C-Reactive Protein As a Marker of Melanoma Progression

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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A B S T R A C T

Purpose

To investigate the association between blood levels of C-reactive protein (CRP) in patients with melanoma and overall survival (OS), melanoma-specific survival (MSS), and disease-free survival.

Patients and Methods

Two independent sets of plasma samples from a total of 1,144 patients with melanoma (587 initial and 557 confirmatory) were available for CRP determination. Kaplan-Meier method and Cox regression were used to evaluate the relationship between CRP and clinical outcome. Among 115 patients who underwent sequential blood draws, we evaluated the relationship between change in disease status and change in CRP using nonparametric tests.

Results

Elevated CRP level was associated with poorer OS and MSS in the initial, confirmatory, and combined data sets (combined data set: OS hazard ratio, 1.44 per unit increase of logarithmic CRP; 95% CI, 1.30 to 1.59; $P < .001$; MSS hazard ratio, 1.51 per unit increase of logarithmic CRP; 95% CI, 1.36 to 1.68; $P < .001$). These findings persisted after multivariable adjustment. As compared with CRP < 10 mg/L, CRP ≥ 10 mg/L conferred poorer OS in patients with any-stage, stage I/II, or stage III/IV disease and poorer disease-free survival in those with stage I/II disease. In patients who underwent sequential evaluation of CRP, an association was identified between an increase in CRP and melanoma disease progression.

Conclusion

CRP is an independent prognostic marker in patients with melanoma. CRP measurement should be considered for incorporation into prospective studies of outcome in patients with melanoma and clinical trials of systemic therapies for those with melanoma.

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INTRODUCTION

It remains important to investigate biomarkers of melanoma progression to assist with selection of high-risk patients for adjuvant therapy, identify those at low risk of recurrence for whom follow-up can be simplified, and suggest mechanisms of disease progression to identify novel treatment strategies.¹ Furthermore, although newer treatments including immune checkpoint blockade are encouraging,²⁻⁵ not all patients benefit. Therefore, it is particularly important to investigate the role of immune and inflammatory mechanisms in melanoma prognosis, in part through continued biomarker evaluation.

C-reactive protein (CRP) is a biomarker synthesized in hepatocytes in response to proinflammatory cytokines; the liver seems to be the major source of CRP in blood.⁶ An elevated level of CRP is a risk factor for cardiovascular disease and death resulting

from cardiovascular causes in both men and women.⁷⁻¹⁴ Prior investigations have demonstrated an association between CRP level and colorectal or lung cancer, but prospective studies have provided inconsistent evidence for an etiologic role of CRP in cancer.^{15,16} Elevated CRP has been correlated with poor prognosis in breast, lung, and other cancers.¹⁷⁻²¹ A small study suggested that CRP may represent a prognostic marker in patients with early-stage melanoma.²² An investigation of the combination of interferon alfa-2b and tremelimumab (anti-CTLA-4) in 37 patients identified a preliminary association between lower baseline CRP level and clinical benefit.²³ The relationship between changes in CRP and melanoma disease progression within the same patient has not been investigated. The primary aim of this study was to investigate whether increased levels of CRP in plasma are associated with disease stage, recurrence, or survival in patients with melanoma.

PATIENTS AND METHODS

Study Design

This study is part of an ongoing prospective investigation designed to identify blood-based, molecular, genetic, and environmental factors that influence melanoma risk and clinical outcome. The study population includes patients with all stages of invasive cutaneous melanoma. All individuals provided informed consent under an institutional review board–approved protocol. Peripheral blood was collected at study entry from 3,189 non-Hispanic white patients and controls recruited at the University of Texas MD Anderson Cancer Center between March 1998 and August 2009. Sequential blood draws were performed in 115 patients at follow-up.

Data were collected from patient records and maintained in the Melanoma Informatics, Tissue Resource, and Pathology Core. American Joint Committee on Cancer 2009 stage¹ was determined by direct physical examination and pathologic review, supplemented by laboratory and radiographic examinations. Length of follow-up and overall survival (OS), melanoma-specific survival (MSS), and disease-free survival (DFS) were measured from the date of blood draw to the date of last contact or death, death resulting from melanoma, or disease recurrence, respectively. Patients were defined as having recurrence if they had subsequent local, in-transit, regional nodal or distant metastasis during follow-up.

Experiments

Plasma was obtained from whole blood and stored at -80°C . A total of 1,144 patients with invasive melanoma had sufficient plasma stocks to be eligible for CRP testing by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN; catalog No. DCRP00). The minimum detection level was 0.010 ng/mL. Study-entry blood samples were drawn after primary surgery but before systemic therapy. The 587 samples in the initial data set represented patients with sufficient plasma stocks collected from August 2004 to December 2007 (storage times, 1.36 to 4.60 years). The 557 samples in the confirmatory data set were tested for CRP after analysis of the initial data set and represented all remaining patients with sufficient plasma stocks collected both before and after those in the initial cohort (August 1998 to July 2004 and December 2007 to August 2009; storage times, 0.69 to 12.47 years).

Genomic DNA was obtained from peripheral-blood samples from 3,156 participants in a genome-wide investigation of patients with melanoma and controls, representing a subset of the study population described previously.²⁴ An Omni1-Quad v1-0 B array (Illumina, San Diego, CA) was used to genotype 1,016,423 probes; after application of quality control, data from 818,237 single-nucleotide polymorphisms (SNPs) remained for 1,804 patients and 1,026 cancer-free controls.

