

ONLINE FIRST

The Efficacy of Afamelanotide and Narrowband UV-B Phototherapy for Repigmentation of Vitiligo

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Background: Vitiligo is characterized by depigmented patches of skin due to loss of cutaneous melanocytes. Many recent studies have demonstrated defects in the melanocortin system in patients with vitiligo, including decreased circulating and lesional skin levels of α -melanocyte-stimulating hormone (α -MSH). Afamelanotide is a potent and longer-lasting synthetic analogue of naturally occurring α -MSH.

Observations: We describe the preliminary results of 4 patients with generalized vitiligo who developed repigmentation using afamelanotide in combination with narrowband UV-B (NB-UV-B) phototherapy. Patients were treated 3 times weekly with NB-UV-B and starting in the second month received a series of 4 monthly implants containing 16 mg of afamelanotide. Afamelanotide induced

faster and deeper repigmentation in each case. All patients experienced follicular and confluent areas of repigmentation within 2 days to 4 weeks after the initial implant, which progressed significantly throughout treatment. All patients experienced diffuse hyperpigmentation.

Conclusions: We propose that afamelanotide represents a novel and potentially effective treatment for vitiligo. The combined therapy of NB-UV-B and afamelanotide appears to promote melanoblast differentiation, proliferation, and eumelanogenesis. Further studies are necessary to confirm these observations.

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VITILIGO IS CHARACTERIZED by depigmented patches of skin due to loss of cutaneous melanocytes. The lesions are cosmetically disfiguring and are often associated with profound emotional trauma.^{1,2} Although multiple mechanisms have been proposed for the pathogenesis of vitiligo, current data suggest that it is indeed an autoimmune disease.²⁻⁶ Humoral and cell-mediated immunologic defects are common in vitiligo. Such defects include organ-specific autoantibodies, antimelanocyte antibodies, quantitative and qualitative alterations in T cells, and aberrant cytokine expression. Melanocyte-specific CD8 T cells have been shown to mediate the destruction of melanocytes in human vitiligo skin.⁶ Key therapies for repigmentation of vitiliginous lesions include topical corticosteroids, calcineurin inhibitors, and narrowband UV-B (NB-UV-B) phototherapy.^{2,7} Narrowband UV-B phototherapy has emerged as the most effective treatment for patients with moderate to severe disease. Although favorable re-

sults can be achieved with each of the aforementioned therapies, none are optimal. Therefore, novel and new therapeutic interventions for vitiligo are urgently needed.

Many studies⁸⁻¹¹ have demonstrated defects in the melanocortin system in patients with vitiligo, including low physiologic plasma α -melanocyte-stimulating hormone (α -MSH) levels, decreased α -MSH levels in the lesional affected skin, and reduced expression of prohormone convertases. The α -MSH is a key regulatory protein that stimulates melanocyte proliferation and melanogenesis.¹²⁻¹⁴ It also has significant protective and anti-inflammatory effects in immune cells. Afamelanotide is a potent and longer-lasting synthetic linear analogue of naturally occurring α -MSH in a controlled release formulation.¹⁵ We herein describe the first clinical observations of 4 patients with generalized vitiligo who developed repigmentation based on the scientific premise of combining a melanocyte agonist (afamelanotide) with NB-UV-B phototherapy in the treatment of nonsegmental vitiligo.

REPORT OF CASES

All the patients in this case series were adults with generalized vitiligo of less than 5 years' duration, with a 15% to 50% body surface involvement. They were part of a larger randomized clinical trial assessing the efficacy and safety of afamelanotide and NB-UV-B phototherapy compared with NB-UV-B monotherapy in 56 patients. Patients were randomized 50:50 into the 2 treatment arms. The patients were treated with NB-UV-B phototherapy 2 to 3 times weekly for 1 month, and starting in the second month they received a series of 4 monthly implants containing 16 mg of afamelanotide. The study design was approved by an institutional review board (IRBco). The implants were administered subcutaneously in the suprailiac crest area using a sterile technique. The area was disinfected with alcohol and povidone-iodine followed by a local injection of 1% lidocaine with epinephrine.

