

# Detection of Clinically Amelanotic Malignant Melanoma and Assessment of Its Margins by In Vivo Confocal Scanning Laser Microscopy

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**Background:** Near-infrared confocal scanning laser microscopy (CSLM) represents a novel imaging technique for in vivo microscopic analysis of skin lesions, including pigmented lesions.

**Objectives:** To investigate the feasibility of detecting a clinically amelanotic malignant cutaneous melanoma using CSLM and to explore the use of this technique for assessing its margins.

**Patients and Methods:** Two lesions from 2 patients were imaged and analyzed using CSLM. Sites suspected to represent melanoma or benign skin on CSLM were marked as such; then, biopsy specimens were obtained for diagnosis using conventional histological analysis. Both lesions were stained for melanin pigment and analyzed immunohistochemically for the expression of melanosomal markers. In 1 case, a biopsy specimen was also examined with electron microscopy.

**Results:** The images obtained using CSLM allowed recognition of an abnormal intraepidermal melanocytic proliferation that was distinctly different from normal skin. Comparison of the sites examined using CSLM and subsequently using conventional histological methods revealed that CSLM correctly identified intraepidermal melanoma and benign skin. Fontana-Masson stains and immunohistochemical and ultrastructural studies showed that clinically amelanotic melanoma cells contained melanosomes and rare melanin granules.

**Conclusions:** We demonstrated, for the first time, the detection of clinically amelanotic melanoma using CSLM. This technique may aid in the early detection of clinically barely visible or nonpigmented melanomas and may facilitate preoperative noninvasive assessment of their margins.

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**M**ALIGNANT melanoma typically manifests clinically as an abnormal pigmented lesion, with irregular contours and colors ranging from light brown to black. On occasion, melanomas lack sufficient pigment to be recognizable as such and may be confused with other tumors, such as basal cell carcinomas, or inflammatory processes. These so-called amelanotic melanomas account for 2% to 8% of cutaneous melanomas.<sup>1-3</sup> They represent an important diagnostic pitfall for clinicians and a significant management problem for surgeons.<sup>4-8</sup> Since the boundaries of an amelanotic lesion often cannot be reliably determined clinically, repeated surgical sessions may be needed to achieve a final negative margin.<sup>4</sup>

A noninvasive imaging tool that would enable clinicians to detect amelanotic melanoma could be of great benefit. Reflectance confocal scanning laser

microscopy (CSLM) represents a novel technique that allows in vivo microscopic examination of the epidermis and papillary dermis.<sup>9,10</sup> It is currently being explored for use in the diagnosis of a variety of cutaneous lesions,<sup>11-17</sup> including pigmented skin lesions.<sup>18</sup> However, since melanin pigment represents an endogenous "contrast" agent that facilitates the recognition of melanocytes by CSLM,<sup>9</sup> it has been unclear whether this technique can also detect amelanotic melanoma.

We report the real-time detection of clinically amelanotic malignant melanomas by in vivo CSLM in 2 female patients. One patient had an unusually large primary lesion on her leg. The other patient presented with an erythematous patch on her cheek. Near-infrared CSLM, along with conventional histological analysis, was used for diagnosis in both cases. In 1 case, CSLM was also investigated for its potential to map the lesion for subsequent surgical management.

## PATIENTS AND METHODS

### PATIENTS

Two patients were recruited from the pigmented lesion clinic at Memorial Sloan-Kettering Cancer Center (MSKCC), New York, NY. They gave informed consent for their lesions to be examined with CSLM.

### CONFOCAL SCANNING LASER MICROSCOPY

Confocal imaging was performed with a commercially available, near-infrared, reflectance confocal laser scanning microscope (Vivascope 1000; Lucid Inc, Henrietta, NY). The instrument uses a diode laser at 830 nm with a power of less than 35 mW at tissue level. A  $\times 30$  water-immersion objective lens of numerical aperture 0.9 was used with either water (refractive index, 1.33) or gel (refractive index, 1.3335) as an immersion medium. It images with a spatial resolution of 0.5 to 1.0  $\mu\text{m}$  in the lateral dimension and 4 to 5  $\mu\text{m}$  in the axial dimension. An automated stepper was used to obtain a grid of 16 contiguous horizontal images in less than 20 seconds to construct a montage image with an effective in vivo field of view of  $1.6 \times 2.0$  mm. Use of a modified skin contact device also allows an oblique imaging mount with an angle of  $5.7^\circ$ . At this angle, the depth of imaging increases approximately 50  $\mu\text{m}$  across the field of view. This allows a single frame to discriminate vertical information  $\pm 25$   $\mu\text{m}$  about the center of the image. The resultant images have the appearance of elongate (oblique) vertical histological sections.

