

# Low recurrence rates for in situ and invasive melanomas using Mohs micrographic surgery with melanoma antigen recognized by T cells 1 (MART-1) immunostaining: Tissue processing methodology to optimize pathologic staging and margin assessment

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**Background:** Various methods of tissue processing have been used to treat melanoma with Mohs micrographic surgery (MMS).

**Objective:** We describe a method of treating melanoma with MMS that combines breadloaf frozen sectioning of the central debulking excision with complete peripheral and deep microscopic margin evaluation, allowing detection of upstaging and comprehensive pathologic margin assessment before reconstruction.

**Methods:** We conducted a retrospective cohort study evaluating for local recurrence and upstaging in 614 invasive or in situ melanomas in 577 patients treated with this MMS tissue processing methodology using frozen sections with melanoma antigen recognized by T cells 1 (MART-1) immunostaining. Follow-up was available in 597 melanomas in 563 patients.

**Results:** Local recurrence was identified in 0.34% (2/597) lesions with a mean follow-up time of 1026 days (2.8 years). Upstaging occurred in 34 of 614 lesions (5.5%), of which 97% (33/34) were detected by the Mohs surgeon before reconstruction.

**Limitations:** Limitations include retrospective study, intermediate follow-up time, and that the recurrence status of 39.6% of patients was self-reported.

**Conclusion:** Treating melanoma with MMS that combines breadloaf sectioning of the central debulking excision with complete peripheral and deep microscopic margin evaluation permits identification of upstaging and consideration of sentinel lymph node biopsy before definitive reconstruction and achieves low local recurrence rates compared with conventional excision. (J Am Acad Dermatol 2015;72:840-50.)

**Key words:** immunostaining; melanoma; melanoma antigen recognized by T cells 1; Mohs micrographic surgery; recurrence; upstaging.

Conventional treatment of melanoma involves excision with a recommended margin of clinically normal-appearing skin.<sup>1</sup> A separate pathologist typically microscopically examines the margins and determines final pathological staging after the surgeon has reconstructed the wound. The

#### Abbreviations used:

AJCC:	American Joint Committee on Cancer
MART:	melanoma antigen recognized by T cells
MMS:	Mohs micrographic surgery
SLNB:	sentinel lymph node biopsy

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excision specimen is typically fixed in formalin, and vertical sections are cut from grossly breadloaf-sectioned pieces. This method of tissue processing allows visualization and staging of the central tumor and its relationship to the peripheral and deep surgical margins. Its primary disadvantages are: (1) the delay between the excision and assessment of the final pathological staging and margin status, so immediate reconstruction may occur before detection of upstaging and before complete removal of the tumor; and (2) that it examines less than 1% of the surgical margin, which increases the risk for false-negative margins.<sup>2</sup>

Mohs micrographic surgery (MMS) also involves excision with a margin of clinically normal-appearing skin; however, the margins may or may not conform to those recommended for conventional excision. MMS includes immediate, rather than delayed, microscopic examination of the entire surgical margin, and pathology is interpreted by the Mohs surgeon, rather than a separate pathologist. Rapid frozen section immunohistochemical stains allow accurate identification and precise excision of subclinical melanoma on the same surgical day.<sup>3-5</sup> Reconstruction commences only after confirming clear margins. The key advantage of MMS is pathological assessment of the entire surgical margin, increasing the likelihood of removing subclinical tumor before reconstruction.<sup>6-8</sup> Potential disadvantages, if only the peripheral and deep surgical margins are examined, are that the surgeon cannot assess the distance between the tumor and the surgical margin, and pathological review of residual tumor will not detect upstaging before reconstruction. If a patient subsequently upstages to candidacy for sentinel lymph node biopsy (SLNB), the accuracy of the technique may be compromised, especially if the wound was repaired with a flap.<sup>9</sup>

Combining breadloaf sectioning and mapping of the debulking excision of melanomas with complete microscopic margin evaluation and mapping of a Mohs layer capitalizes on the strengths and mitigates shortcomings of each technique. This study details this method of tissue processing and reports the short-term local recurrence rates and incidence of upstaging for one of the largest reported cohorts of melanoma in situ and invasive melanoma treated with MMS using melanoma antigen

recognized by T cells (MART)-1 immunohistochemical staining.

## METHODS

### Experimental design

This study was approved by the Institutional Review Board of the Hospital of the University of Pennsylvania.

