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Vitiligo: Mechanisms of Pathogenesis and Treatment

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Abstract

Vitiligo is an autoimmune disease of the skin that targets pigment-producing melanocytes and results in patches of depigmentation that are visible as white spots. Recent research studies have yielded a strong mechanistic understanding of this disease. Autoreactive cytotoxic $CD8^+$ T cells engage melanocytes and promote disease progression through the local production of IFN-γ, and IFN-γ-induced chemokines are then secreted from surrounding keratinocytes to further recruit T cells to the skin through a positive-feedback loop. Both topical and systemic treatments that block IFN-γ signaling can effectively reverse vitiligo in humans; however, disease relapse is common after stopping treatments. Autoreactive resident memory T cells are responsible for relapse, and new treatment strategies focus on eliminating these cells to promote long-lasting benefit. Here, we discuss basic, translational, and clinical research studies that provide insight into the pathogenesis of vitiligo, and how this insight has been utilized to create new targeted treatment strategies.

INTRODUCTION

Clinical Vitiligo

Vitiligo was described 3,500 years ago in Egyptian and Indian texts, and the social stigma associated with this disfiguring disease was evident from the very beginning. The Atharvaveda, an ancient text written in India between 1500 and 1000 BCE, records details of white patches on the skin, as do the Egyptian Ebers Papyrus (1500 BCE) and the book of Leviticus in the Hebrew Bible from approximately the same time. Indian literature indicates that marriage of a son or daughter to one who has these white patches is "abhorred" (1). Early Buddhist literature states that men and women with vitiligo were not eligible for ordainment, and Hindu texts suggest that those who suffered from this disease may have stolen clothes in their former existence (2).

Vitiligo is characterized by patchy skin depigmentation that can be present on any part of the body. It affects ~1% of the world's population without any significant difference in prevalence due to sex, ethnicity, or geographic region (3). As in ancient times, vitiligo negatively influences patients' quality of life by decreasing self-esteem and causing significant psychological distress (4–6). This reduction in quality of life is comparable to other burdensome skin diseases such as psoriasis and eczema (5). Vitiligo skin lesions are observable signs of disease that cause shame, anxiety, and depression (4). Visible sites such as the hands and face are commonly affected, and patients often especially fear the spread and worsening of their disease at these locations (4).

Public misconceptions and negative social stigma associated with vitiligo continue to impact those with the disease. One of my patients (J.E.H.) who flew to our clinic on an airplane was seated next to a woman who asked to change seats because of his vitiligo, despite reassurance that it was not contagious. Another woman with vitiligo was on the subway in New York City when she was approached by a child who said, "You look like a monster, but I know you're not!" A man originally from Pakistan but living in the United Kingdom inquired about getting an amputation of his arm in order to remove his vitiligo, as he worried that his family would reject him due to vitiligo but not if he were missing an arm (7). Thus, developing a better understanding of disease pathogenesis to develop more effective treatments would have an enormous impact on those who suffer from vitiligo.

Current Treatments

Unlike most autoimmune diseases, vitiligo is fully reversible. Vitiligo primarily destroys the pigment-producing cells, melanocytes, located in the epidermis between the hair follicles (interfollicular epidermis). However, the disease commonly spares melanocytes residing within the hair follicle because of immune privilege at this site, similar to other privileged sites that contain melanocytes, such as the brain, eye, and inner ear. Hair follicles also contain melanocyte stem cells that are capable of repopulating the epidermis of vitiligo lesions with functional, newly differentiated melanocytes that possess the capacity to restore normal pigmentation. Thus, clinical repigmentation of vitiligo lesions typically appears in a punctate, perifollicular pattern, and areas of vitiligo lesions containing no hair or white hairs—where autoimmunity has not spared the follicular melanocyte populations—do not repigment (8) (**Figure 1**).

Treatments listed for vitiligo in historical texts include cow dung or urine; elephant stool; cobra snake bones; topical acids; and heavy metals, including arsenic. While these have thankfully been abandoned, one ancient treatment is still used today. At one time, ancient Indians and Egyptians treated vitiligo with select herbs that were applied topically or taken orally, followed by sun exposure. These herbs have been discovered to contain psoralens, which are currently used in purified forms and combined with UVA light to treat skin diseases, a therapy called PUVA, for psoralen

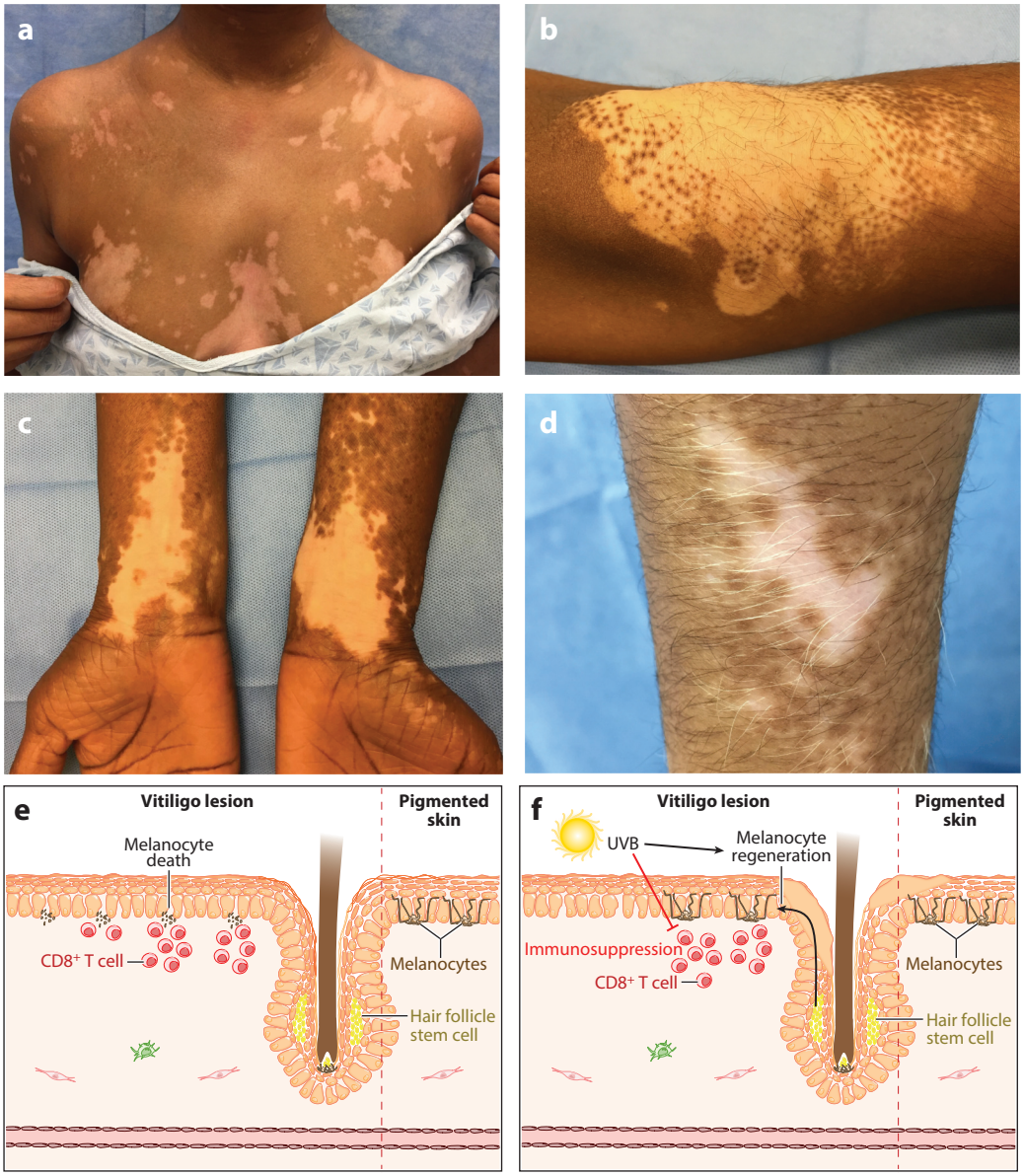

Figure 1

(*a*) Vitiligo presents as patches of depigmentation due to autoimmune melanocyte destruction. Due to the common preservation of melanocyte stem cells among hair follicle stem cells, vitiligo lesions can repigment. (*b*) Repigmentation usually presents in a perifollicular pattern. Certain anatomic sites do not repigment, (*c*,*d*) including sites lacking hair such as the ventral wrists and locations containing white hairs. (*e*) Lesions are characterized by melanocyte death and infiltrates of $CD8^+$ T cells that preferentially localize to melanocytes at the borders of lesions. (*f*) Reversal of disease is achieved through immunosuppression and stimulation of melanocyte stem cells.

plus UVA light. Therefore, a version of this modern vitiligo therapy was used over three millennia ago, and this treatment was then rediscovered in the twentieth century (9). In rare patients, vitiligo may regress without treatment (10, 11); however, this is most likely due to sun exposure. Most patients require interventional treatment to observe significant reversal of vitiligo.

Predictably for an autoimmune disease, immunosuppression is an important component of the clinical management of vitiligo. Topical corticosteroids and calcineurin inhibitors promote repigmentation (12–14), and systemic corticosteroid treatment helps to stabilize very active disease (14–17). However, successful repigmentation of vitiligo requires the accomplishment of two treatment goals: the suppression of autoimmunity, as well as the regeneration of melanocytes from their stem cell niche in the hair follicle. PUVA utilizes UVA light to convert psoralen compounds into DNA-reactive, oxidative chemical products that both suppress immune function and stimulate melanocyte proliferation and pigmentation (18, 19). The combined immunosuppressive and pigment-stimulating properties may explain the strong efficacy of PUVA as a vitiligo treatment.

However, more recent clinical studies reveal that narrow-band UVB (nbUVB) phototherapy provides superior repigmentation of vitiligo lesions with fewer adverse side effects, such as an increased risk of skin cancer noted only with PUVA therapy (20–25). Similar to PUVA, UVB light also suppresses cutaneous immunity while stimulating melanocyte proliferation and pigmentation (26–29). Thus, nbUVB has largely replaced PUVA as first-line therapy for vitiligo, and conventional treatment strategies now incorporate nbUVB phototherapy in combination with topical corticosteroids and/or topical calcineurin inhibitors (25).

Current treatments for vitiligo remain far from ideal, as they are not universally effective in all patients, they do not repigment all anatomic locations, and they can be quite cumbersome for patients to use. Phototherapy requires several weekly visits to a dermatologist, and topical treatments are applied twice daily to all affected skin. Furthermore, all current treatments provide only short-term benefit, as patients relapse at a rate of 40% within the first year after discontinuing treatment (30). Thus, while many of the earlier treatments have been replaced in modern times by safer options, the search for even more effective, targeted therapies has continued.

Vitiligo usually appears in patients before the age of 30 years, and since it rarely regresses spontaneously, it enables close observation of the disease and its progression over decades. This visibility as well as its high incidence and ready access to affected tissues have enabled detailed study of vitiligo through translational research approaches over decades, providing a strong foundation for modern studies to determine detailed mechanisms of pathogenesis (9, 31). Current understanding of vitiligo now offers an unprecedented opportunity to develop better treatments.

Early Studies into the Pathogenesis of Vitiligo—Degenerative Theory

The cause of depigmentation in vitiligo is clearly the loss of melanocytes from the skin, yet it was long debated whether this was through a degenerative or autoimmune process. Difficulty culturing melanocytes derived from human vitiligo patients (32, 33) and increased sensitivity to exogenous insults suggested that degeneration might explain melanocyte loss (34). Melanocytes from vitiligo patients were noted to proliferate more slowly than healthy control melanocytes (32), and they also demonstrated dysregulated redox balance associated with low expression of catalase (35). Catalase protects cells from reactive oxygen species (ROS) by reducing hydrogen peroxide to oxygen and water, and melanocytes in particular produce high levels of ROS as a by-product of melanin production. Thus, the culture of vitiligo patient melanocytes required compensatory media supplements including growth factors or catalase (33, 36).

Vitiligo patient melanocytes were also more susceptible in vitro to oxidative treatments, including cumene hydroperoxide and UVB light (37, 38). Dysregulated redox balance was reported

within vitiligo patient skin, which had elevated hydrogen peroxide levels and increased oxidative by-products (35, 39–41). Treatment of vitiligo patient skin with exogenous catalase (pseudocatalase), hypothesized to benefit vitiligo by normalizing ROS, was, however, ineffective (42); and so it is unclear whether redox imbalance in vitiligo skin is a cause or consequence of vitiligo. Additionally, when human vitiligo lesional skin was grafted onto nude mice, the skin rapidly repigmented—demonstrating that intrinsic melanocyte death was not the sole cause of vitiligo (43). Thus, melanocytes in vitiligo patients are certainly abnormal relative to healthy controls; however, this abnormality does not appear to be sufficient for disease.

Genetics of Vitiligo

The concept that genetic factors contribute to vitiligo was first sparked by the identification of eight families that each had a very high incidence of vitiligo (44–46). In addition, individuals who had first-degree relatives with vitiligo were noted to have an elevated risk for developing the disease: approximately 6% compared to 1% or less in the general population (46, 47). Further studies suggested that polygenic, multifactorial inheritance accounted for the partial heritability of vitiligo (46, 48, 49). Confirming an important role for genetic factors in vitiligo, the twin concordance rate was found to be 23% (47); but since the twin concordance is not 100%, this simultaneously emphasized the contributory significance of other factors. Current understanding suggests that complex interactions of genetic, environmental, and stochastic factors account for development of vitiligo (46).

