

Non-Radical Diagnostic Biopsies Do Not Negatively Influence Melanoma Patient Survival

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Background: In fair-skinned Caucasian populations both the incidence and mortality rates of cutaneous melanoma have been increasing over the past decades. With adjuvant therapies still being under investigation, early detection is the only way to improve melanoma patient survival. The influence of incisional biopsies on melanoma patient survival has been discussed for many years. This study investigates both the influence of diagnostic biopsy type and the presence of residual tumor cells in the re-excision specimen on disease free and overall survival.

Methods: After (partial) removal of a pigmented skin lesion 471 patients were diagnosed with stage I/II melanoma and underwent re-excision and a sentinel node biopsy. All patients were followed prospectively, mean follow up > 5 years. Patients were divided according to their diagnostic biopsy type (wide excision biopsy, narrow excision biopsy, excision biopsy with positive margins and incisional biopsy) and the presence of residual tumor cells in their re-excision specimen. Survival analysis was done using Cox's proportional hazard model adjusted for eight important confounders of melanoma patient survival.

Results: The diagnostic biopsy was wide in 279 patients, narrow in 109 patients, 52 patients underwent an excision biopsy with positive margins and 31 patients an incisional biopsy. In 41 patients residual tumor cells were present in the re-excision specimen. Both the diagnostic biopsy type and the presence of tumor cells in the re-excision specimen did not influence disease free and overall survival of melanoma patients.

Conclusions: Non-radical diagnostic biopsies do not negatively influence melanoma patient survival.

Key Words: Melanoma—Survival—Diagnostic—Initial—Re-excision and Biopsy.

In fair-skinned Caucasian populations cutaneous melanoma is an important growing public health problem, causing a heavy burden on healthcare services. Both its incidence and mortality rates have been increasing in Europe over the past decades.¹ The absolute total number of new cases of melanoma in the Netherlands is expected to be more than 4800

in 2015, compared with around 2400 in 2000.² The Netherlands, as many other countries, has a two-tiered medical care system in which patients need to seek a medical opinion initially from a general practitioner (GP) before referral if necessary, to a specialist.

The mean number needed to treat (NNT), defined as the mean number of pigmented lesions needed to be excised to identify one melanoma, among 468 GPs in Perth, Australia, was 29 and ranged from 83 in the youngest patients (≤ 19 years) to 11 in the oldest patients (≥ 70 years).³ Assuming that for each new case of melanoma another 20–50 patients with pigmented skin lesions will visit the GP, the demand for detection will increase quite markedly. With

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adjuvant therapies still being under investigation, early detection is the only way to improve melanoma patient survival.⁴ Dutch guidelines recommend pigmented skin lesions suspect for melanoma to be removed through a diagnostic excision biopsy with a minimal lateral clearance of 2 mm.⁵ This recommendation is inline with the advice of the National Institutes of Health (NIH) to remove any suspicious lesion through excisional biopsy with a narrow margin of normal-appearing skin.⁶ The influence of an incisional biopsy on melanoma patient survival has been discussed for many years and different investigators have found contradicting results.⁷⁻¹⁵ Two recent publications concluding incisional biopsies not to interfere with melanoma patient survival were not able to end this discussion since patient groups were not fully comparable¹⁶ or follow up (FU) was short.¹⁷

This study investigated both the influence of diagnostic biopsy type and the presence of residual tumor cells in the re-excision specimen on melanoma patient disease free survival (DFS) and overall survival (OS), in 471 patients with a mean FU of more than 5 years. Survival analysis was done using Cox's proportional hazard model adjusted for; gender, age, site of primary melanoma, Breslow thickness, type of melanoma, ulceration, lymphatic invasion and sentinel node (SN) status. Both the diagnostic biopsy type and the presence of tumor cells in the re-excision specimen were found not to influence melanoma patient survival.

METHODS

Patients

Between August 1993 and September 2004, 551 patients were diagnosed with clinical stage I/II cutaneous melanoma according to criteria of the American Joint Committee on Cancer (AJCC) and underwent re-excision of the primary melanoma site and a SN biopsy. If patients were referred to us from another institution the pathologic characteristics of the primary melanoma were reviewed in our hospital before the SN procedure. All patients were treated according to the same protocol; re-excision margins were 1 cm for melanomas with a Breslow thickness of ≤ 2 mm and 2 cm for melanomas with a Breslow thickness of > 2 mm. To identify and retrieve the SN, the triple technique was used as described previously.¹⁸⁻²⁰ In short, the day before surgery patients underwent a dynamic and static lymphoscintigraphy

to determine the lymphatic drainage pattern. Just prior to surgery, Patent Blue V (Laboratoire Guerbet, Aulnay-sous-Bois, France) was injected intradermally next to the initial site of the melanoma. During surgery, guided by a hand held gamma probe and the blue staining of the draining tissues, the SN was removed.