### HISTOPATHOLOGIC ANALYSIS

The biopsy specimen or excised tissue was fixed in formalin and embedded in paraffin. After routine processing, the slides were stained with hematoxylin-eosin. Immunohistochemical studies were performed using the avidin-biotin-peroxidase complex method. The antibodies used in this study included HMB-45 (anti-gp100; 1:200, Dako Corp, Carpinteria, Calif) and A103, which recognizes Melan-A/MART-1 and was described previously.<sup>19,20</sup> For the 2 antibodies, a heat-based antigen retrieval system was used. Melanin pigment was stained with silver granules using the Fontana-Masson method.<sup>21</sup>

For ultrastructural studies, tissue was fixed in 3% glutaraldehyde and processed according to routine procedures.<sup>22</sup>



**Figure 1.** Patient 1. Large amelanotic melanoma of the leg. The perimeter of the lesion as estimated by combined Wood lamp examination and analysis by near-infrared confocal scanning laser microscopy (CSLM) is marked with a blue line (short arrow). The central biopsy site (asterisk) corresponds to microinvasive melanoma. The peripheral biopsy sites (long arrow) represent in situ melanoma on CSLM and histological examination.

effect. After an initial biopsy specimen revealed melanoma, 17 subsequent biopsy specimens were obtained at an outside hospital prior to her visit to MSKCC. The biopsies were performed in an attempt to map the extent of the lesion. Review of the biopsy specimens at MSKCC revealed that they all showed in situ melanoma. Three specimens showed focal invasion into the papillary dermis to a greatest measured thickness of 0.5 mm (Clark level II).

At her visit to MSKCC, the patient appeared well, with no evidence of extracutaneous disease. Her dermatologic history was notable for basal and squamous cell carcinoma, multiple actinic keratoses, and a pigmented lesion on her shin that had been excised 30 years earlier. The biopsy specimen was not available for review. She also had a family history of melanoma: her brother had a cutaneous melanoma 2 years before her visit to MSKCC.

Various areas of erythema and ill-defined hypopigmentation with normal-appearing intervening skin were present on the lower part of the patient's left leg (**Figure 1**). Wood lamp examination revealed light, ill-defined areas of hypopigmentation, with no accentuation of pigment. The proximal and distal margins of the

## RESULTS

### CLINICAL HISTORY AND FINDINGS

#### Patient 1

A 69-year-old white woman presented to MSKCC for a second opinion after a malignant melanoma was unexpectedly diagnosed and found to be unusually large in size. She had a 1-year history of an erythematous rash on her left shin that had been treated as eczema, to no

lesion could not reliably be determined on clinical examination with the Wood lamp.

### Patient 2

A 66-year-old white woman with a history of biopsy-proven and narrowly excised in situ melanoma on her left cheek presented to MSKCC in the summer of 2000 for facial erythema near the scar from the prior biopsy and excision (**Figure 2**). There was no apparent pigmentation in or around the scar on routine visual examination. Wood lamp examination revealed an area of hypopigmentation medial and inferior to the scar.

## HISTOLOGICAL AND CSLM FINDINGS

### Patient 1

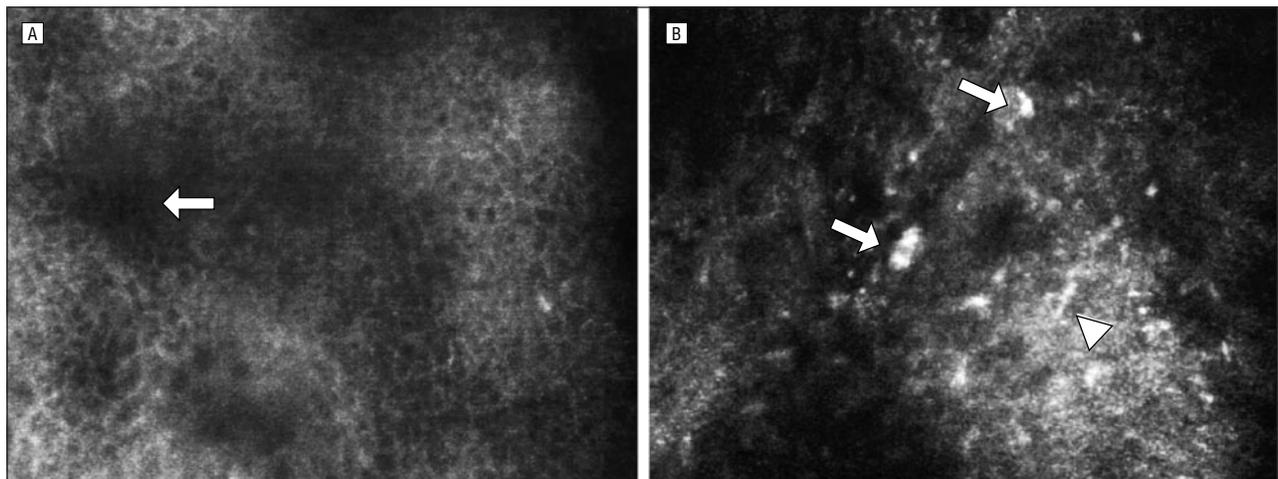
Multiple foci of the skin of the leg of patient 1 were examined with CSLM. Most foci were studied at the periphery of the lesion to distinguish lesional from benign skin. On 5 foci, a preliminary diagnosis was made based on the findings of CSLM: 3 foci were judged to repre-