For this retrospective cohort study, we identified from our prospectively updated MMS database 617 consecutive primary or locally recurrent cutaneous melanomas without clinical evidence of local or distant metastasis at the time of surgery in 580 patients treated at the University of Pennsylvania from March 2006 to September 2012. All lesions were surgically excised with MMS using both frozen section hematoxylin-eosin and MART-1 immunostaining. Follow-up data were obtained via patients' medical

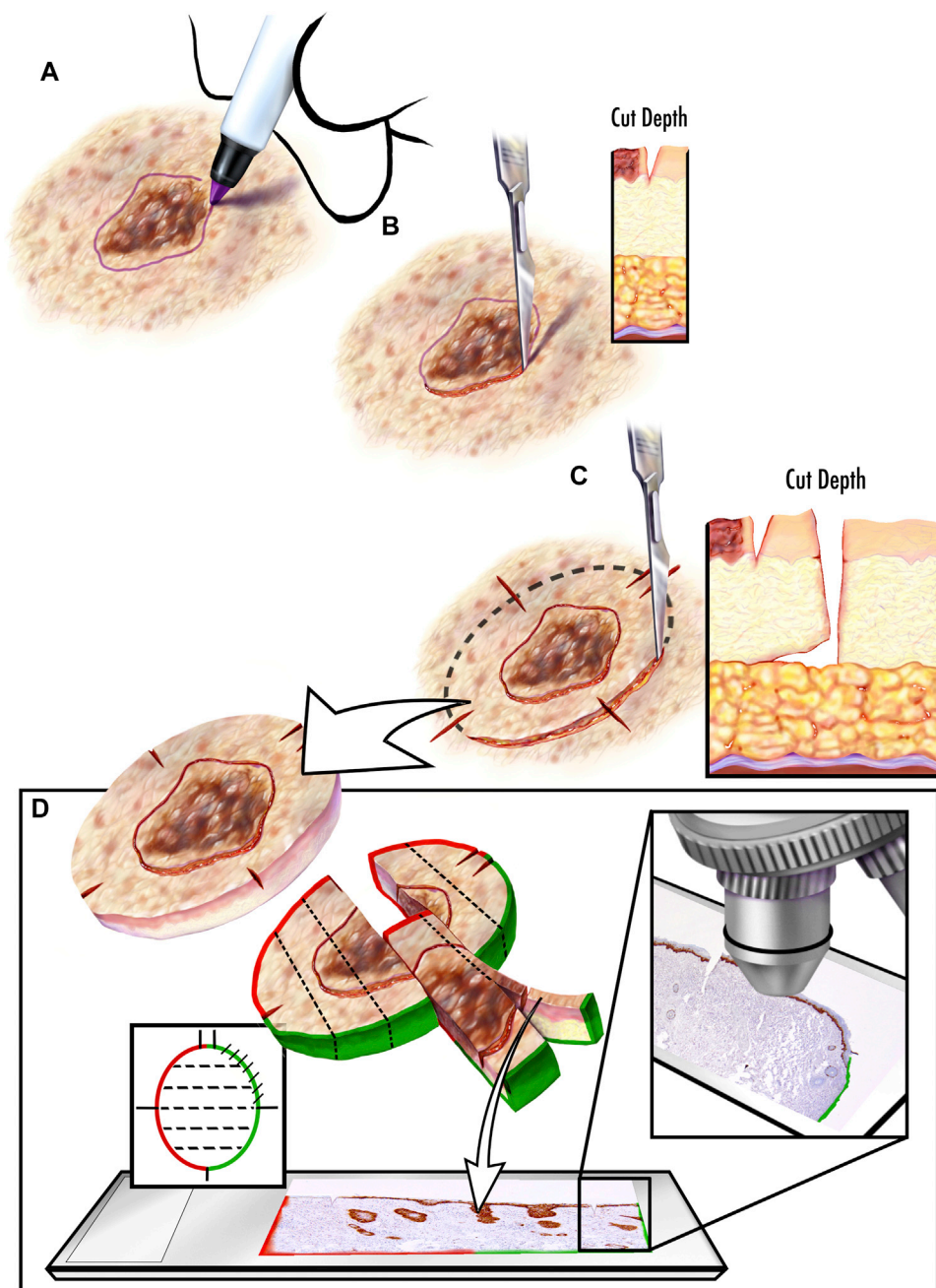
records and a telephone call. At the time of the telephone call, patients were asked for their consent to participate in the study.

Patient-reported information from the telephone call was combined with the chart review to update recurrence data. Patients were asked if pigment was present within the scar or the 2 cm of skin around it, or if their doctor had diagnosed melanoma around the scar. If a patient reported a recurrence, he or she was seen in the clinic to distinguish among a true local recurrence (defined by in situ or invasive melanoma within the scar from treatment of the primary tumor), satellitosis (defined by melanoma without a radial growth phase arising  $\leq 5$  cm from the original primary tumor and discontinuous with the scar), or a second primary tumor (defined by melanoma in situ or invasive melanoma discontinuous with the scar from treatment of the primary tumor). Recurrence status was determined by clinical examination in 63.1% (377/597) of lesions and from telephone follow-up in 36.9% (220/597) of lesions.