To investigate the influence on survival, patients were divided both according to their diagnostic biopsy type; wide excisional biopsy (lateral clearance ≥ 2 mm), narrow excisional biopsy (lateral clearance < 2 mm), excisional biopsy with positive margins and incisional biopsy (includes punch) and the presence of residual tumor cells in their re-excision specimen.

Statistical Analysis

Data were processed with the Statistical Package for the Social Sciences software for Windows 2000 (SPSS 11.5, Chicago, IL). Cox's proportional hazard model was used for survival analysis. *P* values < 0.05 were considered significant.

RESULTS

Patient Population

Between August 1993 and September 2004, 551 patients were diagnosed with clinical stage I/II cutaneous melanoma, 257 male (46.6%) and 294 female (53.4%) with a mean age of 49.9 years (Table 1). Most primary melanomas were located on the trunk (43.7%) or on the lower extremities (36.7%). Breslow thickness was categorized into four groups (≤ 1.00 mm; 1.01–2.00 mm; 2.01–4.00 mm; > 4.01 mm), but due to spontaneous regression of the primary lesion remained unknown in 38 patients. The majority of patients had a superficial spreading melanoma (65.0%) or a nodular melanoma (26.7%). In 46 patients the type of melanoma was different or remained unknown (8.3%). Ulceration, defined as the absence of intact epidermis overlying the major portion of primary melanoma, was diagnosed in 80 patients (14.5%), unknown in 1 patient (0.2%) and absent in 470 patients (85.3%). Lymphatic invasion was present in 25 patients (4.5%), absent in 521 patients (94.6%) and remained unknown in 5 patients (0.9%). The SN was negative in 446 patients (80.9%) and positive in 94 patients (17.1%). In 11 patients the SN was not removed and the SN status remained unknown (2.0%). In total, there were 101 missing

TABLE 1. Patient characteristics

Characteristics	Patients (n = 551)
Follow up (years)	
Mean (SD)	5.1 (2.8)
Gender	
Male	257 (46.6%)
Female	294 (53.4%)
Age (years)	
Mean (SD)	49.9 (15.3)
Site of primary melanoma	
Lower extremity	202 (36.7%)
Upper extremity	63 (11.4%)
Head/Neck	45 (8.2%)
Trunk	241 (43.7%)
Breslow thickness (mm)	
0 < x ≤ 1	153 (27.8%)
1 < x ≤ 2	207 (37.6%)
2 < x ≤ 4	114 (20.7%)
> 4	39 (7.1%)
Unknown (regression)	38 (6.9%)
Type of melanoma	
Superficial spreading	358 (65.0%)
Nodular	147 (26.7%)
Other/Unknown	46 (8.3%)
Ulceration	
No	470 (85.3%)
Yes	80 (14.5%)
Unknown	1 (0.2%)
Lymphatic invasion	
No	521 (94.6%)
Yes	25 (4.5%)
Unknown	5 (0.9%)
Sentinel node status	
Negative	446 (80.9%)
Positive	94 (17.1%)
Unknown	11 (2.0%)

variables in 80 patients; all were excluded from the study.

SN Identification

In 11 of the 551 patients the SN status remained unknown (2.0%), in 5 of these patients the SN was located in the deep lobe of the parotid gland and in one patient the SN was located high in the left axilla, in all cases the decision was made not to remove the SN to avoid potential morbidity associated with the intervention. The SN was not identified in 3 cases due to non-visualization by preoperative lymphoscintigraphy. In one patient the SN was located in the right axilla and could not be removed because the patient was suffering from frozen shoulder syndrome, the physical condition of another patient did not allow further treatment. Therefore, the success rate of SN identification was 98% (540 of 551 patients). Two of the patients with the SN located in the deep lobe of the parotid gland experienced metastasis of the parotid gland, one patient is still alive with disease and one patient is dead of disease. The patient whose