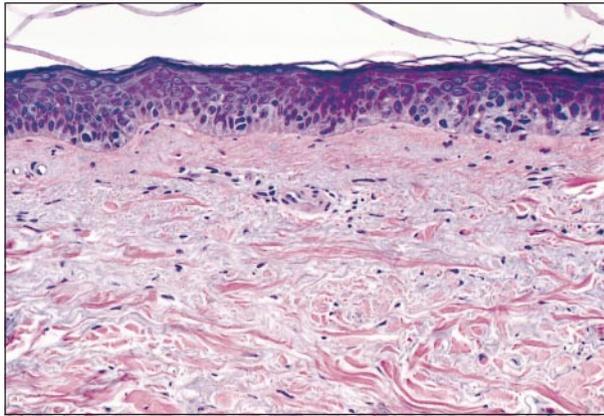
**Figure 2.** Patient 2. Erythematous patch on the cheek.

sent in situ melanoma (1 in the center of the lesion and 2 at its periphery), and 2 peripheral foci were judged to represent normal skin (negative margin). The preliminary diagnoses of in situ melanoma and normal skin were later confirmed histologically. The morphological features of a focus of in situ melanoma on CSLM, as well as a focus of surrounding normal skin, are illustrated in en face (horizontal) sections in **Figure 3**. On CSLM, in situ melanoma was characterized by the presence of an increased number of melanocytes in solitary units at the dermoepidermal junction and above in the spinous cell layer. Individual melanocytes appeared as bright round, oval, or elongated fusiform structures with or without recognizable dendritic processes. Also, small (<1  $\mu\text{m}$  in diameter) bright structures of granular appearance were found. No significantly increased number of melanocytes was seen in normal skin. The epidermis of normal skin showed a honeycomb pattern of keratinocytes with easily recognized cell borders. This pattern was blurred in the foci of in situ melanoma. The histological features of the in situ melanoma corresponding to the focus examined with CSLM are shown in **Figure 4**. An atypical intraepidermal melanocytic proliferation was identified. Melanocytes were dispersed as solitary units and in nests in an irregular asymmetrical fashion along the dermoepidermal junction. Single melanocytes predominated over nests. Cytologically, the melanocytes were epithelioid (large round to oval cells), with enlarged nuclei. Occasional melanocytes were present above the dermoepidermal junction in the midspinous cell layer. The dermis showed marked solar elastosis.

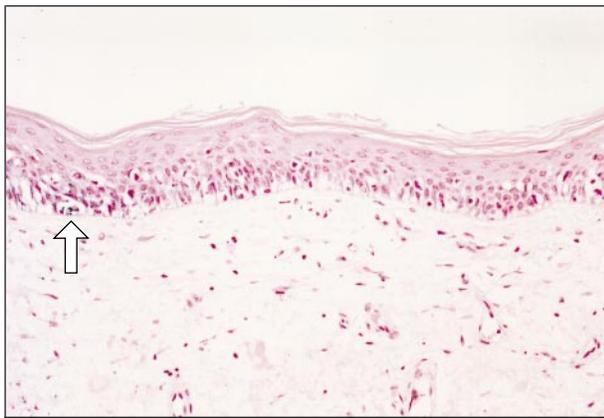
A Fontana-Masson stain revealed focal deposits of silver granules within melanocytes at the dermoepidermal junction (**Figure 5**). Variable amounts of pigment deposition were also present in basilar keratinocytes within the area of skin involved by in situ melanoma. No more and focally even less melanin was present in skin involved by in situ melanoma compared with adjacent uninvolved skin. Immunohistochemical studies with antibodies to gp100 and Melan-A/MART-1 revealed posi-



