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SHEDDING OF DISTINCT CRYPTIC COLLAGEN EPITOPE (HU177) IN SERA OF MELANOMA PATIENTS

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Abstract

Purpose—Extracellular matrix remodelling during tumor growth plays an important role in angiogenesis. Our preclinical data suggest that a newly identified cryptic epitope (HU177) within collagen type-IV regulates endothelial and melanoma cell adhesion *in vitro* and angiogenesis *in vivo*. In this study, we investigated the clinical relevance of HUI77 shedding in melanoma patient sera.

Experimental Design—Serum samples from 291 melanoma patients prospectively enrolled at the New York University Medical Center and 106 control subjects were analyzed for HU177 epitope concentration by a newly-developed sandwich ELISA assay. HU177 serum levels were then correlated with clinical and pathologic parameters.

Results—Mean HU177 epitope concentration was 5.8ng/ml (range=0–139.8 ng/ml). A significant correlation was observed between HU177 concentration and nodular melanoma histological subtype (nodular, 10.3±1.6ng/ml (mean ±SEM); superficial spreading melanoma, 4.5±1.1 ng/ml; all others, 6.1±2.1ng/ml; P=0.01 by ANOVA test). Increased HU177 shedding also

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correlated with tumor thickness (1.00mm, 3.8 ± 1.1 ng/ml; 1.01–3.99mm, 8.7 ± 1.3 ng/ml; 4.00mm, 10.3 ± 2.4 ng/ml; $P=0.003$ by ANOVA). After multivariate analysis controlling for thickness, the correlation between higher HU177 concentration and nodular subtype remained significant ($P=0.03$). The mean HU177 epitope concentration in control subjects was 2.4ng/ml.

Conclusions—We report that primary melanoma can induce detectable changes in systemic levels of cryptic epitope shedding. Our data also support that nodular melanoma might be biologically distinct compared to superficial spreading type melanoma. As targeted interventions against cryptic collagen epitopes are currently undergoing phase I clinical trial testing, these findings indicate that patients with nodular melanoma may be more susceptible to such targeted therapies.

Keywords

cryptic epitope; melanoma; type IV collagen

INTRODUCTION

The increasing incidence of melanoma remains a public health concern, with an estimated 60,000 new cases diagnosed and approximately 8,000 deaths from this disease in the United States in 2007. [1] Metastatic melanoma has long been refractory to existing therapies; even adjuvant treatment of high-risk melanoma patients with interferon- α (IFN- α) has been shown to have only a modest impact on overall survival. [2] The identification of melanoma patient populations with distinct molecular alterations will be a more rational approach in the design of novel treatment modalities and strategies.

The pharmacologic inhibition of extracellular matrix (ECM) protein fragments is a legitimate target for cancer therapy. The proteolytic remodeling of ECM components has been shown to be integral to tumor invasion and angiogenesis. [3–5] In particular, cleavage of type IV collagen, a major component of the vascular basement membrane (BM), generates protein fragments that have been shown to function as endogenous inhibitors of angiogenesis. [6,7] Several of these proteolytic fragments have been identified and evaluated as novel therapeutic targets, and while preliminary investigations with these molecules were promising, phase I and II clinical trials concluded with disappointing results. [8–12]

Distinct cryptic collagen peptides are among the protein fragments exposed by collagen type IV remodeling, and recent data indicate that these cryptic epitopes may facilitate tumor migration and angiogenesis. [3,5,13] Cryptic epitope, HUIV26, within denatured type IV collagen, has been identified to exhibit angiogenic properties in both murine and chick embryo animal models.⁴ Recently, a second novel cryptic epitope, HU177, has been described and also appears to play a functional role in tumor angiogenesis. [14] HU177 was selectively exposed in the interstitial matrix of tumors as well as the extracellular matrix of angiogenic blood vessels. Furthermore, a monoclonal antibody (mAb) directed to the HU177 cryptic site inhibited endothelial cord formation *in vitro* and tumor angiogenesis *in vivo*. [14] These data suggest that the selective targeting of cryptic collagen epitopes may represent an effective anti-angiogenic treatment strategy.