Statistical Analysis

To compare the distribution of characteristics between the initial and confirmatory data sets, we performed χ^2 (categorical factors) and Wilcoxon tests (continuous variables). To evaluate whether clinical parameters affected CRP levels, we performed the Spearman rho test and analysis of variance for correlation between continuous variables and CRP/log-transformed CRP and the Wilcoxon test for association between dichotomous categorical variables and CRP. For trichotomous variables, we applied a Kruskal-Wallis test for nonparametric analyses. We included standard clinicopathologic predictors of outcome for melanoma patients. In addition, because of the often-prolonged sample storage times, we investigated the potential influence of storage time on CRP levels. The distribution of raw plasma CRP data in the initial and confirmatory data sets was skewed (data not shown), but it was symmetric after log transformation. Therefore, log-transformed CRP level was used to observe the relationship between continuous CRP level and OS, MSS, and DFS.¹⁶

To test for a dose effect of CRP on disease progression, we divided the patients into five quintiles with respect to CRP level and computed hazard ratios (HRs) of OS and MSS, with or without adjustment for age at blood draw, sex, stage at blood draw, blood storage time, and data set. We tested for linear trends using the median values of CRP in the quintile categories as a continuous variable.

To determine the best threshold of CRP level for predicting disease progression and determine whether the commonly used cutoff at 10 mg/L was applicable to our data, we performed recursive partitioning of CRP using the combined data set and recorded the split that led to the maximum improvement in the Gini index using the RPART function in R software (version 2.15.0; <http://www.r-project.org/>). The Gini index measures the purity of partitions; Gini index of 0 expresses perfect equality, where all values equally predict outcome between two subsets, whereas a Gini index of 1 expresses maximal inequality in prediction of outcomes between splits of the data.

To evaluate the potential association of CRP levels with disease response or progression in individual patients, we examined the relationship between changes in raw CRP and changes in disease status (using variety of measures of disease response or progression) between two sequential blood draws using nonparametric tests in 115 patients for whom sequential CRP levels were available.

Finally, because CRP levels in blood are under partial genetic control,²⁵ and because if both CRP-related genotypes and CRP levels were associated with melanoma progression it would suggest a direct causal association between CRP genes, CRP levels, and patient outcomes, we performed a genome-wide association study of log-transformed CRP among 1,144 patients with melanoma for whom both genotype and CRP data were available. We extracted all SNPs with *P* values for association with log CRP levels $< 5.0 \times 10^{-8}$. The two SNPs identified, as well as five additional candidate SNPs previously reported to regulate CRP levels (+2147A>G [rs1205], 3'-untranslated region; +942G>C [rs1800947], exon 2 coding region; -717A>G [rs2794521] and -757T>C [rs3093059], promoter region; and rs7553007, CRP locus),²⁶ were investigated for association with melanoma risk (among 1,804 patient cases and 1,026 controls) and for association with stage and clinical outcome (among 1,804 patient cases).

All analyses except recursive partitioning were performed using SAS Enterprise Guide 4.3 (SAS Institute, Cary, NC). All *P* values were two sided. *P* values $< .05$ were considered statistically significant.

RESULTS

Baseline Characteristics of Patients With Melanoma in Data Sets

Baseline characteristics of the 1,144 study participants are listed in Table 1. Compared with the initial data set, patients in the confirmatory data set had younger median age at blood draw, less frequent tumor mitosis, lower percentage of sentinel lymph node evaluation but higher proportion of involved sentinel lymph nodes, advanced stage at blood draw, longer plasma storage time, higher CRP levels, higher overall death rate, and longer follow-up time from blood draw. The median follow-up time from blood draw to patient death or censoring in all patients was 6.23 years. We also compared characteristics of the 115 patients who underwent sequential blood draw with those of the remaining patients in the combined data set (Appendix Table A1, online only). Patients who underwent sequential blood draw had more advanced stage, thicker tumors, more frequent tumor ulceration, and more frequent primary tumor mitosis. Finally, we determined the association of standard clinical and pathologic characteristics with OS, MSS, and DFS using univariable analysis (Appendix Table A2, online only).

Relationship Between Storage Time, Clinical Features, and CRP Level

Factors associated with CRP level (Appendix Table A3, online only) included age at blood draw, tumor thickness, ulceration, presence of an involved sentinel lymph node, and stage at blood draw. Mitosis ($\geq 1 \text{ v} < 1/\text{mm}^2$) was associated with log-transformed CRP

Table 1. Comparison of Characteristics Between Initial and Confirmatory Data Sets

