Published in final edited form as:

Am J Surg Pathol. 2011 February ; 35(2): 235–242. doi:10.1097/PAS.0b013e3182036ccd.

High Lymphatic Vessel Density and Lymphatic Invasion Underlie the Adverse Prognostic Effect of Radial Growth Phase Regression in Melanoma

Sook Jung Yun, MD, PhD^{*}, Phyllis A. Gimotty, PhD^{†, **}, Wei-Ting Hwang, PhD[†], Peter Dawson, MS[†], Patricia Van Belle, PhD[‡], David E. Elder, MB, ChB^{‡, **}, Rosalie Elenitsas, MD[§], Lynn Schuchter, MD^{¶, **}, DuPont Guerry, MD[¶], and Xiaowei Xu, MD, PhD^{†, **}

^{*} Department of Dermatology, Chonnam National University Medical School, Gwangju, Korea

[†] Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

[‡] Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

[§] Department of Dermatology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

[¶] Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

^{**} Melanoma Program of the Abramson Cancer Center, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Abstract

Regression in the radial growth phase (RGP) of primary cutaneous melanomas is common and has been shown to be an adverse prognostic factor. However, the underlying mechanism is unclear. We performed dual immunohistochemical staining of podoplanin and S100 on paraffin tissues from 321 patients with vertical growth phase (VGP) primary melanomas who had 10 years or more of follow-up. Lymphatic density (LD) and Lymphatic invasion (LI) was quantified and documented. The time to first metastasis and melanoma-specific death from the date of definite treatment was analyzed using univariate and multivariate Cox models. Among the 116 VGP melanomas that had regression in the adjacent RGP, 75 (23%) were classified as complete and 41 (13%) as partial. LD was significantly higher ($p < 0.001$) in the 75 lesions with complete regression (mean $\pm$ SD, $23.7 \pm 12.3/\text{mm}^2$) compared to the 41 with partial regression ($15.5 \pm 7.1/\text{mm}^2$) and was lower in 155 areas of adjacent normal dermis ($7.3 \pm 3.5/\text{mm}^2$) and 69 areas of distant normal dermis ($5.5 \pm 2.6/\text{mm}^2$). Patients whose lesions had areas of complete regression with LI and either high or low LD or had no LI with high LD, had shorter TFM (HRs = 2.5, 3.8, and 2.5, respectively) and increased risk of melanoma-specific death (HRs=3.1, 1.3 and 3.0, respectively) than those with no LI and low LD or those without areas of complete regression. These data indicate that complete RGP regression is associated with significantly increased LD, and that the adverse prognostic effect of RGP regression is at least partially mediated through lymphangiogenesis and LI in this area.

Keywords

regression; lymphatic vessel density; lymphatic invasion; melanoma; prognosis

INTRODUCTION

RGP regression is defined histologically and it is different from disappearance/regression of an established pigmented lesions clinically. Histological regression can be seen in lesions without clinical evidence of disappearance or fading. RGP regression of primary melanomas (with or without an associated VGP) has been defined as increased vascularity and delicate fibroplasia in the papillary dermis, a lymphocytic and melanophage infiltrate, and an absence of dermal melanoma cells. In complete regression melanoma cells are absent in the overlying epidermis; whereas in partial regression they are present in the epidermis.⁹ RGP regression is common, particularly in thin lesions. The incidence of regression in melanomas of all thicknesses has been estimated at about 10–35%, and as up to 58% in melanomas with thicknesses of <0.75 mm.^{3,18}

RGP regression has been shown to be associated with an adverse clinical outcome, both in lesions without and with an adjacent VGP.⁹ The presence of complete regression in the RGP of lesions with VGP was associated with poorer survival in a prognostic model published by Clark et al.⁴ The extent of regression in thin melanomas also has been associated with poorer prognosis.^{22,25} The association of RGP regression with a brisk lymphocytic infiltrate and absent melanoma cells has been taken to reflect a host response directed against malignant cells. Paradoxically, the presence of brisk tumor infiltrating lymphocytes (TIL) in the VGP is a favorable prognostic factor. Therefore, it is puzzling that RGP regression is associated with worse clinical outcome and the mechanisms underlying these phenomena have remained obscure.