In this study, we tested the hypothesis that melanoma can induce detectable changes in systemic levels of cryptic epitope shedding, specifically the HU177 epitope. We also correlated the levels of HU177 shedding with clinical and pathologic parameters. Our data identified nodular melanoma patients as a subset of patients who may be biologically distinct and may be more responsive to treatment with anti-ECM agents. Our study supports further investigation of HU177 epitope as a candidate for targeted intervention for treatment of nodular melanoma.

PATIENTS AND METHODS

Study Population

The study cohort consisted of 291 melanoma patients prospectively enrolled in the Interdisciplinary Melanoma Cooperative Group (IMCG) at the New York University (NYU) School of Medicine between August 2002 and November 2006 (119 females, 172 males, mean age 59, range 20 to 96 yrs). Clinicopathologic and demographic data were recorded prospectively for all patients. The NYU Institutional Review Board approved this study and informed consent was obtained from all patients at the time of enrollment.

Sera samples from 106 control subjects, including patients with non-melanoma cancer (bladder cancer (n=56), renal cancer (n=10), and testicular cancer (n=10), and normal volunteers (n=30)) were included. The mean age was 64 (range 23 to 91 yrs). All control subjects signed informed consent prior to inclusion in this study.

Serum Preparation and Determination of HU177 Concentration by ELISA

Blood was collected from primary melanoma patients after primary melanoma excisional surgery (n=174). In 32 patients, the blood was collected prior to surgery, and in 4 patients, time of collection was not recorded. Patients presenting to NYU for treatment of metastatic melanoma (n=81) had blood drawn either during their first consult at NYU. All serum samples were collected in 10 ml BD serum tubes, stored immediately at 4°C, and then centrifuged at 10°C for 10 minutes at 1,500× g. The supernatant serum was then aliquoted into 1.5 ml cryovials and stored at –80°C until further use. All samples examined in the ELISA assay detailed below were subjected to only one freeze-thaw cycle.

HU177 cryptic epitope concentration (ng/ml) was quantified by a newly developed “capture” ELISA assay. A 96-well, flat bottom microtiter plate was coated with monoclonal antibody HU177 (50 ug/ml) and incubated overnight at 4°C. After several phosphate-buffered saline washes, plates were blocked with 2.5% bovine serum albumin at 37°C for 2 hours. Standards were created using native collagen type IV that was denatured by boiling and then diluted in PBS. Patient samples or standards were added to each well (100 ul) in triplicate. After 2-hour incubation, biotinylated anti-collagen IV antibody was added (1:20,000, Southern Biotech). Following 1.5-hour incubation, anti-biotin mAb conjugated to horseradish peroxidase was added (1:20,000, Sigma Aldrich). The plate was then incubated with a substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) for 5–10 minutes at room temperature. Substrate absorbance was determined at 400 nm (Bio-Rad microplate reader). Known concentrations, in duplicate, of denatured collagen were utilized to establish a

standard curve ranging from 5–100 ng/ml, from which the concentration of cryptic epitope within patient samples was extrapolated (Figure 1).

Statistical Analysis

Descriptive statistics were calculated for baseline demographic and clinicopathologic characteristics. Associations between epitope HU177 shedding and age, gender, tumor thickness, ulceration, histological subtype, recurrence, metastatic tumor type, and time of blood draw were evaluated by the t-test (or Wilcoxon rank-sum test), the analysis of variance (ANOVA) test (or Kruskal-Wallis test), and the Spearman-rank correlation coefficient, as appropriate. Multivariate ANOVA was performed to assess the independent effect of histological subtype on epitope HU177 shedding after adjustment for tumor thickness. For univariate and multivariate analysis, tumor thickness was evaluated both as a continuous variable as well as a categorical variable (≤ 1 mm, 1.01 – 3.99 mm, or ≥ 4 mm in Breslow thickness). This classification was selected as it effectively stratifies patients into prognostic groups. [15] For histological subtype analyses, patients were grouped into three categories: those who were diagnosed with nodular melanoma, superficial spreading melanoma, or “other” subtypes. Likewise, age was analyzed both as a continuous variable and as a categorical variable (≥ 60 vs. <60 years). Mean epitope concentrations of patients categorized by each stage of melanoma were also calculated. All p-values are two-sided with statistical significance evaluated at the 0.05 alpha level. All analyses were performed in SAS Version 9.1 (SAS Institute Inc., Cary, North Carolina) and SPSS Version 15.0 (SPSS Inc., Chicago, Illinois).

