

An Immunohistochemical Comparison Study of SOX10, Pan Melanoma Cocktail and S100 in Malignant Melanoma

David Tacha, PhD and Charlie Yu, MD; Biocare Medical, Concord CA

Introduction

Traditionally, melanoma markers such as S100, HMB45, and MART-1 have been used as a panel of antibodies to identify melanoma.¹⁻⁵ The S100 antibody has been primarily used as a first-line screener for melanoma for the last three decades. However, S100 has been shown to lack specificity as it stains interdigitating reticulum cells in the paracortex of lymph nodes, a common site of metastatic melanoma and its mimics.¹⁻⁶ A cocktail of MART-1 and Tyrosinase (known as Pan Melanoma) has been shown to be a very sensitive and specific marker in metastatic melanomas and studies have shown its sensitivity was comparable to that of S100.⁷ However, MART-1 and Tyrosinase have demonstrated a lack of sensitivity in desmoplastic melanoma (DM) and spindle cell melanoma (SCM).^{1, 5} SOX10 is a nuclear transcription factor that plays an important role in melanocytic cell differentiation. It has been shown to be a sensitive marker for melanoma including spindle and desmoplastic subtypes.⁸⁻¹⁰ A mouse monoclonal SOX10 antibody has recently been developed that was shown to stain the majority of melanomas and most importantly, stained 98% (47/48) of DM and SCM.¹⁰

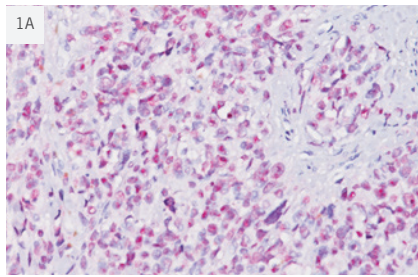
Design

Formalin-fixed, paraffin-embedded tissue microarrays (TMAs) consisting of malignant and metastatic melanoma, including SCM and DM (n = 72), were stained by immunohistochemistry and compared in a direct study using a mouse monoclonal SOX10 [BC34] (nuclear), a Pan Melanoma antibody cocktail (cytoplasmic) (Biocare Medical) and a polyclonal S100 antibody (cytoplasmic/nuclear) (Dako). TMA tissue sections and normal lymph node tissues were deparaffinized and hydrated down to water. Slides were placed in a modified citrate buffer antigen retrieval solution in a pressure cooker and heated to 110°C for 15 minutes. SOX10, Pan Melanoma Cocktail and S100 were optimized for immunohistochemistry and incubated for 30 minutes, followed by an alkaline phosphatase (AP) polymer detection system and visualized with Fast Red chromogen. A melanoma triple cocktail of SOX10, MART-1 and Tyrosinase was applied simultaneously to tissues sections, detected with an AP polymer detection system and visualized with Fast Red. A sequential two-color double stain for a single section was accomplished by incubating SOX10 for 30 minutes, followed by a horseradish peroxidase polymer detection system and visualization with DAB; the Pan Melanoma Cocktail was incubated for 30 minutes followed by an AP polymer detection system and visualization with Fast Red.

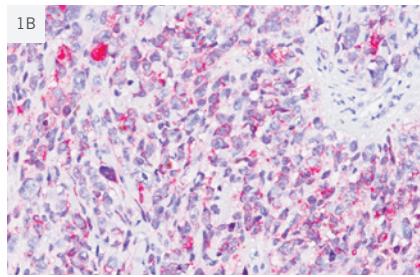
Results

In malignant melanoma cases, SOX10, Pan Melanoma and S100 stained 88%, 90% and 88%, respectively (Figure 1, Table 1). In metastatic melanoma, SOX10, Pan Melanoma and S100 stained 81%, 89% and 81%, respectively (Figures 2A-C, Table 1). The combination of SOX10 and Pan Melanoma stained 92% (24/26) of metastatic melanomas (Figure 2D, data not shown). In addition, SOX10 and S100 both stained 100% of DM and SCM, whereas Pan Melanoma stained 50% (Figure 3, Table 1). Normal lymph node was negative for SOX10 and Pan Melanoma but was strongly positive for S100 (Figure 4). The melanoma triple cocktail (Fast Red only) and the sequential double stain of SOX10 (DAB) and Pan Melanoma (Fast Red) were successfully achieved and could be visualized in a single section (Figure 5).