Characteristic	Combined Data Set (N = 1,144)		Initial Data Set (n = 587)		Confirmatory Data Set (n = 557)		P*
	No.	%	No.	%	No.	%	
Age at blood draw, years							
Median	55.1		55.9		53.9		.0313
Interquartile range	44.3-66.3		45.7-67.0		43.6-65.3		
Female sex	492	43.0	248	42.2	244	43.8	.5949
Tumor thickness, mm							.3278
Median	1.2		1.2		1.1		
Interquartile range	0.7-2.4		0.7-2.5		0.7-2.3		
Presence of ulceration	194	21.0	108	21.6	86	20.4	.6618
> One primary tumor	112	9.8	52	8.9	60	10.8	.2764
≥ One mitosis per mm ²	496	68.4	301	71.5	195	64.1	.0356
SLN biopsy performed	758	66.3	408	69.5	350	62.8	.0171
Positive SLN	145	19.1	65	15.9	80	22.9	.0157
Stage III/IV disease at blood draw	384	33.6	188	32.0	196	35.2	.2577
Storage time from blood draw to CRP testing, years							< .001
Median	3.6		2.6		6.5		
Interquartile range	2.3-6.5		1.9-3.4		5.2-8.4		
CRP level, mg/L							.0022
Median	1.7		1.4		1.9		
Interquartile range	0.7-4.4		0.6-3.9		0.9-4.9		
Logarithmic mean	0.6		0.4		0.7		.0021
CRP ≥ 10 mg/L	122	10.7	56	9.5	66	11.9	.2060
Follow-up time from blood draw to disease relapse or censoring, years							< .001
Median	1.4		1.3		1.6		
Interquartile range	0.5-2.9		0.6-2.2		0.5-4.1		
Follow-up time from blood draw to death or censored point, years							< .001
Median	6.2		5.8		8.0		
Interquartile range	4.9-7.7		5.0-6.6		4.5-9.4		
Subsequent event	280	24.5	130	22.2	150	26.9	.0600
Death	229	20.0	107	18.2	122	21.9	.1205

Abbreviations: CRP, C-reactive protein; SLN, sentinel lymph node.

* χ^2 test for categorical variables and Wilcoxon test for continuous variables.

only after adjustment for confirmatory versus initial data set. Because the liver is considered a primary source of CRP blood levels,⁶ we evaluated for a relationship between CRP and liver metastasis in patients with stage IV disease; we found no association (Appendix Table A3, online only). The correlation between storage time and raw or log-transformed CRP was small (correlation coefficient, 0.0755 [ie, 0.0755-mg/L change in CRP per year of storage time]) but significant (combined data set $P = .0106$; Appendix Table A4, online only). We therefore adjusted for storage time in the multivariable analysis of effect of CRP on outcome measures of patients with melanoma.

CRP As Risk Factor for OS, MSS, and DFS

Elevated CRP was associated with poorer OS in the initial, confirmatory, and combined data sets. In the combined data set, CRP contributed to overall risk of death by a factor of 1.44 (ie, HR; 95% CI, 1.30 to 1.59; $P < .001$) per unit increase of logarithmic CRP. After adjustment for age at blood draw, sex, stage at blood draw, blood storage time, and data set, increased CRP remained associated with poorer OS in both of the individual data sets as well as the combined data set. The association of CRP with MSS (HR, 1.51; 95% CI, 1.36 to 1.68; $P < .001$) was stronger than with OS (Table 2).

To evaluate for a dose effect of raw CRP level on OS and MSS, we divided baseline CRP levels into quintiles. Patients in the highest quintile, as compared with patients in the lowest quintile, had a significantly poorer OS (HR, 4.14; 95% CI, 2.58 to 6.64) and MSS (HR, 5.31; 95% CI, 3.09 to 9.13), with significant trends across quintiles (Appendix Tables A5 and A6, online only). After adjustment for age at blood draw, sex, stage at blood draw, blood storage time, and data set, these associations decreased but remained significant (Appendix Tables A5 and A6, online only).

Univariable recursive partitioning of raw CRP levels using the splitting Gini index demonstrated the highest Gini index when CRP was split at 10.94 mg/L (Appendix Fig A1, online only). Patients with CRP > 10.94 mg/L had an HR 3.64× higher than those with CRP ≤ 10.94 mg/L (95% CI, 2.67 to 4.97; $P < .001$). Further analysis therefore focused on dichotomizing at the clinically standard CRP level of 10 mg/L.

Compared with CRP < 10 mg/L, CRP ≥ 10 mg/L was associated with poorer OS in patients with any-stage, stage I/II, or stage III/IV disease (Figs 1A to 1C) and poorer MSS in patients with any-stage or stage III/IV disease (Appendix Figs A2 to A4, online only). CRP ≥ 10 mg/L was also associated with poorer DFS in patients with stage I/II disease (Fig 1D). These associations remained significant after adjustment for covariates (Table 3).