RESULTS

Table 1 illustrates the clinical and pathologic characteristics of the study population as well as the association with HU177 serum concentration. Serum HU177 epitope concentration was determined for 210 primary melanoma patients (140 Stage I, 41 Stage II, 29 Initial Stage III) and 81 patients with recurrent or metastatic disease (32 Recurrent Stage III, 49 Stage IV). The mean HU177 epitope concentration of the study cohort was 5.8 ng/ml (range, 0 to 139.8 ng/ml) compared to 2.4 ng/ml in control subjects (range, 0 to 6.09 ng/ml, $n=106$). Mean HU177 epitope concentration for primary patients was 6.2 ng/ml (range, 0 to 139.8 ng/ml) compared to 4.9 ng/ml (range, 0 to 39.8 ng/ml) in metastatic patients ($P=0.09$, by Wilcoxon rank-sum test).

HU177 epitope shedding correlates with nodular melanoma, primary tumor thickness, and time of blood draw

Figure 2a displays the mean HU177 epitope concentration for each melanoma histological subtype. Patients who presented with a primary nodular melanoma ($n=51$) demonstrated a higher mean HU177 serum concentration compared to those with superficial spreading melanomas ($n=118$) and “other” subtypes ($n=30$) (Table 1, 10.3 ± 1.69 ng/ml (mean $\pm$ SEM) vs. 4.5 ± 1.1 ng/ml vs. 6.1 ± 2.1 ng/ml, respectively, $P=0.01$, by ANOVA test).

Figure 2b demonstrates the mean HU177 epitope concentration for each of the following 3 subgroups of tumor thickness: ≤ 1.00 mm ($n=113$), 1.01–3.99mm ($n=73$), and ≥ 4.00 mm

(n=22). The mean primary tumor thickness was 2.1 mm (range 0.1 to 30 mm). Our results indicate that increasing tumor thickness correlates with higher levels of HU177 epitope shedding (Table 1, $P=0.003$ by ANOVA test). Evaluation of HU177 levels while holding tumor thickness as a continuous variable further supported the association between HU177 epitope shedding and increasing tumor thickness ($P=0.005$ by Spearman-rank correlation coefficient). Multivariate analysis confirmed the independent correlation between nodular subtype and HU177 epitope concentration after controlling for tumor thickness ($P=0.03$).

We also considered the possibility that the relationship between HU177 epitope shedding and nodular histological subtype may be confounded by an association between the time of blood draw in primary patients and HU177 epitope shedding. However, even after controlling for time of blood draw and tumor thickness, multivariate analysis continued to validate the independent correlation between nodular histological subtype with HU177 epitope concentration ($P=0.04$ by multivariate ANOVA). Multivariate analysis also confirmed the independent correlation between tumor thickness and HU177 epitope concentration after controlling for time of blood draw ($P=0.007$ by multivariate ANOVA).

HU177 epitope shedding in patients with ulcerated melanomas and/or recurrences was elevated, but not statistically significant

Primary melanoma patients with ulcerated tumors showed higher mean HU177 epitope shedding than those whose tumors were not ulcerated (Table 1, 8.06 ng/ml vs. 5.74 ng/ml, $P=0.12$ by Wilcoxon rank-sum test).

The correlation between HU177 epitope shedding and recurrent/metastatic disease was also evaluated. Twenty-three of 210 primary melanoma patients (11%) developed recurrences or metastases during a median follow-up time of 31.5 months (range, 10.3 – 45.2 months). These included 7 cases of lymph node metastases, 8 with skin or subcutaneous metastases, and 8 with visceral metastases. There was a trend towards increased HU177 epitope shedding in patients who developed recurrences when the median HU177 concentration was compared to patients without recurrences ($P=0.07$, by Wilcoxon rank-sum test). On other hand, mean HU177 epitope concentration did not correlate with age, gender, or stage.