Several vitiligo patients were observed to develop multiple autoimmune disorders (46), which led to a study of shared heritable risk for autoimmunity. Indeed, risk for development of vitiligo is greater when either patients or their family members have any of several autoimmune diseases, including type 1 diabetes mellitus, autoimmune thyroiditis, pernicious anemia, Addison disease, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, alopecia areata, or autoimmune gastritis (46, 47, 50, 51). Therefore, genetic factors generally do not appear to predispose patients only to development of vitiligo, but instead confer susceptibility to several related autoimmune diseases.

Consistent with genetic risk for developing several autoimmune diseases, genome-wide association studies (GWAS) identified approximately 50 genetic loci that confer risk for vitiligo, and many of these loci are shared with other autoimmune diseases (46). Polymorphisms in *HLA-A* confer the most significant genetic risk of vitiligo (46, 52), and the second- and third-most significant risk factors for vitiligo also relate to antigen presentation (*HLA-DRB1/DQA1*, *CPVL*) (46, 53). *HLA* genes directly present antigen, and *CPVL* is a peptidase involved in antigen processing. Risk alleles also identified genes that mediate immune target cell lysis (*GZMB*, *FASLG*), regulate adaptive immunity (*FOXP3*, *CTLA4*, *IL2RA*, *BACH2*), drive innate immunity (*TICAM1*, *IFIH1*, *CD80*), and also directly implicate melanocytes (*TYR*, *PMEL*, *MC1R*, *OCA2-HERC2*, *IRF4*) (46, 53).

These same polymorphisms also account for genetic risk of vitiligo regardless of family history for vitiligo, suggesting that the pathogenesis of familial-associated vitiligo is the same as that of sporadic cases (53). The only polymorphism that was enriched in familial vitiligo was associated with *HLA-DRB1/DQA1* (53). These genetic associations support the hypothesis that complex interactions among melanocytes, innate immunity, and adaptive immunity all contribute to initiation of vitiligo. Genetic polymorphisms likely predispose patients to autoimmune dysregulation, and melanocyte abnormalities along with other nongenetic factors probably initiate pathologic killing of melanocytes. Environmental triggers then act upon genetically predisposed individuals to help initiate and exacerbate vitiligo. These factors are discussed in detail below in the context of disease initiation.

T CELL RECRUITMENT AND FUNCTION IN VITILIGO

CD8+ T Cells Cause Vitiligo

Vitiligo patients were found to have elevated serum titers of melanocyte-reactive antibodies (54), and these autoantibodies were found to damage human melanocytes both in culture and when split-thickness human skin was grafted onto nude mice (55, 56). However, antibody-induced melanocyte damage was only mildly induced with the use of vitiligo patient sera compared with healthy control sera (55, 56), and titers of melanocyte-specific antibodies were only modestly elevated in vitiligo patients compared to nonvitiligo controls (54, 57). Importantly, autoantibody titers do not correlate with disease activity (58). Furthermore, vitiligo lesions develop in well-defined patches, which is difficult to explain if driven by a ubiquitous distribution of antibodies. All of these findings suggest that antimelanocyte antibodies are not a primary driver of vitiligo pathogenesis.

Early histology of human vitiligo lesions revealed lymphocytic infiltrates at the border of depigmented lesions, where disease was most active. These infiltrates were comprised predominantly of $CD8^+$ T cells that preferentially localized to the dermal-epidermal borders, adjacent to melanocytes (59–62). Flow cytometry analysis of fluid isolated from human vitiligo skin through suction blistering confirmed that $CD8^+$ T cells are increased in the skin of active vitiligo lesions (63). Melanoma studies first identified several melanocyte-specific antigens that are recognized by self-reactive $CD8^+$ T cells (64), including tyrosinase, Melan-A/MART-1, gp100, TRP-1, and TRP-2; and vitiligo patients have elevated numbers of these cells in their peripheral blood relative to healthy controls (65, 66). Furthermore, peri-lesional skin is highly enriched with melanocyte-specific $CD8^+$ T cells (62), and clones propagated from these cells are capable of killing melanocytes in vitro (62, 66, 67). $CD8^+$ T cells isolated from vitiligo lesions also infiltrated autologous healthy skin explants ex vivo and induced melanocyte apoptosis in a pattern similar to clinical vitiligo pathology. Notably, similarly isolated $CD4^+$ T cells were unable to cause melanocyte apoptosis in autologous skin explants (67). These studies provided strong evidence that $CD8^+$ T cells are both necessary and sufficient for elimination of melanocytes in vitiligo lesions.

Similarly, effective responses following immunotherapy for metastatic melanoma are associated with $CD8^+$ T cells. Melanoma immunotherapy includes blockade of T cell checkpoint inhibitors, which disrupts T cell tolerance in the tissue, and adoptive cell therapy, which expands autologous tumor-infiltrating T cells *ex vivo* for therapeutic reinjection into patients. Notably, $CD8^+$ T cell infiltration of tumors is crucial to the efficacy of both strategies (68, 69), and these cells are believed to control melanomas through perforin-dependent cytolysis (70). Melanoma immunotherapy commonly triggers new-onset vitiligo, occurring in approximately 4% of melanoma patients treated by immunotherapy (71), and vitiligo lesions that are triggered by melanoma immunotherapy are replete with melanocyte-specific $CD8^+$ T cells similar to idiopathic vitiligo lesions (72). $CD8^+$ T cells are, therefore, critical to the elimination of melanoma as well as the pathogenesis of vitiligo.

Vitiligo Requires the IFN-γ-Chemokine Axis for T Cell Recruitment and Function

Regarding the mechanisms by which $CD8^+$ T cells cause vitiligo, their production of the cytokine IFN-γ is central to disease. Gene expression analysis of human lesional skin revealed predominant upregulation of *IFNG* (73, 74), as well as IFN-γ-induced genes, including the T cell chemokine receptor *CXCR3* and its multiple ligands: *CXCL9*, *CXCL10*, and *CXCL11* (74). Consistent with this finding, skin biopsies of vitiligo lesions also contain lymphocytes that are

predominantly CXCR3$^+$ (74–77); melanocyte-specific T cells isolated from the blood and skin of vitiligo patients are enriched for the CXCR3 receptor (74, 77); and CXCL9 is a reliable skin biomarker of vitiligo disease activity (63). Functional studies in vitiligo mouse models confirmed a mechanistic role for this pathway in vitiligo, as IFN-γ, CXCR3, and CXCL10 are all required for disease (74, 78–80). When IFN-γ is neutralized by antibody treatment or when T cells lack expression of *CXCR3*, autoreactive T cells fail to migrate into the skin and thus do not cause vitiligo (74, 80).

Experiments using chemokine reporter mice revealed that keratinocytes produce the bulk of the CXCR3 ligands CXCL9 and CXCL10, and functional experiments revealed that keratinocytes are predominantly responsible for T cell recruitment (81). CXCL9 appears primarily responsible for bulk T cell recruitment, as in its absence the number of melanocyte-reactive T cells within vitiligo lesions is reduced by tenfold (74). However, despite this reduction in T cell number, vitiligo severity is unchanged, suggesting that T cells are over-recruited during vitiligo. In contrast, in the absence of CXCL10, vitiligo incidence and severity are reduced (74); however, bulk T cell recruitment is unchanged. Interestingly, the number of T cells found in the epidermis relative to the dermis in the skin is reduced when CXCL10 is absent, suggesting that CXCL10 is required for localization of T cells within the skin, and possibly their function as well (74).

Therefore, T cells secrete IFN-γ, which induces CXCL9 and CXCL10 production by keratinocytes to recruit additional T cells and promote vitiligo progression. In addition to initiation and progression of vitiligo, the IFN-γ-chemokine pathway is also required for maintenance of established lesions, as both depletion of CXCR3-expressing cells and neutralization of CXCL10 chemokine induced repigmentation of vitiligo (74, 82), positioning the IFN-γ-chemokine axis as a potential therapeutic target.

Clinical JAK Inhibitor Treatments Provide Mechanistic Insight

Consistent with mouse studies that demonstrated the functional significance of IFN-γ to vitiligo, disruption of IFN-γ signaling by Janus kinase (JAK) inhibitors contributes to repigmentation of human vitiligo patients. IFN-γ signals by binding to its cell surface receptor (IFNgR), which forms a heterodimeric protein complex in the presence of IFN-γ that activates gene transcription through associated JAKs. There are four members of the JAK family (JAK1, JAK2, JAK3, TYK2), and inhibitors directed at several combinations of these kinases are being tested as new treatments for many human diseases (83). The IFNgR signals through JAK1 and JAK2, and inhibition of either JAK1 or JAK2 reduces IFN-γ signaling. The first two JAK inhibitors approved for human use, ruxolitinib and tofacitinib, both reduce IFNgR signaling by targeting JAK1/2 (ruxolitinib) or JAK1/3 (tofacitinib) (84).

Two case reports first recognized the potential of these JAK inhibitors to induce repigmentation in human vitiligo patients (85, 86). First, a woman with vitiligo treated with low-dose tofacitinib repigmented her face and hands (85). Next, a man with both vitiligo and alopecia areata, an autoimmune disease that causes hair loss, noted rapid reversal of both diseases following oral treatment with ruxolitinib. In addition, this subject had elevated levels of serum CXCL10 for over one year prior to treatment, and this level dropped precipitously after starting treatment with ruxolitinib. This indicated that not only was ruxolitinib an effective treatment for vitiligo but also its mechanism may involve blocking IFN-γ-induced chemokines (86).

A case series tested topical ruxolitinib as a treatment for vitiligo and supported this approach as an effective treatment strategy (87). Based on the rationale provided through mechanistic experiments in mice and translational studies in human tissues, as well as these preliminary findings, a larger clinical trial was initiated by Incyte to test the efficacy of topical ruxolitinib for vitiligo

(NCT02809976). Early data from this trial indicate impressive responses, suggesting that this will be an effective treatment for vitiligo patients (88). Additional clinical trials have been initiated to test novel JAK inhibitors as well, including a topical study by Aclaris (NCT03468855) as well as an oral study by Pfizer (NCT03715829) (**Table 1**).

Early clinical observations using JAK inhibitors suggested that light exposure might be required for effective reversal of vitiligo. Subjects treated with JAK inhibitors repigmented more rapidly and completely in sun-exposed skin, or when subjects were concomitantly treated with nbUVB phototherapy (89, 90). This was consistent with the idea that JAK inhibitors are effective immunosuppressants but might not provide the appropriate stimulation required for melanocyte regeneration. Because of this, some studies are incorporating nbUVB phototherapy with JAK inhibition into clinical trials. However, early results from the Incyte trial include good repigmentation despite no addition of phototherapy, suggesting that this may not be necessary. Future studies will shed light on this controversy and provide information on how best to treat patients with these new modalities. In addition, it will be important to closely follow the side effect profile of these newer drugs, particularly when tested orally, as they can block signaling of multiple inflammatory cytokines, which my impact infectious and other responses as well.

Testing of second-generation JAK inhibitors in vitiligo patients is likely to provide additional insight into vitiligo pathogenesis. The rationale for blocking JAK1/2 in vitiligo is clear, as it is required for IFN-γ signaling. However, JAKs are required for signaling of multiple cytokines, and additional inflammatory pathways may be important for vitiligo as well. JAK3 is required for signaling of many essential cytokines through the common gamma chain (91), and human JAK3 deficiency results in severe combined immunodeficiency syndrome (SCID) (92). TYK2 is required for cytokine signaling through IFNAR, IL-12R, and IL-23R, which have not been functionally implicated in the pathogenesis of vitiligo, yet these cytokines contribute to several other autoimmune and autoinflammatory conditions (93, 94). One study suggested that IFNAR signaling may promote vitiligo (95), but functional studies to confirm this are still lacking. In addition, small-molecule inhibitors are rarely specific for a single enzyme, and tofacitinib and ruxolitinib are no exception (92). Thus, nonspecific targeting of multiple pathways could be partly responsible for the observed clinical efficacy of these drugs in vitiligo. Pfizer's ongoing clinical vitiligo trial (NCT03715829) is testing a specific JAK3 inhibitor as well as a TYK2/JAK1 inhibitor, and results of this trial may provide additional information about vitiligo pathogenesis.

TNF-α, IL-23, and IL-17 Do Not Drive Vitiligo

TNF-α, IL-23, and IL-17 have been suggested as potential targets for vitiligo treatment, as the US Food and Drug Administration (FDA) has approved several drugs that target these cytokines; these drugs effectively treat psoriasis, psoriatic arthritis, and rheumatoid arthritis (96–98). Interventions that target TNF-α, IL-23, or IL-17 are thereby efficacious for other immune-mediated diseases, including one that affects the skin. Concentrations of IL-17 and TNF-α are mildly elevated in vitiligo patient serum and in skin lesions (99), so it was proposed that these cytokines might also contribute to vitiligo (100). However, these treatments do not appear to reverse vitiligo (101, 102), and they have even been reported to induce or worsen vitiligo (103–106).

