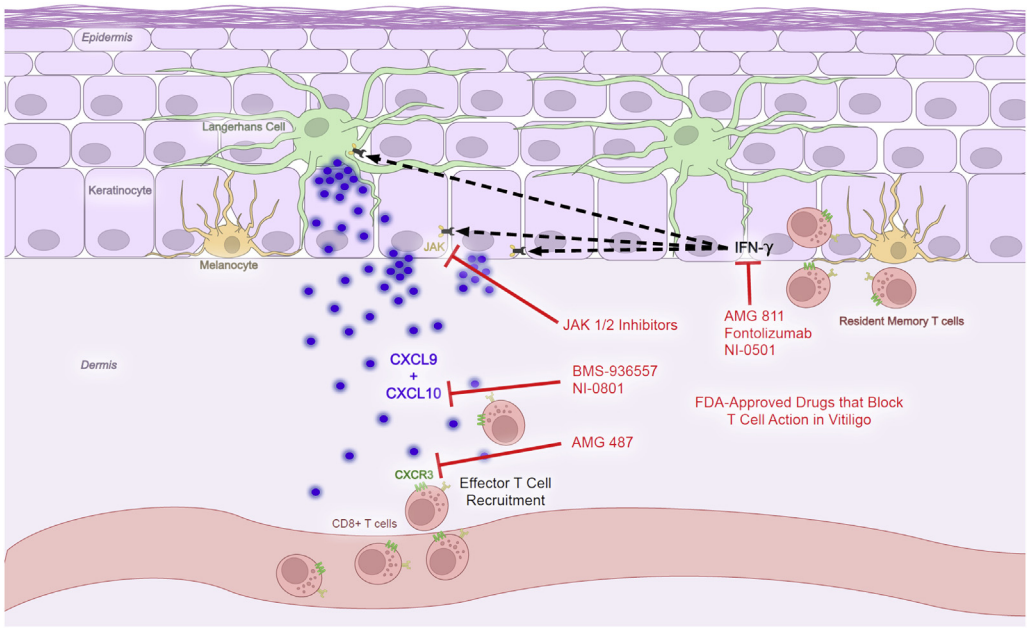

FIG 2. IFN-γ signals to keratinocytes and Langerhans cells, stimulating production of CXCL9 and CXCL10. CXCR3 recognizes these chemokines, leading to recruitment of $CXCR3^+CD8^+$ T cells. Autoreactive T cells kill melanocytes and establish long-lived resident memory within the skin. Drugs that target aspects of this pathway are shown in red.

intracellular Janus kinase (JAK)–signal transducer and activator of transcription signaling pathway. Two case reports found that patients with vitiligo receiving either of the 2 JAK inhibitors tofacitinib or ruxolitinib rapidly repigmented. The patient treated with ruxolitinib was found to have a stable increased level of CXCL10 in his serum, which rapidly decreased after treatment with the JAK inhibitor. This observation was consistent with the hypothesis that the mechanism of improvement was through inhibition of IFN-γ signaling and resulting loss of chemokine production.[19-21] Consistent with the key role of keratinocytes in promoting vitiligo progression in mice, a case series reported recently that topical ruxolitinib was effective in repigmenting a small number of patients with vitiligo, particularly on the face.[22]

Based on these studies, a phase 2 clinical trial has been initiated to test the effectiveness of topical ruxolitinib for vitiligo treatment (NCT02809976). In addition to JAK inhibitors, multiple other drugs have been developed that target the IFN-γ pathway, many having passed phase I trials with a good safety profile, and these could be tested as vitiligo treatments (Fig 2). We have found in our mouse model that blocking any component of the IFN-γ signaling pathway is effective at mitigating disease, and thus multiple options exist for developing novel therapeutics that target autoimmunity in patients with vitiligo.[12,16-18]