**Figure 3.** Patient 1. Near-infrared confocal scanning laser microscopy (horizontal sections). A, Normal skin (adjacent to in situ melanoma, imaged at the level of the dermoepidermal junction). A honeycomb pattern of epidermal keratinocytes with distinct cellular outlines is present. The dark space (arrow) corresponds to papillary dermis. B, In situ melanoma (imaged at similar depth). The honeycomb pattern is blurred. Bright irregular oval structures (arrows) or elongated dendritic processes (arrowhead) are present at the dermoepidermal junction and above.



**Figure 4.** An increased number of epithelioid melanocytes are present at the dermoepidermal junction. Single cells predominate, and there is a confluency of cells.



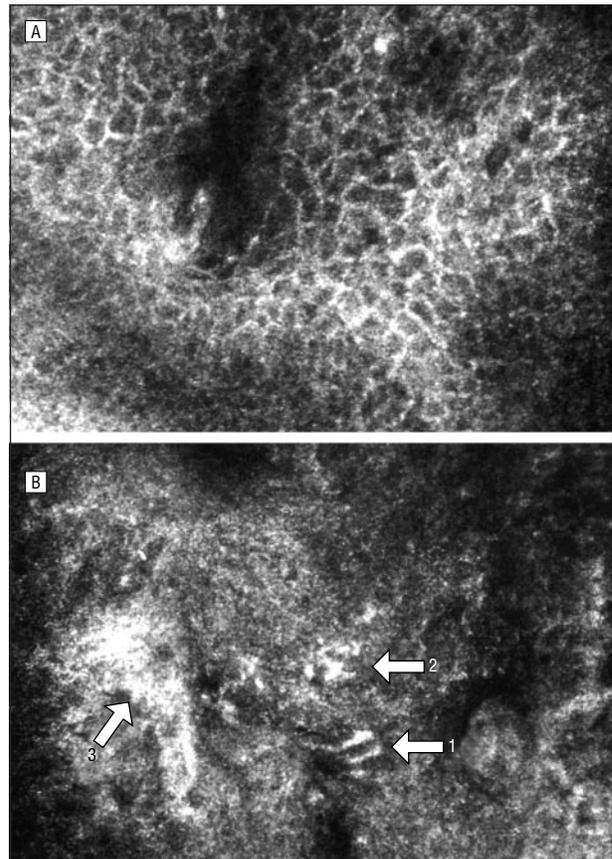
**Figure 5.** Foci of silver granules are seen within melanocytes (arrow) (Fontana-Masson stain).

tive staining of the melanoma cells for both antigens (more intense for Melan-A/MART-1 than for gp100).

After the status of the lesion's periphery was preliminarily assessed by confocal microscopy, it was mapped in more detail by conventional light microscopic examination of multiple punch biopsy specimens. Subsequently, an excision was performed with negative final margins. Histological review of the final excision confirmed the presence of widespread in situ melanoma spanning an area of approximately 25 × 20 cm, involving more than 80% of the excised tissue. Rare foci of microinvasion (Breslow thickness, 0.35 mm; Clark level II) were found on histological examination. These foci of invasion had not been examined with confocal microscopy. No ulceration was seen. Intraepidermal melanoma showed mixed patterns of nested, single-cell lentiginous and pagetoid growth. In situ melanoma was focally separated by lichenoid inflammatory reactions, with loss of intraepidermal melanocytes. There was marked solar elastosis throughout the skin.