TABLE 2. Patient distribution according to (A) diagnostic biopsy type and (B) the presence of residual tumor cells in the re-excision specimen

A	Patients (n = 471)	Follow up (years; mean ± SD)
Diagnostic biopsy type		
Wide excision biopsy (≥ 2 mm)	279 (59.3%)	5.0 ± 3.0
Narrow excision biopsy (0 < x < 2 mm)	109 (23.1%)	6.0 ± 2.7
Excision biopsy with positive margins	52 (11.0%)	5.8 ± 2.9
Incision biopsy	31 (6.6%)	5.4 ± 2.4
B	Patients (n = 441)	Follow up (years; mean ± SD)
Re-excision specimen		
No residual tumor cells	400 (90.7%)	5.1 ± 2.9
Residual tumor cells	41 (9.3%)	5.8 ± 2.6

physical condition did not allow further treatment, passed away soon after re-excision of the primary melanoma site, from massive hematogenic and lymphogenic metastasis. The 8 remaining patients have shown no evidence of disease.

Diagnostic Biopsy Type and Survival

The influence of diagnostic biopsy type on DFS and OS was tested in 471 patients with a mean FU of more than 5 years; 279 patients (59.3%) underwent a wide excision biopsy, 109 patients (23.1%) a narrow excision biopsy, 52 patients (11.0%) an excision biopsy with positive margins and 31 patients (6.6%) an incision biopsy (Table 2A).

In 91/471 patients (19.3%) the SN was positive, 58/279 patients (20.8%) after a wide excision biopsy, 14/109 patients (12.8%) after a narrow excision biopsy, 15/52 patients (28.8%) after an excision biopsy with positive margins and 4/31 patients (12.9%) after an incisional biopsy.

In 79/471 patients (16.8%) a recurrence was found during FU, 45/279 patients (16.1%) after a wide excision biopsy (21 locoregional skin, 8 SN basin and 16 systemic), 17/109 patients (15.5%) after a narrow excision biopsy (7 locoregional skin, 2 SN basin and 8 systemic), 10/52 patients (19.2%) after an excision biopsy with positive margins (5 locoregional skin, 2 SN basin and 3 systemic) and 7/31 patients (22.6%) after an incision biopsy (5 locoregional skin, and 2 systemic). But confounding factors were not equally distributed between the diagnostic biopsy groups. Therefore, survival analysis was done using Cox's proportional hazard model adjusted for; gender, age, site of primary melanoma, Breslow thickness, type of melanoma, ulceration, lymphatic invasion and sentinel node (SN) status.

TABLE 3. Univariate and multivariate Cox regression analysis of disease-free survival and overall survival according to diagnostic biopsy type

Tested variables	Disease Free Survival				Overall Survival			
	Univariate <i>P</i>	Multivariate			Univariate <i>P</i>	Multivariate		
		HR	95% CI	<i>P</i>		HR	95% CI	<i>P</i>
Diagnostic biopsy type								
Wide excision biopsy	0.691	1.00	–	0.204	0.500	1.00	–	0.765
Narrow excision biopsy	0.360	0.71	0.39–1.28	0.255	0.315	0.74	0.37–1.51	0.411
Excision biopsy with positive margins	0.870	0.59	0.29–1.18	0.132	0.748	0.75	0.34–1.65	0.476
Incision biopsy	0.581	0.47	0.19–1.16	0.101	0.387	0.74	0.29–1.89	0.530
Gender	0.018	1.15	0.70–1.89	0.573	0.004	1.44	0.81–2.57	0.215
Age (years)	0.004	1.02	1.00–1.04	0.018	0.002	1.03	1.01–1.05	0.007
Site of primary melanoma								
Lower extremity	0.091	1.00	–	0.023	0.153			
Upper extremity	0.825	0.91	0.41–2.01	0.809	0.071			
Head/Neck	0.014	3.40	1.45–7.97	0.005	0.095			
Trunk	0.731	0.93	0.54–1.61	0.790	0.063			
Breslow thickness (mm)								
0 < x ≤ 1	<0.001	1.00	–	<0.001	<0.001	1.00	–	0.001
1 < x ≤ 2	0.003	13.19	1.77–98.41	0.012	0.016	8.15	1.07–62.19	0.043
2 < x ≤ 4	<0.001	34.11	4.50–258.60	0.001	<0.001	21.43	2.79–164.84	0.003
> 4	<0.001	15.73	1.86–132.81	0.011	<0.001	11.85	1.35–103.70	0.025
Type of melanoma	<0.001	1.58	0.95–2.62	0.078	<0.001	1.57	0.88–2.79	0.128
Ulceration	<0.001	1.83	1.09–3.07	0.023	<0.001	1.64	0.89–3.04	0.116
Lymphatic invasion	<0.001	3.98	2.05–7.72	<0.001	<0.001	2.19	1.07–4.48	0.032
Sentinel node status	<0.001	3.87	2.23–6.70	<0.001	<0.001	3.19	1.75–5.81	<0.001