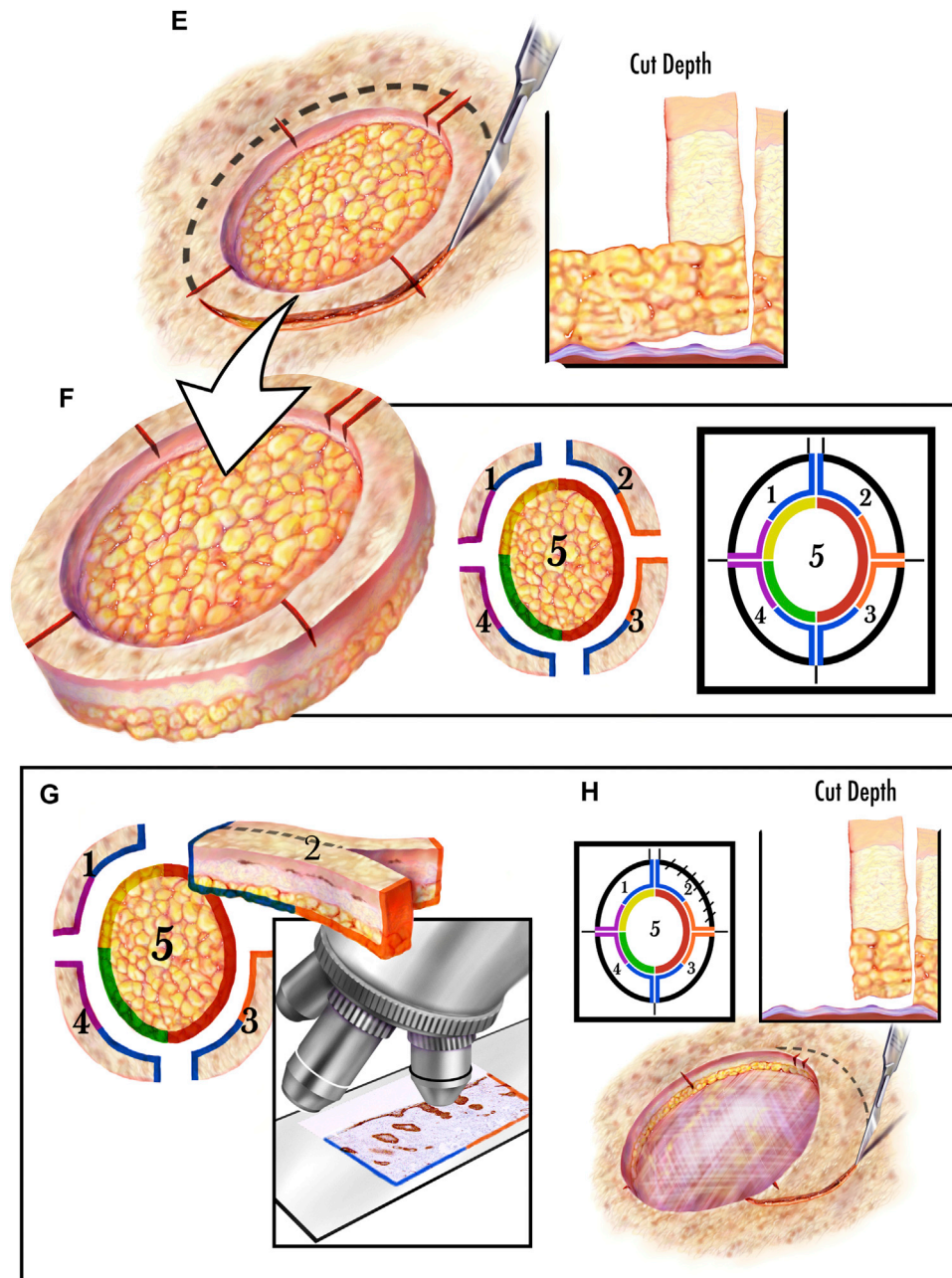
Data for all patients had been prospectively entered at the time of their melanoma treatment in an electronic database that includes patient demographics, preoperative diagnosis, postoperative diagnosis, tumor location, and previous treatment. The medical records of all patients were reviewed to verify the accuracy of the data in the electronic database. All diagnoses were verified by examination of biopsy reports from both the original

### CAPSULE SUMMARY

- Various methods of tissue processing have been used when treating melanoma with Mohs micrographic surgery.
- Frozen section breadloaf processing of the central debulking excision permits immediate identification of upstaging.
- Combining frozen section pathology of the debulking excision with Mohs micrographic surgery optimizes local clearance and pathologic staging before reconstruction.