Our previous studies revealed that lymphatic vessel density (LD) is significantly increased in melanomas compared with either common acquired or dysplastic nevi¹² and that lymphatic invasion (LI) in primary melanomas is much more frequently observed when using dual immunohistochemical (IHC) markers for lymphatic vessels and for melanoma cells rather than routine histology.²⁸ Other groups have shown that lymphangiogenesis in melanoma is associated with an increased incidence of sentinel lymph node metastases and shorter disease-free survival.²¹ Barnhill and Levy¹ reported that areas of RGP regression had strikingly greater vascularity than areas without regression, however, their study did not address whether the increased vascularity was made up of lymphatic or blood vessels. In this study, we used a cohort of 321 patients with vertical growth phase (VGP) primary melanomas who had 10 years or more of follow-up. Patients with invasive RGP without VGP were not included in the study since it has been reported that over 95% of these patient did not have recurrence of disease after over more than 10 years of follow up as described in the Clark Paper⁴. We asked if the prognostic value of RGP regression could be ascribed to increased LD and LI in areas of regression.

PATIENTS AND METHODS

Study Patients and Study Design

The current study is a retrospective cohort study that included patients seen between 1972 and 1991 at the Pigmented Lesion Clinic of the University of Pennsylvania. Patients eligible for this study had VGP primary melanomas and no apparent metastasis at the time of definitive treatment. This study included 346 patients who had at least 10 years of follow-up and had paraffin blocks available for IHC staining. We excluded 25 cases in which stained

tissue sections were technically poor (n=21), had no residual VGP on the IHC stained slides (n=3) and 1 case in which no consensus could be reached by the two pathologists. The study protocol was approved by the institutional review board at the University of Pennsylvania.

IHC Staining

Lymphatic endothelium was visualized with the brown chromogen diaminobenzidine (DAB) (DakoCytomation, Carpinteria, CA) and melanoma cells, with Nova Red (Vector Laboratories, Burlingame, CA). The D2-40 antibody (mouse monoclonal, 1:25 dilution, Signet Laboratories, Dedham, MA) that specifically detects a fixation resistant epitope on podoplanin was used to decorate lymphatic endothelium. Melanoma cells were identified using S-100 antibody (rabbit polyclonal, 1:50 dilution, DakoCytomation). IHC assays were done on 5 µm-thick, formalin-fixed, paraffin-embedded sections. Heat-induced epitope retrieval was performed by boiling the slides in 1x EDTA buffer (LabVision, Fremont, CA) for 20 minutes. Slides were incubated with the D2-40 and S-100 antibodies for 1 hour at room temperature. Staining was done on a DakoCytomation Autostainer using the EnVision + horseradish peroxidase (HRP) DAB system (DakoCytomation) according to manufacturer's recommendations. Normal mouse serum (1:1000 dilution) was substituted for the primary antibody in each case as a negative control.

Pathology Definitions

All slides were originally reviewed for routine histological attributes by 2 pathologists (Elder DE and Clark WH Jr) without knowledge of patients' outcomes as described previously.⁴ Attributes included tumor thickness; dermal mitotic rate, expressed in mitoses per square millimeter; VGP TIL, classified as present or absent; and regression, if present in the invasive or in situ RGP adjacent to the VGP; and ulceration. Subsequently cut and prepared IHC stained slides were reviewed independently by two dermatopathologists (Yun, SJ and Xu, X) who were also blinded to clinical outcome.

Complete and partial regression was defined as noted in the introduction (Figure 1A, 1B, 1E and 1F). LD was quantified by counting the number of lymphatic vessels in 1 mm² in "hot spots" within areas of complete or partial regression. "Hot spots" were discrete areas with easily visible lymphatic vessels positive for podoplanin staining (Figure 2A). Agreement on LD between two reviewers was first established by the Altman-Bland approach,² and the average of LD was used in all the analyses.

We found that double staining for melanoma cells and lymphatic endothelial cells was optimal to identify LI. S-100 (Figure 1C), Melan-A (Figure 1D), or podoplanin alone was not sufficient to identify LI. LI was defined by S-100 positive cell(s) present in lymphatic vessel lumens within the area of regression (Figure 2B and 2C). We also compared LD in regression with that in the normal dermis immediately adjacent to regressed area and "distant" histologically normal dermis (at least 1 mm away from any area of regression, RGP or VGP).