#### Patient 2

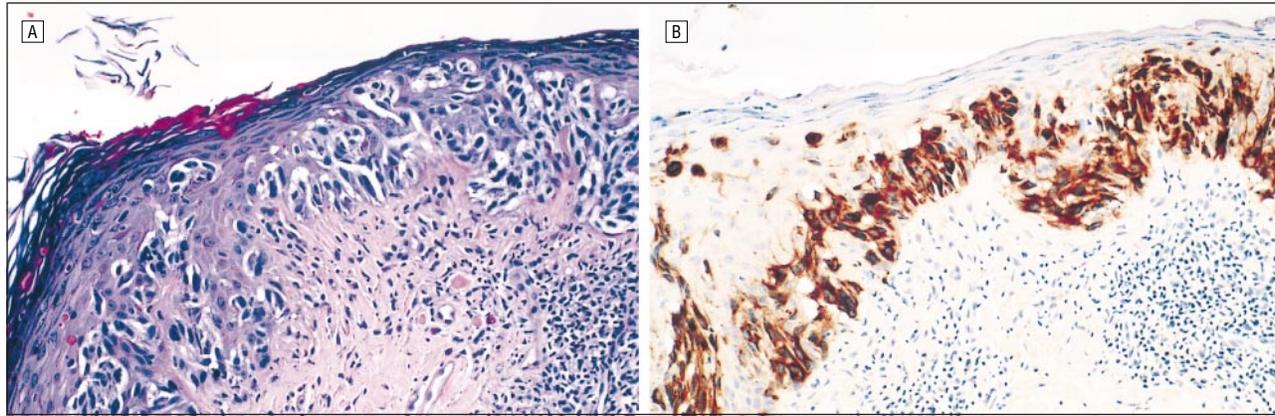
The erythematous patch, as well as a focus of clinically normal-appearing skin lateral from it, was examined with CSLM (**Figure 6**). The focus, judged on CSLM examination to represent in situ melanoma, was characterized



**Figure 6.** Patient 2. Near-infrared confocal scanning laser microscopy (horizontal section). A, Virtual section of normal skin within the spinous cell layer and focally at the dermoepidermal junction (darkened area). Polygonal keratinocytes form a honeycomb pattern. B, In situ melanoma. The honeycomb pattern of epidermal keratinocytes is blurred. Bright irregular fusiform (arrow 1) or round to oval structures with short dendritic processes (arrow 2) are present at the dermoepidermal junction and above. Confluent clusters of melanoma cells form an ill-defined bright structure (arrow 3).

by an irregular proliferation of melanocytes in the epidermis manifesting as bright solitary structures at and above the dermoepidermal junction and by a confluency of bright signals lacking distinct cellular outlines (**Figure 6B**). The shape of individual melanocytes varied, ranging from round/oval to fusiform, with occasional short processes. As in patient 1, the keratinocytic honeycomb pattern seen in the epidermis of normal skin was blurred in the focus of in situ melanoma, with loss of cell demarcation and architectural disarray. After CSLM examination of the lesion, a biopsy was performed, and the site previously imaged with CSLM (**Figure 6**) was examined by conventional histological methods (**Figure 7A**). An irregular proliferation of melanocytes in nests and solitary units is present at all layers of the epidermis (**Figure 7A**). While a Fontana-Masson stain failed to demonstrate melanin pigment deposition in melanocytes, immunohistochemical studies confirmed the presence of Melan-A/MART-1 (**Figure 7B**) and gp100 antigens.

A portion of a biopsy specimen of tissue was also examined with electron microscopy. The ultrastructural findings are illustrated in **Figure 8**. Intraepidermal epithelioid melanocytes are back-to-back at the dermoepidermal junction adjacent to keratinocytes.



**Figure 7.** Patient 2. In situ melanoma as observed on conventional histological examination (vertical sections). A, Hematoxylin-eosin–stained section. B, Immunoreactivity for Melan-A (A103).

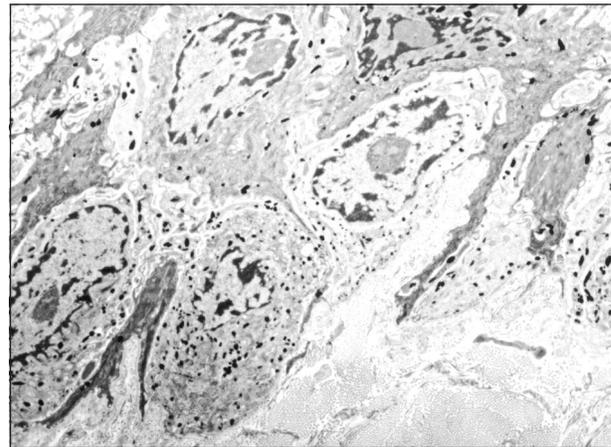
The latter are identified by the presence of desmosomes and tonofilaments. Within the cytoplasm of the melanocytes, several melanosomes were identified. Although most melanosomes were stage II (premelanosomes), a few stage III and rare stage IV melanosomes were also present.

#### COMMENT

One of the most challenging problems in clinical dermatology is the early detection of malignant melanoma. All noninvasive melanomas and the majority of thin invasive melanomas are treated with complete excision. Delayed recognition of melanoma needs to be avoided, as it puts the patient at risk of dying of the disease once the tumor has progressed to competence for metastasis. Early detection of melanoma relies on the critical visual analysis of a new or changing pigmented lesion and subsequent biopsy or excision for diagnosis and treatment.