MEMORY T CELLS IN PATIENTS WITH VITILIGO

As mentioned above, vitiligo lesions are reversible with treatment. However, these lesions frequently return when treatment is discontinued, with relapse occurring in 40% of patients within the first year after stopping treatment. This relapse can be significantly decreased by using periodic treatment with topical calcineurin inhibitors, which strongly suggests that a memory component of autoimmunity persists long-term within lesional skin.[23]

$CD8^+$ resident memory T (Trm) cells remain within the skin and mucosae for very long periods after viral infections to prevent reinfection and are thought to be responsible for controlling herpes virus recurrences, including herpes simplex virus–induced cold sores.[24-28] Trm cells have also been associated with inflammatory skin diseases, including mycosis fungoides, psoriasis, and vitiligo[29,30]; however, their functional contributions to maintain disease are still unclear. Cheuk et al[30] reported that Trm cells are highly enriched in the skin of patients with vitiligo, possess cytotoxic effector function when exposed to inflammatory cytokines, and thus might play a role in the persistence of vitiligo lesions. Another group found that Trm cells are present in vitiligo lesions in a mouse model of melanoma and vitiligo and might be responsible in part for mediating protection against recurrence of melanoma.[31] Future studies might determine that targeting Trm cells in patients with vitiligo could be an effective treatment strategy and could be durable, even after discontinuing treatment.

LYMPHOCYTE REGULATION IN PATIENTS WITH VITILIGO

Abnormal regulation of lymphocytes is an important component of vitiligo pathogenesis. Genome-wide association studies have identified risk alleles related to T- and B-cell regulation and tolerance: *CTLA4*, *FASLG*, *PTPN22*, *UBASH3A*, and others.[3,32] Polymorphisms in *FOXP3*, *BACH2*, and *IL2RA*, all important drivers of regulatory T (Treg) cell development and function, are associated with vitiligo risk, which implicates Treg cells as important factors in determining vitiligo initiation, progression,