Clinical Definitions

The study patients, all derived from a cohort that predated the use of staging with sentinel node biopsies, were followed for 10–35 years per protocol. The primary endpoint was "first metastasis" within 10-years of diagnosis where metastasis was defined to be metastasis to regional nodes, disseminated disease or, in the absence of information on the preceding metastases, melanoma-specific death. Time to first metastasis (TFM) was defined as the time from definitive surgery to the first metastasis, as defined above. For 41 patients who received elective node dissections (ELND), 16 (39%) of these had histologically positive nodes, and these patients were not used in the calculation of the time to first metastasis.

Consequently, for those with an ELND, the time to first metastasis is observed if there is recurrence of disease in non-regional nodes, disseminated disease or melanoma-related death. Patients were censored due to death unrelated to melanoma or if they were alive at the time of last follow up. The median follow up for those alive at last follow up was 20.4 years (n=215, range was 2.2 to 33.5 years). The median time to first metastasis was 2.02 years (n=106 patients, range was 0.05 to 21.6 years).

Statistical Methods

Patient and tumor characteristics were compared between subjects with and without areas of complete regression and between those with partial and no regression using Pearson's chi-squared statistics or Fisher's exact test. LD was compared between the areas of complete and partial regression, and between normal areas adjacent and distant from the tumor using t-tests with Satterthwaite's modification for unequal variances. The primary analysis was to examine the association of TFM and regression with the areas of regression further classified as to its extent (complete, partial or none), density of lymphatic vessels (LD high or low), and the presence of lymphatic invasion (LI present or absent). The secondary analysis looked at time to melanoma-specific death. We determined an optimal cut-point for LD as that associated with the most significant difference between the two survival curves defined by the range of potential cut-points. Kaplan-Meier curves were estimated for TFM, and the log-rank test was used to identify significant differences between curves defined by the variables. Cox proportional hazards regression models were used to obtain unadjusted and adjusted hazard ratios (with associated 95% confidence intervals) and to investigate how more specific classification of the area of regression (extent, LD and LI, as well as the interaction of LD and LI) would mediate the association. A p-value for a pairwise comparison of two survival curves (Figure 3) was considered significant, if it was ≤ 0.01 based on Bonferroni's adjustment for multiple hypothesis testing. These analyses were performed using SAS Version 9.1. P values were based on two-sided tests.

RESULTS

Patient and Lesion Characteristics

The characteristics of the patients and their tumors are presented in Table 1. In this study, 75 patients' lesions (23%) had complete and 41 (13%) had partial regression. There were no significant differences in these characteristics between those with melanomas that had areas of partial regression and those had no area of regression. However, there were significant differences in tumor-related characteristics among those with melanomas that had areas of complete regression and those whose lesions had no complete regression. Ulceration was significantly more likely in lesions with complete regression (29%) compared to those with partial regression (9%) or no regression (19%) ($p=0.029$). In addition, VGP TIL were more often observed in melanomas with complete RGP regression (85%) compared to those with either partial regression (68%) or no regression (65) ($p=0.005$).

LD was increased in the Area of Regression in Melanoma

Most of the vascular channels within areas of RGP regression were lymphatic, staining strongly with podoplanin (Figure 2A). LD significantly decreased in stepwise fashion from the 75 areas of complete regression (mean $\pm$ sd, $23.7 \pm 12.3/\text{mm}^2$) to the 41 areas of partial regression ($15.5 \pm 7.1/\text{mm}^2$), to the 155 areas of adjacent normal dermis ($7.3 \pm 3.5/\text{mm}^2$) and to 69 areas of distant normal dermis ($5.4 \pm 2.6/\text{mm}^2$) (Table 2). Patients with melanomas with areas of complete regression were classified into two groups: 44 with low LD ($\leq 24.5/\text{mm}^2$) and 31 with high LD ($>24.5/\text{mm}^2$), using the optimal cut-point for LD equal to 24.5, a value that was close to the median value of LD which was $22.5/\text{mm}^2$.

