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Mohs micrographic surgery using Mart-1 immunostains in the treatment of invasive melanoma and melanoma in situ

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Abstract

Background—Mohs Micrographic Surgery (MMS) with melanoma antigen recognized by T cells (MART-1) immunostaining is an effective treatment for cutaneous melanoma.

Objective—Determine the efficacy of MMS with MART-1 immunostains in the management of invasive and in-situ melanoma.

Methods and Materials—A retrospective cohort study evaluated 2, 114 melanomas in 1,982 patients excised using MMS and MART-1 immunostains. Margins required for excision were calculated based on Breslow thickness, location and size. Survival and local recurrence rates were calculated and compared with those of historical controls.

Results—The mean follow-up was 3.73 years. Local recurrence was identified in 0.49 % (7/1,419) of primary melanomas. ~82% of melanomas were excised with 6mm margins. The surgical margin was significantly related to tumor location and size, but not to Breslow thickness. The five-year Kaplan-Meier local recurrence and disease-specific survival were 0.59 ± 0.30 and

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98.53 ± 0.42, respectively. MMS with MART-1 immunostains achieved lower local recurrence rates, and equivalent or higher Kaplan-Meier survival rates when compared with conventional wide local excision.

Conclusion—MMS with MART-1 immunostain is an effective treatment for melanoma as evidenced by low local recurrence rates. It offers the advantage of more tissue conserving margins than those recommended for conventional excision.

Introduction

The incidence of cutaneous melanoma (CM) continues to increase at a fast rate with the number of new cases rising annually across all age groups.¹ Therefore, identifying safe and cost effective therapy is increasingly important. According to the *American Academy of Dermatology* guidelines, “the primary treatment modality for cutaneous melanoma is surgical excision” and “the primary goal of surgical excision of melanoma, of any thickness, is to achieve histologically negative margins and prevent local recurrence because of persistent disease”.²

The challenge nowadays lies in finding the most effective way to confirm histologically negative margins and the ideal approach to achieve this continues to be a matter of debate. Multiple surgical modalities for CM, including wide surgical excision, staged excision, and Mohs micrographic surgery (MMS), have been reported with variable success.^{3–17} Mohs surgery offers relevant advantages over traditional or staged excision. It examines 100% of the surgical margins and it is a time-efficient process completed in one day allowing same day confirmation of tumor-free margins prior to reconstruction. Furthermore, it is a cost-effective method in which surgical, technical, and pathologic costs are bundled.^{18,19}

The most common method used today for the treatment of CM is wide local excision with traditional (vertical sectioning) pathology examination. The most significant weakness of this method is that it allows examination of less than 1% of the margins.²⁰ Moreover, studies have demonstrated that bread-loaf cross sections of excised melanoma in situ specimens at 4mm intervals have only a 19% chance of detecting a positive margin and recommend the use of complete en face examination of the surgical margins.²¹

The use of Mohs surgery for melanoma has been reported by multiple authors who have demonstrated a high success rate, usually defined as equivalent or superior melanoma-specific survival rates, as well as equivalent or lower local recurrence and metastasis rates, when compared to historical controls treated with wide surgical excision.^{4,5} There is little or no evidence based controversy about the value of Mohs surgery. However, despite the evidence in support of MMS, only a minority of Mohs surgeons use this technique in the treatment of melanoma due to the perceived difficulty of interpreting atypical melanocytic proliferations on frozen section slides.

One of the most important advances in margin exam has been the use of immunostains. Their use, particularly melanoma-associated antigen recognized by T cells (MART-1), has revolutionized the interpretation of surgical margins when treating melanoma. Its melanocyte specificity, and easy distinction from surrounding background tissue, makes

MART-1 frozen sections easy to interpret and a significant advance in the accurate determination of surgical margins.^{7, 22, 23}

The use of MART-1 on frozen sections during Mohs surgery has been proven to offer a more precise evaluation of the surgical margins than hematoxylin and eosin (H&E) staining of permanent sections, and to provide equivalent information from that obtained in MART-1 permanent sections.²⁴ Characterization and definitions of a positive surgical margin, as compared to normal, sun-damaged skin, have additionally contributed to overcoming the challenging task of evaluating melanoma on frozen sections.^{25, 26}

We evaluated our experience from a single institution using Mart-1 immunostains to test the hypothesis that MMS with MART-1 would provide more accurate removal of melanoma compared to conventional surgery. The current study reports the results of the largest cohort to date of the use of MMS with MART-1 immunostain in the surgical management of invasive and in-situ melanomas. Survival and local recurrence rates are reported and compared with those of historical controls.