Consistent with the conclusion that these cytokines are dispensable for vitiligo, we have not observed significant expression of this pathway in vitiligo lesions, in contrast to IFN-γ and its induced genes (74). In addition, T cells isolated from human psoriatic skin produce large amounts of IL-17A when stimulated ex vivo, whereas T cells isolated from human vitiligo skin and stimulated the same way produce IFN-γ without any IL-17A (77, 107). These studies provide consistent, strong evidence that TNF-α, IL-23, and IL-17 do not significantly contribute to vitiligo

Table 1 Clinical trials for emerging vitiligo treatments

Study start date	NCT number	Sponsor	Centers (n)	Trial phase	Treatment groups	Drug type	Mechanism of action	Subjects (n)	Allocation	Status	Results
June 2011	NCT01430195	Clinuvel	2	1	Afamelanotide + nbUVB versus nbUVB alone	Mc1R agonist	Stimulation of melanocyte stem cells	56	Randomized	Completed	Combination therapy with afamelanotide improved repigmentation by ~1.5-fold on the face and upper extremities ($P < 0.05$) relative to nbUVB alone
September 2011	NCT01382589	Clinuvel	Not Listed	2	Afamelanotide + nbUVB versus nbUVB alone	Mc1R agonist	Stimulation of melanocyte stem cells	15	Randomized	Completed	None available
None Listed	None Listed	Clinuvel	Not Listed	2	Afamelanotide + nbUVB versus nbUVB alone	Mc1R agonist	Stimulation of melanocyte stem cells	Not listed	Randomized	Completed	Combination therapy with afamelanotide improved repigmentation on the whole body ($P < 0.05$)
January 2012	NCT01517893	Investigator initiated	1	2	Simvastatin versus placebo	HMG-CoA reductase antagonist	Reduction of IFN-γ production	15	Randomized	Completed	Simvastatin did not improve repigmentation relative to placebo
January 2016	NCT02809976	Investigator initiated	1	2	Topical ruxolitinib cream (no placebo)	JAK1/2 inhibitor	Inhibition of cytokine signaling	12	Single group assignment	Completed	With twice-daily, topical application of ruxolitinib for 20 weeks, patients observed a mean improvement of 23% in overall VASI score ($P = 0.02$). Four patients had baseline lesions on the face greater than 0.5% body surface area, and these patients observed a 76% improvement of facial VASI score at week 20 ($P = 0.001$).

(*Continued*)

Table 1 (*Continued*)

Study start date	NCT number	Sponsor	Centers (n)	Trial phase	Treatment groups	Drug type	Mechanism of action	Subjects (n)	Allocation	Status	Results
April 2017	NCT03099304	Incyte	26	2	Topical ruxolitinib cream versus placebo	JAK1/2 inhibitor	Inhibition of cytokine signaling	157	Randomized	Active, not recruiting	None available
March 2018	NCT03468855	Aclaris	4	2	ATI-50002 topical solution versus placebo	JAK3 inhibitor	Inhibition of cytokine signaling	34	Randomized	Active, not recruiting	None available
November 2018	NCT03715829	Pfizer	37	2b	PF-06651600 versus placebo; experimental extension for PF-06651600 versus placebo versus PF06700841 versus nbUVB	JAK1/TYK2 inhibitor	Inhibition of cytokine signaling	Estim. 330	Randomized	Recruiting	None available

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl–coenzyme A; nbUVB, narrow-band UVB; NCT, National Clinical Trial.

pathogenesis. In fact, clinical reports of induction or worsening of disease after receiving drugs that target this pathway suggest that these cytokines may actually suppress autoimmunity in vitiligo, possibly through a process known as cytokine cross regulation (108).

AUTOIMMUNE RESIDENT MEMORY T CELLS CAUSE VITILIGO RELAPSE

Resident Memory T Cells in Vitiligo

Relapse of vitiligo is common, approximately 40% within the first year after stopping treatments (30), and the vitiligo patient who rapidly responded to oral ruxolitinib also relapsed immediately after discontinuing his treatment (89). Importantly, relapse occurs at the exact same areas that were previously involved, suggesting a role for autoimmune memory within the skin that is not cleared with existing treatments. Resident memory T (Trm) cells are a long-lived subset of T cells that remain within most nonlymphoid tissues following T cell–driven inflammation (109, 110). They were first described in the context of viral infections and are required to mediate recall responses during reinfection (109, 111–114).

Skin Trm cells are characterized by their long-lived residence within the skin, patrolling the epidermis and papillary dermis, where they arrest upon encounter of their cognate antigen (115). They are marked by the expression of surface markers CD69, CD103, and CD49a (107, 116, 117). Residence in skin and mucosal tissues requires special adaptations to these environments, as the prevalence of glucose and oxygen required for energy production is more limited than in the blood and lymph. Thus, Trm cells have unique transcriptional programs and different metabolic needs than naive and effector memory T cells (118–120). The tissue of residence also impacts Trm gene expression of these cells, as Trm cells from the skin, lung, and gut are each very different (121, 122). Based on their ability to reside in tissues for long periods and rapidly induce immune responses against viruses, they were strong candidates for inducing relapse of vitiligo lesions.

Multiple groups identified $CD8^+$ T cells possessing a Trm cell phenotype within vitiligo lesions, in both mouse models of disease and human patients (77, 107, 123, 124). These cells express the characteristic markers CD69, CD103, and CD49a and are more prevalent in vitiligo lesions. They also express CXCR3 at high levels (77), as well as IFN-γ and TNF-α after stimulation (107). We found that Trm cells were specific for autoantigens using melanocyte antigen-specific pentamers, identifying these cells as autoreactive Trm cells (123).

Functional Role of Trm Cells in Vitiligo

The functional role of Trm cells has been a matter of debate. In some contexts, these cells are sufficient for controlling viral titers during reinfection, and recirculating cells are not required (111, 125). In other models, they primarily produce cytokines for recruitment of effector T cells from the circulation (112, 113, 126). For example, T cell receptor stimulation activates skin Trm cells to produce cytokines similar to effector memory T cells (127), yet in most studies $CD8^+$ Trm cells appear to have poor cytotoxic capacity. In healthy human skin, $CD8^+$ Trm cells do not express the transcription factors T-bet or Eomes, have low levels of granzyme B and perforin, and highly express the checkpoint inhibitor PD-1 (127). Skin $CD8^+$ Trm cells isolated from healthy human skin are significantly worse than circulating effector memory T cells at lysis of allogeneic target cells (107, 128), unless incubated with IL-15 (107). Trm cells provide immunity to melanoma through prevention of tumor outgrowth rather than tumor elimination, suggesting that Trm cells lack cytotoxic capacity (129, 130). This limited cytotoxicity of Trm cells and efficient production of cytokines suggest that they serve as sentinels to recruit effectors from the circulation.

In a mouse model of vitiligo, we found that selective depletion of recirculating memory T cells or inhibition of T cell migration into skin using the S1P inhibitor FTY720 each caused rapid repigmentation of vitiligo, despite the fact that these approaches do not affect the number of Trm cells (123). Therefore, we concluded that Trm cells are incapable of independently maintaining vitiligo in the absence of additional T cell recruitment, consistent with their role as sentinels that recruit effectors from the circulation, rather than cytotoxic effectors. In this model, a large number of melanocyte-specific $CD8^+$ Trm cells in the skin produce IFN-γ as well as CXCL10, likely using the IFN-γ-chemokine pathway for maintenance of vitiligo lesions, similar to the initiation and progression of vitiligo that is described above (123). Therefore, Trm cells likely mediate long-term maintenance and potential relapse of vitiligo in human patients through cytokine-mediated recruitment of T cells from the circulation. Treatments that inhibit this pathway without affecting Trm cell number, such as conventional treatments and JAK inhibitors, effectively reverse disease, but relapse occurs after they are discontinued (**Figure 2**).

Autoreactive Trm Cell Survival Requires IL-15

Because Trm cells appear to be responsible for relapse of vitiligo after stopping current treatments, they were proposed as potential treatment targets to generate durable, long-lasting reversal of disease. The initial formation of $CD8^+$ Trm cells requires IL-15, IL-7, and TGF-β (121, 131), yet IL-15 has been the only identified cytokine required for their maintenance (124, 132). IL-15 is constitutively produced in many peripheral tissues by myeloid and stromal cells, and its signaling is complex (133). IL-15 binds to three receptor chains, CD215, CD122, and CD132 (the common gamma chain), which can all be expressed by lymphocytes to bind IL-15 as a soluble cytokine (133, 134). However, most commonly IL-15 is *trans*-presented to lymphocytes on the surface of myeloid and stromal cells that express CD215, which is used to anchor the cytokine to the cell surface membrane and prevent disbursal throughout the body (133). Lymphocyte recognition of IL-15 most often occurs by binding to CD122 and CD132, and this signal can stimulate antigen-independent proliferation (135) as well as cell survival (133).

We found that mouse and human autoreactive Trm cells in vitiligo expressed high levels of CD122 in the blood and lesional skin, and lesional keratinocytes induced expression of CD215, suggesting that this pathway was active in vitiligo lesions (124). Interestingly, CD122 expression was significantly higher on melanocyte-specific T cells in both mouse and human vitiligo compared to endogenous memory T cells, suggesting that autoreactive T cells were more dependent on this cytokine than non-autoreactive T cells. We found that anti-CD122 blocking antibody inhibited IL-15-mediated T cell survival but not IL-2-mediated proliferation in vitro. This is consistent with an important role of IL-15 in mediating T cell survival, but not proliferation, which appears to be driven by IL-2. Systemic treatment with anti-CD122 blocking antibody reversed vitiligo and depleted autoreactive, melanocyte-specific Trm cells from the skin, spleen, and lymph nodes, without affecting endogenous memory T cells. Anti-CD122 blocking antibody, therefore, had a specific effect on autoreactive T cells. Short-term treatment had long-lasting effects, and local treatment through skin injection was effective as well (124). These observations are consistent with an important role for IL-15 in maintaining autoreactive Trm cells in vitiligo and suggest this could be an effective targeted treatment strategy for vitiligo patients.

REGULATION MITIGATES VITILIGO SEVERITY

Regulatory T Cells Suppress Autoreactive Effectors to Limit Vitiligo

The identification of a regulatory gene signature in GWAS suggests that immune regulators such as T regulatory (Treg) cells play an important role in restraining autoimmunity in vitiligo. Treg

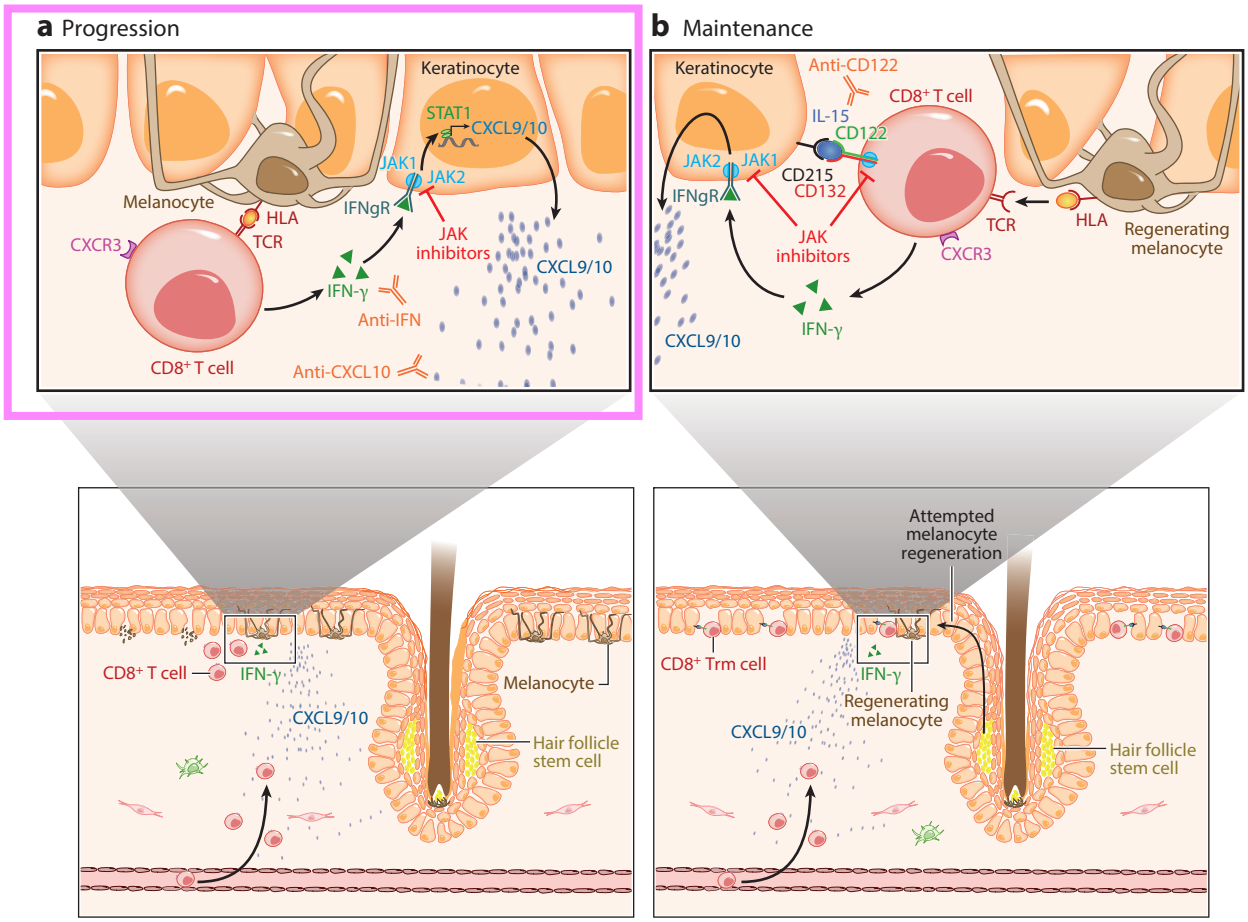

Figure 2

(*a*) Vitiligo progression occurs through a positive-feedback loop that requires continued T cell recruitment. Melanocyte-reactive CD8$^+$ T cells produce IFN-γ upon encounter of melanocyte antigen, which induces local keratinocytes to produce CXCL9 and CXCL10, leading to additional recruitment through the CXCR3 chemokine receptor. (*b*) Established vitiligo lesions are maintained by melanocyte-reactive, Trm cells, which remain long-lived in skin through IL-15-dependent survival signals. Emerging drugs capable of interrupting vitiligo pathogenesis are labeled in orange. Abbreviations: TCR, T cell receptor; Trm, resident memory T.

cells comprise a subset of CD4$^+$ T cells characterized by expression of the FOXP3 transcription factor, and they potently suppress T effector activity to play a crucial role in preventing autoimmunity. Patients with immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome lack functional Treg cells due to a mutation in the *FOXP3* gene and as a result suffer from various autoimmune disorders, including vitiligo (8). Similarly, scurfy mice with dysfunctional FOXP3 lack Treg cells and exhibit widespread autoimmunity (136), highlighting an important role for Treg cells in maintaining tolerance to self-tissues.