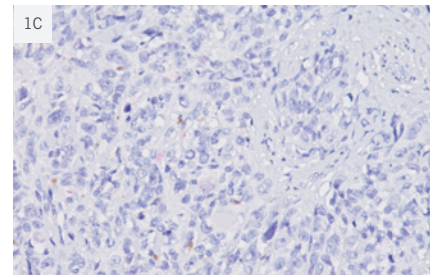
Figure 1: Melanoma



Melanoma stained with SOX10

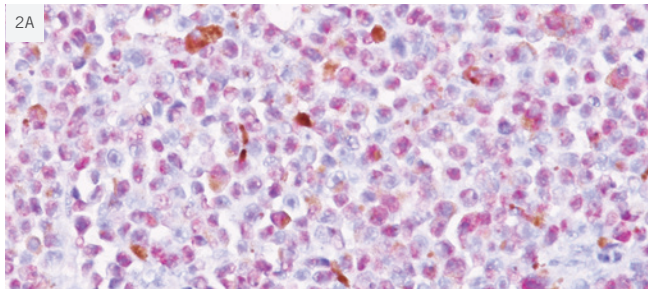


Melanoma stained with Pan Melanoma Cocktail

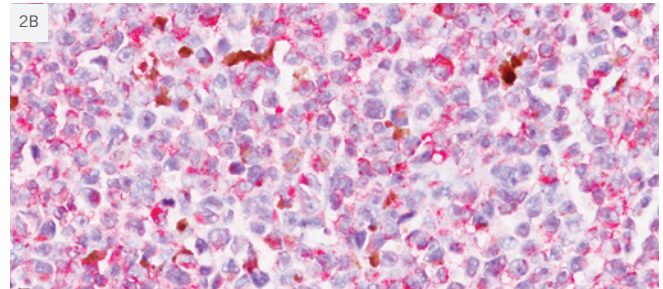


Melanoma stained with S100

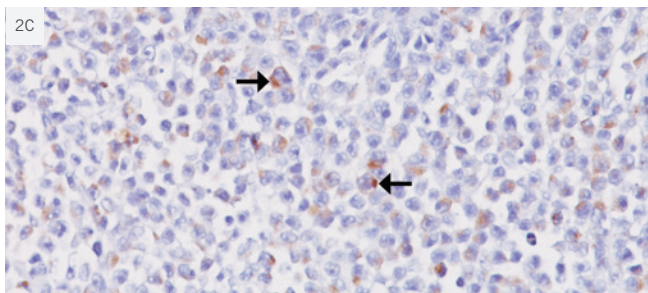
Figure 2: Metastatic Melanoma



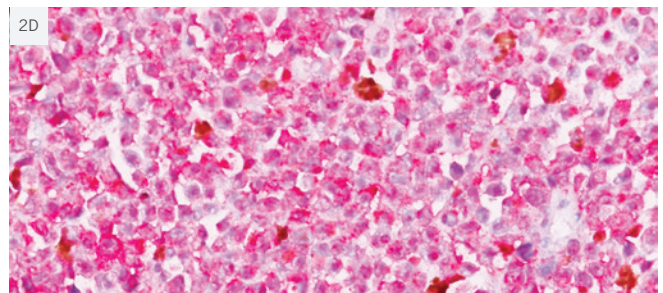
Metastatic melanoma stained with SOX10



Metastatic melanoma stained with Pan Melanoma Cocktail

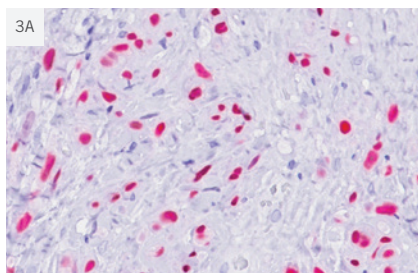


Metastatic melanoma stained with S100 (Arrows: melanin pigment)