In univariate analysis; gender, age, Breslow thickness, type of melanoma, ulceration, lymphatic invasion and SN status were all significantly related to both DFS and OS (Table 3). Site of primary melanoma on the head/neck was in univariate analysis only significantly related to DFS (Table 3). Diagnostic biopsy type did not have a significant relation with either DFS or OS (Table 3).

Even though, diagnostic biopsy type did not have a significant relation with either DFS or OS in univariate Cox regression analysis it was also tested in multivariate Cox regression analysis together with all significant variables from univariate analysis, to investigate its influence on DFS and OS after correction for the confounding factors. In multivariate analysis, the only significant and independent predictors of both DFS and OS were; age, Breslow thickness, lymphatic invasion and SN status (Table 3). Site of primary melanoma on the lower extremities or head/neck and ulceration were in multivariate analysis only significant and independent predictors of DFS (Table 3). Also in multivariate analysis, diagnostic biopsy type did not have a significant relation with either DFS or OS (Fig. 1A, B).

The same analysis was done after combining the groups; the wide excision biopsy group was joined with the narrow excision biopsy group and compared to the excision biopsy group with positive margins joined with the incision biopsy group. Still, diagnostic

biopsy type did not have a significant influence on either DFS or OS (data not shown).

Residual Tumor Cells in the Re-Excision Specimen and Survival

441/471 Patients (93.6%) underwent re-excision of the primary melanoma site and in 30 patients (6.4%) the diagnostic biopsy was found to be sufficient. In 41/441 patients (9.3%) residual tumor cells were found in the re-excision specimen (Table 2B). All 41 patients with residual tumor cells in the re-excision specimen underwent either an excision biopsy with positive margins or an incision biopsy. In none of the patients with a wide or narrow excision biopsy residual tumor cells were found.

In univariate analysis; gender, age, Breslow thickness, type of melanoma, ulceration, lymphatic invasion and SN status were all significantly related to both DFS and OS (Table 4). Site of primary melanoma on the lower extremity or head/neck was in univariate analysis significantly related to DFS and site of primary melanoma on the head/neck or trunk was significantly related to OS (Table 4). Residual tumor cells in the re-excision specimen did not have a significant relation with either DFS or OS (Table 4).

Even though, residual tumor cells in the re-excision specimen did not have a significant relation with either DFS or OS in univariate Cox regression analysis

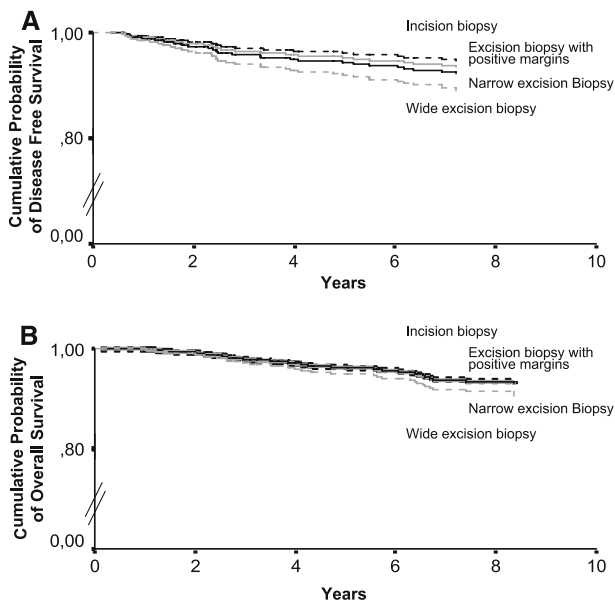


FIG. 1. **A** Disease free survival rates and **B** overall survival rates according to the initial biopsy type.

it was also tested in multivariate Cox regression analysis together with all significant variables from univariate analysis, to investigate its influence on DFS and OS after correction for the confounding factors. In multivariate analysis, the only significant and independent predictors of both DFS and OS were; age, site of primary melanoma on the head/neck, Breslow thickness, lymphatic invasion and SN status (Table 4). Also in multivariate analysis, residual tumor cells in the re-excision specimen did not have a significant relation with either DFS or OS (Fig. 2A, B).