Consistent with a role for Treg cells in suppressing vitiligo, studies in different mouse models of vitiligo demonstrated that increased Treg cell number in the skin correlates with reduced disease severity. Two groups reported increased severity of vitiligo when Treg cells were depleted with either CD4 or CD25 antibodies (78, 137). In one study, induced expression of CCL22 in the skin led to increased cutaneous Treg cell infiltration and decreased depigmentation (138). Another

group found that adoptively transferring exogenous Treg cells to three-week-old vitiligo-prone mice increased numbers of Treg cells in the skin and prevented disease (137). Miao et al. (139) injected vitiligo-prone mice with PD-L1-Fc, which enhanced Treg cell accumulation in the skin and markedly reversed depigmentation. These results support the hypothesis that Treg cell number in skin is important for reducing T effector–driven depigmentation, and thus helping to control the progression of vitiligo.

Several groups report disrupted Treg cell function in human patients with vitiligo, although there is no consensus on exactly where the disruption lies: in Treg cell number, suppressive activity, or ability to migrate to the skin. One group reported that Treg cells isolated from the peripheral blood mononuclear cells of vitiligo patients exhibited a reduced ability to suppress the proliferation and activation of $CD8^+$ T cells in vitro (140); however, another reported normal activity of Treg cells from vitiligo patients but reduced number in the skin. They suggested that reduced skin homing of Treg cells, instead of impaired Treg cell function, contributes to disease pathogenesis (141). Other studies reported no significant decrease in the number of skin Treg cells in vitiligo lesions when using immunohistochemistry (142, 143), while another reported a significant reduction in these cells (144). Thus, it is unclear exactly how Treg cells are disrupted in human vitiligo, yet the phenotype of effector T cells in vitiligo patients also suggests the presence of a Treg cell deficiency. Treg cells naturally reduce the proliferation and activation of self-reactive, effector T cells, a phenomenon termed anergy; and phenotype analysis of peripheral blood mononuclear cells suggests that melanocyte-reactive $CD8^+$ T cells escape anergy within vitiligo patients (145). Additional studies will be required to confidently determine how Treg cell deficits contribute to vitiligo, and to investigate the potential of bolstering Treg cell function as a new method of treating vitiligo.

IMPORTANT QUESTIONS STILL TO BE ANSWERED

Thus, we have gained significant insight into vitiligo pathogenesis through effective animal models, translational research, and clinical trials. In fact, these insights have launched several clinical trials and will likely transform our approach to the management of patients who suffer from this disease. However, despite this significant progress, many questions still remain about the pathogenesis of vitiligo, providing an opportunity for more research to better understand vitiligo specifically, as well as autoimmunity in general.

Mechanisms of Autoimmune Killing

While cytotoxicity of $CD8^+$ T cells is suspected to be required for melanocyte elimination in both vitiligo and melanoma immunotherapy, the exact mechanism used by these cells is still unclear. Multiple effector proteins can be used to kill target cells, including perforin, granzyme, Fas Ligand, TRAIL, cytokines, and others (70). Classically, cytotoxic T cells are believed to primarily use perforin and granzyme as a rapid mechanism of killing tumor cells or virus-infected cells, while cytotoxicity through Fas ligand may serve as a synergistic, slower-acting alternative method (146). However, different intracellular signaling pathways drive T cell killing through perforin, granzyme, and FasL, and thus how they are selectively used by cytotoxic T cells and how they interact are currently not clear (70). In addition, the mechanisms that mediate T cell killing in autoimmunity may also be different than that for tumors and viral-infected cells. Thus, it is still unknown which mechanisms are used to eliminate melanocytes in vitiligo, and further studies will be required to elucidate this process in detail.

Melanocyte Abnormalities and Neoantigen Formation

Although the mechanisms responsible for the progression of autoimmunity in vitiligo are well characterized, it remains unknown how vitiligo begins and why melanocytes are selectively targeted. As mentioned above, melanocytes from the unaffected skin of vitiligo patients do not appear completely normal, and a melanocyte abnormality likely initiates autoimmune targeting of melanocytes. One hypothesis suggests that abnormal melanocyte antigens induce inflammation that leads to autoimmunity. Alleles that affect antigen presentation confer the highest genetic risk for vitiligo (46), and altered proteins known as neoantigens can be highly immunogenic.

Melanoma is immunogenic in part due to somatic DNA mutations that result in altered proteins, a common mechanism of neoantigen formation (147). The immune system is more likely to mount an attack against neoantigens, as thymic epithelial cells responsible for educating T cells do not express these proteins (148). Consequently, central tolerance mechanisms do not remove highly self-reactive T cells that target neoantigens (148), which results in generation of T cells with high-affinity receptors for neoantigens, similar to foreign antigens (148). However, it is unlikely that self-reactive T cells in vitiligo target highly mutated proteins, since untransformed melanocytes do not have the same opportunity to mutate their DNA. Several biochemical processes can generate neoantigens as well, and untransformed beta islet cells have been reported to do this through citrullination, deamidation, oxidation, carbonylation, alternative mRNA splicing, peptide fusion, and defective ribosomal initiation of translation (149). Fusion peptides in particular have been described as a significant target of autoimmunity in type 1 diabetes (150, 151). Thus, while neoantigens have not yet been identified in association with vitiligo, it is plausible that melanocyte neoantigens contribute to onset of vitiligo similar to those described in type 1 diabetes and melanoma.

Melanocyte Abnormalities and Cellular Stress

As mentioned above, elevated cellular stress in melanocytes may also contribute to disease. Intrinsic signs of cell stress in melanocytes from unaffected skin of vitiligo patients include a dilated endoplasmic reticulum and increased ROS (152, 153). Cell stress has been directly associated with formation of neoantigens within beta islet cells (149), and melanocyte stress may form neoantigens as well. Increased ROS within melanocytes of vitiligo patients has been associated with lipid peroxidation (41), and it is possible that ROS forms melanocyte neoantigens through protein oxidation and carbonylation.

Cell stress can also activate innate immunity through the release of damage-associated molecular patterns (DAMPs). It is plausible that DAMPs are constantly released from stressed melanocytes and lead to subclinical skin inflammation in vitiligo patients (154). Consistent with this, nonlesional skin of vitiligo patients contains elevated numbers of lymphocytes compared to healthy controls (155). Thus, melanocyte stress can likely contribute to initiation of autoimmunity through both neoantigen formation and activation of innate immunity, and more studies are required to determine how intrinsic melanocyte stress most significantly contributes to vitiligo pathogenesis.

Chemical Triggers of Melanocyte Stress

As discussed above, environmental factors also contribute to vitiligo, and specific chemical exposures have been identified as clear triggers of the disease. The first chemical trigger of vitiligo was described in 1939 when 52% of factory workers developed vitiligo after skin exposure to monobenzyl ether of hydroquinone (MBEH) (156). In fact, this chemical is now FDA approved for use in

vitiligo patients who have extensive depigmentation and wish to gain a uniform skin tone through rapid worsening of their disease and loss of their remaining pigment (157). Several other environmental chemicals and commercial chemical products have also been associated with vitiligo (158, 159), and in 26% of cases the depigmentation involves remote locations other than the site of direct chemical contact—strongly implicating these chemicals as inducers of autoimmunity, rather than acting through direct toxicity (158). Often, chemical-induced vitiligo occurs subsequent to an initial allergic contact dermatitis reaction, yet it can also occur without any preceding dermatitis (159). Additionally, chemical-induced vitiligo is indistinguishable from idiopathic vitiligo by histologic examination.

One common feature of all vitiligo-triggering chemicals is structural homology to tyrosine, the initial substrate for melanogenesis (159). Expression of melanocyte-specific enzymes, including tyrosinase and TRP1, is required for in vitro melanocyte toxicity of chemicals associated with triggering vitiligo (160, 161). Therefore, it is likely that chemical-induced vitiligo occurs through a common mechanism that is dependent on chemical interactions with melanocyte-specific enzymes.

Chemical inducers of vitiligo are also reported to induce melanocyte stress. MBEH has been the best studied of these, and within melanocytes it induces ROS, the unfolded protein response, cellular autophagy, and increased secretion of microvesicles called exosomes (160). Exosomes contain many types of cargo, including heat shock proteins (162), and it is most likely within exosomes that chemical triggers of vitiligo induce melanocyte secretion of inducible heat shock protein 70 (HSP70i) (163), a well-characterized DAMP. DAMPs induce inflammation through pattern recognition receptors such as Toll-like receptors and nucleotide oligomerization domain (NOD)-like receptors (NLRs), and one NLR in particular, NLRP1, has been associated with vitiligo in a linkage study (164). Chemical-induced secretion of HSP70i stimulates dendritic cells to increase expression of coactivation markers CD80 and CD86 (163, 165), and topical application of MBEH in mice leads to macrophage recruitment within the skin (166). These findings suggest that chemical triggers of vitiligo may function by inducing melanocyte stress and subsequent innate immune activation, yet it remains unclear how these events possibly lead to the autoimmune T cell responses that mediate vitiligo (**Figure 3**).

Innate Immunity in Vitiligo

In addition to identification of multiple innate immune genes by vitiligo GWAS, several observations provide evidence that innate immunity contributes to vitiligo pathogenesis. While vitiligo-triggering chemicals induce features of melanocyte stress, addition of HSP70i alone exacerbates a mouse model of vitiligo, possibly through the activation of dendritic cells in the skin (167). Furthermore, delivery of a mutant form of HSP70i that interferes with endogenous HSP70i signaling is capable of dampening disease in mouse and swine models of vitiligo (168, 169). Therefore, innate immune activation by DAMPs can directly influence vitiligo in animal models, and intentional delivery of mutant HSP70i to the skin provides a potential new treatment for vitiligo by modulating innate immunity.

There are signs of innate immune activation in human vitiligo as well. Active vitiligo lesions demonstrate infiltration of several types of innate immune cells, including dendritic cells, Langerhans cells, macrophages, and natural killer (NK) cells (60, 155, 170, 171). CLEC2B is an activating ligand of NK cell receptors that is increased in active vitiligo skin (155). Inflammasomes, key sensors in innate immune cells that frequently incorporate NLRs, are additionally activated in vitiligo lesions, and a cytokine processed by these sensors, IL-1β, is also increased (170, 172). These

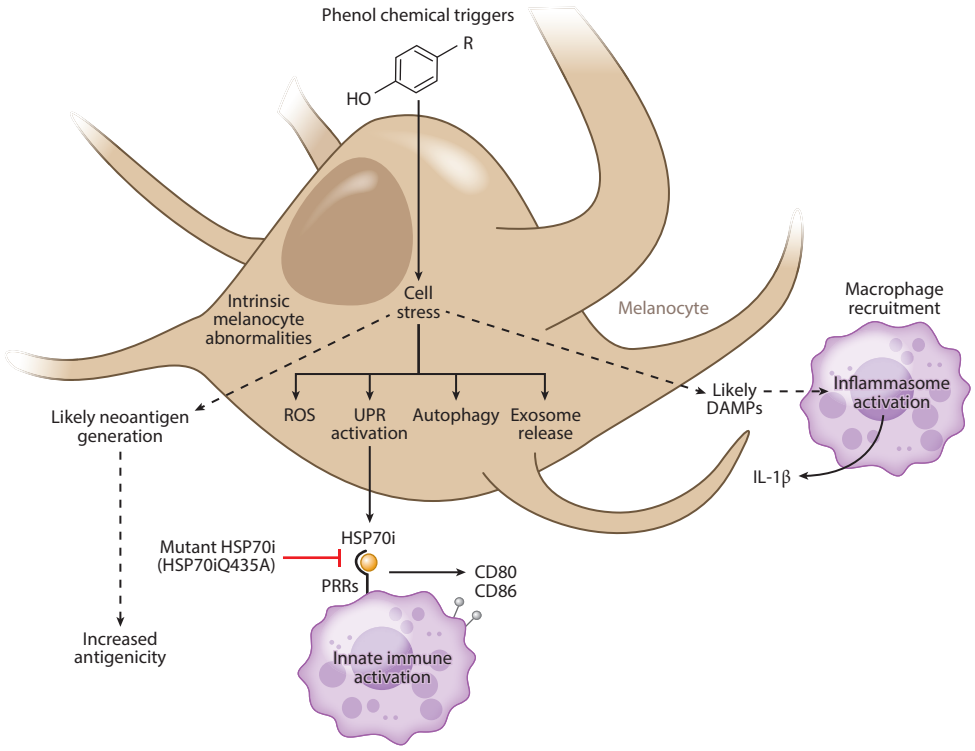

Figure 3

Melanocyte stress likely contributes to vitiligo through release of DAMPs and possible formation of neoantigens. Intrinsic abnormalities and environmental phenols both induce melanocyte stress, leading to elevated ROS, UPR activation, autophagy, and exosome release. HSP70i stimulates PRRs to activate innate immune cells. Macrophages also infiltrate active vitiligo lesions and release IL-1β through inflammasome activation. Abbreviations: DAMP, damage-associated molecular pattern; HSP70i, inducible heat shock protein 70; PRR, pattern recognition receptor; ROS, reactive oxygen species; UPR, unfolded protein response.

findings all implicate innate immune activation with vitiligo, yet the details connecting melanocytes, innate immune cells, and cytotoxic T cells are still unclear.