Amelanotic melanomas often escape early detection.<sup>6</sup> Owing to the lack of distinct pigmentation, the clinical suspicion for melanoma is low, and biopsy and/or excision is frequently delayed. Ancillary noninvasive screening tools may be a significant advance for detection of these enigmatic tumors at an early and curable stage.

Near-infrared reflectance CSLM is a novel imaging technique that allows *in vivo* examination of the epidermis and superficial dermis at a resolution that permits visualization of microanatomical structures.<sup>9,10</sup> It sets a new paradigm of instant noninvasive quasihistological examination of skin lesions *in vivo*.<sup>23</sup> Reflectance CSLM operates by detecting single back-scattered photons from the illuminated in-focus plane of interest.<sup>24-26</sup> High-resolution optical sections are achieved by means of a pinhole placed in an optical conjugated plane in front of the detector that rejects photons coming from out-of-focus planes. Contrast in confocal images is provided by refraction index differences of organelles and other microstructures from the background. This means that highly refractile structures appear white on screen, while low-refractile structures appear dark gray to black.<sup>9,10,26</sup> This technique has been used to image keratinocytic and inflammatory skin lesions<sup>11-17</sup> and is



**Figure 8.** Patient 2. Ultrastructural studies. Melanosomes of various stages are present within melanoma cells at the dermoepidermal junction.

also being investigated for the diagnosis of pigmented skin lesions.<sup>18</sup>

Melanin pigment was found to provide a natural contrast for confocal scanning.<sup>9</sup> Its presence results in a bright-white image signal that illuminates the cytoplasm of melanin-containing cells (pigmented keratinocytes, melanocytes, and melanophages). Individual melanocytes are best recognized by the presence of bright dendritic processes. However, many times, little or no dendritic processes are detectable, and the melanocytes are round to oval or elongated and fusiform. These melanocytes can be discriminated from keratinocytes because of the bright finely granular signal intensity throughout the cells and their spatial distribution as solitary or clustered cells that are round/oval or fusiform in shape. Keratinocytes, on the other hand, appear as cohesive polygonal cells in a characteristic honeycomb arrangement. When they are pigmented, bright granular signals highlight their cytoplasm on CSLM examination. A reliable distinction of individual pigmented keratinocytes from solitary melanocytes can, on occasion, be very difficult. If a melanocyte lacks dendritic processes, one often needs to take architectural context into consideration (single-cell or nested growth pattern vs cohesive growth pattern) to distinguish it from a pigmented keratinocyte.

Although it was known that CSLM is capable of identifying melanocytes in clinically pigmented lesions,<sup>9,18</sup> it was unclear whether CSLM would also be able to visualize the cells of a clinically amelanotic tumor. In this study, we demonstrated for the first time that CSLM can indeed accomplish this. Melanocytes in a clinically amelanotic lesion can still be detected by CSLM, because residual melanin pigment or melanosomes provide cytoplasmic contrast. It has been known from previous ultrastructural studies that amelanotic melanomas still contain premelanosomes.<sup>22,27-30</sup> Such stage II melanosomes are ellipsoidal structures with a striated core (lamellar arrangement of membranes) but no dense melanin granules.

In the first patient's lesion, foci with small amounts of melanin pigment could be demonstrated by Fontana-Masson stain, which sufficiently explains the presence of scattered bright signals in the cytoplasm of melanocytes on CSLM. The lesion of the second patient lacked melanin pigment on Fontana-Masson stain. However, in this case, immunohistochemical studies revealed that the melanoma cells were positive for melanosome-associated glycoproteins (gp100 and Melan-A/MART-1), and ultrastructural studies confirmed the presence of melanosomes in the cytoplasm of melanoma cells. Although most melanosomes were stage II (premelanosomes), stage III and rare stage IV ("mature") melanosomes were also seen in occasional melanocytes. Thus, the fact that some melanocytes gave a bright signal on CSLM can be explained by 2 phenomena: (1) the high refractile index of melanin ( $n=1.7$ )<sup>9</sup> relative to that of epidermis ( $n=1.34$ )<sup>31</sup> and (2) the size of melanosomes (0.6-1.2  $\mu\text{m}$ ) seen at the ultrastructural level, which is similar to the illumination wavelength.