Methods

In this retrospective study, patients with biopsy-proven invasive and in-situ melanoma referred for Mohs excision from January 2003 through December 2011 were assessed. A prospective database established in 1982 that includes patient and tumor characteristics was used. MART-1 immunostaining was begun in 2003, thus only patients in whom MART-1 immunostains were used were included. Patients with an equivocal diagnosis of melanoma (e.g. moderate to severely atypical dysplastic nevi) or desmoplastic melanoma (n=5) were excluded. The surgical methods, as well as the technique of MART-1 immunostaining, have been previously described.^{4-6, 22}

Positive margins were identified as those having one or more of the following^{5, 6, 25, 26}: (1) nests of at least 3 atypical melanocytes, (2) melanocytes above the dermoepidermal junction, (3) confluence of more than 9 adjacent melanocytes, (4) “vertical stacking” of melanocytes, (5) melanocytic hyperplasia significantly different in one area as compared to the rest of the margin, and (6) presence of nests of atypical cells in the dermis. (Figure 1) Isolated findings such as mild to moderate confluence of melanocytes, focal pagetosis, superficial follicular extension (<1mm), and mild or moderate cytologic atypia were not interpreted as melanoma since these can be observed in non-lesional, sun-exposed skin.^{24, 25}

Follow-up, outcome and analyses

Patients with melanoma in situ were followed annually, and patients with invasive melanoma were followed at least semi-annually or more depending on the Breslow thickness. Patients that did not receive follow-up examination, either in our office or with their primary dermatologist after MMS, were contacted by phone. The cause of death of patients that died during the follow-up period was obtained from the patient’s primary care physician or family member.

Local recurrence was defined in two ways. First, the reappearance of tumor within or adjacent to the surgical scar, with an intra-epidermal component, was used as the definition of inadequate initial excision. Second, it was defined as any recurrence within 2cm of the surgical scar, which may include satellite metastasis, and was used to be able to compare local recurrence rates to those of historical controls, although known not to be a precise measure of surgical success. Mortality was disease-specific and defined as mortality from metastatic melanoma.

Cases were stratified using updated American Joint Commission for Cancer (AJCC) Breslow thickness criteria²⁷ and the Kaplan-Meier method was used to calculate 5-year local recurrence and disease-specific survival rates after MMS with MART-1 immunostain. Earlier Breslow thickness criteria²⁸ were used to re-stratify the tumors in order to compare local recurrence rates and disease-specific survival rates to those of historical controls.

Margins required to completely excise melanomas were calculated based on AJCC Breslow thickness, tumor location and tumor size. The minimum surgical margin required to completely excise 97% of all tumors was calculated for each subgroup.²⁹

Statistical Analysis

Descriptive analysis was performed to characterize our study group. We evaluated the statistical association between the following variables: thickness, size, and location with the margins of excision. The assessment of the statistical significance of these associations between categorical variables was performed using either Chi-squared probability distribution or Fisher Exact test, as considered appropriate. For quantitative variables, the Student's t-test was performed to assess the mean difference between two groups. P-values less than 0.05 were considered statistically significant.

Local recurrence rates, disease-specific survival rates and mortality rates were described using Kaplan-Meier method. The local recurrence rate, including satellite metastasis, was calculated for melanoma in situ and invasive melanoma and compared to those receiving conventional treatment (control groups) in a series of studies.^{30–42} We also compared the survival rates of patients treated with MMS and MART-1 immunostain to those of 15,798 patients treated with conventional treatment using wide local excision.⁴³ Patients lost to follow-up (n=513), mostly because they had outdated contact information, were excluded from the recurrence, survival, and mortality analysis. STATA (Version 12.0, College Station, TX, USA) was used to perform data management and all statistical analyses.