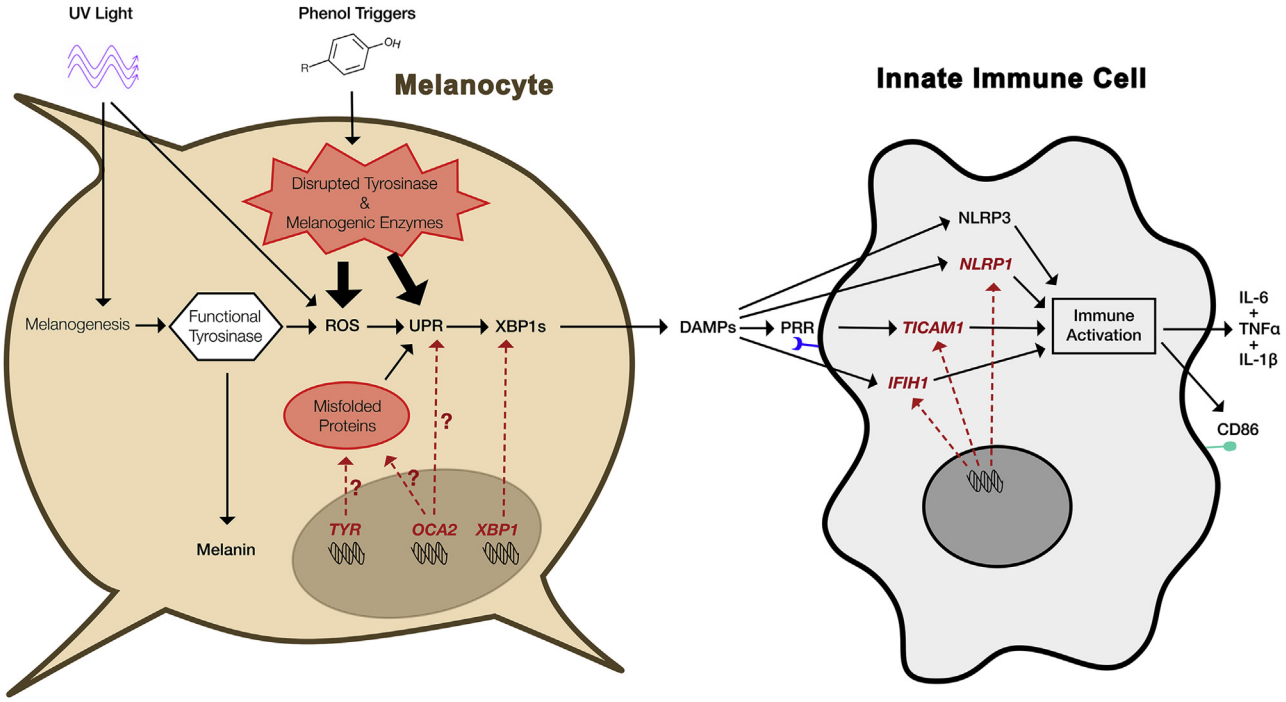

FIG 3. UV light, melanogenesis, and vitiligo-associated genetic polymorphisms (shown in *red*) can contribute to intrinsic melanocyte stress. Several environmental phenols also interact with melanogenic enzymes to induce stress, which then leads to secretion of damage-associated molecular patterns *(DAMPs)* that stimulate nearby innate immune cells through pattern recognition receptors *(PRRs)*. Shown in *red*, vitiligo-associated genetic polymorphisms can affect both melanocyte stress and innate immune response. Hypothesized pathways are denoted with *?*, and significant stress from exogenous insult is denoted by *thick arrows*.

or both.[33] The incidence of vitiligo is also increased in patients with immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, who lack Treg cells.[34] Autoreactive effector T cells in patients with vitiligo have a dysregulated phenotype compared with healthy control subjects, suggesting ineffective Treg cell function.[35] However, it is unclear exactly how Treg cells might be dysfunctional in patients with vitiligo. Studies are conflicting on whether Treg cells are present in normal or decreased numbers, whether there is a defect in their skin-homing potential, and whether they possess abnormal regulatory function.[36-40]

MELANOCYTE STRESS IN PATIENTS WITH VITILIGO

Although $CD8^+$ T cells mediate autoimmunity in patients with vitiligo, the question remains why melanocytes are selectively targeted for autoimmune attack. In patients with melanoma, T cells target tumor cells because of the immunogenicity of neoantigens produced by somatic mutations, yet no current evidence of protein modification has been found to explain T-cell autoimmune attack in patients with vitiligo.[41] However, melanocyte stress appears to initiate autoimmune inflammation in patients with vitiligo. Translational and clinical research studies suggest that intrinsic and/or extrinsic factors induce the cellular stress response in melanocytes, which then activates innate immunity within the skin to trigger the initial inflammation that leads to autoimmunity.[42-44] Melanocytes from nonlesional skin biopsy specimens of patients with vitiligo demonstrate a dilated endoplasmic reticulum, which represents structural evidence of cell stress.[45] Both lesional and nonlesional skin of patients with vitiligo also show increased reactive oxidative species, indicating that melanocyte stress and redox aberrations pre-exist within the skin before autoimmune onset.[46]

Intrinsic factors capable of promoting melanocyte stress include genetic risk alleles, which have been implicated through genome-wide association studies. Several non-HLA single nucleotide polymorphisms associated with vitiligo lie within the melanocyte-specific enzyme tyrosinase *(TYR)*.[32] Although these polymorphisms have been suggested to primarily promote presentation of autoantigen for autoimmune targeting, they can also induce stress through protein misfolding or mark abnormal enzymatic activity during melanogenesis, which has been strongly associated with promoting cellular stress through production of reactive oxidative species.[47,48] Another single nucleotide polymorphism associated with an increased risk for vitiligo is X-box binding protein 1 *(XBP1)*, which can augment the cell stress response in patients with vitiligo (Fig 3).[33,49]