DISCUSSION

Our study reveals several important observations. First, it demonstrates for the first time the feasibility of detecting and quantifying circulating levels of cryptic collagen-IV epitope (HU177) in the sera of melanoma patients using a newly developed assay. We show that even single primary tumors can induce detectable changes in systemic levels of degraded collagen. Second, our data support a hypothesis that primary nodular melanoma has distinct genetic and/or phenotypic characteristics that can be exploited for targeted therapy. Lastly, the increased HU177 epitope concentration observed in primary but not metastatic melanomas supports a restricted, tissue specific, shedding of cryptic collagen epitopes.

Nodular melanoma is characterized by rapid vertical growth and clinical features that are unaccounted for in the ABCD acronym. [16] As such, these patients pose a challenge, often presenting with high risk, thick lesions at the time of diagnosis. [17] In fact, reviews of four

separate melanoma databases found that approximately two-thirds of all thick melanomas (3mm) were of nodular histological subtype. [18–20] Furthermore, the median thickness of nodular melanomas has not changed over the last decade. [16] In contrast, superficial spreading melanomas, the most common histological subtype according to SEER data, are typically diagnosed as thin lesions (median 0.54mm). [16] The relative higher frequency of nodular melanoma amongst thick tumors is particularly alarming considering its poor prognosis for all stages of disease when compared to superficial spreading melanoma. [21] The worse prognoses of nodular melanoma has also been demonstrated in cutaneous head and neck melanomas, portending a significantly increased risk of death when compared to all other histological subtypes. [22]

Our data support that nodular melanoma might represent a distinct biologic entity. Additional support is found in a recent microarray study of primary and metastatic melanoma specimens. [23] These authors identified a characteristic gene expression profile unique to nodular melanomas. Specifically, nodular melanomas displayed up-regulation of genes implicated in tumor invasion and cell adhesion, namely matrix metalloproteinase 16 (MMP16), BCL2-related protein A (BCL2A1), intercellular adhesion molecule 1 (ICAM1), and carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1). [23] The molecular signature of superficial spreading melanomas demonstrated a differential profile, with upregulation of genes involved in alternative signaling pathways, specifically cell cycle regulation and cell-cell communication. [23] Studies examining alterations in DNA copy number or somatic mutations also support distinct genetic differences between melanoma subtypes. In one study, nodular melanomas were more likely to have loss of p16 expression compared to superficial spreading melanomas. [24] There are also differences in BRAF mutation frequency among melanoma subtypes, with the largest studies having reported BRAF mutation frequencies in the range of 27–63% for nodular melanomas. [25] This knowledge of the genetic alterations underlying distinct melanoma subtypes may provide opportunities to tailor treatment regimens based on genetic and/or phenotypic profiles. In a limited study subset, we examined serum metalloproteinase activity from patients with high and low HU177 serum collagen levels but did not find a correlation.

Our findings suggest that the HU177 epitope could serve as a novel target for drug development. Previously, studies have shown that treatment with a monoclonal antibody directed against HU177 leads to the significant inhibition of tumor growth and angiogenesis *in vivo*. [14] Consequently, a phase I clinical trial was approved to evaluate the safety, tolerability, pharmacokinetics, and anti-tumor activity of D93 (TRC093), a humanized monoclonal antibody directed to the HU177 site. This study is currently being conducted at several medical centers in patients with advanced cancers (Tracoon Pharma). Given the increased shedding of HU177 epitope in nodular melanoma patients and its potential role in angiogenesis, a rational therapeutic approach could be to specifically examine the response of nodular melanoma patients to inhibitors of angiogenesis, allowing for the selection of patients who are more likely to benefit from this particular modality of cancer therapy. In fact, both vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 2 α (HIF2 α) have been shown to be independent negative prognostic variables in nodular

melanoma, further supporting the potential efficacy of antiangiogenic strategies on melanomas of this histological subtype. [26]

Given the dismal prognosis of advanced stage nodular melanoma and the lack of available adjuvant treatment modalities, the testing of angiogenesis inhibitors on an adjuvant basis in this population is worth further examination. An increasing number of these agents are currently under clinical investigation, with anti-VEGF strategies as the most actively pursued. In particular, bevacizumab, a humanized anti-VEGF monoclonal antibody approved for colorectal cancer in 2005, is now being tested in phase II trials of melanoma. [27,28] The clinical evaluation of anti-angiogenic therapies in combination with standard treatments is also ongoing. [29]