RESULTS

CASE 1

A 62-year-old African American woman (Fitzpatrick skin type VI) presented with stable vitiligo of 5 years' duration. She was in excellent health. A benign thyroid nodule was diagnosed in May 2010. Cutaneous examination revealed myriad depigmented patches on the face, trunk, and extremities. Total body surface area affected was 30%. She developed a few follicular freckles after 4 NB-UV-B treatments. However, 14 days after her first afamelanotide implant and 11 NB-UV-B treatments, she was noted to have confluent and follicular areas of repigmentation of the head, neck, upper extremities, trunk, and lower extremities. This pattern continued throughout the observation period (**Figure 1**). She achieved 75% repigmentation over all of the affected body surface areas (**Table 1** and **Table 2**). Adverse effects included transient nausea and diffuse hyperpigmentation of her normal skin. The diffuse hyperpigmentation appeared 2 days after the first implant and persisted throughout the observation period.

CASE 2

A 55-year-old African American woman (Fitzpatrick skin type V) presented with slowly progressive, generalized, confettillike vitiligo of 2 years' duration. She had no previous treatment, including NB-UV-B. Cutaneous examination revealed myriad confettillike, depigmented lesions on the trunk and upper and lower extremities. Total body surface area affected was 15%. No repigmentation was evident before the first afamelanotide implant while receiving NB-UV-B only for the first month (12 treatments). However, 4 days after the first implant, she had confluent areas of repigmentation of the hands (**Figure 2**). After 22 NB-UV-B treatments, significant repigmentation was evident on all affected areas, including the head, neck, trunk, and upper and lower extremities (Tables 1 and 2). After 4 implants and 57 NB-UV-B

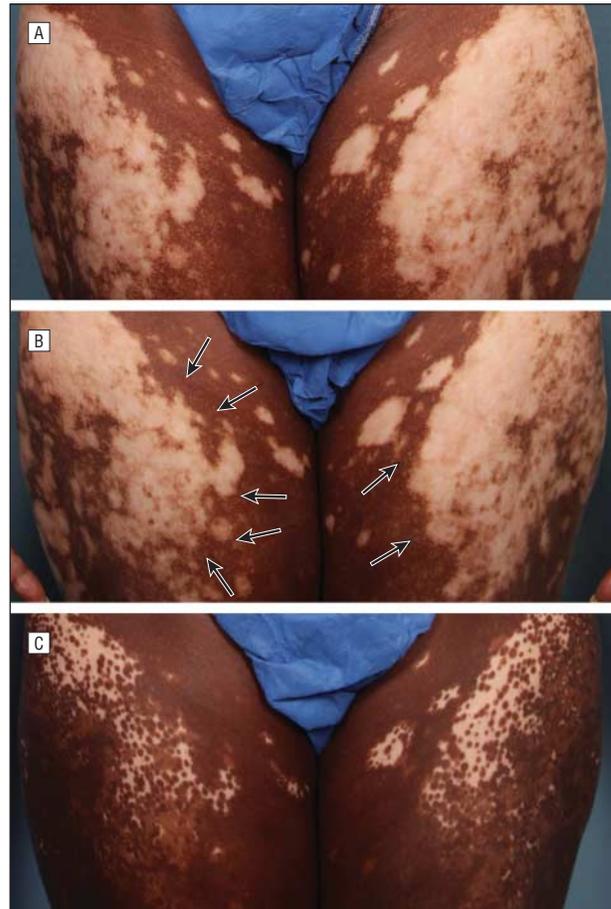


Figure 1. Case 1. A, Before initiation of treatment. B, After 11 narrowband UV-B (NB-UV-B) treatments and 14 days after the first 16-mg afamelanotide implant, improvement is seen in the follicular areas of pigment loss. Arrows indicate areas of repigmentation. C, After 55 NB-UV-B treatments and the fourth implant, marked improvement of the thigh area is seen.

treatments, she achieved 90% repigmentation. Diffuse hyperpigmentation of the face, trunk, and extremities appeared after the first implant and persisted throughout the observation period. The patient also experienced some nausea and dizziness after each implant. She had 1 episode of palpitations.