Checkpoint Inhibitors Trigger Vitiligo

Drugs that interfere with immunoregulatory pathways on T cells, known as checkpoint inhibitors, are highly effective as cancer treatments, and vitiligo is a frequent side effect of this therapeutic strategy. FDA-approved cancer immunotherapies include pembrolizumab and nivolumab, which target PD-1, as well as ipilimumab and tremelimumab, which target CTLA4. Both PD-1 and CTLA4 are inhibitory receptors on T cells that suppress T cell receptor signaling, and treatments that block these receptors have been reported to trigger and worsen vitiligo in an average of 2% of patients (71). However, the incidence of new-onset vitiligo following pembrolizumab treatment has been reported by several studies to be between 4% and 26%, which demonstrates that immunotherapy-induced vitiligo is a common phenomenon (173). Onset of vitiligo is a positive prognostic sign that correlates with improved tumor response and longer survival of cancer

patients (71, 174, 175), and associations of vitiligo with single-target immunotherapies provide insight into pathways involved in restraining autoimmunity. The propensity for checkpoint inhibitors to trigger vitiligo strongly implicates immune checkpoint proteins as critical factors for maintenance of peripheral tolerance to self-tissues. Future studies to dissect the events that lead to vitiligo during therapy with checkpoint inhibitors may reveal new pathways involved in the pathogenesis of vitiligo as well as other autoimmune diseases.

Mechanisms of Immune Privilege in the Hair Follicle

Melanocyte stem cells reside in the bulge region of hair follicles in close proximity to epithelial hair follicle stem cells, and they are identifiable as nonpigmented, $CD34^+$, DCT^+, c-Kit^-, $Tyrosinase^-$ cells with low MITF expression (176–179). These cells are frequently protected from autoimmune attack in vitiligo, a phenomenon described as immune privilege of the hair follicle, which is shared by other organs such as the brain, eye, and inner ear. While the pathways directly responsible for protecting the melanocyte stem cells are unknown, a number of hypotheses exist (180). One explanation is that melanocyte stem cells do not express the antigens targeted in vitiligo and therefore hide from the autoimmune attack. However, loss of hair pigment does occur in vitiligo with some regularity, suggesting that hair follicle melanocytes are capable targets of autoimmunity. Thus, enhanced immune suppression within the follicular microenvironment likely prevents autoimmune attack at these locations.

An absence of lymphatics within the follicle may limit efficient trafficking of immune cells in or out of the location. Low expression of MHC-I and MHC-II (181) and few Langerhans cells have been reported in the hair follicle (182), suggesting that decreased antigen presentation may exist there. Elevated expression of TGF-β and IL-10 has been reported (180), as well as expression of receptors that suppress NK cell activation (183). The hair follicle reportedly recruits Treg cells and mast cells (184), which may help to suppress immune responses. These and other mechanisms have been suggested to drive immune privilege of the hair follicle, yet functional studies to determine which pathways are protective in the context of autoimmunity are lacking. Future studies to define these key pathways that protect melanocyte stem cells within the hair follicle may offer therapeutic strategies to treat the interfollicular epidermis as well.

Regenerating Melanocytes to Enhance Repigmentation

Once autoimmune attack on melanocytes is achieved, clinical repigmentation of vitiligo lesions requires the proliferation, migration, and differentiation of new melanocytes within lesions. Thus, mechanisms to promote this process would synergize with immunosuppression to treat vitiligo. UV radiation promotes repopulation of the epidermis with melanocyte stem cells from the hair follicle (185, 186), which is likely why it is such an effective treatment for vitiligo, providing both immunosuppression and melanocyte stem cell stimulation. The mechanism for this process appears to rely on signaling of melanocyte stem cells through Mc1r, endothelin receptors, and wnt receptors, which can all be stimulated by keratinocyte-derived ligands and can induce melanocyte migration to the interfollicular epidermis following UVB exposure (185, 187–190). These receptors induce melanocyte activation through signaling pathways that are all dependent on β-catenin activation (191, 192). Thus, treatments that modulate these pathways may synergize with immunosuppressants for vitiligo, potentially substituting for treatment with UVB.

Consistent with this hypothesis, addition of ACTH, a ligand for Mc1r, synergized with UVB treatment to enhance the generation of epidermal melanocytes in mice (185). Clinical trials with vitiligo patients also reported that a synthetic Mc1r ligand, afamelanotide, enhanced phototherapy-induced repigmentation (193, 194). However, side effects of this treatment included

Table 2 Emerging Vitiligo Treatments

Drug class	Treatments	Treatment goal	Mechanism
JAK inhibitors	Ruxolitinib (JAK1/2)	Immunosuppression	Disruption of cytokine signaling (IFN-γ, IL-15)
	Tofacitinib (JAK1/3)		
	ATI-50002 (JAK1/3)		
	PF-06651600 (JAK3)		
	PF06700841 (JAK1/TYK2)		
Anti-IL-15 biologics	Anti-IL-15 mAb	Immunosuppression and Trm cell elimination	Blocking of IL-15 signaling
	Anti-CD122 mAb		
Plasmid HSP70i gene therapy	HSP70iQ435A	Block endogenous innate immune activation	Mutant HSP70i counteracts innate immune activation by endogenous HSP70i
Mc1R agonist	Afamelanotide	Melanocyte regeneration	Stimulation of melanocyte stem cell proliferation and migration
wnt agonist	SKL2001		

Abbreviations: mAb, monoclonal antibody; Trm, T resident memory.

hyperpigmentation, which accentuated the visible contrast of depigmented lesions, as well as headaches and nausea, and several participants dropped out of the study (194).

Importantly, targeting melanocyte stem cells through wnt receptors also induces repigmentation and may not induce clinical hyperpigmentation. Topical application of the chemical phorbol 12-myristate 13-acetate (PMA) stimulates repigmentation in mice through wnt-dependent activation of β-catenin (195, 196), and evidence suggests that pharmacologic stimulation of β-catenin induces the same repigmentation process within humans. Specific wnt activators as well as inhibitors of glycogen synthase 3β (GSK3β), the critical negative regulator of β-catenin activation, were both found to induce melanocyte proliferation and differentiation within biopsies of vitiligo patient lesions that were cultured ex vivo (197). Additional studies will be required to determine whether targeting melanocyte activation will enhance clinical responses seen with immunosuppressants, and whether this approach can replace cumbersome treatments with nbUVB.

CONCLUSIONS

Vitiligo has been diagnosed and treated for millennia; however, new discoveries in vitiligo pathogenesis within recent years promise to usher in more targeted, effective, and safe treatments for patients who suffer from this devastating disease (**Table 2**). The IFN-γ-chemokine signaling axis is responsible for T cell recruitment during both the progression and maintenance of vitiligo, and promising results from clinical trials with JAK inhibitors suggest that these may become the first approved medical treatments. Relapse of disease after stopping treatment is mediated by autoreactive Trm cells, and targeting their maintenance in the skin through IL-15 or other approaches may prove to be a more durable treatment strategy. Future methods promoting regulation in the skin, such as Treg cell activation or use of factors that confer immune privilege in the hair follicle, may reverse disease by resetting skin homeostasis rather than simply inhibiting inflammation. Stimulation of melanocyte stem cell regeneration may replace cumbersome phototherapy treatments and synergize with immune therapies to produce more effective treatment strategies. Many unanswered questions regarding vitiligo initiation and progression still exist, providing innumerable opportunities for additional discovery. Finally, shared pathogenesis between vitiligo and other autoimmune diseases suggests that insights gained from significant advancements in vitiligo, which is uniquely accessible to translational research, may accelerate research in diseases that are more difficult to study.

DISCLOSURE STATEMENT

J.E.H. is an investigator for recent and ongoing clinical trials to test JAK inhibitors as treatments for vitiligo, including those by Incyte, Aclaris, Dermavant, and Pfizer. J.E.H. has a patent application pending that protects the use of targeting IL-15 as a treatment for vitiligo. J.E.H. is the scientific founder of Villaris Therapeutics, Inc., and holds equity in Villaris Therapeutics, Inc., a company focused on developing new treatments for vitiligo. K.E. holds equity in Villaris Therapeutics, Inc.

LITERATURE CITED

1. Barman S. 1995. Switra and its treatment in Veda. *Ancient Sci. Life* 15:71–74
2. Singh G, Ansari Z, Dwivedi RN. 1974. Vitiligo in ancient Indian medicine. *Arch. Dermatol.* 109:913
3. Zhang Y, Cai Y, Shi M, Jiang S, Cui S, et al. 2016. The prevalence of vitiligo: a meta-analysis. *PLOS ONE* 11(9):e0163806-17
4. Salzes C, Abadie S, Seneschal J, Whitton M, Meurant J-M, et al. 2016. The Vitiligo Impact Patient scale (VIPs): development and validation of a vitiligo burden assessment tool. *J. Investig. Dermatol.* 136(1):52–58
5. Linthorst Homan MW, Spuls PI, de Korte J, Bos JD, Sprangers MA, van der Veen JPW. 2009. The burden of vitiligo: patient characteristics associated with quality of life. *J. Am. Dermatol.* 61(3):411–20
6. Elbuluk N, Ezzedine K. 2017. Quality of life, burden of disease, co-morbidities, and systemic effects in vitiligo patients. *Dermatol. Clin.* 35(2):117–28
7. Ezzedine K, Eleftheriadou V, Whitton M, van Geel N. 2015. Vitiligo. *Lancet* 386(9988):74–84
8. Frisoli ML, Harris JE. 2017. Vitiligo: Mechanistic insights lead to novel treatments. *J. Allergy Clin. Immunol.* 140(3):654–62
9. Nordlund JJ. 2017. The medical treatment of vitiligo. *Dermatol. Clin.* 35(2):107–16
10. Koranne RV, Sachdeva KG. 1988. Vitiligo. *Int. J. Dermatol.* 27(10):676–81
11. Ezzedine K, Harris JE. 2019. Vitiligo. In *Fitzpatricks Dermatology in General Medicine*, ed. S Kang, M Amagai, AL Bruckner, AH Enk, DJ Margolis, et al., chap. 76, pp. 1330–50. New York: McGraw-Hill Educ. 9 ed.
12. Bleehen SS. 1976. The treatment of vitiligo with topical corticosteroids. *Br. J. Dermatol.* 94:1–9
13. Njoo MD, Spuls PI, Bos JD, Westerhof W, Bossuyt PMM. 1998. Nonsurgical repigmentation therapies in vitiligo. *Arch. Dermatol.* 134:1532–40
14. Passeron T. 2017. Medical and maintenance treatments for vitiligo. *Dermatol. Clin.* 35(2):163–70
15. Radakovic-Fijan S, Fürnsinn-Friedl AM, Hönigsmann H, Tanew A. 2001. Oral dexamethasone pulse treatment for vitiligo. *J. Am. Acad. Dermatol.* 44(5):814–17
16. Kanwar AJ, Mahajan R, Parsad D. 2013. Low-dose oral mini-pulse dexamethasone therapy in progressive unstable vitiligo. *J. Cutan. Med. Surg.* 17(4):259–68
17. Pasricha JS, Khaitan BK. 1993. Oral mini-pulse therapy with betamethasone in vitiligo patients having extensive or fast-spreading disease. *Int. J. Dermatol.* 32:753–57
18. Pathak MA, Fitzpatrick TB. 1992. The evolution of photochemotherapy with psoralens and UVA (PUVA): 2000 BC to 1992 AD. *J. Photochem. Photobiol. B* 14(1–2):3–22
19. Abdel-Naser MB, Liakou AI, Elewa R, Hippe S, Knolle J, Zouboulis CC. 2016. Increased activity and number of epidermal melanocytes in lesional psoriatic skin. *Dermatology* 232(4):425–30
20. Westerhof W, Nieuweboer-Krobotova L. 1997. Treatment of vitiligo with UV-B radiation versus topical psoralen plus UV-A. *Arch. Dermatol.* 133(12):1525–28
21. Yones SS, Palmer RA, Garibaldinos TM, Hawk J. 2007. Randomized double-blind trial of treatment of vitiligo. *Arch. Dermatol.* 143:578–84
22. Parsad D, Kanwar AJ, Kumar B. 2006. Psoralen-ultraviolet A versus narrow-band ultraviolet B phototherapy for the treatment of vitiligo. *J. Eur. Acad. Dermatol. Venerol.* 20(2):175–77
23. Mofty ME, Mostafa W, Esmat S, Youssef R, Azzam O, et al. 2006. Narrow band ultraviolet B 311 nm in the treatment of vitiligo: two right-left comparison studies. *Photodermatol. Photoimmunol. Photomed.* 22(1):6–11