In this study, we found that primary melanomas, as a whole, had higher levels of HU177 shedding compared to metastatic cases, although this observation did not reach statistical significance. It is possible that exposure of the HU177 epitope is specific to the extracellular matrix of the skin, with minimal expression in extracutaneous sites. We evaluated the shedding of HU177 in patients with other tumor types, including renal, bladder, and testicular cancers. In general, we found that the serum levels of HU177 in these patients were lower than that found in either primary or metastatic melanoma patients.

The prognostic relevance of HU177 epitope expression in melanoma requires further investigation. Despite the relatively short median follow-up time of patients enrolled in the NYU-Interdisciplinary Melanoma Cooperative Group (31.5 months), we observed a trend towards increased HU177 epitope shedding in patients who developed recurrences of their disease. Evidence supporting the predictive potential of HU177 epitope shedding has been demonstrated in a phase II trial of sorafenib (BAY 43-9006) in malignant melanoma, where serial serum HU177 levels were shown to correlate with tumor response as monitored by PET/CT SUV changes. [30] These findings are promising and support the further exploration of HU177 as a prognostic marker in melanoma.

In summary, we demonstrated that circulating levels of cryptic collagen-IV epitope HU177 can be detected in the sera of melanoma patients; primary nodular melanoma may be a candidate for targeted therapy design as it displays distinct genetic and/or phenotypic characteristics; and that shedding of cryptic collagen epitopes appears to be restricted and tissue specific. In the context of prior basic and clinical work, this study supports further investigation of HU177 epitope as a candidate for targeted intervention for treatment of nodular melanoma.