CASE 3

A 54-year-old African American woman (Fitzpatrick skin type V) presented with a 3-year history of slowly progressive, generalized vitiligo. She had a 13-year history of hypothyroidism and had no previous treatments for her vitiligo. She had multiple depigmented patches on the face, trunk, and extremities. Total body surface affected was 21%. During the first month of phototherapy, the patient received 13 NB-UV-B treatments and was noted to have only a few small areas of follicular repigmentation of her face and neck. The first afamelanotide implant was given 4 weeks after the initiation of NB-UV-B; clinical evaluation 2 days later revealed multiple follicular areas of repigmentation of the eyelids (**Figure 3**). Twenty-two days after implantation, the patient had received 22 NB-UV-B treatments and was noted to have new areas of follicular and confluent repigmen-

Table 1. Therapeutic Responses and Adverse Events

Case No.	Total No. of NB-UV-B Sessions	Overall Repigmentation, %	Follow-up After Treatment	Adverse Events
1	55	75	3 mo, stable	Nausea
2	57	90	3 mo, stable	Headache, dizziness, nausea, 1 episode of heart palpitation
3	62	50	3 mo, stable	None
4	49	50	3 mo, 10% regression of areas of repigmentation	Fatigue, nausea

Abbreviation: NB-UV-B, narrowband UV-B.

Table 2. Time to Onset of Repigmentation

Date	No. of Days After Implant	No. of NB-UV-B Treatments	Areas
Case 1			
7/11/11	0	4	Head and neck
8/3/11	14 (Implant #1)	11	Head and neck, upper extremities, trunk, lower extremities
10/3/11	40 (Implant #2)	33	All body parts
Case 2			
10/14/11	4 (Implant #1)	13	Hands
10/28/11	18 (Implant #1)	19	Head and neck, hands
11/4/11	25 (Implant #1)	22	Head and neck, upper extremities, trunk, lower extremities, hands
Case 3			
8/31/11	0	13	Head and neck
9/2/11	2 (Implant #1)	14	Head and neck, upper extremities, trunk
9/22/11	22 (Implant #1)	22	Head and neck, upper extremities, trunk, lower extremities.
Case 4			
10/14/11	25 (Implant #1)	19	Head and neck, upper extremities, trunk, lower extremities

Abbreviation: NB-UV-B, narrowband UV-B.

tation of the lower extremities and continued improvement to the eyelids, neck, upper extremities, and trunk. No repigmentation was observed on the hands or feet. The patient tolerated the treatment well with no reports of adverse events except for diffuse hyperpigmentation of her normal skin. The treatment phase ended with the patient receiving an overall improvement of nearly 50% (Tables 1 and 2).

CASE 4

A 42-year-old white woman (Fitzpatrick skin type III) presented with a 5-year history of slowly progressive vitiligo. She had no history of other autoimmune diseases and had never received NB-UV-B phototherapy. Cutaneous examination revealed depigmented patches on the face, trunk, and upper and lower extremities. Total body surface area affected was approximately 15%. Although no repigmentation occurred during the first month of NB-UV-B phototherapy,¹⁰ repigmentation became evident with

multiple follicular and confluent areas after 19 NB-UV-B treatments and 25 days after the first afamelanotide implant. After 4 months of NB-UV-B treatment (and 3 implants), she had experienced more than 50% repigmentation of her affected areas (Tables 1 and 2). Adverse effects included transient nausea and fatigue. Intense diffuse hyperpigmentation of her normal skin was noted within 5 days of each implant, which usually began to subside after 20 to 25 days.

COMMENT

To our knowledge, this report describes for the first time the use of a melanocortin (afamelanotide) in combination with NB-UV-B phototherapy for treatment of generalized vitiligo. The results of this case series suggest that several doses of the 16-mg afamelanotide implant administered at 4-week intervals beginning 28 to 30 days after 1 month of triweekly NB-UV-B phototherapy induced faster and deeper repigmentation. All patients experienced follicular and/or confluent areas of repigmentation within 1 to 4 weeks of the initial implant.

Repigmentation of vitiligo requires the presence of melanocytes originating from the hair follicle, the edge of vitiliginous areas, or residual lesional unaffected melanocytes. However, the primary and best method of repigmentation is from the hair follicle.

Both NB-UV-B phototherapy and psoralen phototherapy induce follicular repigmentation. Previous studies¹⁶⁻¹⁸ suggest that melanocytes are recruited from the outer root sheet of the hair follicle. This inactive reservoir of melanocytes undergoes activation, proliferation, and migration to the depigmented affected areas. Recent data suggest that a key source of immature pigment cells capable of full differentiation reside in the bulge region or niche of the hair follicle.¹⁹ This area is known as the melanocyte reservoir.