Consistent Confounders of Melanoma Patient Survival

Age, Breslow thickness, lymphatic invasion and SN status were the most consistent, independent and significant confounders of melanoma patient DFS and OS (Table 3, 4). Site of the primary melanoma was not always an independent and significant confounder of melanoma patient survival, but location on the head/neck region consistently carried the highest hazard ratio (HR) (Table 3, 4). Surprisingly, ulceration was not an independent and significant confounder of OS (Table 3, 4).

DISCUSSION

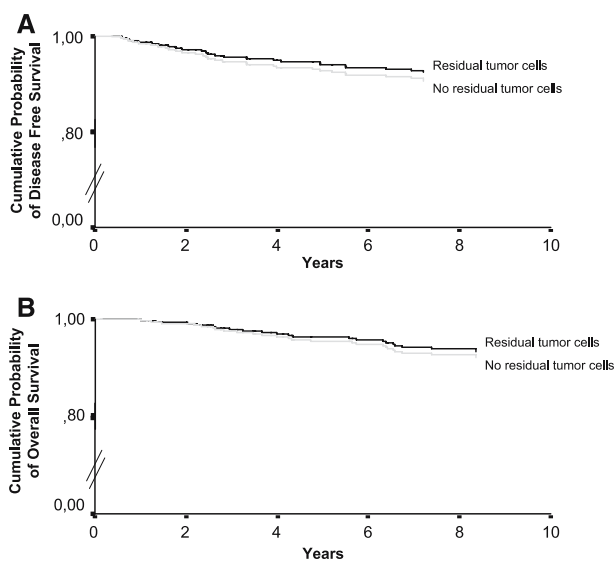
Numerous investigators have studied the influence of incisional biopsy on melanoma patient survival

and found contradicting results. Fitzpatrick et al. found a five-year OS rate of 30% in the incisional biopsy group as compared to 48% in the excisional biopsy group but the different biopsy groups were not matched for important prognostic factors.⁸ Epstein et al. found a more favorable ten-year OS in the biopsy group (65%) as compared to the primary wide excision group (56%) but biopsy was loosely defined as less than optimal or complete surgical excision.⁹ Ironside et al. found a five-year OS rate of 66% in the excision- and 43% in the incision biopsy group but failed to describe the distribution of prognostic factors between both groups.¹⁰ Rampen et al. also found a worse prognosis for patients after an incision biopsy (14 patients) but the study was small and retrospective.¹¹ Griffiths et al. found no difference in survival but 7/19 incisional biopsy patients were excluded because of missing histopathological data.¹² Survival rates were not significantly different between the incision- and excision biopsy groups of Lederman et al., but patient groups were matched for Breslow thickness only.¹³ Lees et al. also indicated no significant adverse effect of incisional biopsy in 96 patients, but 40% of the histopathological data was not assessable.¹⁴ Austin et al. did find a significantly reduced survival in the incisional biopsy group, but patients in the incisional biopsy group were also significantly older.¹⁵ The two most recent publications found no negative influence of incisional biopsies on melanoma patient survival but in Bong et al. patients groups were not fully matched and in Martin et al. median FU was only 28 months.^{16,17}

This study not only investigated the influence of diagnostic biopsy type but also the presence of residual tumor cells in the re-excision specimen on melanoma patient survival in patient groups adjusted for 8 important confounders of survival with a mean FU > 5 years. Excision biopsies with positive margins and incisional biopsies were found not to influence melanoma patient survival. Interestingly, DFS and OS even seemed slightly better in the non-radical biopsy groups (Fig. 1A, B). In line with this, patients with residual tumor cells in their re-excision specimen also had a slightly better survival as compared to patients without residual tumor cells in their re-excision specimen (Fig. 2A, B). Melanoma is the most immunogenic tumor identified to date; melanoma specific T cells are detectable both in the blood and in tumor draining lymph nodes from melanoma patients and their frequency can be increased by specific vaccination.²¹⁻²³ This intrinsic immunogenicity makes melanoma lesions particularly amenable to therapeutic approaches aimed at strengthening