Results

Patient population

From 2003 to 2011, 1,982 patients with 2,114 melanomas were treated with MMS and MART-1 immunostain. The mean patient age was 66 (Range: 12–99) years; most of the patients were male (60.7%). Patient and tumor characteristics are detailed in Table 1. The mean time of follow-up, excluding those patients that were lost to follow-up, was 3.73 ($\pm$ 2.40) years (minimum = 0 years, maximum = 9.54 years).

Among all the melanomas treated, a total of 1,882 (89.1%) were primary melanomas whereas 230 (10.9%) had been previously treated. The most common location was the face (44.7%), followed by the extremities (22.3%), and trunk (21.5%). 53.2% of the melanomas were located on the head and neck. The mean size of the tumor (i.e., diameter) was 1.66 cm (± 0.96). Among the invasive tumors, the mean Breslow thickness was 0.9mm (± 1.1 mm). In situ and thin (≤ 1 mm) melanomas comprised 90.5% of the melanomas treated.

Surgical margins

Most melanomas (76%) were excised in one stage. The mean margin for excision overall was 7.2 mm (± 3.3 mm). A significant difference in margin size for excision was observed between primary and recurrent melanomas (6.86 mm vs 9.57 mm, respectively; $p < 0.0001$). Also, the margin size was significantly different between melanomas on the head and neck and other locations (7.56 mm vs. 6.69 mm, respectively; $p < 0.0001$). The margin size did not show significant difference between in situ and invasive melanomas (7.10 mm vs. 7.23 mm, respectively; $p = 0.39$).

Figure 2 shows the cumulative margin required for complete excision among primary melanomas according to their thickness. About 82% of these melanomas were completely excised with a ≤ 6 mm margin. Increasing margin size showed a higher proportion of melanomas being completely excised; however, no significant difference was observed between the different Breslow thickness subgroups ($p > 0.05$).

The size of the surgical margin needed to remove the melanoma completely was significantly related to tumor location and size ($p < 0.001$). Margins of ≤ 9 mm successfully removed 97% (95% confidence interval [CI]: 96 – 98) of the melanomas on the trunk and extremities. On the other hand, a similar proportion of melanomas were removed by using margins ≤ 12 mm on the hands and feet ($\sim 98\%$, 95%CI: 95 – 101) and ≤ 15 mm on the head and neck ($\sim 97\%$, 95%CI: 96 – 98). (Figure 3) Similarly, when all cases were analyzed by tumor size, a ≤ 9 mm margin completely removed $\sim 95\%$ (95%CI: 92 – 97) of melanomas < 1 cm in diameter, while larger margins were required for larger tumors (data not shown).

Local recurrence rates

There were a total of 11 local recurrences, as defined by the reappearance of tumor within or adjacent to the surgical scar, 7 in primary tumors and 4 in recurrent tumors treated with MMS using MART-1 immunostain. (Table 2) The crude local recurrence rates are detailed in Table 3. All but one patient with recurrent disease were treated with MMS and MART-1 immunostains and were free of disease at last follow-up. One patient chose wide local excision and was also free of disease at last follow-up.

We compared our results to those of historical controls with large cohorts and valid follow-up.^{30–42} The local recurrence rate for our group was calculated to include satellite metastasis, because historical controls in a series of studies for comparison used the same definition. Among melanomas in situ and invasive melanomas, the recurrence rate was significantly lower in patients treated with MMS and MART-1 immunostain than those treated with conventional wide local excision. (Table 4) Local recurrence rates by location were lower for patients treated with MMS using MART-1 immunostain when compared to

historical controls. Among some of the series analyzed for melanomas on the head and neck or the trunk and extremities, recurrence rates were significantly lower with MART-1 immunostain as compared to wide local excision. (Table 5)

Overall and disease-specific survival and mortality

The five-year Kaplan-Meier local recurrence and disease-specific survival for primary tumors treated with MMS and MART-1 immunostain were $0.59\% \pm 0.30$ and $98.53\% \pm 0.42$, respectively. (Table 6; Figure 4)

During the maximum follow-up period of 9.54 years, there were a total of 121 deaths for an overall mortality of 7.6% (121/1,601). Of these, 16 were due to melanoma metastasis with a disease-specific mortality of 1.0% (16/1,601). The mean Breslow thickness of the patients who died from melanoma was 2.38mm (± 1.98 mm).