Table 2. Association Between Log-Transformed CRP Levels and OS or MSS

CRP Value	Initial Data Set (n = 587)			Confirmatory Data Set (n = 557)			Combined Data Set (N = 1,144)		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
OS									
Univariable analysis									
Logarithmic CRP	1.59	1.38 to 1.83	< .001	1.30	1.14 to 1.50	< .001	1.44	1.30 to 1.59	< .001
Multivariable analysis									
Logarithmic CRP	1.40	1.22 to 1.62	< .001	1.17	1.03 to 1.33	.0140	1.28	1.16 to 1.41	< .001
Blood storage time	1.07	0.86 to 1.33	.5660	1.15	1.07 to 1.24	< .001	1.14	1.06 to 1.22	< .001
Age at blood draw	1.01	1.00 to 1.03	.0693	1.03	1.02 to 1.05	< .001	1.02	1.01 to 1.03	< .001
Sex (male v female)	1.33	0.89 to 2.00	.1695	2.16	1.42 to 3.28	< .001	1.68	1.26 to 2.24	< .001
Disease stage at blood draw (III/IV v I/II)	6.57	4.23 to 10.20	< .001	7.28	4.76 to 11.15	< .001	6.98	5.14 to 9.48	< .001
Data set (confirmatory v initial)	—			—			0.61	0.41 to 0.91	.0162
MSS									
Univariable analysis									
Logarithmic CRP	1.62	1.39 to 1.88	< .001	1.41	1.22 to 1.64	< .001	1.51	1.36 to 1.68	< .001
Multivariable analysis									
Logarithmic CRP	1.42	1.22 to 1.64	< .001	1.24	1.09 to 1.42	.0016	1.33	1.20 to 1.47	< .001
Blood storage time	1.00	0.79 to 1.26	.9880	1.09	1.01 to 1.18	.0234	1.08	1.01 to 1.16	.0329
Age at blood draw	1.01	0.99 to 1.02	.4344	1.03	1.01 to 1.04	< .001	1.02	1.01 to 1.03	.0021
Sex (male v female)	1.24	0.81 to 1.89	.3282	2.55	1.59 to 4.09	< .001	1.75	1.28 to 2.39	< .001
Stage at blood draw (III/IV v I/II)	8.85	5.36 to 14.62	< .001	8.36	5.16 to 13.56	< .001	8.65	6.11 to 12.25	< .001
Data set (confirmatory v initial)	—			—			0.70	0.46 to 1.06	.0914

Abbreviations: CRP, C-reactive protein; HR, hazard ratio; MSS, melanoma-specific survival; OS, overall survival.

Correlation of Sequential CRP Levels With Disease Burden

To evaluate whether elevated CRP level was associated with progression in individual patients, change in CRP was compared with change in clinical status in 115 patients for whom sequential blood samples were available. Table 4 summarizes correlations between change in CRP levels obtained a median of 17.12 months apart (range, 0.33 to 87.79 months) and several alternative measures of disease progression: response to treatment (stable, responding, or progressing), progression of disease (no progression v progression), increase in cancer stage (no increase v increase), and increase in number of metastases (no increase v increase as well as no increase, one additional metastasis, and > one additional metastasis). Response to treatment ($P < .001$), progression of disease ($P < .001$), increase in cancer stage ($P = .0065$), and increase in number of metastases (two levels, $P = .0013$; three levels, $P = .0044$) were all highly associated with change in CRP level. We also ran logistic regression models for outcome variables with two categories and an ordinal regression model for variables with three groups and included change in log-transformed CRP as a predictor variable, with or without adjustment for age, sex, stage at first blood draw, and duration between the two blood draws. These parametric tests resulted in findings similar to those from nonparametric analysis (Table 4).

Evaluation of CRP-Associated SNPs With CRP Levels and Patient Outcome Measures

To investigate one important potential mechanism linking CRP blood levels with melanoma progression, CRP-associated SNPs from a genome-wide scan as well as previously reported candidate CRP SNPs were evaluated for association with melanoma risk, melanoma clinical features, and patient outcome. Two SNPs located near the

FAM5C gene region reached genome-wide significance ($P < 5 \times 10^{-8}$) with regard to predicting CRP blood levels (rs7514392 $P = 2.31 \times 10^{-8}$; rs7536465 $P = 3.05 \times 10^{-8}$) and were also significantly associated with melanoma risk, OS, MSS, and DFS. Of five previously identified candidate SNPs, three were found to be related to CRP levels in our study, but none of them was significantly associated with melanoma risk or outcome (Appendix Table A7, online only).

DISCUSSION

In this study, we found that an elevated CRP blood level independently predicted disease recurrence and poorer survival among patients with early- or advanced-stage melanoma. We observed a dose effect between CRP level and survival. Sequential blood draw data in a subset of patients identified associations between CRP level and measures of melanoma disease progression.

CRP has been suggested as a prognostic marker in several cancers, including melanoma.^{20,27-29} Deichman et al³⁰ found that among 216 patients with melanoma, CRP was superior to lactate dehydrogenase in discriminating between those with progressive and nonprogressive disease. Findeisen et al²² found that among 596 patients with melanoma, CRP—in combination with amyloid A—could predict relapse-free survival and OS in those with early-stage disease. In an investigation of the combination of interferon alfa-2b and tremelimumab (anti-CTLA-4) in 37 patients, Tarhini et al²³ identified a preliminary association between lower baseline CRP level and clinical benefit.

In our study, which included CRP determinations for 1,144 patients with melanoma, we found an association between CRP level and disease severity; CRP levels were strongly associated with Breslow tumor thickness, presence of primary tumor ulceration, and disease

Fig 1. Kaplan-Meier survival curves and No. of patients at risk at each time point for patients with C-reactive protein (CRP) ≥ 10 versus < 10 mg/L. Overall survival of patients with (A) all stages of disease, (B) stage I/II disease, and (C) stage III/IV disease. (D) Disease-free survival of patients with stage I/II disease.

stage. We detected an association between elevated values of the logarithm of the CRP level and OS, with an HR of 1.44. After adjusting for covariates that included standard clinicopathologic predictors of melanoma progression, this risk was slightly attenuated to 1.28 but remained significant. Quintile analysis identified a dose-effect relationship between CRP level and OS in both univariable and multivariable models.