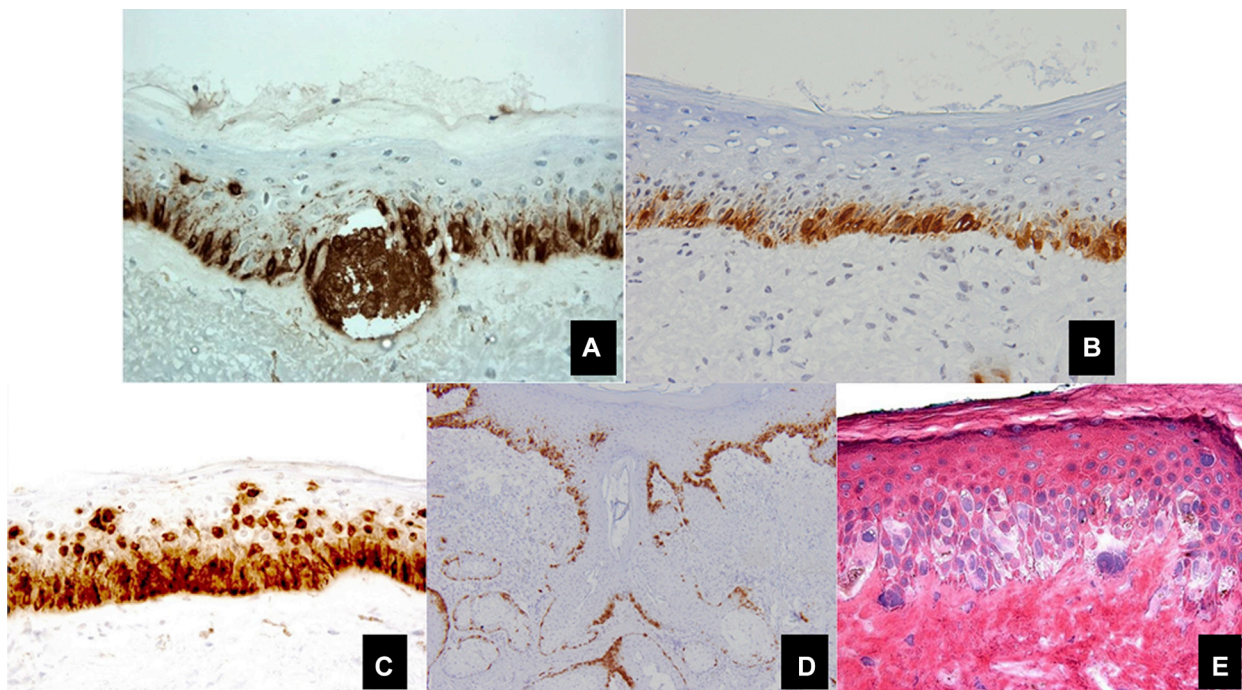
**Fig 1.** Steps for Mohs micrographic surgery technique for melanoma at the Hospital of the University of Pennsylvania. **A**, The scar and clinically visible residual melanoma at the site of the original biopsy are outlined. Additional pigmented lesions near the primary melanoma are also outlined and documented with photography in case they would collide with either the surgical margin or reconstruction. **B**, An incision is made to the level of the papillary dermis at the exact clinical margin of the melanoma. **C**, The visible tumor is excised to the superficial fat with a peripheral margin of at least 2 to 3 mm of clinically normal-appearing skin (larger margins may be excised for higher risk tumors). **D**, The peripheral margins of the debulking specimen are inked with tissue dye and a map is drawn to record the grossing strategy. The debulking excision is grossly sectioned in breadloaf fashion at 2- to 3-mm intervals and vertical sections are cut for microscopic examination. The inset demonstrates tumor extending beyond the hash mark at the clinical margin of the tumor (made in step **B**) to the green-dyed edge. **E**, The Mohs layer is excised around the entire defect from the debulking excision to the fascia with an additional peripheral margin of at least 2 to 3 mm of clinically normal-appearing skin (larger margins may be excised for higher risk tumors). Hash marks are made on the skin surface to



**Fig 1.** (continued).

maintain orientation relative to the patient. **F**, The Mohs layer specimen is grossly sectioned to separate the epidermis, dermis, and a thin layer of subcutaneous fat from the deep fatty margin. Free cut edges of all grossly sectioned specimens are inked, and surgical maps are drawn to represent the method of gross sectioning. **G**, Microscopic frozen sections are cut from the complete peripheral and deep margins for evaluation by the Mohs surgeon. In this example, piece 2, which corresponds to the site of the positive margin on the debulking excision (see step **D**), has tumor at the margin. **H**, The presence of tumor at the margins is indicated on the Mohs map, and additional layers around the positive margin are excised until there is no evidence of microscopic disease. A minimum of a 2- to 3-mm peripheral margin was excised on subsequent stages, but larger margins were sometimes excised if the previous stage was strongly positive.





**Fig 2.** Examples of criteria for positive margins on Mohs micrographic surgery frozen sections. **A**, Nesting of 3 or more melanocytes that did not all contact the basement membrane. **B**, Confluence of 10 or more melanocytes in direct contact with the basement membrane. **C**, Pagetoid spread of melanocytes at or above the level of the mid epidermis in the presence of increased melanocyte density. **D**, Confluent extension of melanocytes deep to the follicular infundibulum. **E**, Severe melanocytic atypia defined by large atypical nuclei and/or significant pleomorphism. (A to D, Frozen section, melanoma antigen recognized by T cells 1 stain; original magnification:  $\times 40$ ; E, frozen section, hematoxylin-eosin stain; original magnification:  $\times 40$ .)

diagnostic biopsy specimen and from the debulking specimen taken at the time of MMS. Upstaging was defined as an increase in the T category in the 7th edition of the American Joint Committee on Cancer (AJCC) melanoma staging classification<sup>10</sup> when comparing the pathology of the initial biopsy specimen and complete debulking excision specimen.

### Surgical procedure

All patients were treated under local anesthesia with a similar protocol (Fig 1).