The detection of rare mature melanosomes in the 2 lesions described herein does not disqualify them from being designated *amelanotic melanoma*. The term *amelanotic melanoma* is used to refer to the clinical appearance of a lesion, ie, its lack of shades of tan, brown, or black on routine visual inspection. This is the clinically most relevant use of the term, because it corresponds to the diagnostic pitfall amelanotic melanomas represent in patient care. It is the melanomas, whose sum of pigment does not exceed the pigmentation of the surrounding normal skin, which are most problematic to detect and manage. If a lesion were completely devoid of any pigment, it would likely receive more clinical attention as an area of marked hypopigmentation.

The lesions were, as illustrated, not completely amelanotic on special stains or at the ultrastructural level. This finding is in keeping with prior observations on amelanotic melanoma. In their study of 15 melanomas, which were amelanotic on routine histological examination, Gibson and Goellner<sup>30</sup> found melanosomes in various stages of melanization in all but 2 cases of metastatic melanoma. They concluded that "amelanotic melanomas generally produce some pigment; thus 'amelanotic' is used because of convention, not because pigmentation is totally absent." To our knowledge, complete lack of any mature melanosomes has not been documented yet in a primary cutaneous melanoma and has been reported only for histologically amelanotic metastatic tumors.

Near-infrared, reflectance CSLM is currently an investigational tool approved by the Food and Drug Administration for human use. Although initial results are promising and encourage further exploration of CSLM, more experience is needed to determine its future role in the practice of clinical dermatology and dermatologic surgery. At its current state of technology, CSLM has 2 major limitations compared with conventional histological methods. First, its level of resolution is inferior to that of conventional histology: nuclear features, such as chromatin pattern, cannot be evaluated by CSLM. Second, CSLM can assess microanatomical structures only to a depth of approximately 300  $\mu\text{m}$ . Thus, reticular dermal process cannot be reliably analyzed by CSLM.

Nonetheless, CSLM merits evaluation for use in the screening of pigmented lesions. As the cases reported herein suggest, CSLM may emerge not only as an ancillary tool for diagnosis, but may also be of value for assessing the margins of a biopsy-proven lesion. It has its best resolution within the epidermis and superficial dermis, which makes it suitable for assessing the margins of in situ melanomas. If future studies confirm that there is good correlation between the findings of assessment of margins by CSLM and by histological examination, the surgical planning of clinically poorly recognizable melanomas would be greatly facilitated. Precise mapping of the perimeter of an in situ melanoma may be accomplished by noninvasive means in 1 clinical visit, and numerous punch biopsies and subsequent visits may no longer be necessary.

In conclusion, we have shown that in 2 cases of clinically amelanotic melanoma reflectance CSLM allowed the in vivo recognition of an abnormal intraepidermal melanocytic proliferation by noninvasive means. The correlation between confocal images and histological findings was excellent. Reflectance CSLM shows promise as a noninvasive enhanced screening tool for the detection of melanoma, including amelanotic melanoma, and it may be of great value in mapping the margins of in situ melanoma. However, future studies are needed to determine the sensitivity and specificity of CSLM in detecting melanoma and in outlining its perimeter.

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## REFERENCES

1. Huvos AG, Shah JP, Goldsmith HS. A clinicopathologic study of amelanotic melanoma. *Surg Gynecol Obstet.* 1972;135:917-920.
2. Ariel IM. Amelanotic melanomas: an analysis of 77 patients. *Curr Surg.* 1981; 38:151-155.