We performed recursive partitioning using the entire pool of patients and found the best cutoff value of CRP was 10.94 mg/L, which is close to a commonly used clinical cutoff value of 10 mg/L.²² Our stratification analysis determined that CRP ≥ 10 mg/L predicted poorer survival among patients with early- or advanced-stage melanoma and a higher rate of recurrence among those with early-stage disease. In analysis of a subset of 115 patients, we detected strong (although preliminary) correlations between a rise in CRP and individual-patient disease progression. Finally, our genetic investigation identified an association between candidate CRP SNPs and melanoma disease susceptibility and progression, suggesting that genetic regulation of CRP could be partly responsible for the association between CRP level and patient outcome. We acknowledge these genetic associations must be validated.

Our study has some limitations. Patients with melanoma were recruited from a population undergoing evaluation at a large cancer hospital and might not be representative of the general melanoma population. Although CRP ≥ 10 mg/L was strongly associated with poorer OS and DFS among patients with stage I/II disease and poorer MSS among all patients and those with stage III/IV disease, we were unable to demonstrate a statistically significant relationship between CRP level and MSS among patients with stage I/II disease, likely in part because of the small number of deaths resulting from melanoma in this subset as well as competing causes of CRP-associated mortality. It could be beneficial to investigate alternative mechanisms for CRP-associated mortality. Although several important confounders of CRP levels were included in our models, additional potential cofounders, including body mass index,³¹ smoking,³² and systemic treatment variables, were not included. However, CRP levels did not vary by age, sex, or body weight across individuals.²⁹ In addition, because patients underwent initial blood draw before initiation of any systemic therapy, because of the multiplicity of treatments provided to patients with advanced disease, and because patients in this study were treated before the widespread introduction of modern targeted therapies or immune checkpoint inhibitors, no attempt was made to adjust for

Table 3. HRs for DFS, OS, and MSS Among Patients With CRP Level ≥ 10 Versus < 10 mg/L in Combined Data Set

Disease Stage	Univariable Analysis			Multivariable Analysis		
	HR	95% CI	P	HR	95% CI	P
OS						
All*	3.42	2.53 to 4.64	< .001	2.62	1.93 to 3.56	< .001
I/II†	2.63	1.29 to 5.37	.0078	3.70	1.35 to 10.14	.0110
III/IV†	2.45	1.75 to 3.44	< .001	3.30	1.90 to 5.72	< .001
MSS						
All*	3.58	2.60 to 4.93	< .001	2.67	1.93 to 3.69	< .001
I/II†	1.49	0.53 to 4.18	.4496	2.52	0.72 to 8.84	.1495
III/IV†	2.73	1.93 to 3.84	< .001	3.42	1.97 to 5.94	< .001
DFS						
I/II†	2.53	1.17 to 5.44	.0179	3.29	1.17 to 9.20	.0236

Abbreviations: CRP, C-reactive protein; DFS, disease-free survival; HR, hazard ratio; MSS, melanoma-specific survival; OS, overall survival.

*Adjusted for age, sex, stage, blood storage time, and data set in multivariable analysis.

†Adjusted for age, sex, thickness, ulceration, mitoses, blood storage time, and data set in multivariable analysis.

systemic therapies administered. Blood samples were obtained over many years and in some cases stored for more than a decade; however, we evaluated effects of storage time on change in CRP levels over time and found that the magnitude of change in CRP levels was small; we adjusted for this small effect in our analyses.

In conclusion, these data provide strong evidence that CRP is an independent prognostic biomarker in patients with melanoma, including those with early-stage disease as well as those with advanced-stage disease. A markedly elevated CRP level in particular seems to identify a subgroup of patients at high risk for disease recurrence and

death. On the basis of the data presented here, we believe it is reasonable to include measurement of CRP in prospective investigations of outcomes of patients with melanoma, including in trials of systemic therapy. Furthermore, although these data cannot determine whether interventions to reduce inflammation and/or CRP could benefit selected patients with melanoma, they do suggest that preclinical investigation of such interventions is justified. Statins reduce CRP levels and have an established clinical role in the prevention of cardiovascular disease among patients with high CRP levels.³³ Although one large population-based study in the United States suggested that long-term statin use reduced melanoma risk,³⁴ a recent pooled meta-analysis did not confirm this association.³⁵ The role of statins in the prevention of cancer is promising but remains controversial; a recent Danish population study reported lower cancer-related mortality among statin users,³⁶ and a large population-based investigation in the United Kingdom identified reduced prostate cancer–associated death among statin users.³⁷ Although there is as yet no defined role for clinical use of statins in cancer treatment, our data suggest that preclinical evaluation of statin therapy in melanoma models is reasonable to pursue.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

AUTHOR CONTRIBUTIONS

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Table 4. Correlation of Sequential CRP Levels With Disease Progression (n = 115)

Clinical Factor	No. of Patients	Change in CRP Level		Change in Log(CRP)		
		Median	Nonparametric Test P*	Median	P†	P‡
Response status			< .001		.0143	.0981
Stable	65	−0.22		−0.17		
Response	17	0.22		0.16		
Progression	33	2.67		0.44		
Progression status			< .001		.0028	.0445
No progression	64	−0.25		−0.23		
Progression	51	0.71		0.28		
Increase in cancer stage			.0065		.0101	.0159
No increase	87	−0.19		−0.15		
Increase	28	0.565		0.34		
Increase in No. of metastases			.0013		.0024	.0147
No increase	80	−0.22		−0.16		
Increase	35	1.11		0.39		
Increase in No. of metastases			.0044		.0073	.0391
None	80	−0.22		−0.16		
One additional metastasis	16	0.675		0.27		
> One additional metastasis	19	1.29		0.42		

Abbreviation: CRP, C-reactive protein.