24. Bhatnagar A, Kanwar AJ, Parsad D, De D. 2007. Comparison of systemic PUVA and NB-UVB in the treatment of vitiligo: an open prospective study. *J. Eur. Acad. Dermatol. Venerol.* 21(5):638–42
25. Esmat S, Hegazy RA, Shalaby S, Chu-Sung Hu S, Lan C-CE. 2017. Phototherapy and combination therapies for vitiligo. *Dermatol. Clin.* 35(2):171–92
26. Poon TSC, Barnetson RSC, Halliday GM. 2005. Sunlight-induced immunosuppression in humans is initially because of UVB, then UVA, followed by interactive effects. *J. Investig. Dermatol.* 125(4):840–46
27. Schwarz T. 2005. Mechanisms of UV-induced immunosuppression. *Keio J. Med.* 54(4):165–71
28. Choi W, Miyamura Y, Wolber R, Smuda C, Reinhold W, et al. 2010. Regulation of human skin pigmentation in situ by repetitive UV exposure: molecular characterization of responses to UVA and/or UVB. *J. Investig. Dermatol.* 130(6):1685–96
29. van Schanke A, Jongsma MJ, Bisschop R, van Venrooij GMCAL, Rebel H, de Gruijl FR. 2005. Single UVB overexposure stimulates melanocyte proliferation in murine skin, in contrast to fractionated or UVA-1 exposure. *J. Investig. Dermatol.* 124(1):241–47
30. Cavalié M, Ezzedine K, Fontas E, Montaudié H, Castela E, et al. 2015. Maintenance therapy of adult vitiligo with 0.1% tacrolimus ointment: a randomized, double blind, placebo-controlled study. *J. Investig. Dermatol.* 135(4):970–74
31. Millington GWM, Levell NJ. 2007. Vitiligo: the historical curse of depigmentation. *Int. J. Dermatol.* 46:990–95
32. Puri N, Mojamdar M, Ramaiah A. 1987. In vitro growth characteristics of melanocytes obtained from adult normal and vitiligo subjects. *J. Investig. Dermatol.* 88(4):434–38
33. Puri N, Mojamdar M, Ramaiah A. 1989. Growth defects of melanocytes in culture from vitiligo subjects are spontaneously corrected in vivo in repigmenting subjects and can be partially corrected by the addition of fibroblast-derived growth factors in vitro. *Arch. Dermatol. Res.* 281:178–84
34. Dell'Anna ML, Mastrofrancesco A, Sala R, Venturini M, Ottaviani M, et al. 2007. Antioxidants and narrow band-UVB in the treatment of vitiligo: a double-blind placebo controlled trial. *Clin. Exp. Dermatol.* 32:631–36
35. Schallreuter KU, Moore J, Wood JM, Beazley WD, Gaze DC, et al. 1999. In vivo and in vitro evidence for hydrogen peroxide (H_2O_2) accumulation in the epidermis of patients with vitiligo and its successful removal by a UVB-activated pseudocatalase. *J. Investig. Dermatol. Symp. Proc.* 4(1):91–96
36. Medrano EE, Nordlund JJ. 1990. Successful culture of adult human melanocytes obtained from normal and vitiligo donors. *J. Investig. Dermatol.* 95:441–45
37. Maresca V, Roccella M, Roccella F, Camera E, Del Porto G, et al. 1997. Increased sensitivity to peroxidative agents as a possible pathogenic factor of melanocyte damage in vitiligo. *J. Investig. Dermatol.* 109(3):310–13
38. Jimbow K, Chen H, Park J-S, Thomas PD. 2000. Increased sensitivity of melanocytes to oxidative stress and abnormal expression of tyrosinase-related protein in vitiligo. *Br. J. Dermatol.* 144:55–65
39. Shalbaf M, Gibbons NCJ, Wood JM, Maitland DJ, Rokos H, et al. 2008. Presence of epidermal allantoin further supports oxidative stress in vitiligo. *Exp. Dermatol.* 17(9):761–70
40. Koca R, Armutcu F, Altinyazar C, Gurel A. 2004. Oxidant-antioxidant enzymes and lipid peroxidation in generalized vitiligo. *Exp. Dermatol.* 29:406–9
41. Dell'Anna ML, Ottaviani M, Albanesi V, Vidolin AP, Leone G, et al. 2007. Membrane lipid alterations as a possible basis for melanocyte degeneration in vitiligo. *J. Investig. Dermatol.* 127(5):1226–33
42. Gawkrodger DJ. 2009. Pseudocatalase and narrowband ultraviolet B for vitiligo: clearing the picture. *Br. J. Dermatol.* 161(4):721–22
43. Gilhar A, Pillar T, Eidelman S, Etzioni A. 1989. Vitiligo and idiopathic guttate hypomelanosis: repigmentation of skin following engraftment onto nude mice. *Arch. Dermatol.* 125:1363–66
44. Stuttgen G. 1950. Die vitiligo in erbbiologischer betrachtung. *Z. Haut Geschlechtskr.* 9:451–57
45. Teindel H. 1950. Familiare vitiligo. *Z. Haut Geschlechtskr.* 9:457–62
46. Spritz RA, Andersen GHL. 2017. Genetics of vitiligo. *Dermatol. Clin.* 35(2):245–55
47. Alkhateeb A, Fain PR, Thody A, Bennett DC, Spritz RA. 2003. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. *Pigment Cell Res.* 16(3):208–14
48. Hafez M, Sharaf L, Abd el-Nabi SM. 1983. The genetics of vitiligo. *Acta Derm. Venerol.* 63:249–51

49. Das SK, Majumder PP, Majumdar TK, Haldar B. 1985. Studies on vitiligo. II. Familial aggregation and genetics. *Genet. Epidemiol.* 2:255–62
50. Hadi A, Wang JF, Uppal P, Penn LA, Elbuluk N. 2019. Comorbid diseases of vitiligo: a 10-year cross-sectional retrospective study of an urban US population. *J. Am. Acad. Dermatol.* In press
51. Gill L, Zarbo A, Isedeh P, Jacobsen G, Lim HW, Hamzavi I. 2016. Comorbid autoimmune diseases in patients with vitiligo: a cross-sectional study. *J. Am. Dermatol.* 74(2):295–302
52. Li Z, Ren J, Niu X, Xu Q, Wang X, et al. 2016. Meta-analysis of the association between vitiligo and human leukocyte antigen-A. *BioMed. Res. Int.* 2016(5):1–13
53. Roberts GHL, Paul S, Yorgov D, Santorico SA, Spritz RA. 2019. Family clustering of autoimmune vitiligo results principally from polygenic inheritance of common risk alleles. *Am. J. Hum. Genet.* 105(2):364–72
54. Naughton GK, Eisinger M, Bystryn J-C. 1983. Antibodies to normal human melanocytes in vitiligo. *J. Exp. Med.* 158:246–51
55. Cui J, Arita Y, Bystryn J-C. 1993. Cytolytic antibodies to melanocytes in vitiligo. *J. Investig. Dermatol.* 100(6):812–15
56. Gilhar A, Zelickson B, Ulman Y, Etzioni A. 1995. In vivo destruction of melanocytes by the IgG fraction of serum from patients with vitiligo. *J. Investig. Dermatol.* 105(5):683–86
57. Merimsky O, Shoenfeld Y, Baharav E, Altomonte M, Chaitchik S, et al. 1996. Melanoma-associated hypopigmentation: Where are the antibodies? *Am. J. Clin. Oncol.* 19(6):613–18
58. Kroon MW, Kemp EH, Wind BS, Krebbers G, Bos JD, et al. 2012. Melanocyte antigen-specific antibodies cannot be used as markers for recent disease activity in patients with vitiligo. *J. Eur. Acad. Dermatol. Venerol.* 27(9):1172–75
59. Badri AMTA, Todd PM, Garioch JJ, Gudgeon JE, Stewart DG, Goudie RB. 1993. An immunohistological study of cutaneous lymphocytes in vitiligo. *J. Pathol.* 170:149–55
60. Le Poole IC, van der wijngaard RM, Westerhof W, Das P. 1996. Presence of T cells and macrophages in inflammatory vitiligo skin parallels melanocyte disappearance. *Am. J. Pathol.* 148:1219–28
61. Ahn SK, Choi EH, Lee SH, Won JH, Hann SK, Park Y-K. 1994. Immunohistochemical studies from vitiligo. *Yonsei Med. J.* 35:404–10
62. Wankowicz-Kalinska A, van den Wijngaard RMJGJ, Tigges BJ, Westerhof W, Ogg GS, et al. 2003. Immunopolarization of $CD4^+$ and $CD8^+$ T cells to Type-1-like is associated with melanocyte loss in human vitiligo. *Lab. Investig.* 83(5):683–95
63. Strassner JP, Rashighi M, Ahmed Refat M, Richmond JM, Harris JE. 2017. Suction blistering the lesional skin of vitiligo patients reveals useful biomarkers of disease activity. *J. Am. Dermatol.* 76(5):847–855.e5
64. Kirkin AF, Dzhandzhugazyan K, Zeuthen J. 1998. Melanoma-associated antigens recognized by cytotoxic T lymphocytes. *AMPIS* 106:665–79
65. Ogg GS, Dunbar PR, Romero P, Chen J-L, Cerundolo V. 1998. High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. *J. Exp. Med.* 21:1203–8
66. Palermo B, Campanelli R, Garbelli S, Mantovani S, Lantelme E, et al. 2001. Specific cytotoxic T lymphocyte responses against Melan-A/MART1, tyrosinase and gp100 in vitiligo by the use of major histocompatibility complex/peptide tetramers: the role of cellular immunity in the etiopathogenesis of vitiligo. *J. Investig. Dermatol.* 117(2):326–32
67. van den Boorn JG, Konijnenberg D, Dellemijn TAM, van der Veen JPW, Bos JD, et al. 2009. Autoimmune destruction of skin melanocytes by perilesional t cells from vitiligo patients. *J. Investig. Dermatol.* 129(9):2220–32
68. Edwards J, Wilmott JS, Madore J, Gide TN, Quek C, et al. 2018. $CD103^+$ Tumor-resident $CD8^+$ T cells are associated with improved survival in immunotherapy-naive melanoma patients and expand significantly during anti-PD-1 treatment. *Clin. Cancer Res.* 24:3036–45
69. Wu R, Forget M-A, Chacon J, Bernatchez C, Haymaker C, et al. 2012. Adoptive T-cell therapy using autologous tumor-infiltrating lymphocytes for metastatic melanoma. *Cancer J.* 18(2):160–75
70. Martinez-Lostao L, Anel A, Pardo J. 2015. how do cytotoxic lymphocytes kill cancer cells? *Clin. Cancer Res.* 21(22):5047–56

71. Teulings H-E, Limpens J, Jansen SN, Zwinderman AH, Reitsma JB, et al. 2015. Vitiligo-like depigmentation in patients with stage III-IV melanoma receiving immunotherapy and its association with survival: a systematic review and meta-analysis. *JCO* 33(7):773–81
72. Yee C, Thompson JA, Roche P, Byrd DR, Lee PP, et al. 2000. Melanocyte destruction after antigen-specific immunotherapy of melanoma. *J. Exp. Med.* 192(11):1637–44
73. Grimes PE, Morris R, Avaniss-Aghajani E, Soriano T, Meraz M, Metzger A. 2004. Topical tacrolimus therapy for vitiligo: therapeutic responses and skin messenger RNA expression of proinflammatory cytokines. *J. Am. Acad. Dermatol.* 51(1):52–61
74. Rashighi M, Agarwal P, Richmond JM, Harris TH, Dresser K, et al. 2014. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. *Sci. Transl. Med.* 6(223):223ra23
75. Bertolotti A, Boniface K, Vergier B, Mossalayi D, Taïeb A, et al. 2014. Type I interferon signature in the initiation of the immune response in vitiligo. *Pigment Cell Melanoma Res.* 27(3):398–407
76. Wang XX, Wang QQ, Wu JQ, Jiang M, Chen L, et al. 2016. Increased expression of CXCR3 and its ligands in patients with vitiligo and CXCL10 as a potential clinical marker for vitiligo. *Br. J. Dermatol.* 174(6):1190–91
77. Boniface K, Jacquemin C, Darrigade A-S, Dessarthe B, Martins C, et al. 2018. Vitiligo skin is imprinted with resident memory CD8 T cells expressing CXCR3. *J. Investig. Dermatol.* 138(2):355–64
78. Gregg RK, Nichols L, Chen Y, Lu B, Engelhard VH. 2010. Mechanisms of Spatial and Temporal Development of Autoimmune Vitiligo in Tyrosinase-Specific TCR Transgenic Mice. *J. Immunol.* 184(4):1909–17
79. Amos SM, Pegram HJ, Westwood JA, John LB, Devaud C, et al. 2011. Adoptive immunotherapy combined with intratumoral TLR agonist delivery eradicates established melanoma in mice. *Cancer Immunol. Immunother.* 60(5):671–83
80. Harris JE, Harris TH, Weninger W, Wherry EJ, Hunter CA, Turka LA. 2012. A mouse model of vitiligo with focused epidermal depigmentation requires IFN-γ for autoreactive $CD8^+$ T-cell accumulation in the skin. *J. Investig. Dermatol.* 132(7):1869–76
81. Richmond JM, Bangari DS, Essien KI, Currimbhoy SD, Groom JR, et al. 2017. Keratinocyte-derived chemokines orchestrate T-cell positioning in the epidermis during vitiligo and may serve as biomarkers of disease. *J. Investig. Dermatol.* 137(2):350–58
82. Richmond JM, Masterjohn E, Chu R, Tedstone J, Youd ME, Harris JE. 2017. CXCR3 depleting antibodies prevent and reverse vitiligo in mice. *J. Investig. Dermatol.* 137(4):982–85
83. Damsky W, King BA. 2017. JAK inhibitors in dermatology: The promise of a new drug class. *J. Am. Dermatol.* 76(4):736–44
84. Jackson SW, Jacobs HM, Arkatkar T, Dam EM, Scharping NE, et al. 2016. B cell IFN-γ receptor signaling promotes autoimmune germinal centers via cell-intrinsic induction of BCL-6. *J. Exp. Med.* 213(5):733–50
85. Craiglow BG, King BA. 2015. Tofacitinib citrate for the treatment of vitiligo. *JAMA Dermatol.* 151(10):1110–13
86. Harris JE, Rashighi M, Nguyen N, Jabbari A, Ulerio G, et al. 2016. Rapid skin repigmentation on oral ruxolitinib in a patient with coexistent vitiligo and alopecia areata (AA). *J. Am. Dermatol.* 74(2):370–71
87. Rothstein B, Joshipura D, Saraiya A, Abdat R, Ashkar H, et al. 2017. Treatment of vitiligo with the topical Janus kinase inhibitor ruxolitinib. *J. Am. Dermatol.* 76(6):1054–60.e1
88. Rosmarin D, Pandya A, Lebwohl M, Grimes P, Hamzavi I, et al. 2019. Efficacy and safety of ruxolitinib cream for the treatment of vitiligo: results of a 24-week randomized, double-blind, dose-ranging, vehicle-controlled study. Presented at 24th World Congress of Dermatology, Milan
89. Liu LY, Strassner JP, Refat MA, Harris JE, King BA. 2017. Repigmentation in vitiligo using the Janus kinase inhibitor tofacitinib may require concomitant light exposure. *J. Am. Dermatol.* 77(4):675–82.e1
90. McKesey J, Pandya AG. 2019. A pilot study of 2% tofacitinib cream with narrowband ultraviolet B for the treatment of facial vitiligo. *J. Am. Acad. Dermatol.* 81(2):646–48
91. Wang H, Brown J, Gao S, Liang S, Jotwani R, et al. 2013. The role of JAK-3 in regulating TLR-mediated inflammatory cytokine production in innate immune cells. *J. Immunol.* 191(3):1164–74