The cutaneous melanocortin system represents a family of bioactive peptides, including α -MSH, adrenocorticotrophic hormone, β -endorphin, and other peptides, all derived from the precursor peptide proopiomelanocortin. Melanocortins are expressed in the pituitary, but more relevant to cutaneous pigmentation, melanocortin synthesis occurs in keratinocytes and melanocytes.²⁰⁻²² In view of the spectrum of deficiencies of the melanocortin system reported in patients with vitiligo, restoring the system by use of exogenous melanocortin peptides theoretically should be therapeutically beneficial for patients. Alternatively, an α -MSH defect could

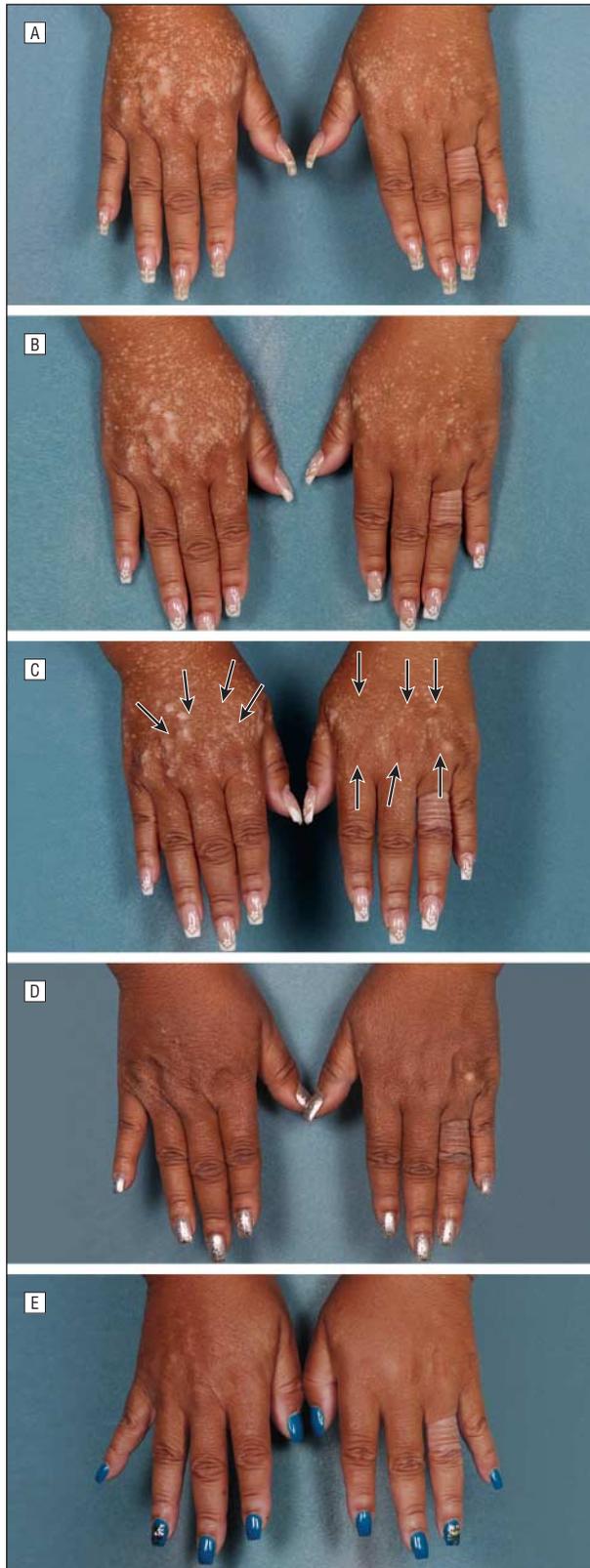


Figure 2. Case 2. A, Before initiation of treatment. B, After 11 narrowband UV-B (NB-UV-B) treatments and before first implant, minimal improvement is seen compared with baseline. C, After 13 NB-UV-B treatments and 4 days after the first implant, follicular and confluent areas of repigmentation are seen predominantly on the right hand. D, After 28 NB-UV-B treatments and second afamelanotide implant, near-complete repigmentation of the hands is seen. E, After no NB-UV-B treatments for 3 months and no implant for 5 months, persistence of repigmentation is seen.