For melanoma in situ and AJCC 7th edition tumor stage T1a, a minimum total margin (debulking specimen plus Mohs layer) of 5 to 6 mm was excised on the first stage of MMS. On rare occasions in critical cosmetic or functional anatomic locations (eg, distal nasal tip or ala, vermilion lip, eyelid margin), the tumor was removed with clinical margins smaller than 5 mm. No tumor was excised with a clinical margin less than 3 mm. For high-risk tumors, defined as AJCC 7th edition tumor stage T1b or greater, more generous margins were taken to equal a total of a 1-cm margin, unless the wider margin would compromise aesthetic or functional outcomes.

Microscopic frozen sections containing epidermis and dermis were cut with a thickness of 4 to 6  $\mu\text{m}$ . Specimens consisting entirely of fat were often cut with thicker sections of 8 to 10  $\mu\text{m}$  to avoid any holes in the specimen. All specimens were stained immediately with both hematoxylin-eosin and MART-1 immunostains. Fig 2 demonstrates criteria for a positive margin.<sup>11-13</sup>

The Mohs surgeon immediately reconstructed most wounds after declaring clear margin status, except in cases where patients upstaged to candidacy for a SLNB or when desmoplastic melanoma was present on the preoperative biopsy specimen or detected on the debulking specimen.

After confirming clear microscopic margins, the entire debulking excision was thawed and sent for paraffin-embedded sections. Mohs layer specimens were not sent for paraffin-embedded sections unless they contained invasive tumor or another incidental melanocytic lesion.

When upstaging to candidacy for SLNB (defined as a Breslow depth of  $\geq 0.76$  mm) was detected with frozen sections, the Mohs surgeon engaged each patient in a discussion regarding SLNB. If the patient

elected to undergo SLNB, MMS continued until tumor-free margins were achieved, but reconstruction was delayed until after the SLNB, so that lymphatic drainage patterns would be preserved to the greatest extent possible. If desmoplastic melanoma was present on the preoperative biopsy specimen or frozen sections, MMS continued until clear margins were attained with frozen sections. The debulking and Mohs layer specimens were thawed, fixed in formalin, and sent for paraffin-embedded sections. Reconstruction was delayed until clear margins status was confirmed with paraffin sections and S100 and/or SOX-10 immunostains.

## RESULTS

Of 580 patients, 3 patients with 3 melanomas elected not to participate in the study when consent was sought via a telephone call. Follow-up data were available for 597 lesions in 561 patients. In all, 436 lesions (73%) were melanoma in situ, and 161 (27%) were invasive melanomas. The mean number of stages required for clearance was 1.4, with a range of 1 to 7 stages. Desmoplastic melanoma was present in 8 lesions, for which clear margins were obtained on the Mohs frozen sections, then confirmed with paraffin-embedded sections of the Mohs layer. There was 100% agreement between the Mohs surgeon's interpretation of the frozen sections and the dermatopathologist's interpretation of the paraffin sections. Characteristics of the study population are outlined in [Table I](#).

### Local recurrence

Follow-up time was a median of 941 days (2.6 years) and a mean of 1026 days (2.8 years). Local recurrence was identified in 2 of 597 lesions (0.34%).

The first local recurrence was detected 154 days after MMS for a melanoma in situ located on the right zygoma of a patient with chronic lymphocytic leukemia. The recurrent lesion was also a melanoma in situ and was treated with MMS. The second local recurrence was detected by the patient 1484 days after MMS for a melanoma in situ on the right plantar foot. The recurrent lesion was also a melanoma in situ and was retreated with MMS.

### Patients lost to follow-up

Seventeen melanomas (2.8%) were lost to follow-up. The characteristics of the associated patients and their lesions are displayed in [Table II](#).

### Upstaging

Upstaging (defined as an increase in the T category in the 7th edition of the AJCC melanoma staging classification) occurred in 34 of 614 lesions

(5.5%), of which 97% (33/34) were detected by the Mohs surgeon before reconstruction. Of these cases, 23.5% (8/34) upstaged to a Breslow depth of 0.97 mm or greater and met criteria for SLNB. The Mohs surgeon identified 87.5% (7/8) of these cases and offered SLNB before reconstruction. Patients elected to undergo SLNB and delay reconstruction in 3 of the 7 cases (43%), only 1 of which was positive for disease in the sentinel lymph node. In 1 of the 8 lesions that upstaged to candidacy to SLNB, the invasive component was not detected by the Mohs surgeon; while the in situ component of this lesion was highlighted with MART-1 immunostaining, the invasive component of this lesion was composed of a subtle desmoplastic melanoma that was not highlighted with MART-1 immunostaining and was not detected on the hematoxylin-eosin frozen sections. Detailed information on the characteristics of upstaged lesions is presented in [Table III](#).