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References

1. American Cancer Society. Cancer Facts and Figures 2007. Atlanta, GA: 2007.

2. Kirkwood JM, Manola J, Ibrahim J, et al. A pooled analysis of eastern cooperative oncology group and intergroup trials of adjuvant high-dose interferon for melanoma. *Clin Cancer Res.* 2004; 10:1670–1677. [PubMed: 15014018]
3. Roth JM, Caunt M, Cretu A, et al. Inhibition of experimental metastasis by targeting the HUIV26 cryptic epitope in collagen. *Am J Pathol.* 2006; 168:1576–1586. [PubMed: 16651624]
4. Stamenkovic I. Extracellular matrix remodelling: the role of matrix metalloproteinases. *J Pathol.* 2003; 200:448–464. [PubMed: 12845612]
5. Xu J, Rodriguez D, Petitclerc E, et al. Proteolytic exposure of a cryptic site within collagen type IV is required for angiogenesis and tumor growth in vivo. *J Cell Biol.* 2001; 154:1069–1079. [PubMed: 11535623]
6. Clamp AR, Jayson GC. The clinical potential of antiangiogenic fragments of extracellular matrix proteins. *Br J Cancer.* 2005; 93:967–972. [PubMed: 16234821]
7. Nyberg P, Xie L, Kalluri R. Endogenous inhibitors of angiogenesis. *Cancer Res.* 2005; 65:3967–3979. [PubMed: 15899784]
8. Eder JP Jr, Supko JG, Clark JW, et al. Phase I clinical trial of recombinant human endostatin administered as a short intravenous infusion repeated daily. *J Clin Oncol.* 2002; 20:3772–3784. [PubMed: 12228197]
9. Hansma AH, Broxterman HJ, van der Horst I, et al. Recombinant human endostatin administered as a 28-day continuous intravenous infusion, followed by daily subcutaneous injections: a phase I and pharmacokinetic study in patients with advanced cancer. *Ann Oncol.* 2005; 16:1695–1701. [PubMed: 16012180]
10. Herbst RS, Hess KR, Tran HT, et al. Phase I study of recombinant human endostatin in patients with advanced solid tumors. *J Clin Oncol.* 2002; 20:3792–3803. [PubMed: 12228199]
11. Kulke MH, Bergsland EK, Ryan DP, et al. Phase II study of recombinant human endostatin in patients with advanced neuroendocrine tumors. *J Clin Oncol.* 2006; 24:3555–3561. [PubMed: 16877721]
12. Thomas JP, Arzooarian RZ, Alberti D, et al. Phase I pharmacokinetic and pharmacodynamic study of recombinant human endostatin in patients with advanced solid tumors. *J Clin Oncol.* 2003; 21:223–231. [PubMed: 12525513]
13. Gagne PJ, Tihonov N, Li X, et al. Temporal exposure of cryptic collagen epitopes within ischemic muscle during hindlimb reperfusion. *Am J Pathol.* 2005; 167:1349–1359. [PubMed: 16251419]
14. Cretu A, Roth JM, Caunt M, et al. Disruption of endothelial cell interactions with the novel HU177 cryptic collagen epitope inhibits angiogenesis. *Clin Cancer Res.* 2007; 13:3068–3078. [PubMed: 17505010]
15. Balch CM, Buzaid AC, Soong SJ, et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. *J Clin Oncol.* 2001; 19:3635–3648. [PubMed: 11504745]
16. Demierre MF, Chung C, Miller DR, et al. Early detection of thick melanomas in the United States: beware of the nodular subtype. *Arch Dermatol.* 2005; 141:745–750. [PubMed: 15967921]
17. Chamberlain AJ, Fritschi L, Kelly JW. Nodular melanoma: patients' perceptions of presenting features and implications for earlier detection. *J Am Acad Dermatol.* 2003; 48:694–701. [PubMed: 12734497]
18. Chamberlain AJ, Fritschi L, Giles GG, et al. Nodular type and older age as the most significant associations of thick melanoma in Victoria, Australia. *Arch Dermatol.* 2002; 138:609–614. [PubMed: 12020221]
19. Hanrahan PF, Hersey P, D'Este CA. Factors involved in presentation of older people with thick melanoma. *Med J Aust.* 1998; 169:410–414. [PubMed: 9830387]
20. Kopf AW, Welkovich B, Frankel RE, et al. Thickness of malignant melanoma: global analysis of related factors. *J Dermatol Surg Oncol.* 1987; 13:345–390. 401–420. [PubMed: 3558930]
21. Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and noncutaneous melanoma: a summary of 84,836 cases from the past decade. The American College of Surgeons Commission on Cancer and the American Cancer Society. *Cancer.* 1998; 83:1664–1678. [PubMed: 9781962]

22. Golger A, Young DS, Ghazarian D, et al. Epidemiological features and prognostic factors of cutaneous head and neck melanoma: a population-based study. *Arch Otolaryngol Head Neck Surg.* 2007; 133:442–447. [PubMed: 17515502]
23. Jaeger J, Koczan D, Thiesen HJ, et al. Gene expression signatures for tumor progression, tumor subtype, and tumor thickness in laser-microdissected melanoma tissues. *Clin Cancer Res.* 2007; 13:806–815. [PubMed: 17289871]
24. Pavey SJ, Cummings MC, Whiteman DC, et al. Loss of p16 expression is associated with histological features of melanoma invasion. *Melanoma Res.* 2002; 12:539–547. [PubMed: 12459643]
25. Lang J, MacKie RM. Prevalence of exon 15 BRAF mutations in primary melanoma of the superficial spreading, nodular, acral, and lentigo maligna subtypes. *J Invest Dermatol.* 2005; 125:575–579. [PubMed: 16117801]
26. Giatromanolaki A, Sivridis E, Kouskoukis C, et al. Hypoxia-inducible factors 1alpha and 2alpha are related to vascular endothelial growth factor expression and a poorer prognosis in nodular malignant melanomas of the skin. *Melanoma Res.* 2003; 13:493–501. [PubMed: 14512791]
27. Becker JC, Kirkwood JM, Agarwala SS, et al. Molecularly targeted therapy for melanoma: current reality and future options. *Cancer.* 2006; 107:2317–2327. [PubMed: 17039502]
28. Scappaticci FA. Mechanisms and future directions for angiogenesis-based cancer therapies. *J Clin Oncol.* 2002; 20:3906–3927. [PubMed: 12228212]
29. Solti M, Berd D, Mastrangelo MJ, et al. A pilot study of low-dose thalidomide and interferon alpha-2b in patients with metastatic melanoma who failed prior treatment. *Melanoma Res.* 2007; 17:225–231. [PubMed: 17625452]
30. Pavlick A, Liebes L, Brooks P. BAY 43–9006 (sorafenib-BAY) alters proliferation pathways and mutant specific-PCR (MS-PCR) improves detection of BRAF mutations in metastatic melanoma (MM). *Journal of Clinical Oncology.* 2007; 25 (suppl; abstr 8534).