For each thickness subgroup, the five-year Kaplan-Meier survival rates after MMS were compared to those of standard surgery. MMS achieved statistically significant higher five-year survival rates than those of the control group in four of the five groups compared. (Figure 5)

Discussion

The evidence in this study confirms that MMS with MART-1 immunostain is an effective treatment for melanoma in situ and invasive melanoma. Previous studies of MMS for the treatment of CM, with, or without the use of immunostains support these findings.⁴⁻¹⁷ A recently published series with a large cohort and long-term follow-up also demonstrates a very low recurrence rate (0.34%) with MMS and MART-1 immunostain.¹⁷ We now have two large studies of adequate follow-up with separate investigators and distinct training validating similar results (recurrence rates $<1\%$), significantly better than wide surgical excision.¹⁷

When compared to traditional wide local excision for CM, MMS offers the ability to excise melanoma completely with histologically negative margins, and therefore offer the highest cure rates and the best tissue conservation of any surgical method. Additionally, same day confirmation of negative margins prior to reconstruction represents a significant advantage.

Frozen-section interpretation

We have previously reported that frozen sections have a sensitivity of 100% in detecting melanoma in the surgical margins when present and a specificity of 90%.⁴⁴ Further studies have found a 100% concordance between frozen sections and formalin-fixed, paraffin-embedded permanent sections.¹²

Immunohistochemical stains considerably aid in the detection of melanocytes and residual melanoma on permanent sections, as well as frozen sections.⁴⁵ MART-1 has been shown to be effective and useful in the identification of residual tumor within MMS margins, demonstrating superior sensitivity compared to Mel-5 and superior specificity to S-100 immunostains.^{7, 8}

The low recurrence rate in the current study, combined with the fact that we were able to achieve narrow margins (<1cm) in ~94% of the cases validate the accuracy of frozen sections with MART-1 immunostains in detecting melanoma at the margins. It is our experience, as well as that of others, that Mart-1 immunostains aid in the speed and certainty of reading frozen sections as compared to routine hematoxylin and eosin-stained frozen sections, and determination of a positive or negative margin can often be done on low-power examination.^{7, 46} Variations in the density of melanocytes along the margin also aid when interpreting margin status, something that cannot be assessed with vertical sectioning.

Surgical margins

Evidence to date confirms that very wide surgical margins aimed at removing satellite micrometastasis do not offer a survival advantage or reduce local recurrence rates.^{47–51} Our data also demonstrates that margins that are narrower, but also confirmed to be histologically negative, than “standard” margins do not increase the risk of recurrence or negatively affect survival.

In the current study, ~82% of all melanomas were completely excised with 6mm margins; ~94% with 9mm margins; and ~97% with 12mm margins. Similarly, for melanoma in situ and thin melanoma, which comprised most of our sample, 12mm margins were required to excise ~97% of the tumors, although, in the current study, there was no significant relationship between tumor thickness and the width of excision merging necessary for histologically tumor-free margins. Our data shows that MIS must be treated with the same margin as thin melanomas, which differs from what is suggested by the NIH consensus panel (i.e. 5mm margin is adequate for MIS).⁵²

The evidence in this study is useful to propose surgical margins for traditional wide local excision. In this study, 97% of the melanomas on the trunk and extremities were excised with a 9mm margin but on the head and neck, and hands and feet, a 15mm and 12mm margin, respectively, is necessary.

Most of the patients who required >1cm margin had melanomas either on the head and neck or hands and feet, similar to previous findings, and confirming that tumors on these locations often have wider subclinical extensions than melanomas on the trunk or proximal extremities.^{4,5} On the other hand, MMS with MART-1 immunostain allowed us to successfully remove ~77% and ~85% of melanoma on the head and neck and hands and feet, respectively, with 6mm margins and to identify those outlier patients that required wider margins in order to minimize local recurrence. This demonstrates the substantial tissue conserving value of Mohs surgery in an area where conventional wide margins often cannot be taken because of cosmetic or functional limits.