LI was observed in the Area of Regression in Melanoma

Among patients with melanomas that had either an area of complete or partial regression (n=116), 21.6% were observed to have LI in the area of regression (Figure 2B and 2C). We note that LI was significantly more likely to be observed in the areas of complete regression (24%) than it was in areas of partial regression (12%) ($p<0.001$).

High LD and LI in the Area of Complete Regression were Associated with Poor Prognosis

Patients with lesions exhibiting complete regression had shorter TFM (HR=1.63, $p=0.021$) and shorter time to MSD (HR=1.63, $p=0.036$) than those with lesions having partial or no regression (Table 3, Model 1). Patients with lesions having high LD in areas of complete regression had shorter TFM (HR=2.8, $p<0.001$) and shorter time to MSD (HR=2.6, $p=0.001$) compared to patients whose lesions did not have areas of complete regression (Table 3, Model 2). Interestingly, patients with low LD in RGP areas of complete regression had similar clinical outcomes compared to patients whose melanomas did not have complete regression (HR=1.0, $p=0.882$ for TFM and HR=1.1, $p=0.720$ for MSD) (Table 3, Model 2). Patients whose lesions had LI in the area of complete regression had shorter TFM (HR=2.9, $p<0.001$) and shorter time to MSD (HR=2.4, $p=0.016$) than those whose lesions had areas of complete regression without LI (HR=1.3, $p=0.295$ for TFM and HR=1.4, $p=0.204$ for MSD) compared with those without complete regression (Table 3, Model 3). Fig. 2 presents the 4 survival curves for patients with lesions with an area of complete regression subdivided into groups defined by LD (high and low) and LI (present and absent), as well as the survival curve for those whose lesions were without an area of complete regression. Based on the hazard ratios from the Cox model (Table 3, Model 2), patients whose lesions had areas of complete regression with LI (and either high or low LD) or with no LI with high LD had shorter TFM (HRs = 2.5, 3.8, 2.5, respectively) than those with no LI and low LD (unadjusted HR=0.7, $p=0.291$) or those without areas of complete regression.

DISCUSSION

This study demonstrates for the first time that regression in the RGP adjacent to the VGP of primary melanomas is associated with lymphangiogenesis and LI. Further, high LD or the presence of LI in the area of RGP regression may be used to stratify patients having areas of complete regression into high or low risk groups. In addition, partial RGP regression does not appear to be associated with worse prognosis.

We and others have shown that RGP regression is an adverse prognostic factor.^{4,10,22,25} However, more recent studies are inconsistent on the association of regression with metastasis to sentinel lymph nodes.^{11,15,20} Olah et al²⁰ reported a significant association of SLN metastasis and regression, while Fontaine et al¹¹ reported no association. Kaur et al¹⁵ used a histopathological staging system for regression and concluded that regression is associated with a favorable prognosis in thin melanomas. The variable results in these studies may be explained by the different criteria used to define regression and whether partial regression was included in the analyses. In the current report, we compared complete and partial regression as defined by Clark et al⁴ and found that complete regression is an adverse prognostic factor for both TFM and MSD whereas partial regression is not. It is unclear why partial regression was not associated with adverse prognosis. We postulate that partial regression is an intermediate state and it may evolve to complete regression with time. The extra time to reach complete regression may allow further lymphangiogenesis and LI within the lesion.

A strength of the study is that time to metastasis was not confounded by sentinel node biopsy, which finds metastases that are not clinically evident. However, in the era from

which these cases derived, some patients received elective node dissections (ELND). The results of the ELND were not used in the calculation of the time to first metastasis. Consequently, for those with an ELND, the time to first metastasis was observed if there was recurrence of disease in non-regional nodes, disseminated disease or melanoma-related death. We computed the hazard ratio for those with and without ELND and there was no significant difference in the hazard of disease recurrence between the two groups.

One of the key histological features in regression is an increased number of vascular channels. We found that most of these microvessels were lymphatic and that LD in a regressed area was significantly higher than immediate adjacent normal or distant normal dermis, likely indicating active lymphangiogenesis in areas of regression. There are a number of studies that have showed that increased LD in melanoma is associated with poor prognosis.^{6,7,17,24,27} One study demonstrated that LD in primary melanomas with VGP was a significant independent prognostic indicator for the presence of metastases in sentinel lymph nodes, having a stronger statistical associations than tumor thickness.⁷ High peritumoral or intratumoral LD was associated with SLN metastasis and shorter survival.¹⁷ Our results echo these findings and showed that high LD in the regressed area is also associated with poorer prognosis.