*Nonparametric Wilcoxon two-sample test was used for outcome variables with two categories, and Kruskal-Wallis test was used for variables with three groups.

†Logistic regression model for outcome variables with two categories, and ordinal logistic regression model for variables with three groups (univariable analysis).

‡Logistic regression model for outcome variables with two categories, and ordinal logistic regression model for variables with three groups, adjusted for age, sex, stage at first blood draw (III/IV v I/II), and duration between two blood draws.

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GLOSSARY TERMS

disease-free survival: the survival period spanning the time from surgery to a recurrence of cancer.

exon: segment of a gene that consists of a sequence of nucleotides that encodes amino acids in the protein. Genes are often made up of multiple exons separated by introns that do not encode amino acids in the protein.

genome-wide association study: hypothesis-free studies that evaluate the association of genetic variations throughout the entire genome with traits, using high throughput genotyping technologies to assay single nucleotide polymorphisms.

immune checkpoint: immune inhibitory pathway that negatively modulates the duration and amplitude of immune responses. Examples include the CTLA-4:B7.1/B7.2 pathway, and the PD-1:PD-L1/PD-L2 pathway.

overall survival: the duration between random assignment and death.

recursive partitioning: multivariable analysis that generates a clinically intuitive decision tree model in which the population is divided into prognostic subgroups. This is achieved through multiple dichotomous divisions on the basis of a set of independent variables.

sentinel lymph node: the lymph node that is anatomically located such that it is the first site of lymph drainage from the location of the primary tumor. It is suspected and assumed that if a malignancy is going to disseminate via the lymphatic system, metastases will first be evident in the sentinel lymph node. In this manner, this lymph node is said to stand guard or sentinel over the metastatic state of the tumor. For many cancers, the sentinel lymph node is biopsied as part of the staging process and presence of macro- or micrometastases in the sentinel lymph node is a negative prognostic factor.

single nucleotide polymorphism (SNP): natural variations in the genomic DNA sequence present in greater than 1% of the population, with single nucleotide polymorphisms representing DNA variations in a single nucleotide. Single nucleotide polymorphisms are being widely used to better understand disease processes, thereby paving the way for genetic-based diagnostics and therapeutics.

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C-Reactive Protein As a Marker of Melanoma Progression

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Appendix

Table A1. Comparison of Characteristics Between Patients With Sequential Blood Draw and Those in Combined Data Set

Characteristic	Sequential Data Set (n = 115)		Combined Data Set (n = 1,053)		P*
	No.	%	No.	%	
Age at blood draw, years					.1030
Mean	56.8		54.7		
SD	15		15		
Median	58.5		54.9		
Tumor thickness, mm					.0052
Mean	2.9		2.0		
SD	3.5		2.6		
Median	1.5		1.1		
Female sex	47	40.9	453	43.0	.6581
Present of ulceration	23	28.8	176	20.6	.0901
> One primary tumor	14	12.2	104	9.9	.4376
≥ One mitosis per mm ²	51	86.4	453	67.2	.0022
SLN biopsy performed	62	53.9	707	67.1	.0045
Positive SLN	11	17.7	135	19.1	.7945
Stage III/IV at blood draw	62	53.9	331	31.4	< .001

Abbreviations: SD, standard deviation; SLN, sentinel lymph node.

* χ^2 test for categorical variables, and Wilcoxon test for continuous variables.

C-Reactive Protein and Melanoma Progression

Table A2. Univariable Analysis of Association of Demographic Characteristics and Clinical Parameters With Melanoma Progression