3. Clark WH, From L, Bernardino EA, Mihm MC. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. *Cancer Res.* 1969; 29:705-726.
4. Adler MJ, White CR Jr. Amelanotic malignant melanoma. *Semin Cutan Med Surg.* 1997;16:122-130.
5. Ulmer A, Dietl J, Schaumburg-Lever G, Fierlbeck G. Amelanotic malignant melanoma of the vulva: case report and review of the literature. *Arch Gynecol Obstet.* 1996;259:45-50.
6. Koch SE, Lange JR. Amelanotic melanoma: the great masquerader. *J Am Acad Dermatol.* 2000;42:731-734.
7. Conrad N, Jackson B, Goldberg L. Amelanotic lentigo maligna melanoma: a unique case presentation. *Dermatol Surg.* 1999;25:408-411.
8. Holder JE, Colloby PS, Fletcher A, Camp RD. Amelanotic superficial spreading malignant melanoma mimicking Bowen's disease. *Br J Dermatol.* 1996;134:519-521.
9. Rajadhyaksha M, Grossman M, Esterowitz D, Webb RH, Anderson RR. In vivo confocal scanning laser microscopy of human skin: melanin provides strong contrast. *J Invest Dermatol.* 1995;104:946-952.
10. Rajadhyaksha M, Gonzalez S, Zavislan JM, Anderson RR, Webb RH. In vivo confocal scanning laser microscopy of human skin II: advances in instrumentation and comparison with histology. *J Invest Dermatol.* 1999;113:293-303.
11. Gonzalez S, Gonzalez E, White MW, Rajadhyaksha M, Anderson RR. Allergic contact dermatitis: correlation of in vivo confocal imaging to routine histology. *J Am Acad Dermatol.* 1999;40:708-713.
12. Gonzalez S, Rajadhyaksha M, Gonzalez-Serva A, White WM, Anderson RR. Confocal reflectance imaging of folliculitis in vivo: correlation with routine histology. *J Cutan Pathol.* 1999;26:201-205.
13. Gonzalez S, Rajadhaksa M, Rubinstein G, Anderson RR. Characterization of psoriasis in vivo by reflectance confocal microscopy. *J Med Clin Exp Mol.* 1999;30: 337-356.
14. Gonzalez S, Rajadhyaksha M, White WM, Anderson RR, Gonzalez E. Confocal imaging of sebaceous gland hyperplasia in vivo to assess mechanism and efficacy of pulsed dye laser treatment. *Lasers Surg Med.* 1999;25:8-12.
15. Gonzalez S, Rubinstein G, Mordovtseva V, Rajadhyaksha M, Anderson RR. In vivo abnormal keratinization of Darier-White's disease as viewed by real-time confocal imaging. *J Cutan Pathol.* 1999;26:504-508.
16. Aghassi D, Anderson RR, Gonzalez S. Confocal laser microscopic imaging of actinic keratose in vivo: a preliminary report. *J Am Acad Dermatol.* 2000;43:42-48.
17. Hongcharu W, Dwyer P, Gonzalez S, Anderson RR. Confirmation of onychomycosis by in vivo confocal microscopy. *J Am Acad Dermatol.* 2000;42:214-216.
18. Langley RGB, Rajadhyaksha M, Dwyer PJ, Anderson RR, Sober AJ. Confocal scanning laser microscopy of pigmented skin lesions. *J Invest Dermatol.* 1996; 106:836. Abstract 185.
19. Chen YT, Stockert E, Jungbluth A, et al. Serologic analysis of Melan-A (MART-1), a melanocyte-specific protein homogeneously expressed in human melanomas. *Proc Natl Acad Sci U S A.* 1996;93:5915-5919.
20. Busam KJ, Jungbluth AA. Melan-A: a novel melanocytic differentiation marker. *Adv Anat Pathol.* 1999;6:12-18.
21. Luna LG. Fontana-Masson stain. In: *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology.* 3rd ed. New York, NY: McGraw Hill Co; 1968:104-105.
22. Erlandson RA. Ultrastructural diagnosis of amelanotic malignant melanoma: aberrant melanosomes, myelin figures or lysosomes? *Ultrastruct Pathol.* 1987; 9:51-57.
23. Pierard GE. In vivo confocal microscopy: a new paradigm in dermatology. *Dermatology.* 1993;186:4-5.
24. Wilson T, ed. *Confocal Microscopy.* Orlando, Fla: Academic Press Inc; 1990.
25. Webb RH. Confocal optical microscopy. *Rep Prog Phys.* 1996;59:427-471.
26. Dunn AK, Smithpeter C, Welch AJ, Richards-Kortum R. Sources of contrast in confocal reflectance imaging. *Appl Opt.* 1996;35:3441-3446.
27. Persky B, Meyskens PL Jr, Hendrix MJC. Diagnostic electron microscopy for amelanotic melanoma: correlation of patient biopsy, soft agar assay, and xenograft. *J Pathol.* 1983;141:17-27.
28. Curran RC, McCann BG. The ultrastructure of benign pigmented naevi and melanocarcinomas in man. *J Pathol.* 1976;119:135-146.
29. Drezewiecki KT. Cutaneous malignant melanoma: studies on the fine structure of cells and their surface morphology in relation to tumour type and clinical course. *Scand J Plast Reconstr Surg.* 1979;13:321-332.
30. Gibson LE, Goellner JR. Amelanotic melanoma: cases studied by Fontana stain, S-100 immunostain, and ultrastructural examination. *Mayo Clin Proc.* 1988;63: 777-782.
31. Tearney GT, Brezinski ME, Souther JF, Bouma BE, Hee MR, Fujimoto JG. Determination of the refractive index of highly scattering human tissue by optical coherence tomography. *Opt Lett.* 1995;20:2258-2260.