RGP regression is associated with dense lymphohistiocytic infiltrates. Although T cells do not expression VEGFC, it has been shown that tumor-infiltrating macrophages secrete VEGF-C and VEGF-D,^{7,17,23} key growth factors regulating lymphangiogenesis¹⁴. The number of lymphatic vessels has been shown to directly correlate with the intensity of the inflammatory infiltrate in and around the tumor.^{6,17,26} Therefore, it is plausible that the inflammatory cells in the area of regression may play a significant role in lymphangiogenesis.

It is interesting that melanoma cells can be seen within lymphatic vessels in areas of regression. We have previously shown that LI was common (33%) in primary melanomas with VGP using dual IHC staining rather than routine morphology (4.7%).²⁸ Doeden et al⁸ reported LI was associated with sentinel lymph node metastasis. In this study, we found that LI was detected more frequently in areas of complete regression (23.5%) than in areas of partial regression area (14.6%). Lesions having areas of complete regression that included LI, with or without high LD, had a poorer prognosis than those without an area of complete regression (HR=2.5 and 3.8, p=0.019 and 0.004, respectively).

These results suggest that the adverse prognostic effect of RGP regression is mediated through the increased LD and/or LI in the area of complete regression; whereas partial regression does not appear to be associated with worse prognosis.

Acknowledgments

Supported provided by: Specialized Program of Research Excellence (SPORE) on Skin Cancer (P50-CA-093372) and Cancer Biostatistics Training Grant (T32-CA093283)

References

1. Barnhill RL, Levy MA. Regressing thin cutaneous malignant melanomas (<1.0 mm) are associated with angiogenesis. *Am J Pathol.* 1993; 143:99–104. [PubMed: 7686347]
2. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* 1986; 1:307–310. [PubMed: 2868172]
3. Blessing K, McLaren KM. Histological regression in primary cutaneous melanoma: recognition, prevalence and significance. *Histopathology.* 1992; 20:315–322. [PubMed: 1577409]