## DISCUSSION

The American Academy of Dermatology, American College of Mohs Surgery, American Society for Mohs Surgery, and American Society for Dermatologic Surgery Association have determined MMS to be appropriate for the treatment of primary and locally recurrent melanoma in situ and lentigo maligna on the head and neck, genitalia, acral sites, and the pretibial leg; however, they omit commentary on invasive melanoma.<sup>14</sup> Previous authors have demonstrated low rates of local recurrence after MMS for both melanoma in situ<sup>7</sup> and invasive melanoma.<sup>6,8,15,16</sup> Data from this study corroborate the efficacy of MMS for both melanoma in situ and invasive melanoma and contribute the largest published cohort in which the pathology for every patient was evaluated with both hematoxylin-eosin and MART-1 immunostains. The low rate of local recurrence occurred despite the fact that the cohort consisted primarily of subsets of melanoma that have notoriously high rates of local recurrence after conventional excision. The majority (474/597 [79.4%]) of the melanomas in the cohort were located on the head and neck, an anatomic site known to be an independent risk factor for local recurrence.<sup>17-19</sup> Moreover, a substantial number (16.4% [98/597]) of the melanomas had been previously treated, another characteristic associated with local recurrence. The low local recurrence rates after MMS compare favorably with much higher published local recurrence rates after conventional excision of melanoma, although the definition of local recurrence in comparative studies varies and was not always defined ([Table IV](#)).

Numerous previous authors have defined local recurrence as the presence of melanoma within

**Table I.** Data for patients with follow-up (n = 597)

Characteristics	Melanoma type	
	In situ	Invasive
Age, y		
Range	18-92	27-93
Mean	65	67
Median	66	67
Sex		
Male	61.9% (270/436)	62.7% (101/161)
Female	38.1% (166/436)	37.3% (60/161)
Tumor location		
Head and neck		
Scalp or mastoid	5.7% (25/436)	12.4% (20/161)
Upper third of face*	12.6% (55/436)	12.4% (20/161)
Nose	13.1% (57/436)	9.9% (16/161)
Ears	7.8% (34/436)	9.3% (15/161)
Periocular	4.6% (20/436)	4.3% (7/161)
Perioral	1.4% (6/436)	0% (0/161)
Lower two thirds of face†	31.0% (135/436)	24.2% (39/161)
Neck	3.4% (15/436)	6.8% (11/161)
Total	79.6% (347/436)	79.5% (128/161)
Trunk and extremities		
Trunk	5.5% (24/436)	6.8% (11/161)
Proximal upper extremity	3.9% (17/436)	2.5% (4/161)
Distal upper extremity	2.3% (10/436)	1.2% (2/161)
Hand	1.6% (7/436)	0% (0/161)
Proximal lower extremity	0.2% (1/436)	0.6% (1/161)
Distal lower extremity	2.8% (12/436)	7.5% (12/161)
Foot	4.1% (18/436)	1.9% (3/161)
Total	20.4% (89/436)	20.5% (33/161)
Thickness		
In situ	100% (436/436)	n/a
0.01-1.0 mm	n/a	85.1% (137/161)
1.01-2.0 mm	n/a	9.3% (15/161)
2.01-4.0 mm	n/a	3.7% (6/161)
>4.0 mm	n/a	1.9% (3/161)
Previously treated		
Yes		
Recent incomplete excision	44.2% (34/77)	38.1% (8/21)
Recurrent after prior excision	29.9% (23/77)	47.6% (10/21)
Recurrence after other nonexcisional therapy (eg, laser, cryosurgery, imiquimod)	26.0% (20/77)	14.3% (3/21)
Total	17.7% (77/436)	13.0% (21/161)
No	82.3% (359/436)	87.0% (140/161)
Follow-up, d		
Mean	1058	938
Median	941	938
Range	4-3167	6-2666

n/a, Not applicable.

\*Locations include the following: forehead, brow, suprabrow, and temple.

†Locations include the following: chin and cheek, including the preauricular, mandibular, zygomatic, malar, infraorbital, maxillary, and buccal regions.

distances of up to 5 cm or more from the scar of the primary excision,<sup>18</sup> a definition that may include either epidermal or intralymphatic metastases/satellites. Local recurrence that includes intralymphatic metastases near the scar is not an accurate

measure of surgical success; evidence indicates that intralymphatic metastases occur independently of the size of the excision margin, disputing the unsubstantiated dogma that wider margins “capture microsateclites.”<sup>17,20,21</sup>

**Table II.** Data for patients lost to follow-up (n = 17)

Characteristics	Melanoma type	
	In situ	Invasive
Age, y		
Range	27-83	53-81
Mean	60	65
Median	67	62
Sex		
Male	46% (6/13)	25% (1/4)
Female	54% (7/13)	75% (3/4)
Tumor location		
Head and neck		
Scalp or mastoid	0% (0/13)	0% (0/4)
Upper third of face*	0% (0/13)	25% (1/4)
Nose	15.4% (2/13)	0% (0/4)
Ears	15.4% (2/13)	0% (0/4)
Periocular	23.1% (3/13)	25% (1/4)
Perioral	7.7% (1/13)	0% (0/4)
Lower two thirds of face†	15.4% (2/13)	0% (0/4)
Neck	7.7% (1/13)	0% (0/4)
Total	84.6% (11/13)	50% (2/4)
Trunk and extremities		
Trunk	7.7% (1/13)	0% (0/4)
Proximal upper extremity	0% (0/13)	25% (1/4)
Distal upper extremity	0% (0/13)	25% (1/4)
Hand	0% (0/13)	0% (0/4)
Proximal lower extremity	0% (0/13)	0% (0/4)
Distal lower extremity	7.7% (1/13)	0% (0/4)
Foot	0% (0/17)	0% (0/4)
Total	15.3% (2/13)	50% (2/4)
Thickness		
In situ	100% (17/17)	n/a
0.01-1.0 mm	n/a	100% (4/4)
1.01-2.0 mm	n/a	0% (0/4)
2.01-4.0 mm	n/a	0% (0/4)
>4.0 mm	n/a	0% (0/4)
Previously treated		
Yes		
Recent incomplete excision	0% (0/3)	100% (1/1)
Recurrent after prior excision	33% (1/3)	0% (0/1)
Recurrence after other nonexcisional therapy (eg, laser, cryosurgery, imiquimod)	67% (2/3)	0% (0/1)
Total	23.1% (3/13)	25% (1/4)
No	76.9% (10/13)	75% (3/4)