Several experimental vitiligo treatments have sought to reduce melanocyte stress. Aimed at compensating for diminished expression of redox enzymes within the skin of patients with vitiligo, a topical metal ion formulation with antioxidative properties, termed pseudocatalase, was tested for its ability to boost the effectiveness of NB-UVB phototherapy.[46,50] However, this study was unblinded and uncontrolled, and controlled studies have since failed to show a benefit of pseudocatalase treatment.[51-53] Some antioxidant dietary supplements reportedly provide a mild enhancement of NB-UVB phototherapy effectiveness.[54,55] Pooled antioxidant supplementation with α-lipoic acid,

vitamin C, vitamin E, and polyunsaturated fatty acids was found to improve the response to NB-UVB phototherapy.[56] Similarly, *Polypodium leucotomos* extract can quench oxidative free radicals and has been reported to enhance the NB-UVB response.[57] *Gingko biloba* possesses both antioxidant properties and anti-inflammatory effects.[58] This might explain its mild efficacy reported in 2 small clinical studies.[59,60] Therefore the use of antioxidant therapies has demonstrated limited efficacy, yet future studies might identify more effective therapies as the pathways that contribute to cellular stress in melanocytes are better defined.

Although genetic factors clearly play a role in vitiligo risk, they do not account for all of this risk. In fact, identical twins are concordant for vitiligo only 23% of the time, strongly implicating other factors as well, such as environmental exposures that can act as extrinsic factors to influence vitiligo onset.[61] Exposure to a number of chemicals has been reported to induce and exacerbate vitiligo, including monobenzyl ether of hydroquinone, 4-tert-butylphenol, 4-tert-butylcatechol, and others.[62] These chemicals are similar in that they are all phenols and likely act as analogs of the melanogenesis substrate tyrosine, also a phenol. Toxicity to these vitiligo-associated chemicals also depends on interaction with tyrosinase and other melanogenic enzymes, which increases cellular stress in the melanocyte and initiates inflammation through activation of innate immunity (Fig 3).[62,63] A number of common household products have also been implicated in vitiligo induction, including permanent hair dyes, detergents, and others.[62,64] Exposure to a cosmetic skin-lightening cream led to the development of vitiligo in more than 18,000 users in Japan in 2013, and the chemical culprit in the cream was likely to be the tyrosine analogue rhododendrol.[62,65] Therefore management of vitiligo includes counseling patients about these risks so they can consider avoidance of these products, and future studies to identify key chemicals in these products could lead to safer formulations.

INNATE IMMUNE RESPONSE IN PATIENTS WITH VITILIGO

Inflammation responsible for initiating autoimmunity in patients with vitiligo appears to result from immune activation directly by stressed melanocytes (Fig 3). These cells produce damage-associated molecular patterns, secretion of which leads to activation of nearby dendritic cells and their production of proinflammatory cytokines.[42,66,67] The damage-associated molecular pattern most associated with vitiligo, inducible heat shock protein 70, is induced within perilesional and lesional skin of patients with vitiligo and functionally worsens disease in a mouse model of vitiligo by inducing and/or recruiting activated inflammatory dendritic cells.[66,68-70] Furthermore, mutant inducible heat shock protein 70 with an altered C-terminal domain was able to prevent disease by reducing dendritic cell activation and subsequent T-cell responses. Therefore inhibiting melanocyte-induced innate immune responses represents another potential new therapeutic strategy.[70]

A recent study found that skin inflammation caused by chemically induced melanocyte stress in a mouse model required the innate immune receptor NLR family pyrin domain containing 3 (NLRP3), suggesting that this receptor, its associated proinflammatory inflammasome complex, or its downstream effector cytokine IL-1β, might be possible therapeutic targets to prevent the initiation of disease or its spread.[71] In addition to activation of