4. Clark WH Jr, Elder DE, Guerry DIV, et al. Model predicting survival in stage I melanoma based on tumor progression. *J Natl Cancer Inst.* 1989; 81:1893–1904. [PubMed: 2593166]
5. Cook MG. The significance of inflammation and regression in melanoma. *Virchows Archiv A Pathol Anat.* 1992; 420:113–115.
6. Dadras SS, Paul T, Bertocini J, et al. Tumor lymphangiogenesis: a novel prognostic indicator for cutaneous melanoma metastasis and survival. *Am J Pathol.* 2003; 162:1951–1960. [PubMed: 12759251]
7. Dadras SS, Lange-Asschenfelt B, Velasco P, et al. Tumor lymphangiogenesis predicts melanoma metastasis to sentinel lymph nodes. *Mod Pathol.* 2005; 18:1232–1242. [PubMed: 15803182]
8. Doeden K, Ma Z, Nasasimhan, et al. Lymphatic invasion in cutaneous melanoma is associated with sentinel lymph node metastasis. *J Cutan Pathol.* 2009; 36:772–780. [PubMed: 19032379]
9. Elder, DE.; Elenitsas, R.; Murphy, GF., et al. Benign pigmented lesions and malignant melanoma. In: Elder, DE.; Elenitsas, R.; Johnson, B., Jr, et al., editors. *Lever's histopathology of the skin.* 10. Philadelphia, PA: Lippincott Williams & Wilkins; 2008. p. 699-789.
10. Emanuel PO, Mannion M, Phelps RG. Complete regression of primary malignant melanoma. *Am J Dermatopathol.* 2008; 30:178–181. [PubMed: 18360126]
11. Fontaine D, Parkhill W, Greer W, et al. Partial regression of primary cutaneous melanoma. Is there an association with sub-clinical sentinel lymph node metastasis? *Am J Dermatopathol.* 2003; 25:371–376. [PubMed: 14501285]
12. Giorgadze TA, Zhang PJ, Pasha T, et al. Lymphatic vessel density is significantly increased in melanoma. *J Cutan Pathol.* 2004; 31:672–677. [PubMed: 15491327]
13. Kahn HJ, Marks A. A new monoclonal antibody, D2-40, for detection of lymphatic invasion of primary tumors. *Lab Invest.* 2002; 82:1255–1257. [PubMed: 12218087]
14. Karpanen T, Egeblad M, Karkkainen MJ, et al. Vascular endothelial growth factor C promotes tumor lymphangiogenesis and intralymphatic tumor growth. *Cancer Res.* 2001; 61:1786–1790. [PubMed: 11280723]
15. Kaur C, Thomas RJ, Desai N, et al. The correlation of regression in primary melanoma with sentinel lymph node status. *J Clin Pathol.* 2008; 61:297–300. [PubMed: 17675538]
16. Little D, Said JW, Siegel RJ, et al. Endothelial cell markers in vascular neoplasms: an immunohistochemical study comparing factor VIII-related antigen, blood group specific antigens, 6-Keto-PGF1 alpha, and Ulex europaeus 1 lectin. *J Pathol.* 1986; 149:89–95. [PubMed: 2426432]
17. Massi D, Puig S, Franchi A, et al. Tumor lymphangiogenesis is a possible predictor of sentinel lymph node status in cutaneous melanoma: a case-control study. *J Clin Pathol.* 2006; 59:166–173. [PubMed: 16443733]
18. McGovern VJ, Shaw HM, Milton GW. Prognosis in patients with thin malignant melanoma: influence of regression. *Histopathology.* 1983; 7:673–680. [PubMed: 6629343]
19. Niakosari FN, Kahn H, Marks A, et al. Detection of lymphatic invasion in primary melanoma with monoclonal antibody D2-40: A new selective immunohistochemical marker of lymphatic endothelium. *Arch Dermatol.* 2005; 141:440–444. [PubMed: 15837861]
20. Olah J, Gyulai R, Korom I, et al. Tumor regression predicts higher risk of sentinel node involvement in thin cutaneous melanomas. *Br J Dermatol.* 2003; 149:662–663. [PubMed: 14511013]
21. Rinderknecht M, Detmar M. Tumor lymphangiogenesis and melanoma metastasis. *J Cell Physiol.* 2008; 216:347–354. [PubMed: 18481261]
22. Ronan SG, Eng AM, Briele HA, et al. Thin malignant melanomas with regression and metastases. *Arch Dermatol.* 1987; 123:1326–1330. [PubMed: 3662564]
23. Schoppman SF, Birmer P, Stockl J, et al. Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. *Am J Pathol.* 2002; 161:947–956. [PubMed: 12213723]
24. Shields JD, Borsetti M, Rigby H, et al. Lymphatic density and metastatic spread in human malignant melanoma. *Br J Cancer.* 2004; 90:693–700. [PubMed: 14760386]
25. Sondergaard K, Hou Jensen K. Partial regression in thin primary cutaneous malignant melanomas clinical stage I. A study of 486 cases. *Virchows Arch A.* 1985; 408:241–247.

26. Straume O, Jackson DG, Akslen LA. Independent prognostic impact of lymphatic vessel density and presence of low-grade lymphangiogenesis in cutaneous melanoma. *Clin Cancer Res.* 2003; 9:250–256. [PubMed: 12538477]
27. Valencak J, Heere-Ress E, Kopp T, et al. Selective immunohistochemical staining shows significant prognostic influence of lymphatic and blood vessels in patients with malignant melanoma. *Eur J Cancer.* 2004; 40:358–364. [PubMed: 14746853]
28. Xu X, Gimotty PA, Guerry D, et al. Lymphatic invasion revealed by multispectral imaging is common in primary melanomas and associates with prognosis. *Hum Pathol.* 2008; 39:901–909. [PubMed: 18440591]

FIGURE 1.

Complete and partial RGP regression. A-B) Complete regression (H&E, magnification, x50 and 200). Arrows point to the open vessels within the inflammatory cell infiltrate. C-D) Complete regression (S-100 and Melan-A staining, magnification, x200), no apparent melanoma cells in the dermis. E-F) Partial regression (H&E, magnification, x50 and 200). Arrows point to the residual melanoma cells in the epidermis.