Figure 1.
Typical standard curve generated with denatured type IV collagen showing the linearity in the range of the sandwich ELISA assay over the range of 5–100 ng/ml.

Figure 2. HU177 Correlation with Histological Subtype and Primary Tumor Thickness: Scatter graphs of shed HU177 collagen epitope

A. HU177 and Histological subtype. Patients who presented with a primary nodular melanoma demonstrated a higher mean HU177 serum concentration compared to those with superficial spreading melanomas and “other” histological subtypes ($P=0.01$ by ANOVA). **B. HU177 and Primary tumor thickness.** The mean tumor thickness for primary patients was 2.1 mm (range 0.1 to 30 mm). Increasing tumor thickness correlated with higher levels of mean HU177 epitope shedding ($P=0.003$ by ANOVA test). Mean $\pm$ SEM are indicated for each subgroup.

Table 1

Clinicopathologic Characteristics of Study Population (n=291) and Association With HU177 Serum Concentration

	Patients	Mean HU177	Univariate Analysis
Variable	No. (%)	Conc. $\pm$ SE	P-value
(ng/mL)			
Sex			
Female	119 (41)	5.34 $\pm$ 0.65	0.47
Male	172 (59)	6.16 $\pm$ 0.93	
Age, years			
Mean $\pm$ SD	59 $\pm$ 16.3	r = -0.05	0.39
Primary Tumor Histological Type			
Superficial Spreading Melanoma	118 (59)	4.53 $\pm$ 0.45	0.01 *
Nodular Melanoma	51 (26)	10.27 $\pm$ 2.84	
Acral Lentiginous	6 (3)	2.03 $\pm$ 0.78	
Desmoplastic Melanoma	6 (3)	13.55 $\pm$ 7.07	
Lentigo Maligna Melanoma	6 (3)	2.41 $\pm$ 0.68	
Other	8 (4)	5.28 $\pm$ 2.48	
Unknown	4 (2)	8.52 $\pm$ 3.86	
Thickness			
1.00 mm	113 (54)	3.81 $\pm$ 0.37	0.003
1.01 – 3.99 mm	73 (35)	8.75 $\pm$ 2.04	
4.00 mm	22 (11)	10.28 $\pm$ 2.23	
Primary Tumor Ulceration			
Present	35 (18)	8.06 $\pm$ 1.73	0.28
Absent	163 (82)	5.74 $\pm$ 0.93	
CDUS Stage at Blood Draw			
Stage I	140 (48)	5.84 $\pm$ 1.06	0.76
Stage II	41 (14)	7.01 $\pm$ 1.47	
Stage III	61 (21)	6.01 $\pm$ 0.93	
Stage IV	49 (17)	4.65 $\pm$ 1.05	
Primary Tumor Location			
Axial	113 (55)	5.67 $\pm$ 0.64	0.44
Extremity	94 (45)	6.99 $\pm$ 1.59	
Metastatic Tumor Type			
Regional Lymph Node	20 (25)	6.08 $\pm$ 1.62	0.19
Regional Skin or Subcutaneous	18 (22)	3.33 $\pm$ 0.73	

Variable	<u>Patients</u>	<u>Mean</u> <u>HU177</u>	<u>Univariate</u> <u>Analysis</u>
	No. (%)	Conc. $\pm$ SE	P-value
		(ng/mL)	
Distant Lymph Node	3 (4)	4.33 $\pm$ 4.23	
Visceral Organ	19 (23)	7.60 $\pm$ 2.43	
Multiple Sites	21 (26)	2.91	

* P=0.01 for nodular vs. superficial spreading vs. all others