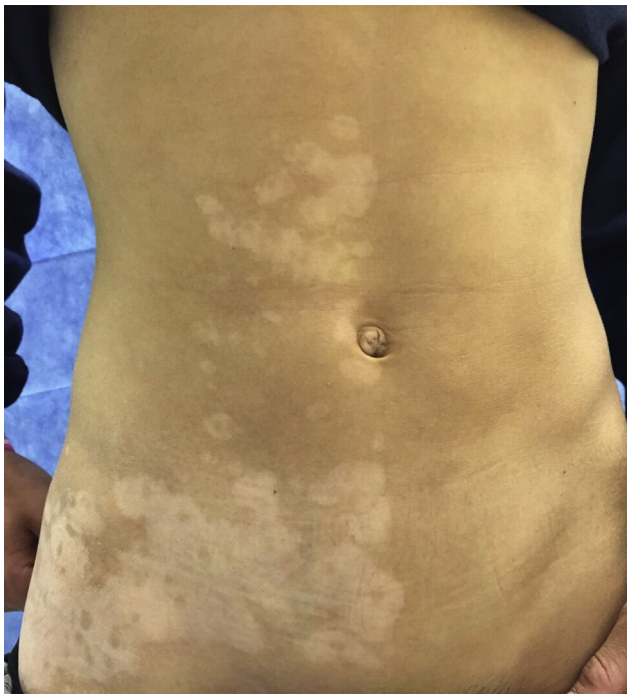

FIG 4. Segmental vitiligo is characterized by unilateral depigmentation that does not cross the midline.

NLRP3 by chemical-induced melanocyte stress, the inflammasome receptor *NLRP1* has been associated with vitiligo in genetic studies.[72] Vitiligo-associated NLRP1 increases inflammasome processing of pro–IL-1β to functional IL-1β, which further implicates inflammasome activation and innate inflammation with vitiligo pathogenesis.[73] Perilesional sin of patients with vitiligo, in which disease is most active, also has increased IL-1β expression, suggesting that the IL-1β pathway might contribute to vitiligo progression.[74,75] To our knowledge, anti–IL-1β targeted therapies have not been tested in patients with vitiligo.

IL-17 PATHWAY CYTOKINES IN PATIENTS WITH VITILIGO

Levels of TNF-α and IL-17, which are key targets for immunotherapy in patients with psoriasis, are also mildly increased in vitiligo lesions and have been proposed to be potential treatment targets for vitiligo.[76] However, currently, it is unclear whether they play any functional role in vitiligo pathogenesis. Diseases mediated by IL-17 and TNF-α, such as psoriasis, the prototypic T_H17 skin disease, typically have epidermal acanthosis, prominent neutrophil recruitment, and symptomatic inflammation with erythema and scale, features that are absent in patients with vitiligo.[77] In addition, targeted immunotherapies that disrupt TNF-α, IL-17, and other cytokines in this pathway are thus far reportedly ineffective for vitiligo, and some have even initiated new-onset vitiligo in patients receiving treatment for other diseases.[78-81] Furthermore, we found limited production of IL-17 and related cytokines in vitiligo lesions compared with IFN-γ–induced cytokines, and Trm cells found in psoriasis lesions produced large amounts of IL-17, whereas Trm cells in vitiligo lesions produced IFN-γ without any increased IL-17.[12,30] These observations suggest that TNF-α and IL-17 are not major drivers of vitiligo pathogenesis.