FIGURE 2.

Lymphatic density and lymphatic invasion in RGP regression. A) D2-40 positive lymphatic vessels in complete RGP regression (D2-40 and S-100 double staining, magnification, x100). B-C) Lymphatic invasion in complete RGP regression (D2-40 and S-100 double staining, magnification, x400), S100(+) melanoma cells in D2-40 positive lymphatic vessels.

FIGURE 3.
A) Kaplan-Meier survival curves for time to first metastasis by LI (present and absent) and LD status (high versus low). B) P-values for the log-rank test for pairwise comparisons of the survival curves.

Table 1

Patient and Lesional Characteristics by Type of Regression

	Complete (n=75) Percent		Partial (n=41) Percent	None (n=205) Percent	p-value	All (n=321) Percent
Lymphatic Invasion in Area of Regression [/]						
Sex	Present	24.0	12.2	--	<0.001	--
	Absent	76.0	87.8	--		--
Anatomic Site	Male	56.0	46.3	51.7	0.602	52.0
	Female	44.0	53.7	48.3		48.0
Thickness	Axial	68.0	68.3	60.0	0.352	62.9
	Extremity	32.0	31.7	40.0		37.1
Mitotic Rate	<=1.00 mm	33.3	51.2	44.4	0.170	42.7
	1–2 mm	26.7	26.8	26.3		26.5
	2–4 mm	24.0	17.1	22.4		22.1
	>4 mm	16.0	4.9	6.8		8.7
Ulceration	0–0.99	26.7	43.9	40.0	0.081	37.4
	≥ 1.00	73.3	56.1	60.0		62.6
VGP TILS	Present	29.3	9.8	18.5	0.029	19.9
	Not present	70.7	90.2	81.5		74.5
Age at Diagnosis	Present	85.3	68.3	65.4	0.005	70.4
	Absent	14.7	31.7	34.6		29.6
	< 60	61.3	70.7	70.7	0.308	68.5
	≥60	38.7	29.3	29.3		31.5

[/] LI not assessed in those without regression

Table 2

LD in Areas of Regression and Normal (Adjacent and Distant) Tissue

	n	Mean	SD
Area of Regression			
Complete	75	23.7	12.3
Partial	41	15.5	7.1
Area Adjacent to Tumor	155	7.3	3.5
Area Distant from the Tumor	69	5.5	2.6

Table 3

Unadjusted and Adjusted Hazard Ratios from a Cox Proportional Hazards Models

	n	First Metastasis			Melanoma-specific Death		
		Unadjusted HR	p-value	Adjusted* HR	Unadjusted HR	p-value	Adjusted* HR
Model 1							
Regression							
Not Complete	246	1.00		1.00	1.00		1.00
Complete	75	1.63	0.021	1.40	1.63	0.036	1.40
Model 2							
LD on Regression							
No Complete Regression	246	1.00		1.00	1.00		1.00
Complete Regression w/low LD	56	1.00	0.882	0.88	1.10	0.720	0.95
Complete Regression w/high LD	19	2.80	<0.001	2.50	2.60	0.001	2.20
Model 3							
LI on Regression							
No Complete Regression	246	1.00		1.00	1.00		1.00
Complete Regression w/o LI	37	1.30	0.295	1.20	1.40	0.204	1.30
Complete Regression w/LI	38	2.90	<0.001	2.10	2.40	0.016	1.60
Model 4							
LI and LD on Regression							
No Complete Regression	246	1.00		1.00	1.00		1.00
Complete Regression w/o LI, low LD	23	0.66	0.291	0.61	0.59	0.260	0.56
Complete Regression w/o LI, high LD	33	2.5	0.002	2.2	3	<0.001	2.6
Complete Regression w/LI, low LD	10	2.5	0.019	1.6	3.1	0.005	1.9
Complete Regression w/LI, high LD	9	3.8	0.004	4.1	1.3	0.716	1.1

* Multivariate models included the indicators for lymphatic invasion, extent of lymphatic density, and their interaction as indicated in the description of the models above, as well as the 2010 American Joint Commission on Cancer Stage indicators for Stages IA and IB with Stage II as the reference group.