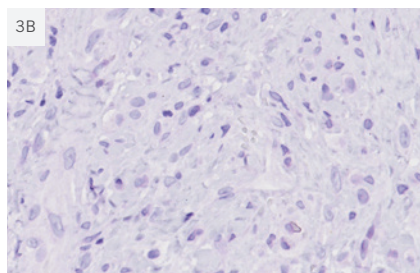


Metastatic melanoma stained with SOX10 and Pan Melanoma Cocktail

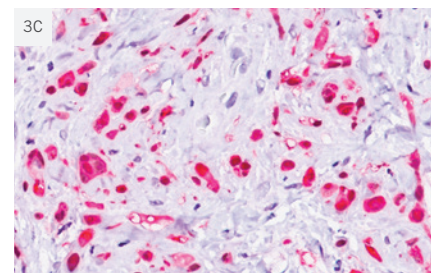
Figure 3: Desmoplastic Melanoma



Desmoplastic melanoma stained with SOX10

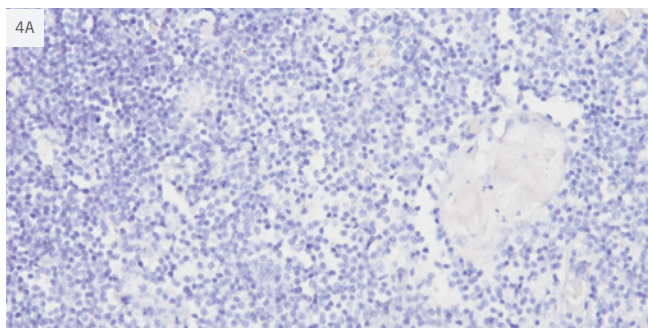


Desmoplastic melanoma stained with Pan Melanoma Cocktail

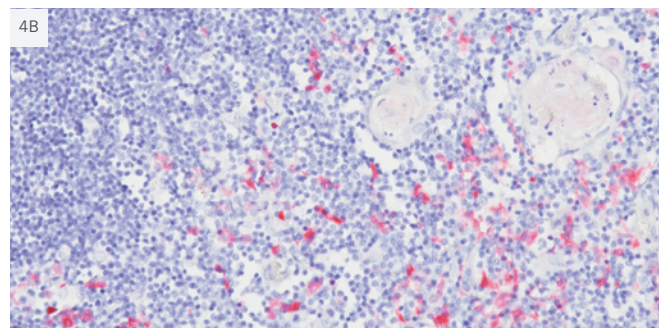


Desmoplastic melanoma stained with S100

Figure 4: Normal Lymph Node

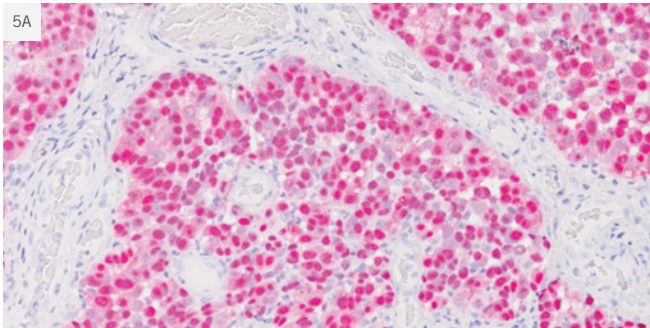


Normal lymph node stained with SOX10 and Pan Melanoma Cocktail (Arrow)