CRP Value	Initial Data Set (n = 587)			Confirmatory Data Set (n = 557)			Combined Data Set (N = 1,144)		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
OS									
Logarithmic CRP	1.59	1.38 to 1.83	< .001	1.30	1.14 to 1.50	< .001	1.44	1.30 to 1.59	< .001
Blood storage time	0.98	0.79 to 1.21	.8160	1.15	1.07 to 1.24	< .001	1.08	1.03 to 1.13	.0014
Age at blood draw	1.02	1.01 to 1.03	.0043	1.04	1.02 to 1.05	< .001	1.03	1.02 to 1.04	< .001
Sex (male v female)	1.50	1.00 to 2.24	.0477	2.72	1.79 to 4.13	< .001	2.02	1.52 to 2.69	< .001
Breslow thickness, mm	1.10	1.07 to 1.13	< .001	1.12	1.09 to 1.16	< .001	1.11	1.08 to 1.13	< .001
Ulceration (present v absent)	4.59	2.92 to 7.20	< .001	5.71	3.67 to 8.90	< .001	5.07	3.70 to 6.96	< .001
Mitoses (≥ 1 v $< 1/\text{mm}^2$)	5.45	2.19 to 13.55	< .001	3.35	1.58 to 7.09	.0016	4.14	2.33 to 7.36	< .001
Stage at blood draw (III/IV v I/II)	7.77	5.04 to 11.96	< .001	8.15	5.34 to 12.44	< .001	7.98	5.90 to 10.80	< .001
Data set (confirmatory v initial)	—			—			1.13	0.87 to 1.47	.3525
MSS									
Logarithmic CRP	1.62	1.39 to 1.88	< .001	1.41	1.22 to 1.64	< .001	1.51	1.36 to 1.68	< .001
Blood storage time	0.90	0.72 to 1.14	.3892	1.10	1.03 to 1.18	.0227	1.05	1.00 to 1.10	.0760
Age at blood draw	1.01	1.00 to 1.03	.0752	1.03	1.02 to 1.05	< .001	1.02	1.01 to 1.03	< .001
Sex (male v female)	1.39	0.92 to 2.11	.1261	3.20	2.00 to 5.12	< .001	2.08	1.53 to 2.83	< .001
Breslow thickness, mm	1.11	1.08 to 1.14	< .001	1.13	1.09 to 1.17	< .001	1.11	1.09 to 1.14	< .001
Ulceration (present v absent)	5.26	3.43 to 8.93	< .001	6.70	4.12 to 10.91	< .001	6.02	4.28 to 8.47	< .001
Mitoses (≥ 1 v $< 1/\text{mm}^2$)	8.66	2.71 to 27.62	< .001	3.58	1.61 to 7.95	< .001	5.10	2.66 to 9.76	< .001
Stage at blood draw (III/IV v I/II)	10.49	6.40 to 17.18	< .001	9.67	5.99 to 15.62	< .001	10.07	7.14 to 14.21	< .001
Data set (confirmatory v initial)	—			—			1.08	0.82 to 1.43	.5821
DFS									
CRP (≥ 10 v < 10 mg/L)	2.76	0.80 to 9.53	.1090	2.42	0.91 to 6.43	.0758	2.53	1.17 to 5.44	.0179
Blood storage time	0.93	0.54 to 1.61	.7844	1.28	1.04 to 1.58	.0177	1.07	0.96 to 1.21	.2300
Age at blood draw	1.05	1.02 to 1.09	.0054	1.03	1.00 to 1.05	.0729	1.04	1.01 to 1.06	.0016
Sex (male v female)	2.15	0.77 to 6.03	.1468	3.93	1.48 to 10.93	.0060	3.05	1.50 to 6.17	.0020
Breslow thickness, mm	1.14	1.09 to 1.20	< .001	1.10	1.02 to 1.18	.0101	1.12	1.08 to 1.17	< .001
Ulceration (present v absent)	5.08	2.00 to 12.90	< .001	7.65	3.14 to 18.61	< .001	6.22	3.27 to 11.83	< .001
Mitoses (≥ 1 v $< 1/\text{mm}^2$)*	—			7.43	0.98 to 56.62	.0528	17.89	2.44 to 131.07	.0045
Data set (confirmatory v initial)	—			—			0.95	0.50 to 1.81	.8759

Abbreviations: CRP, C-reactive protein; DFS, disease-free survival; HR, hazard ratio; MSS, melanoma-specific survival; OS, overall survival.
 *HR was not estimated in initial data set because of missing data.

Table A3. Association of Demographic Characteristics and Clinical Parameters With Plasma CRP Levels in Combined Data Set

Clinical Factor	No. of Patients	Raw CRP			Log CRP			
		Median	Interquartile Range	P*	Mean	SD	P†	P‡
Age at blood draw	1,144	1.69	0.69-4.39	.0018	0.56	1.37	.0012	< .001
Sex				.0534			.3613	.3711
Male	652	1.50	0.64-3.97		0.52	1.34		
Female	492	1.99	0.73-4.74		0.60	1.41		
Tumor thickness	1,020	1.63	0.63-4.05	< .001	0.48	1.34	.0028	.0023
Ulceration				.0015			< .001	< .001
Absent	729	1.50	0.60-3.61		0.40	1.33		
Present	194	2.04	0.96-5.40		0.79	1.36		
Mitotic rate, per mm ²				.1928			.0629	.0379
< 1	229	1.58	0.57-3.66		0.37	1.41		
≥ 1	496	1.65	0.77-4.15		0.57	1.30		
No. of primary tumors				.6146			.4292	.3910
1	1,032	1.71	0.69-4.39		0.56	1.36		
> 1	112	1.63	0.61-4.42		0.48	1.48		
SLNs				< .001			.0181	—
Negative	613	1.51	0.64-3.66		0.45	1.29		
Positive	145	1.83	0.82-4.25		0.62	1.31		
Not performed	386	1.84	0.73-5.24		0.70	1.50		
Stage at blood draw				< .001			< .001	< .001
I, I/II, or II	760	1.43	0.60-3.51		0.40	1.30		
III or IV	384	2.15	0.97-6.23		0.88	1.46		
Liver metastasis (IV)				.0782			.069	.068
No	60	3.93	1.37-19.12		1.56	1.60		
Yes	18	13.20	2.97-28.01		2.32	1.29		

Abbreviations: CRP, C-reactive protein; SD, standard deviation; SLN, sentinel lymph node.

*Spearman rho test for Spearman correlation between continuous variables and CRP, and Wilcoxon test for association between categorical variables and CRP.

†Analysis of variance test.