Our data also confirms that the margins recommended for invasive melanomas on the trunk and proximal extremities published in five randomized controlled trials are reasonable when margins are determined without immediate, complete microscopic margin evaluation.^{47–51} These studies evaluated only trunk and proximal extremities and cannot be extrapolated to guidelines for the head and neck. For this practice gap, we show that wider margins are necessary for invasive melanomas on the head and neck as compared to the trunk and

extremities, and thus recommend a 1.5 and 1.2 cm margin for CM on the head and neck and hands and feet, respectively.

Local recurrences and disease-specific survival

The efficacy of Mohs surgery is confirmed by demonstrating a significantly lower recurrence rate compared to historical controls. This was achieved by microscopic margin evaluation for tumor clearance, rather than the use of pre-determined, wider margins. Our local recurrence rate was very low; 0.56 ± 0.40 and 0.66 ± 0.47 , for melanoma in situ and invasive melanoma, respectively. All but one of our local recurrences appeared on the head and neck, a location associated with very high local recurrence rates.^{30–33, 36–41} Reasons for our higher local recurrence rate may be the inherent difficulty distinguishing early melanoma from normal, sun-damaged skin of the head and neck, as well as a referral bias cohort with treatment of very challenging and/or previously treated tumors. In either case, the rate was low and MMS provides a significant advantage with a reduced rate of local recurrence.

The efficacy of MMS for the treatment of melanoma is also demonstrated by non-inferiority of melanoma specific survival. The finding of a higher melanoma specific survival may be explained, in part, as a consequence of decreased local recurrence.

A previous study has shown that 23% of MIS recurrences appear as invasive disease with a mean Breslow thickness of approximately 1mm.⁵⁴ In this study, the MIS local recurrence rate for conventional surgery is 20 times higher than MMS (.41 vs 9.0^{31, 32}). Thus, for every 1,000 patients, conventional surgery will result in 90 recurrences, and 21 (23%) of these patients will reappear with melanomas of approximately 1mm. One of these patients is predicted to die from recurrent melanoma that may not be seen in the MMS group. Similarly, in the invasive melanoma group, for every 1,000 patients treated with conventional surgery, 30 (3%) will develop recurrent disease and 10 (33%) will reappear with melanoma deeper than the thickness of the original primary.⁵⁴ One or two of these patients can be predicted to die of recurrent disease. Thus, although the magnitude of a survival benefit for MMS may be small, it may be reflected in the melanoma-specific survival.

The large cohort of patients and the length of follow-up demonstrate the main strengths of this study. On the other hand, limitations of this study include that it is a single-site retrospective study with lack of a control arm. Also, some of the local recurrence data relied on patient reporting, which could cause a falsely low rate of local recurrence. A prospective, multi-center, long-term follow-up study with a control arm using traditional wide local excision would greatly help further validate these findings.

Conclusion

Our study demonstrates the accuracy and effectiveness of MART-1 immunostaining when used for margin evaluation during MMS for melanoma. We show the ability to completely remove melanoma as demonstrated by our low local recurrence rates. We have furthermore showed the advantage of being able to remove melanoma in the majority of cases with more tissue conserving margins than the standard margins recommended for wide local excision.

Melanoma-specific survival is at least as high as wide excision. With lower recurrence rates, there is a theoretical survival advantage absent some melanomas that recur with an increased Breslow thickness and risk of metastasis.

Surgical margin data helps to establish evidence based margins for the head and neck melanomas and melanoma in situ, and validates the randomized controlled trials margin data for trunk and extremity melanomas.^{47–51} Lastly, the data presented should be considered in future revisions of the Appropriate Use Criteria for Mohs Surgery regarding melanoma in the future.⁵⁵

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Figure 1. Melanoma in situ, positive margin. (Melanoma antigen recognized by T cell 1 staining, original magnification x 100)

Figure 2.
Cumulative margin required for complete excision among primary melanomas according to their thickness.