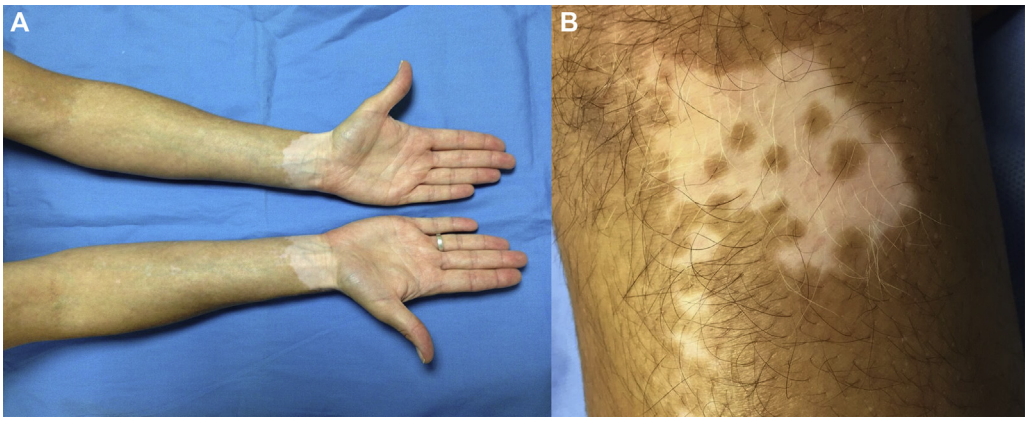

FIG 5. Repigmentation is impaired at anatomic sites where there is little hair growth. **A,** Lack of repigmentation on the volar wrists in a patient whose entire forearms were otherwise successfully treated. **B,** White hairs do not support perifollicular repigmentation relative to pigmented hairs.

PROMOTING MELANOCYTE REGENERATION IN PATIENTS WITH VITILIGO

Although inhibition of autoimmunity alone is an effective treatment strategy, promoting melanocyte regeneration can further improve treatment responses. The best example of this is the efficacy of surgical procedures that transplant melanocytes from an unaffected site to depigmented lesions of patients with stable vitiligo. This includes older approaches, such as split-thickness skin grafting or punch minigrafts, as well as transfer of blister roofs from nonlesional to lesional skin sites. A newer approach is to create a single-cell suspension from nonlesional donor epidermis and transfer the combined melanocyte and keratinocyte cellular mixture to lesional skin prepared through dermabrasion or laser ablation. However, because active autoimmunity will quickly destroy newly transplanted melanocytes, procedural approaches are only effective when the disease is stable, typically characterized by the lack of spread of existing lesions or the appearance of new lesions within the previous 1 to 2 years. Segmental vitiligo, a particularly stable subtype of vitiligo characterized by unilateral depigmentation that is limited by the midline (Fig 4), is uniquely responsive to surgical approaches, with excellent success rates of between 58% and 84%.[82-84]

α-Melanocyte stimulating hormone activates melanocyte proliferation, migration, differentiation, and function. A randomized clinical trial demonstrated that a synthetic analogue of the α-melanocyte stimulating hormone afamelanotide increased the efficacy of NB-UVB phototherapy.[85] However, treatment with afamelanotide induced hyperpigmentation of nonlesional skin, causing some patients to withdraw from the study because of the enhanced contrast of vitiligo lesions. Aimed more specifically at promoting differentiation of melanocyte stem cells without causing hyperpigmentation, Regazzetti et al[86] reported that oxidative stress impairs melanoblast differentiation by mitigating wingless-related integration site (WNT)–β-catenin signaling. Treatment of vitiligo lesional skin explants with a synthetic WNT agonist stimulated melanocyte differentiation.[86] Inhibition of glycogen synthase kinase-β, a negative regulator of WNT-β–catenin signaling, showed identical effects as the WNT agonist, demonstrating that multiple targets within the WNT-β–catenin pathway could represent viable options for an adjunct vitiligo treatment.[86]