‡Linear regression model, adjusted for data set resource and blood storage time.

Table A4. Correlation Between Storage Time and Measured Plasma CRP Level

Data Set	Storage Time (years)		Raw CRP*		Log CRP†	
	Mean	SD	Coefficient	P	Coefficient	P
Initial (n = 587)	2.72	0.89	0.04976	.2287	0.04205	.3091
Confirmatory (n = 557)	6.41	2.73	0.03575	.3997	0.01259	.7669
Combined (N = 1,144)	4.51	2.73	0.0755	.0106	0.06979	.0182

Abbreviations: CRP, C-reactive protein; SD, standard deviation.

*Spearman rho test for Spearman correlation.

†Pearson's correlation.

Table A5. HRs for OS According to Quintile of CRP Plasma in Patients With Any-Stage Disease

Quintile of Plasma Level	Median (mg/L)	Quintile Value (mg/L)	Univariable Analysis			Multivariable Analysis*		
			HR	95% CI	Trend P	HR	95% CI	Trend P
1	0.33	< 0.55	1.00		< .001	1.00		< .001
2	0.86	0.55-1.18	2.22	1.34 to 3.67		1.57	0.94 to 2.60	
3	1.69	1.18-2.35	1.73	1.03 to 2.93		1.23	0.73 to 2.10	
4	3.46	2.35-5.38	1.95	1.16 to 3.27		1.41	0.83 to 2.37	
5	10.57	≥ 5.38	4.14	2.58 to 6.64		2.67	1.65 to 4.31	

Abbreviations: CRP, C-reactive protein; HR, hazard ratio; OS, overall survival.

*Adjusted for age, sex, stage at blood draw, storage time, and data set.

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Table A6. HRs for MSS According to Quintile of CRP Plasma Level in Patients With Any-Stage Disease

Quintile of Plasma Level	Median (mg/L)	Quintile Value (mg/L)	Univariable Analysis			Multivariable Analysis*		
			HR	95% CI	Trend <i>P</i>	HR	95% CI	Trend <i>P</i>
1	0.33	< 0.55	1.00		< .001	1.00		< .001
2	0.86	0.55-1.18	2.68	1.51 to 4.75		1.93	1.08 to 3.44	
3	1.69	1.18-2.35	2.13	1.18 to 3.86		1.55	0.85 to 2.82	
4	3.46	2.35-5.38	2.23	1.24 to 4.03		1.64	0.90 to 2.98	
5	10.57	≥ 5.38	5.31	3.09 to 9.13		3.44	1.99 to 5.94	

Abbreviations: CRP, C-reactive protein; HR, hazard ratio; MSS, melanoma-specific survival.

*Adjusted for age, sex, stage at blood draw, storage time, and data set.

Table A7. Association of Significant SNPs From GWAS and All Candidate Polymorphisms for CRP With Risk for Melanoma Susceptibility, OS, MSS, and DFS

SNP	Minor/ Major	MAF	Chromosome	Position	<i>P</i>								Gene
					Log(CRP) ^a	Disease ^b	Log (thickness) ^c	Ulceration ^d	Disease Stage (III/IV v I/II) ^e	OS ^f	MSS ^g	DFS ^h	
rs7514392	G/C	0.0005	1	188323178	2.31×10^{-8}	.037	.240	.207	.120	.052	.043	.041	FAM5C
rs7536465	A/G	0.0005	1	188323329	3.05×10^{-8}	.036	.248	.345	.107	.049	.040	.015	FAM5C
rs1205	T/C	0.3276	1	157948857	.001	.063	.255	.388	.260	.687	.724	.742	CRP
rs1800947	G/C	0.0627	1	157950062	.065	.674	.065	.629	.218	.287	.544	.138	CRP
rs2794521	G/A	0.2838	1	157951720	.119	.729	.373	.892	.151	.833	.891	.856	CRP
rs3093059	G/A	0.058	1	157951760	.012	.495	.809	.132	.680	.609	.963	.183	CRP
rs7553007	A/G	0.3369	1	157965173	.001	.134	.201	.298	.210	.781	.925	.241	CRP

Abbreviations: CRP, C-reactive protein; DFS, disease-free survival; GWAS, genome-wide association study; MAF, minor allele frequency; MSS, melanoma-specific survival; OS, overall survival; SNP, single-nucleotide polymorphism.

^aAssociation of each SNP with logarithmic transformed CRP.

^bAssociation of each SNP with disease status.

^cAssociation of each SNP with log-transformed thickness.

^dAssociation of each SNP with presence or absence of ulceration.

^eAssociation of each SNP with stage III/IV versus I/II disease.

^fAssociation of each SNP with OS.

^gAssociation of each SNP with MSS.

^hAssociation of each SNP with DFS among patients with stage I/II disease.

Fig A1. Recursive partitioning demonstrating best cutoff for C-reactive protein (CRP) at 10.94 mg/L. HR, hazard ratio.

Fig A2. Kaplan-Meier curve of melanoma-specific survival for patients with any-stage disease. CRP, C-reactive protein.

Fig A3. Kaplan-Meier curve of melanoma-specific survival for patients with stage I/II disease. CRP, C-reactive protein.

Fig A4. Kaplan-Meier curve of melanoma-specific survival for patients with stage III/IV disease. CRP, C-reactive protein.