Figure 3. Case 3. A, Near-complete periorbital depigmentation and no change after 12 narrowband UV-B (NB-UV-B) sessions. B, After 15 NB-UV-B treatments and 2 days after the first implant. C, After 62 NB-UV-B treatments and fourth implant, near-complete repigmentation is seen.

be secondary to a loss of melanocytes in vitiligo because melanocytes and keratinocytes are a major source of MSH. Afamelanotide ([Nle4-D-Phe7]- α -MSH) is a potent linear analogue of naturally occurring α -MSH that produces more prolonged physiologic effects compared with the parent molecule. The chemical structure of the analogue is modified at 2 places, providing a smaller dissociation constant and a stronger binding affinity to the melanocortin 1 receptor (MC1R) and resulting in a longer half-life and therefore longer pharmacologic activity than the biological hormone.^{15,23} Similar to α -MSH, afamelanotide activates the synthesis, proliferation, and transport of eumelanin within the melanosome. Afamelanotide acts on melanocytes and keratinocytes present in the epidermis, hair follicles, and possibly MC1R, expressing inflammatory cells (neutrophils and lymphocytes), to restore a balanced cytokine environment. This pharmacologic agent exhibits a unique ability to exclusively target multiple cutaneous effector cells, including those linked with immunologic aberrations in vitiligo. Afamelanotide is currently under investigation in human trials to evaluate its safety and efficacy for several photodermatoses, such as erythropoietic protoporphyria, actinic keratoses, and vitiligo. By mimicking the physiologic effects of α -MSH, afamelanotide may counter deficiencies or defects in the melanocortin system that seem to occur in patients with vitiligo.

In our case series, afamelanotide provided a direct source of α -MSH that appeared to significantly accelerate the repigmentation process induced and initiated by NB-UV-B phototherapy. In this pilot proof-of-concept observation, our premise was to evaluate the effect of afamelanotide as an adjunct therapy compared with

NB–UV-B alone, which is currently being evaluated in the larger multisite database. Hair follicle melanoblasts are devoid of a melanocortin receptor system. Hence, patients were treated with NB–UV-B phototherapy alone for a 1-month induction period to stimulate undifferentiated melanoblasts and stem cells in the bulge (niche) region of the hair follicle to express MC1R receptors for binding of afamelanotide. All patients experienced moderate to rapid repigmentation after the afamelanotide implants compared with our patients receiving NB–UV-B monotherapy, suggesting an enhanced and more efficacious repigmenting effect of afamelanotide (ongoing data analysis). Myriad studies^{2,24} document the efficacy of NB–UV-B as a monotherapy treatment. However, compared with our historical NB–UV-B database, the patients in this small series achieved a much more rapid onset of repigmentation. Given these observations, future studies will further assess afamelanotide monotherapy vs NB–UV-B monotherapy and combination therapy.

All patients experienced enhanced follicular and confluent areas of repigmentation after the afamelanotide implants. Hence, our initial clinical observations suggest that the pharmacologic action of afamelanotide involved efficiently priming the niche area within the hair follicle for subsequent melanocyte maturation and proliferation. We propose a multistep process that seems to take place at the follicular level in patients who have received the combination therapy of NB–UV-B and afamelanotide. This process involves the initial induction of differentiation of melanocyte stem cells and melanoblasts by NB–UV-B phototherapy, which stimulates the expression of MC1R receptors.²⁵ Afamelanotide, 16 mg, further assists in melanoblast and stem cell differentiation. Moreover, we postulate that the combination of afamelanotide and NB–UV-B acts synergistically to promote migration of follicular melanocytes to the epidermis.

The rate and degree of repigmentation were most notable in the patients with darker skin types (Fitzpatrick skin types IV through VI). After 6 months of the combination of afamelanotide and NB–UV-B phototherapy, our patients experienced moderate to excellent repigmentation (Tables 1 and 2). All patients have been followed up for 3 months after termination of treatment. Three of the 4 remain totally stable, whereas 1 patient (patient 4) has experienced mild regression in areas of repigmentation. Stability of treatment will be reported in all the 56 patients at 6 months in the overall study analysis.