PLANNING FOR EFFICIENT AND INFORMATIVE CLINICAL TRIALS

Clinical trials to test some of the new targeted therapies described above are being planned. Careful selection of patient populations will be important for designing successful trials that are able to accurately measure treatment responses. For example, patients with vitiligo located primarily in hairless skin, and those with lesions containing white hair do not have a strong capacity for repigmentation (Fig 5) and thus might need to be excluded from these studies, at least initially. Duration of disease has been reported previously to negatively affect prognosis to treatment[87]; however, this can occur indirectly through increased presence of white hairs with long-term disease. In our experience patients with long-duration disease have repigmented well, as long as their lesions contain hair with normal pigment. Patients with the segmental variant of vitiligo are frequently excluded from clinical trials because this variant is less responsive to medical treatments, probably because of its early development of hair depigmentation. However, as mentioned above, these patients are ideal for surgical treatment. Thus patients with active inflammation might represent the best population to test immunomodulators, whereas those with stable disease might be best for therapies that promote melanocyte regeneration.

Vitiligo is slowly responsive to treatment, with early signs of repigmentation visible clinically within 2 to 3 months of initiating treatment and statistically measurable improvement taking up to 6 months to observe. Outcome measures must be carefully selected, and options include the vitiligo activity scoring index, vitiligo European task force measurement, the newly developed vitiligo extent score, and the developing photography-based objective scoring methods.[88,89] Biomarkers of disease activity can help identify subjects with active inflammation and might serve as excellent early indicators of treatment responses.[12,14,90-93] These include both serum biomarkers and biomarkers that can be directly measured within lesional skin, which might have better sensitivity and specificity. Recently, we described a minimally invasive skin-sampling technique through induction of blisters in lesional and nonlesional skin, which provides blister fluid for analysis of infiltrating immune cells and inflammatory cytokines that reflect both disease activity, as well as early treatment responses.[15]

CONCLUSION

Vitiligo is a unique autoimmune disease caused by the reservoir of melanocyte stem cells that provides ability for disease reversal. Although the US Food and Drug Administration has not yet approved any medical treatments for repigmentation of vitiligo, existing off-label therapies are moderately effective, whereas basic and translational research have provided insights into vitiligo pathogenesis that offer promising targets for new drug development. Vitiligo appears to be initiated by the melanocytes themselves, which exhibit cellular stress that can be influenced by both intrinsic and extrinsic factors. Genetic risk alleles can increase endogenous stress or decrease the threshold that can be tolerated by the cells, whereas environmental factors, such as chemicals and household products, can exacerbate stress pathways to initiate disease in predisposed subjects. Cytotoxic T cells are necessary and sufficient for melanocyte killing in vitiligo, and targeting their recruitment appears to be an effective therapeutic strategy (Table I). Innate immune activation can provide the connection between melanocyte stress and adaptive immune responses, which could potentially be targeted as well to mitigate initial activation of autoimmunity. Clinical trials to test these emerging therapies are on the horizon and must be carefully planned for efficient and informative outcomes. We are witnessing a revolution in our understanding of vitiligo pathogenesis, and this revolution is sure to lead to better treatments for our patients in the near future.

What do we know?

- **Vitiligo is an autoimmune skin disease mediated by $CD8^+$ T cells that kill melanocytes in the epidermis.**
- **T-cell recruitment to the skin requires IFN-γ and IFN-γ–induced chemokines secreted from keratinocytes, which can be targeted to develop new treatments.**
- **Chemicals found in common household products can induce or exacerbate vitiligo through induction of melanocyte stress.**
- **Melanocyte stem cell reservoirs are often protected within hair follicles in patients with vitiligo and are responsible for repigmentation of the skin after treatment.**
- **Directly sampling immune markers within lesional skin in patients with vitiligo might provide sensitive and specific biomarkers of disease activity and early treatment response.**

What is still unknown?

- **What is the initial trigger of vitiligo in the absence of chemical exposure?**
- **How does innate inflammation in patients with vitiligo result in loss of self-tolerance and T-cell activation?**
- **Do autoreactive T cells in patients with vitiligo target neoantigens and, if so, how are they formed?**
- **What are the functional roles of genetic risk alleles in patients with vitiligo?**
- **How will the efficacy of new targeted therapies in patients with vitiligo compare with existing treatments?**

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