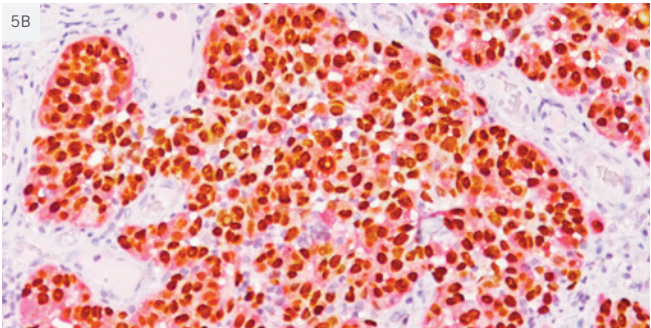


Normal lymph node stained with S100 (Arrow)

Figure 5: Melanoma



Melanoma stained with SOX10, MART-1 and Tyrosinase (FR)



Melanoma stained with a SOX10 (DAB) and Pan Melanoma Cocktail (FR) sequential double stain

Table 1: Comparison of SOX10, Pan Melanoma and S100 Expression in Melanoma

| Melanoma Type | SOX10 | Pan Melanoma | S100 |
|------------------------------------|-------------|--------------|-------------|
| Malignant Melanoma | 35/40 (88%) | 36/40 (90%) | 35/40 (88%) |
| Metastatic Melanoma | 21/26 (81%) | 23/26 (89%) | 21/26 (81%) |
| Desmoplastic/Spindle Cell Melanoma | 6/6 (100%) | 3/6 (50%) | 6/6 (100%) |

Discussion

S100 has been shown to be one of the most sensitive markers for melanocytic differentiation, being diffusely positive in all benign, primary and secondary malignant lesions, as well as in most desmoplastic and spindle cell melanomas.^{5, 6} However, S100 often reacts with a host of non-melanocytic tumors that may share cytomorphological or histomorphological features with malignant melanomas and may lead to misdiagnosis in certain cases.¹¹ Conversely, S100 has been shown to lack sensitivity in epithelioid melanomas when compared to MART-1 and HMB45.¹² Fernandes *et al* showed MART-1 and Tyrosinase to be positive in 91.7% (33/36) and 97.2% (35/36) of uveal melanomas, respectively.¹³ Moreover, MART-1 and Tyrosinase when used together as Pan Melanoma Cocktail stained 100% of uveal melanomas.¹³ Uveal melanomas differ from cutaneous melanomas in the respect that the expression of these immunotherapy antigens was much more heterogeneous.¹⁴ In another study, 100% (50/50), 98% (49/50) and 92% (46/50) of primary cutaneous malignant melanomas were shown to be positive with S100, Pan Melanoma Cocktail and HMB45 respectively, thus demonstrating the high sensitivity of Pan Melanoma when compared to S100.⁷ Nonaka *et al* showed SOX10 nuclear expression in 97% (76/78) of melanomas, whereas S100 was expressed in 91% (71/78) of melanomas.⁸

Our study demonstrates a strong correlation to other comparable studies.⁷⁻¹⁰ The Pan Melanoma antibody cocktail was slightly more sensitive than SOX10 and S100 in malignant and metastatic melanomas. However, it is well known that MART-1 and Tyrosinase stain a much lower percentage of DM and SCM when compared to SOX10 or S100.^{5, 6, 10} Our study reflected these findings as SOX10 and S100 stained 100% of DM and SCM compared to Pan Melanoma, which stained 50% (Figure 3).

Overall, S100 stained 88% of malignant melanomas (Table 1). When examining the literature, some publications have shown higher staining percentages in melanomas. Our results could be due to TMA tissue sampling. However, there were clear cases of melanoma that were strongly positive for SOX10 and Pan Melanoma and were completely negative for S100 (Figures 1C and 2C). The combination of both SOX10 and Pan Melanoma stained 93% (67/72) of all melanomas vs. S100 which stained 88% (63/72). We only observed one case of melanoma that was negative for both SOX10 and Pan Melanoma and positive for S100.