92. Gadina M. 2013. Janus kinases: an ideal target for the treatment of autoimmune diseases. *J. Investig. Dermatol. Symp. Proc.* 16(1):S70–72
93. Sohn SJ, Barrett K, Van Abbema A, Chang C, Kohli PB, et al. 2013. A restricted role for TYK2 catalytic activity in human cytokine responses revealed by novel TYK2-selective inhibitors. *J. Immunol.* 191(5):2205–16
94. Fensome A, Ambler CM, Arnold E, Banker ME, Brown MF, et al. 2018. Dual inhibition of TYK2 and JAK1 for the treatment of autoimmune diseases: discovery of ((*S*)-2,2-difluorocyclopropyl)((1*R*,5S)-3-(2-((1-methyl-1*H*-pyrazol-4-yl)amino)pyrimidin-4-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (PF-06700841). *J. Med. Chem.* 61(19):8597–612
95. Jacquemin C, Rambert J, Guillet S, Thiolat D, Boukhedouni N, et al. 2017. Heat shock protein 70 potentiates interferon alpha production by plasmacytoid dendritic cells: relevance for cutaneous lupus and vitiligo pathogenesis. *Br. J. Dermatol.* 177(5):1367–75
96. Nirula A, Nilsen J, Klekotka P, Kricorian G, Erondu N, et al. 2016. Effect of IL-17 receptor A blockade with brodalumab in inflammatory diseases. *Rheumatology* 55(Suppl. 2):ii43–55
97. Kavanaugh A, Puig L, Gottlieb AB, Ritchlin C, You Y, et al. 2016. Efficacy and safety of ustekinumab in psoriatic arthritis patients with peripheral arthritis and physician-reported spondylitis: post-hoc analyses from two phase III, multicentre, double-blind, placebo-controlled studies (PSUMMIT-1/PSUMMIT-2). *Ann. Rheum. Dis.* 75(11):1984–88
98. Lebwohl M, Strober B, Menter A, Gordon K, Weglowska J, et al. 2015. Phase 3 studies comparing brodalumab with ustekinumab in psoriasis. *N. Engl. J. Med.* 373(14):1318–28
99. Bassiouny DA, Shaker O. 2010. Role of interleukin-17 in the pathogenesis of vitiligo. *Clin. Exp. Dermatol.* 36(3):292–97
100. Singh RK, Lee KM, Vujkovic-Cvijin I, Ucmak D, Farahnik B, et al. 2016. The role of IL-17 in vitiligo: a review. *Autoimmun. Rev.* 15(4):397–404
101. Speeckaert R, Mylle S, Geel N. 2019. IL-17A is not a treatment target in progressive vitiligo. *Pigment Cell Melanoma Res.* 31(2):330–37
102. AlGhamdi KM, Khurrum H, Taieb A, Ezzedine K. 2017. Treatment of generalized vitiligo with anti-TNF-α agents. *J. Drugs Dermatol.* 11(4):534–39
103. Bae JM, Kim M, Lee HH, Kim K-J, Shin H, et al. 2018. Increased risk of vitiligo following anti-tumor necrosis factor therapy: a 10-year population-based cohort study. *J. Investig. Dermatol.* 138(4):768–74
104. Méry-Bossard L, Bagny K, Chaby G, Khemis A, Maccari F, et al. 2016. New-onset vitiligo and progression of pre-existing vitiligo during treatment with biological agents in chronic inflammatory diseases. *J. Eur. Acad. Dermatol. Venerol.* 31(1):181–86
105. Toussirot É, Aubin F. 2016. Paradoxical reactions under TNF-α blocking agents and other biological agents given for chronic immune-mediated diseases: an analytical and comprehensive overview. *RMD Open*. 2(2):e000239-12
106. Webb KC, Tung R, Winterfield LS, Gottlieb AB, Eby JM, et al. 2015. Tumour necrosis factor-α inhibition can stabilize disease in progressive vitiligo. *Br. J. Dermatol.* 173(3):641–50
107. Cheuk S, Schlums H, Sérézal IG, Martini E, Chiang SC, et al. 2017. CD49a expression defines tissue-resident CD8$^+$ T cells poised for cytotoxic function in human skin. *Immunity* 46(2):287–300
108. Palucka AK, Blanck J-P, Bennett L, Pascual V, Banchereau J. 2005. Cross-regulation of TNF and IFN-α in autoimmune diseases. *PNAS* 102:3372–77
109. Mueller SN, Gebhardt T, Carbone FR, Heath WR. 2013. Memory T cell subsets, migration patterns, and tissue residence. *Annu. Rev. Immunol.* 31(1):137–61
110. Steinbach K, Vincenti I, Merkler D. 2018. Resident-memory T cells in tissue-restricted immune responses: for better or worse? *Front. Immunol.* 9:2827
111. Jiang X, Clark RA, Liu L, Wagers AJ, Fuhlbrigge RC, Kupper TS. 2012. Skin infection generates non-migratory memory CD8$^+$ T_{RM} cells providing global skin immunity. *Nature* 483(7388):227–31
112. Schenkel JM, Fraser KA, Beura LK, Pauken KE, Vezys V, Masopust D. 2014. Resident memory CD8 T cells trigger protective innate and adaptive immune responses. *Science* 346(6205):98–101
113. Ariotti S, Hogenbirk MA, Dijkgraaf FE, Visser LL, Hoekstra ME, et al. 2014. Skin-resident memory CD8$^+$ T cells trigger a state of tissue-wide pathogen alert. *Science* 346(6205):101–5

114. Zhu J, Peng T, Johnston C, Phasouk K, Kask AS, et al. 2014. Immune surveillance by CD8αα$^+$ skin-resident T cells in human herpes virus infection. *Nature* 497(7450):494–97
115. Dijkgraaf FE, Matos TR, Hoogenboezem M, Toebes M, Vredevoogd DW, et al. 2019. Tissue patrol by resident memory CD8$^+$ T cells in human skin. *Nat. Immunol.* 20(6):756–64
116. Mueller SN, Mackay LK. 2015. Tissue-resident memory T cells: local specialists in immune defence. *Nat. Rev. Immunol.* 16(2):79–89
117. Topham DJ, Reilly EC. 2018. Tissue-resident memory CD8$^+$ T cells: from phenotype to function. *Front. Immunol.* 9:515
118. Szabo PA, Levitin HM, Miron M, Snyder ME, Senda T, et al. 2019. A single-cell reference map for human blood and tissue T cell activation reveals functional states in health and disease. bioRxiv 555557. **https://doi.org/10.1101/555557**
119. Pan Y, Tian T, Park CO, Lofftus SY, Mei S, et al. 2017. Survival of tissue-resident memory T cells requires exogenous lipid uptake and metabolism. *Nature* 543(7644):252–56
120. Pan Y, Kupper TS. 2018. Metabolic reprogramming and longevity of tissue-resident memory T cells. *Front. Immunol.* 9:745–47
121. Mackay LK, Rahimpour A, Ma JZ, Collins N, Stock AT, et al. 2013. The developmental pathway for CD103$^+$CD8$^+$ tissue-resident memory T cells of skin. *Nat. Immunol.* 14(12):1294–301
122. Miragaia RJ, Gomes T, Chomka A, Jardine L, Riedel A, et al. 2019. Single-cell transcriptomics of regulatory T cells reveals trajectories of tissue adaptation. *Immunity* 50(2):493–97
123. Richmond JM, Strassner JP, Rashighi M, Agarwal P, Garg M, et al. 2019. Resident memory and recirculating memory T cells cooperate to maintain disease in a mouse model of vitiligo. *J. Investig. Dermatol.* 139(4):769–78
124. Richmond JM, Strassner JP, Zapata L Jr., Garg M, Riding RL, et al. 2018. Antibody blockade of IL-15 signaling has the potential to durably reverse vitiligo. *Sci. Transl. Med.* 10:1–10
125. Mackay LK, Stock AT, Ma JZ, Jones CM, Kent SJ, et al. 2012. Long-lived epithelial immunity by tissue-resident memory T (T_{RM}) cells in the absence of persisting local antigen presentation. *PNAS* 109:7037–42
126. Schenkel JM, Fraser KA, Vezys V, Masopust D. 2013. Sensing and alarm function of resident memory CD8$^+$ T cells. *Nat. Immunol.* 14(5):509–13
127. Seidel JA, Vukmanovic-Stejic M, Muller-Durovic B, Patel N, Fuentes-Duculan J, et al. 2018. Skin resident memory CD8$^+$ T cells are phenotypically and functionally distinct from circulating populations and lack immediate cytotoxic function. *Clin. Exp. Immunol.* 194(1):79–92
128. McMaster SR, Wilson JJ, Wang H, Kohlmeier JE. 2015. Airway-resident memory CD8 T cells provide antigen-specific protection against respiratory virus challenge through rapid IFN-γ production. *J. Immunol.* 195(1):203–9
129. Malik BT, Byrne KT, Vella JL, Zhang P, Shabaneh TB, et al. 2017. Resident memory T cells in the skin mediate durable immunity to melanoma. *Sci. Immunol.* 2(10):eaam6346
130. Park SL, Buzzai A, Rautela J, Hor JL, Hochheiser K, et al. 2019. Tissue-resident memory CD8$^+$ T cells promote melanoma-immune equilibrium in skin. *Nature* 565(7739):366–71. Correction. 2019. *Nature* 566(7745):E10
131. Adachi T, Kobayashi T, Sugihara E, Yamada T, Ikuta K, et al. 2015. Hair follicle-derived IL-7 and IL-15 mediate skin-resident memory T cell homeostasis and lymphoma. *Nat. Med.* 21(11):1272–79
132. Baumann NS, Torti N, Welten SPM, Barnstorf I, Borsa M, et al. 2018. Tissue maintenance of CMV-specific inflationary memory T cells by IL-15. *PLOS Pathog.* 14(4):e1006993–23
133. Budagian V, Bulanova E, Paus R, Bulfone-Paus S. 2006. IL-15/IL-15 receptor biology: a guided tour through an expanding universe. *Cytokine Growth Factor Rev.* 17(4):259–80
134. Vamosi G, Bodnar A, Vereb G, Jenei A, Goldman CK, et al. 2004. IL-2 and IL-15 receptor α-subunits are coexpressed in a supramolecular receptor cluster in lipid rafts of T cells. *PNAS* 101:11082–87
135. Alves NL. 2003. IL-15 induces antigen-independent expansion and differentiation of human naive CD8$^+$ T cells in vitro. *Blood* 102(7):2541–46
136. Lahl K, Loddenkemper C, Drouin C, Freyer J, Arnason J, et al. 2007. Selective depletion of Foxp3$^+$ regulatory T cells induces a scurfy-like disease. *J. Exp. Med.* 204(1):57–63