n/a, Not applicable.

\*Locations include the following: forehead, brow, suprabrow, and temple.

†Locations include the following: chin and cheek, including the preauricular, mandibular, zygomatic, malar, infraorbital, maxillary, and buccal regions.

A true local recurrence, defined as in situ or invasive melanoma arising in the scar from treatment of the primary tumor, represents a surgical failure, and

it occurs as a result of incomplete removal of melanoma.<sup>18</sup> Prognosis of a true local recurrence in the absence of metastatic disease depends on the AJCC tumor stage at the time it is detected. True local recurrences often have a greater depth of invasion compared with the tumor at the time of initial treatment,<sup>22</sup> and they may portend a worse prognosis.<sup>19</sup> Our local recurrence rate of 0.34% with a mean follow-up time of 2.8 years is the most accurate measure of the efficacy of our technique. Although we recognize that we may detect more local recurrences with longer follow-up, this study's mean follow-up time of 2.8 years is comparable to numerous publications citing much higher local recurrence rates after conventional surgery of melanoma (Table IV).

Breadloafing and mapping the debulking excision provide staging information that can affect patient treatment, facilitate margin interpretation, and build on the well-described surgical technique of previous authors.<sup>6</sup> First, immediate microscopic examination of the debulking specimen allows accurate measurement of Breslow depth<sup>23</sup> and timely detection of upstaging, so that SLNB can be offered before tissue rearrangement and disruption of lymphatic drainage from reconstruction. In this cohort, 1.3% (8/614) of the melanomas became candidates for SLNB after evaluation of the breadloafed debulking specimen. In 7 of 8 of these cases, a discussion about SLNB ensued before reconstruction, and the patient elected to delay reconstruction and undergo SLNB in 3 of these cases. In the 4 patients who declined SLNB, the mean age was 83 years (range 75–90 years). Previous authors have published cohorts in which patients upstaged to candidacy for SLNB after excision of partially sampled melanomas with frequencies ranging from 0.6% to 10%.<sup>24,25</sup> Second, breadloaf processing of the debulking excision combined with scoring the clinical margin of the melanoma of the debulking specimen (Fig 1, B) provides a positive control and permits precise evaluation of the relationship between the clinical and pathologic surgical margins. Although breadloaf processing of the debulking specimen requires more time for both the surgeon and the histotechnologists, this information assists in the pathologic interpretation of the Mohs layer in heavily sun-damaged skin, a notoriously challenging task even with paraffin sections.<sup>26</sup>

Whereas previous authors have applied uniform peripheral excision margins to melanomas,<sup>6,7,27,28</sup> the size of the peripheral margin in this study varied according to the stage of the tumor (see "Methods" section). This technique also included a deep margin that extended through the entire subcutaneous fat to the fascia or deeper (Fig 1, E). Although there is



**Table III.** Characteristics of tumors that upstaged

Initial diagnosis	Initial T stage	Breslow depth range, mm	Final T stage	Breslow depth range, mm	No. of lesions	Notes
AIMP	n/a	n/a	1a	0.28	1	
Atypical nevus	n/a	n/a	1a	0.23	1	
Lentiginous compound melanocytic nevus with severe atypia	n/a	n/a	2a	1.1	1	
MIS	0	n/a	1a	0.17-0.97	20	Final Breslow $\geq$ 0.75 mm in 1 lesion (0.97 mm)
	0	n/a	2a	1.1-1.2	2	
	0	n/a	4a	Invading cranium	1	Desmoplastic melanoma detected during Mohs micrographic surgery
Invasive melanoma	1a	0.2	1b	0.75	1	Mitoses detected on debulking specimen
	1a	0.22	2a	1.1	1	
	1a	0.67	4a	>4	1	
	1b	0.6	2a	1.3	1	
	1b	0.78	3a	2.2	1	
	2a	1.32-1.8	3a	2.7-3.1	2	
	2b	1.1	3b	4	1	

AIMP, Atypical intraepidermal proliferation; MIS, melanoma in situ.