Adverse effects observed during the study period are listed in Table 1. All patients experienced diffuse hyperpigmentation. Other adverse effects included mild fatigue, nausea, headaches, dizziness, and abdominal cramps. During the 3-month posttreatment observation period, the diffuse hyperpigmentation had faded significantly.

In summary, on the basis of these initial findings, we believe that we may have identified in afamelanotide a new potentially effective treatment for vitiligo. The combined therapy of NB–UV-B and afamelanotide promotes melanoblast differentiation and eumelanogenesis, much like the physiologic UV response.²⁵ It may also modu-

late aberrant immune responses in patients with vitiligo.^{2,14} Moreover, NB–UV-B may induce immunosuppression.²⁶ The potential therapeutic efficacy may be further enhanced by the ability of afamelanotide to scavenge reactive oxygen species.²⁷ Further studies are indeed necessary to elucidate whether afamelanotide combined with NB–UV-B or as monotherapy can be used to potentially reverse pathogenic mechanisms in patients with vitiligo.

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Author Contributions: All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Grimes, Hamzavi, Lebwohl, Ortonne, and Lim. Acquisition of data: Grimes. Analysis and interpretation of data: Grimes, Hamzavi, Lebwohl, Ortonne, and Lim. Drafting of the manuscript: Grimes, Hamzavi, Lebwohl, Ortonne, and Lim. Critical revision of the manuscript for important intellectual content: Grimes, Hamzavi, Lebwohl, Ortonne, and Lim. Obtained funding: Grimes, Hamzavi, Lebwohl, Ortonne, and Lim. Study supervision: Grimes, Hamzavi, Lebwohl, Ortonne, and Lim.

Conflict of Interest Disclosures: Drs Grimes, Hamzavi, Lebwohl, and Lim are clinical investigators for Clinuvel Pharmaceuticals Ltd.

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Notable Notes

Dermatology in the Artwork of Leonardo Da Vinci

Leonardo da Vinci (1452-1519) was a multifaceted genius and one of the greatest artists of the Renaissance. For 500 years, people have marveled at the beauty of his artwork, with all its creative imagination and wide-ranging areas of inquiry. Among the many academic fields touched on by Leonardo was dermatology, as the following examples from his art illustrate.

As many readers know, Leonardo's famous portrait, "Mona Lisa," lacks eyebrows and eyelashes. There are 2 explanations for this finding: (1) during those times, it may have been fashionable for women to pluck their eyebrows into thin lines or even completely remove them, as appears to be the case with Mona Lisa; and (2) Mona Lisa originally was painted with eyebrows, but they faded away over time and with attempts to clean the painting. Supporting this theory is a recent report that Pascal Cotte, a French engineer, used a high-definition camera to photograph Mona Lisa and found evidence of a left eyebrow hair.¹

Only someone with the brilliant mind of Leonardo da Vinci could have envisioned a comparison between an onion and the human scalp. Leonardo demonstrated this in his anatomical drawing "The Layers of the Scalp Compared to an Onion, and Other Studies." Leonardo, in accompanying notes writ-

ten on the drawing, explains as follows: "If you cut an onion down the centre you will be able to see and count all the coatings or rinds which form concentric circles round the centre of this onion. Similarly if you cut a man's head down the centre you will cut through the hair first, then the skin and the muscular flesh and the pericranium, then the cranium and within the dura mater and the pia mater and the brain, then pia and dura mater again and the rete mirabile and the bone which is the foundation of these."²

Leonardo's portrait of Ginevra de' Benci was featured on the cover of *JAMA* in May 1987. In an accompanying discussion, William H. Crosby, MD, took note of Ginevra's pale complexion, which seems faintly tinted green, suggesting that she suffered from iron deficiency anemia and chlorosis. There may, however, be another explanation for Ginevra's "anemia." It appears that some of the red glazes in her cheeks and lips have faded over time, contributing to her pale look.³ Therefore, Ginevra's pallor and greenish tint may have resulted not only from a lack of iron but also from a deficiency of paint pigment!

Leonardo's magnificent artwork, which even touches on dermatology, still brightens our world with its beauty. As a Renaissance man, Leonardo da Vinci continues to inspire us with all his fabulous dreams and endeavors.

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