TABLE 4. Univariate and multivariate Cox regression analysis of disease-free survival and overall survival according to the presence of residual tumor cells in the re-excision specimen

Tested variables	Disease Free Survival				Overall Survival			
	Univariate <i>P</i>	Multivariate			Univariate <i>P</i>	Multivariate		
		HR	95% CI	<i>P</i>		HR	95% CI	<i>P</i>
Residual tumor cells	0.094	0.79	0.38–1.64	0.532	0.230	0.83	0.36–1.92	0.668
Gender	0.025	1.14	0.68–1.92	0.611	0.009	1.22	0.65–2.28	0.531
Age (years)	0.007	1.02	1.00–1.04	0.038	0.007	1.02	1.00–1.04	0.040
Site of primary melanoma								
Lower extremity	0.032	1.00	–	0.033	0.105	1.00	–	0.123
Upper extremity	0.830	0.95	0.41–2.20	0.910	0.069	2.21	0.85–5.71	0.102
Head/Neck	0.004	3.22	1.35–7.66	0.008	0.040	3.22	1.03–10.09	0.045
Trunk	0.647	0.96	0.54–1.74	0.903	0.049	2.02	0.98–4.20	0.059
Breslow thickness (mm)								
0 < x ≤ 1	<0.001	1.00	–	<0.001	<0.001	1.00	–	0.004
1 < x ≤ 2	0.005	12.82	1.71–96.07	0.013	0.020	7.99	1.04–61.49	0.046
2 < x ≤ 4	<0.001	30.16	3.97–229.22	0.001	<0.001	20.13	2.59–156.25	0.004
> 4	<0.001	15.91	1.86–136.25	0.012	<0.001	11.84	1.32–106.54	0.028
Type of melanoma	<0.001	1.61	0.95–2.73	0.076	<0.001	1.63	0.87–3.04	0.128
Ulceration	<0.001	1.55	0.90–2.67	0.119	<0.001	1.61	0.85–3.05	0.141
Lymphatic invasion	<0.001	4.16	2.13–8.14	<0.001	<0.001	2.24	1.04–4.80	0.038
Sentinel node status	<0.001	3.30	1.87–5.83	<0.001	<0.001	3.05	1.56–5.96	0.001

**FIG. 2.** A Disease free survival rates and B overall survival rates according to the presence of residual tumor cells in the re-excision specimen.

tumor immune surveillance. Did residual tumor cells combined with biopsy induced wound healing trigger the melanoma patient's immunosystem? In non-melanoma skin cancer (NMSC) biopsy induced tumor regression has previously been described, Swetter et al reported that 24% of NMSCs transected on the initial biopsy showed no residual tumor in the excision specimens and suggested biopsy induced wound healing to play an important role.²⁴

Even though non-radical diagnostic biopsies and residual tumor cells in the re-excision specimen do not have a negative influence on melanoma patient survival, the routine use of incision biopsies is not recommended. Incisional biopsies often consist only of a small percentage of the surface area of the pigmented skin lesion making it difficult to sample a representative area within the tumor. Somach et al found that 40% of the histopathological features were more pronounced in the re-excision specimen as compared to the incision biopsy and in 20% areas of invasive melanoma not detected in the incisional biopsy were observed in the re-excision specimen.²⁵ Further more when melanoma is diagnosed, attempting to evaluate the depth of invasion in an incisional biopsy is treacherous and may lead to over- or underestimation of the invasion.^{26,27} Of course these problems are less prominent in excision biopsies with positive margins, here the majority of the lesion has been removed and only the outer borders are compromised making a sampling error highly unlikely.

In conclusion; age, Breslow thickness, lymphatic invasion and SN status were the most consistent, independent and significant confounders of melanoma patient DFS and OS. The site of primary melanoma and ulceration were also important confounders of survival. Both the diagnostic biopsy type and the presence of residual tumor cells in the re-excision specimen did not have a negative influence on melanoma patient DFS and OS. With melanoma incidence rates rising¹ and early detection of mela-

noma still being the only way to improve melanoma patient survival,⁴ it is important for all physicians to feel confident about removing a pigmented skin lesion suspect for melanoma. Incisional biopsies are not recommended but there is no cause for concern when an excision biopsy turns out to have positive margins.

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