Figure 3.
Cumulative margin required for complete excision among primary melanomas according their location.

Figure 4. Kaplan-Meier curve for disease-specific survival after treatment with Mohs micrographic surgery.

Figure 5.
Five-year Kaplan-Meier survival rates after Mohs micrographic surgery (n=1, 376) compared with historical controls (n= 15, 798).

Table 1

Patient and tumor characteristics

	All cases (n=2114)	All patients (n=1982)
Age, years		
Mean	66.3 (± 15.1)	66.0 (± 15.2)
Median	69	69
Range	12 – 99	12–99
Sex, n (%)		
Male	1304 (61.7)	1201 (60.7)
Female	810 (38.3)	780 (39.4)
Location, n (%)		
Face	944 (44.7)	
Neck	82 (3.9)	
Trunk	454 (21.5)	
Extremity	472 (22.3)	
Hands/feet	60 (2.8)	
Scalp	98 (4.6)	
Genital	1 (0.1)	
Unspecified	1	
Head & neck	1124 (53.2)	
Other location	988 (46.8)	
Tumor size (cm)		
Mean ($\pm$ SD)	1.66 (± 0.96)	
Median	1.5	
Range	0.2 – 9.0	
Tumor thickness, overall (mm)		
Mean ($\pm$ SD)	0.38(± 0.90)	
Median	0.0	
Range	0–9	
Unspecified	4	
Tumor thickness, invasive (mm)		
Mean ($\pm$ SD)	0.9 (± 1.1)	
Median	0.5	
Range	0.03–9	
Tumor thickness by categories (mm), n (%)		
0	1264 (59.9)	
0.01–1.00	646 (30.6)	
1.01–2.00	108 (5.1)	
2.01–3.99	60(2.8)	
4.00	32 (1.5)	

	All cases (n=2114)	All patients (n=1982)
0.01–0.75	551 (26.1)	
0.76–1.49	160 (7.6)	
1.50–3.99	103 (4.9)	
4.00	32 (1.5)	
Unspecified	4	

Table 2

Patient and tumor characteristics for local recurrences *

Age/sex	Thickness (mm)	Location	Time to recurrence (years)	Thickness at recurrence (mm)
Primary tumors (n=7)				
65/F	0	Cheek	0.59	0
63/M	0	Nose	1.03	0
79/M	0	Cheek	3.99	0
65/M	0.3	Nose	0.27	0
74/M	0.38	Scalp	5.31	0
78/M	2.92	Scalp	5.71	1.35
73/M	4	Scalp	2.40	0.62
Recurrent tumors (n=4)				
63/F	0	Cheek	6.32	0
66/M	0.98	Cheek	0.90	0.33
73/F	1.5	Nose	1.94	0.85
84/M	3.35	Foot	2.12	1

* Defined as the reappearance of tumor within or adjacent to the surgical scar, with an intra-epidermal component.

Table 3

Crude local recurrence rates

Thickness	Primary		Recurrent		Total
	MMS (n)/N	MMS % ($\pm$ SD)	MMS (n)/N	MMS% ($\pm$ SD)	MMS %
MIS	3/863	0.35 ($\pm$ 0.007)	1/119	0.84 ($\pm$ 0.08)	0.41
Invasive	4/556	0.72 ($\pm$ 0.02)	3/59	5.1 ($\pm$ 0.38)	1.14
Total	7/1419	0.49 ($\pm$ 0.005)	4/178	2.25 ($\pm$ 0.08)	0.69

Mean time to recurrence was 2.76 yrs ($\pm$ 2.26yrs) for primary tumors and 2.82 yrs ($\pm$ 2.40yrs) for recurrent tumors.