137. Chatterjee S, Eby JM, Al-Khami AA, Soloshchenko M, Kang H-K, et al. 2014. A quantitative increase in regulatory T cells controls development of vitiligo. *J. Investig. Dermatol.* 134(5):1285–94
138. Eby JM, Kang H-K, Tully ST, Bindeman WE, Peiffer DS, et al. 2015. CCL22 to activate Treg migration and suppress depigmentation in vitiligo. *J. Investig. Dermatol.* 135(6):1574–80
139. Miao X, Xu R, Fan Bin, Chen J, Li X, et al. 2018. PD-L1 reverses depigmentation in Pmel-1 vitiligo mice by increasing the abundance of Tregs in the skin. *Sci. Rep.* 8(1):1605
140. Lili Y, Yi W, Ji Y, Yue S, Weimin S, Ming L. 2012. Global activation of $CD8^+$ cytotoxic T lymphocytes correlates with an impairment in regulatory T cells in patients with generalized vitiligo. *PLOS ONE* 7(5):e37513-10
141. Klarquist J, Denman CJ, Hernandez C, Wainwright DJ, Strickland FM, et al. 2010. Reduced skin homing by functional Treg in vitiligo. *Pigment Cell Melanoma Res.* 23(2):276–86
142. Ben Ahmed M, Zaraa I, Rekik R, Elbeldi-Ferchiou A, Kourda N, et al. 2011. Functional defects of peripheral regulatory T lymphocytes in patients with progressive vitiligo. *Pigment Cell Melanoma Res.* 25(1):99–109
143. Terras S, Gambichler T, Moritz RK, Altmeyer P, Lambert J. 2014. Immunohistochemical analysis of $FOXP3^+$ regulatory T cells in healthy human skin and autoimmune dermatoses. *Int. J. Dermatol.* 53:294–99
144. Abdallah M, Lotfi R, Othman W, Galal R. 2014. Assessment of tissue $FoxP3^+$, $CD4^+$ and $CD8^+$ T-cells in active and stable nonsegmental vitiligo. *Int. J. Dermatol.* 53:940–46
145. Maeda Y, Nishikawa H, Sugiyama D, Ha D, Hamaguchi M, et al. 2014. Detection of self-reactive $CD8^+$ T cells with an anergic phenotype in healthy individuals. *Science* 346(6216):1536–40
146. Hassin D, Garber OG, Meiraz A, Schiffenbauer YS, Berke G. 2011. Cytotoxic T lymphocyte perforin and Fas ligand working in concert even when Fas ligand lytic action is still not detectable. *Immunology* 133(2):190–96
147. Leisegang M, Kammertoens T, Uckert W, Blankenstein T. 2016. Targeting human melanoma neoantigens by T cell receptor gene therapy. *J. Clin. Investig.* 126:854–58
148. Bethune MT, Joglekar AV. 2017. Personalized T cell-mediated cancer immunotherapy: progress and challenges. *Curr. Opin. Biotechnol.* 48:142–52
149. James EA, Pietropaolo M, Mamula MJ. 2018. Immune recognition of β-cells: neoepitopes as key players in the loss of tolerance. *Diabetes* 67(6):1035–42
150. Wiles TA, Delong T, Baker RL, Bradley B, Barbour G, et al. 2017. An insulin-IAPP hybrid peptide is an endogenous antigen for CD4 T cells in the non-obese diabetic mouse. *J. Autoimm.* 78:11–18
151. Babon JAB, DeNicola ME, Blodgett DM, Crèvecoeur I, Buttrick TS, et al. 2016. Analysis of self-antigen specificity of islet-infiltrating T cells from human donors with type 1 diabetes. *Nat. Med.* 22(12):1482–87
152. Boissy RE, Liu Y-Y, Medrano EE, Nordlund JJ. 1991. Structural aberration of the rough endoplasmic reticulum and melanosome compartmentalization in long-term cultures of melanocytes from vitiligo patients. *J. Investig. Dermatol.* 97(3):395–404
153. Schallreuter KU, Salem MAEL, Holtz S, Panske A. 2013. Basic evidence for epidermal H_2O_2/$ONOO^-$ mediated oxidation/nitration in segmental vitiligo is supported by repigmentation of skin and eyelashes after reduction of epidermal H_2O_2 with topical NB-UVB-activated pseudocatalase PC-KUS. *FASEB J.* 27(8):3113–22
154. Richmond JM, Frisoli ML, Harris JE. 2013. Innate immune mechanisms in vitiligo: danger from within. *Curr. Opin. Immunol.* 25(6):676–82
155. Yu R, Broady R, Huang Y, Wang Y, Yu J, et al. 2012. Transcriptome analysis reveals markers of aberrantly activated innate immunity in vitiligo lesional and non-lesional skin. *PLOS ONE* 7(12):e51040-12
156. Oliver EA, Schwartz L, Warren LH. 1939. Occupational leukoderma. *JAMA* 113:927–28
157. Mosher DB, Parrish JA, Fitzpatrick TB. 1977. Monobenzylether of hydroquinone. *Br. J. Dermatol.* 97:669–79
158. Ghosh S, Mukhopadhyay S. 2009. Chemical leucoderma: a clinico-aetiological study of 864 cases in the perspective of a developing country. *Br. J. Dermatol.* 160(1):40–47
159. Harris JE. 2017. Chemical-induced vitiligo. *Dermatol. Clin.* 35(2):151–61

160. van den Boorn JG, Picavet DI, van Swieten PF, van Veen HA, Konijnenberg D, et al. 2011. Skin-depigmenting agent monobenzone induces potent T-cell autoimmunity toward pigmented cells by tyrosinase haptenation and melanosome autophagy. *J. Investig. Dermatol.* 131(6):1240–51
161. Yang F, Sarangarajan R, Le Poole IC, Boissy RE, Medrano EE. 2000. The cytotoxicity and apoptosis induced by 4-tertiary butylphenol in human melanocytes are independent of tyrosinase activity. *J. Investig. Dermatol.* 114(1):157–64
162. Lancaster GI, Febbraio MA. 2005. Exosome-dependent trafficking of HSP70. *J. Biol. Chem.* 280(24):23349–55
163. Mosenson JA, Flood K, Klarquist J, Eby JM, Koshoffer A, et al. 2014. Preferential secretion of inducible HSP70 by vitiligo melanocytes under stress. *Pigment Cell Melanoma Res.* 27(2):209–20
164. Levandowski CB, Mailloux CM, Ferrara TM, Gowan K, Ben S, et al. 2013. NLRP1 haplotypes associated with vitiligo and autoimmunity increase interleukin-1β processing via the NLRP1 inflammasome. *PNAS* 110(8):2952–56
165. Kroll TM, Bommiasamy H, Boissy RE, Hernandez C, Nickoloff BJ, et al. 2005. 4-Tertiary butyl phenol exposure sensitizes human melanocytes to dendritic cell-mediated killing: relevance to vitiligo. *J. Investig. Dermatol.* 124(4):798–806
166. van den Boorn JG, Jakobs C, Hagen C, Renn M, Luiten RM, et al. 2016. Inflammasome-dependent induction of adaptive NK cell memory. *Immunity* 44(6):1406–21
167. Denman CJ, McCracken J, Hariharan V, Klarquist J, Oyarbide-Valencia K, et al. 2008. HSP70i accelerates depigmentation in a mouse model of autoimmune vitiligo. *J. Investig. Dermatol.* 128(8):2041–48
168. Mosenson JA, Zloza A, Nieland JD, Garret-Mayer E, Eby JM, et al. 2013. Mutant HSP70 reverses autoimmune depigmentation in vitiligo. *Sci. Transl. Med.* 5(174):174ra28
169. Henning SW, Fernandez MF, Mahon J, Duff R, Azarafrooz F, et al. 2018. HSP70I$_{Q435A}$-encoding DNA repigments vitiligo lesions in Sinclair swine. *J. Investig. Dermatol.* 138(12):2531–39
170. Wang CQF, Cruz-Inigo AE, Fuentes-Duculan J, Moussai D, Gulati N, et al. 2011. Th17 cells and activated dendritic cells are increased in vitiligo lesions. *PLOS ONE* 6(4):e18907–11
171. Tulic MK, Cavazza E, Cheli Y, Jacquel A, Luci C, et al. 2019. Innate lymphocyte-induced CXCR3B-mediated melanocyte apoptosis is a potential initiator of T-cell autoreactivity in vitiligo. *Nat. Commun.* 10(1):2178
172. Marie J, Kovacs D, Pain C, Jouary T, Cota C, et al. 2014. Inflammasome activation and vitiligo/nonsegmental vitiligo progression. *Br. J. Dermatol.* 170(4):816–23
173. Kosche C, Mohindra N, Choi JN. 2018. Vitiligo in a patient undergoing nivolumab treatment for non-small cell lung cancer. *JAAD Case Rep.* 4(10):1042–44
174. Hua C, Boussemart L, Mateus C, Routier E, Boutros C, et al. 2016. Association of vitiligo with tumor response in patients with metastatic melanoma treated with pembrolizumab. *JAMA Dermatol.* 152(1):45–47
175. Fecher LA, Agarwala SS, Hodi FS, Weber JS. 2013. Ipilimumab and its toxicities: a multidisciplinary approach. *Oncologist* 18(6):733–43
176. Barbulescu CC, Goldstein NB, Roop DR, Norris DA, Birlea SA. 2019. Harnessing the power of regenerative therapy for vitiligo and alopecia areata. *J. Investig. Dermatol.* In press
177. Nishimura EK. 2011. Melanocyte stem cells: a melanocyte reservoir in hair follicles for hair and skin pigmentation. *Pigment Cell Melanoma Res.* 24(3):401–10
178. Joshi SS, Tandukar B, Pan L, Huang JM, Livak F, et al. 2019. CD34 defines melanocyte stem cell subpopulations with distinct regenerative properties. *PLOS Genet.* 15(4):e1008034-25
179. Mort RL, Jackson IJ, Patton EE. 2015. The melanocyte lineage in development and disease. *Development* 142(4):620–32. Erratum. 2015. *Development* 142(7):1387
180. Paus R, Nickoloff B, Ito T. 2005. A "hairy" privilege. *Trends Immunol.* 26(1):32–40
181. Harrist TJ, Ruiter DJ, Mihm MJ, Bhan AK. 1983. Distribution of major histocompatibility antigens in normal skin. *Br. J. Dermatol.* 109:623–33
182. Christoph T, Muller-Rover S, Audring H, Tobin DJ, Hermes B, et al. 2000. The human hair follicle immune system: cellular composition and immune privilege. *Br. J. Dermatol.* 142:862–73

183. Ito T, Ito N, Saatoff M, Hashizume H, Fukamizu H, et al. 2008. Maintenance of hair follicle immune privilege is linked to prevention of NK cell attack. *J. Investig. Dermatol.* 128(5):1196–206
184. Ali N, Zirak B, Rodriguez RS, Pauli ML, Truong H-A, et al. 2017. Regulatory T cells in skin facilitate epithelial stem cell differentiation. *Cell* 169(6):1119–1123.e11
185. Chou WC, Takeo M, Rabbani P, Hu H, Lee W, et al. 2013. Direct migration of follicular melanocyte stem cells to the epidermis after wounding or UVB irradiation is dependent on Mc1r signaling. *Nat. Med.* 19:924–31
186. Moon H, Donahue LR, Choi E, Scumpia PO, Lowry WE, et al. 2017. Melanocyte stem cell activation and translocation initiate cutaneous melanoma in response to UV exposure. *Stem Cell* 21(5):665–66
187. Horikawa T, Norris DA, Yohn JJ, Zekman T, Travers JB, Morelli JG. 1995. Melanocyte mitogens induce both melanocyte chemokinesis and chemotaxis. *J. Investig. Dermatol.* 104(2):256–59
188. Yamada T, Hasegawa S, Inoue Y, Date Y, Yamamoto N, et al. 2013. Wnt/β-catenin and kit signaling sequentially regulate melanocyte stem cell differentiation in UVB-induced epidermal pigmentation. *J. Investig. Dermatol.* 133:2753–62
189. Tagashira H, Miyamoto A, Kitamura S-I, Tsubata M, Yamaguchi K, et al. 2015. UVB stimulates the expression of endothelin B receptor in human melanocytes via a sequential activation of the p38/MSK1/CREB/MITF pathway which can be interrupted by a French maritime pine bark extract through a direct inactivation of MSK1. *PLOS ONE* 10(6):e0128678-17
190. Hachiya A, Kobayashi A, Yoshida Y, Kitahara T, Takema Y, Imokawa G. 2010. Biphasic expression of two paracrine melanogenic cytokines, stem cell factor and endothelin-1, in ultraviolet B-induced human melanogenesis. *Am. J. Pathol.* 165(6):2099–109
191. Takeo M, Lee W, Rabbani P, Sun Q, Hu H, et al. 2016. EdnrB governs regenerative response of melanocyte stem cells by crosstalk with Wnt signaling. *Cell Rep.* 15(6):1291–302
192. Sun Q, Rabbani P, Takeo M, Lee S-H, Lim CH, et al. 2018. Dissecting Wnt signaling for melanocyte regulation during wound healing. *J. Investig. Dermatol.* 138(7):1591–600
193. Grimes PE, Hamzavi I, Lebwohl M, Ortonne J-P, Lim HW. 2013. The efficacy of afamelanotide and narrowband UV-B phototherapy for repigmentation of vitiligo. *JAMA Dermatol.* 149(1):68–73
194. Lim HW, Grimes PE, Agbai O, Hamzavi I, Henderson M, et al. 2015. Afamelanotide and narrowband UV-B phototherapy for the treatment of vitiligo. *JAMA Dermatol.* 151(1):42–49
195. Qiu W, Yang K, Lei M, Yan H, Tang H, et al. 2015. SCF/c-kit signaling is required in 12-O-tetradecanoylphorbol-13-acetate-induced migration and differentiation of hair follicle melanocytes for epidermal pigmentation. *Cell Tissue Res.* 360(2):333–46
196. Qiu W, Tang H, Guo H, Lei M, Yan H, et al. 2016. 12-O-tetradecanoylphorbol-13-acetate activates hair follicle melanocytes for hair pigmentation via Wnt/β-catenin signaling. *Cell Tissue Res.* 366(2):329–40
197. Regazzetti C, Joly F, Marty C, Rivier M, Mehul B, et al. 2015. Transcriptional analysis of vitiligo skin reveals the alteration of WNT pathway: a promising target for repigmenting vitiligo patients. *J. Investig. Dermatol.* 135(12):3105–14