Conclusion

This is the first study comparing SOX10 [BC34], Pan Melanoma Cocktail and S100 in malignant melanoma. The combination of SOX10 and Pan Melanoma Cocktail demonstrated equal or superior sensitivity in melanomas when compared with S100 alone, and may prove to be a better choice for screening of metastatic and malignant melanoma due to the specificity and sensitivity demonstrated in our study. A potential double stain that may include SOX10, MART-1 and Tyrosinase may be suitable for tumors of unknown origin or in differential diagnosis of melanoma and its mimics.

References

1. Blessing K, Sanders DS, Grant JJ. Comparison of immunohistochemical staining of the novel antibody melan-A with S100 protein and HMB-45 in malignant melanoma and melanoma variants. *Histopathology*. 1998 Feb; 32(2):139-46.
2. Heegaard S, Jensen OA, Prause JU. Immunohistochemical diagnosis of malignant melanoma of the conjunctiva and uvea: comparison of the novel antibody against melan-A with S100 protein and HMB-45. *Melanoma Res*. 2000 Aug; 10(4):350-4.
3. de Vries TJ, *et al*. Expression of gp100, MART-1, tyrosinase, and S100 in paraffin-embedded primary melanomas and locoregional, lymph node, and visceral metastases: implications for diagnosis and immunotherapy. A study conducted by the EORTC Melanoma Cooperative Group. *J Pathol*. 2001 Jan; 193(1):13-20.
4. Zubovits J, *et al*. HMB-45, S-100, NK1/C3, and MART-1 in metastatic melanoma. *Hum Pathol*. 2004 Feb; 35(2):217-23.
5. Clarkson KS, Sturdess IC, Molyneux AJ. The usefulness of tyrosinase in the immunohistochemical assessment of melanocytic lesions: a comparison of the novel T311 antibody (anti-tyrosinase) with S-100, HMB45, and A103 (anti-melan-A). *J Clin Pathol*. 2001 Mar; 54(3):196-200.
6. Nakajima T, *et al*. Immunohistochemical demonstration of S100 protein in malignant melanoma and pigmented nevus, and its diagnostic application. *Cancer*. 1982 Sep 1; 50(5):912-8.
7. Orchard G. Evaluation of melanocytic neoplasms: application of a pan-melanoma antibody cocktail. *Br J Biomed Sci*. 2002; 59(4):196-202.
8. Nonaka D, Chiriboga L, Rubin BP. Sox10: a pan-schwannian and melanocytic marker. *Am J Surg Pathol*. 2008 Sep; 32(9):1291-8.
9. Mohamed A, *et al*. SOX10 expression in malignant melanoma, carcinoma, and normal tissues. *Appl Immunohistochem Mol Morphol*. 2013 Dec; 21(6):506-10.
10. Tacha D, *et al*. A Newly Developed Mouse Monoclonal SOX10 Antibody is a Highly Sensitive and Specific Marker for Malignant Melanoma, Including Spindle Cell and Desmoplastic Melanomas. Accepted for publication in *Archives of Pathology*; 2014 0077-OA.
11. Sheffield, *et al*. Comparison of five antibodies as markers in the diagnosis of melanoma in cytologic preparations. *Am J Clin Pathol*. 2002 Dec; 118(6):930-6.
12. Busam KJ, *et al*. Immunohistochemical distinction of epithelioid histiocytic proliferations from epithelioid melanocytic nevi. *Am J Dermatopathol*. 2000 Jun; 22(3):237-41.
13. Fernandes BF, *et al*. Immunohistochemical expression of melan-A and tyrosinase in uveal melanoma. *J Carcinog*. 2007 Apr 20; 6:6.
14. de Vries TJ, *et al*. High expression of immunotherapy candidate proteins gp100, MART-1, tyrosinase and TRP-1 in uveal melanoma. *Br J Cancer*. 1998 Nov; 78(9): 1156-61.