MIS, Melanoma in situ

Table 4

Recurrence rates ^{*} after MMS and conventional surgery for primary tumors

Thickness	MMS n/N (%)	Conventional n/N (%) [‡]	P value
MIS	4/863 (0.5)	16/81 (20.0) ³⁰	<0.001
		2/22 (9.0) ³¹	<0.0001
		2/23 (9.0) ³²	<0.0001
Invasive	7/556 (1.3)	95/3445 (2.8) ³³	0.039
		25/593 (4.2) ³⁴	0.003
		48/638 (7.5) ³⁵	< 0.0001

^{*} Includes satellite metastasis. Mean follow-up= 3.71 years; Mean time to recurrence= 3.15yrs (SD= 2.41yrs)

[‡] Any tumor growth that re-appeared in the scar, under the skin graft, or within 3cm of the margins of the wide excision. Mean follow-up= 3.5 years ³⁰, 3.2 years ³¹, 3 years ³², 8 years ³³, 3 years ³⁴, 5 years ³⁵

MIS, Melanoma in situ

Table 5Recurrence rates ^{*} after MMS and conventional surgery among primary tumors by location

Location	MMS n/N (%)	Conventional n/N [‡] (%)	Follow-up, years	P value
Head and Neck	5/204 (2.4) [‡]	23/533 (4.4) [‡] ³³	8, Median	0.236
	5/204 (2.4) [‡]	130/998 (13.0) [‡] ³⁶	NS	<0.0001
	5/204 (2.4) [‡]	29/584 (5.0) [‡] ³⁷	5, Median	0.1281
	5/204 (2.4) [‡]	7/131 (5.3) [‡] ³⁸	3.2, Median	0.1645
	9/712 (1.3) ^Ψ	80/867 (9.2) ^Ψ ³⁹	NS	<0.0001
	9/712 (1.3) ^Ψ	22/563 (3.9) ^Ψ ⁴⁰	8, Median	0.0023
	2/58 (3.4) [¥]	6/64 (9.3) [¥] ⁴¹	10, Median	0.1866
Hands and Feet	1/22 (4.5) [‡]	15/132 (11.5) [‡] ³³	8, Median	0.3319
	1/22 (4.5) [‡]	15/295 (5.08) [‡] ³⁷	5, Median	0.9112
Trunk and Extremities	1/332 (0.3) [‡]	64/2,738 (2.3) [‡] ³³	8, Median	0.0149
	1/332 (0.3) [‡]	25/3,068 (0.8) [‡] ³⁷	5, Median	0.3074
	1/77 (1.3) [¥]	22/676 (3.25) [¥] ⁴¹	10, Median	0.3447
	1/77 (1.3) [¥]	31/678 (4.57) [¥] ⁴²	7.7, Mean	0.1766
	1/666 (0.1) ^Ψ	26/3,143 (0.8) ^Ψ ⁴⁰	8, Median	0.0585

^{*} Includes satellite metastasis. Mean follow-up= 3.73 (±2.39) years; Mean time to recurrence= 3.05 (SD= 2.08) years.

[‡] Any tumor growth that re-appeared in the scar, under the skin graft, or within 3cm of the margins of the wide excision.

[‡] margin thickness of 0.01mm - >4.0mm

^Ψ margin thickness of 0.0mm - >4.0mm

[¥] margin thickness of 1.0mm - 4.0mm

NS, not specified

Table 6

Five-year Kaplan-Meier local recurrence and disease-specific survival after MMS among primary tumors

Thickness	LR (% $\pm$ SE)	DSS (% $\pm$ SE)
Entire group (n=1,422)	0.59 $\pm$ 0.30	98.53 $\pm$ 0.42
MIS (n=863)	0.56 $\pm$ 0.40	100 $\pm$ 0.00
Invasive (n=556)	0.66 $\pm$ 0.47	96.42 $\pm$ 1.01
<1 mm (n=420)		98.71 $\pm$ 0.75
1.01–2.00 mm (n=75)		93.53 $\pm$ 3.65
2.01–4.00 mm (n=49)		85.38 $\pm$ 6.15
>4.00 mm (n=12)		67.50 $\pm$ 20.74

LR, local recurrence (defined as recurrence within or immediately adjacent to scar because of inadequate excision and excludes local recurrences from satellite and in-transit metastasis); *DSS*, Disease-specific survival; *MIS*, Melanoma in situ (Recurrences: MIS n=3; <1mm n=1, 1.01–2.00mm n=0, 2.01–4.00mm n=1, >4.00mm n=0, excluding lost to follow-up)