**Table IV.** Published standard excision local recurrence rates in studies that allowed delineation of recurrence location between head or neck lesions and trunk or extremity lesions

Study	LR/total patients	LR rate, %	Follow-up, y	Definition of LR
Trunk and extremity melanomas				
Heaton et al, <sup>38</sup> 1998	29/234	12.4	2.3	$\leq$ 3 cm from the WLE surgical scar
Agnese et al, <sup>39</sup> 2007	21/624	3.4	2.8, Median	NS
Balch et al, <sup>20</sup> 2001	22/676	3.3	10, Median	$\leq$ 2 cm from the scar or graft
Neades et al, <sup>40</sup> 1993	6/356	1.7	10, Median	In the scar or graft
Moehrle et al, <sup>18</sup> 2004	40/3376	1.2	5, Median	In the scar or graft
Cohn-Cedermark et al, <sup>41</sup> 1997	26/3143	0.8	8, Median	In the scar or graft
Head and neck melanomas				
Fisher et al, <sup>42</sup> 1992	252/900	28	NS	NS
Harish et al, <sup>43</sup> 2013*	12/56	21.4	3.1, Median	NS
Berdahl et al, <sup>44</sup> 2006	5/40	12.5	3.1, Mean	NS
Jones et al, <sup>45</sup> 2013	6/50	12	3.1, Median	NS
Bogle et al, <sup>37</sup> 2001	4/35	11.4	3.5, Mean	NS
Heaton et al, <sup>38</sup> 1998	5/44	11.3	2.3	$\leq$ 3 cm from the WLE surgical scar
Ravin et al, <sup>46</sup> 2006 <sup>†</sup>	21/199	10.6	3.3, Median	NS
Balch et al, <sup>20</sup> 2001	6/64	9.3	10, Median	$\leq$ 2 cm from the scar or graft
Gibbs et al, <sup>34</sup> 2001	11/168	6.5	NS	In the scar or graft
Neades et al, <sup>40</sup> 1993	5/78	6.4	10, Median	In the scar or graft
Agnese et al, <sup>39</sup> 2007	8/131	6.1	2.8, Median	NS
Moehrle et al, <sup>18</sup> 2004	29/584	5.0	5, Median	In the scar or graft
Cohn-Cedermark et al, <sup>41</sup> 1997	22/563	3.9	8, Median	In the scar or graft
Sullivan et al, <sup>47</sup> 2012	2/72	2.8	5.2, Mean	NS

Studies are arranged in descending order of LR rates.

LR, Local recurrence; NS, not specified; WLE, wide local excision.

\*Eyelid melanomas.

<sup>†</sup>Ear melanomas.

variation in depth of excision of melanoma among physicians,<sup>29</sup> excision to fascia ensures removal of melanoma extending along adnexa to the dermal-subcutaneous fat junction.

Whereas the cohorts of several previous studies have not used immunostaining for all patients,<sup>6,7</sup> this cohort is entirely composed of melanomas treated with MMS aided by frozen section MART-1 immunostaining, which has superior sensitivity and ease of interpretation compared with other melanocytic immunostains.<sup>28,30</sup> MART-1 frozen section immunostains have proven to be as accurate as formalin-fixed paraffin-embedded immunohistochemical sections.<sup>3-5</sup> Because MART-1 will not stain a purely desmoplastic melanoma, delaying reconstruction to confirm margins status with paraffin sections and S100 or SOX-10 immunostains may be prudent when treating desmoplastic melanoma.<sup>31,32</sup> In all 8 of the patients in this cohort with desmoplastic melanomas, paraffin-embedded sections corroborated the clear margin status determined by the frozen sections.

This study has limitations. First, the study was retrospective and lacked a comparison treatment arm, so the efficacy of the technique could only be compared with published rates of local recurrence after conventional wide local excision (Table IV). Second, follow-up time was short, with a mean time of 2.8 years. However, this follow-up time is likely to capture the majority of true local recurrences, because previous authors have shown a median interval ranging from 13.4 to 22 months between excision of the primary tumor and diagnosis of the local or locoregional recurrence,<sup>18,33,34</sup> and two thirds of all local recurrences are detected within 24 months of treatment of the primary melanoma.<sup>35</sup> Third, the local recurrence data relied on patient reporting in 36.9% of the melanomas included in this study, which could lead to a falsely low rate of local recurrence. To minimize the risk of underreporting the local recurrence rate, patients were asked specifically if pigment was visible within the scar or the 2 cm of skin around it or if their doctor had diagnosed melanoma around the scar, and any patients who were uncertain or reported pigment were evaluated in clinic. Patient reporting may be reliable, because up to 88% of melanomas, including notoriously challenging nodular melanomas, are detected by patients or their partners.<sup>36</sup> Finally, the majority of patients in this cohort had melanoma in situ or melanoma with a depth less than 1 mm. Although some may argue that the low local recurrence rate reflected that fact that the majority of melanomas were thin, evidence indicates that melanoma thickness does not correlate

with the risk of true local recurrence,<sup>17,37</sup> and microscopic margin control resulted in low local recurrence rates for melanomas of all depths in this cohort.

MMS with frozen section evaluation aided by MART-1 immunostaining achieves low local recurrence rates for both melanoma in situ and invasive melanoma. MMS with tissue processing that combines breadloaf sectioning of the central debulking excision with complete peripheral and deep microscopic margin evaluation permits identification of upstaging and consideration of SLNB before definitive reconstruction.

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