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Tumor-Infiltrating Lymphocytes and Colorectal Cancer Survival in African American and Caucasian Patients

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Abstract

Background: Compared with Caucasian Americans (CAs), African Americans (AAs) with colorectal cancer have poorer survival, especially younger-age patients. A robust lymphocytic reaction within colorectal cancers is strongly associated with better survival, but whether immune response impacts the disparity in colorectal cancer survival is unknown.

Methods: The study population was comprised of 211 histologically confirmed colorectal cancers at the Medical University of South Carolina (Charleston, SC; 159 CAs and 52 AAs) diagnosed between Jan 01, 2000, and June 30, 2013. We constructed a lymphocyte score based on blinded pathologic assessment of the four different types of lymphocytic reactions. Cox

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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proportional hazards regression was used to evaluate the association between the lymphocyte score and risk of death by race.

Results: Colorectal cancers in AAs (vs. CAs) had a stronger lymphocytic reaction at diagnosis. A high lymphocyte score (vs. the lowest) was associated with better survival in AAs [HR 0.19; 95% confidence interval (CI), 0.04–0.99] and CAs (HR 0.47; 95% CI, 0.15–1.45). AAs with no lymphocytic reaction (vs. other categories) had poor survival HR 4.48 (1.58–12.7) whereas no difference was observed in CAs. The risk of death in AAs (vs. CA) was more pronounced in younger patients (HR 2.92; 95% CI, 1.18–7.22) compared with older (HR 1.20; 95% CI, 0.54–2.67), especially those with lymphocytic poor colorectal cancers.

Conclusions: The lymphocytic reaction in tumor impacted the racial disparity in survival.

Impact: Our results confirm the importance of the lymphocytic score on survival and highlight the need to fully characterize the immune environment of colorectal cancers by race.

Introduction

Colorectal cancer is the third most common malignancy among men and women in the United States and the second leading cause of cancer-related death (1). Compared with Caucasian Americans (CAs), African Americans (AAs) have a higher incidence of colorectal cancer and poorer stage-specific survival (1), especially among younger patients (2–5). These survival disparities persist even after adjustment for factors such as age, sex, stage, comorbidities, socioeconomic status, insurance status, and tumor characteristics (6–10).

AAs are more likely than CAs to present at diagnosis with poor prognostic pathomolecular characteristics, such as proximal colorectal cancer and microsatellite stable (MSS) cancers. These tumor features are known to differ immunologically (11–13) raising the possibility that the immune tumor microenvironment (TME) might play a role in the etiology of the racial differences. Moreover, African ancestry is associated with clear differences in systemic immune response, such as a more efficient antigen-presenting capacity, a stronger, more robust proinflammatory response, and an enhanced wound healing, profibrotic response than those with European ancestry (14–18).

The impact of the immune response within the TME on containing the growth of established colorectal cancer and limiting metastasis is well documented (19–24). Tumor-infiltrating lymphocytes (TILs) have been shown to be associated with lower recurrence and case fatality, independent of stage (20, 21, 24–28). In addition, the immune composition within colorectal cancers differs by molecular phenotype and anatomic location. For example, microsatellite instability high (MSI-H) colorectal cancer has a higher concentration of CD8⁺ cytotoxic and Th1 T cells than MSS cancers, contributing to the better prognoses of these cancers (11, 26). The lymphocytic density in colorectal cancers differs by anatomic location of the tumor and appears to play an important role in colon cancer survival (13, 28).

While a few studies have examined racial differences in immune infiltrates within the colorectal cancers (29, 30) by race none have examined how differences in the lymphocytic reaction contributes to the racial disparity in survival. Therefore, in the current study, we

examined the differences in the lymphocyte reaction score in the colorectal cancers of AAs and CAs, and evaluated the impact on survival while adjusting for potential confounders such as age, sex, stage, treatment, and colorectal cancer tumor-related features. Because many have identified a greater survival disparity in younger AAs compared with younger CAs, we also evaluated the association of the lymphocyte score on survival by race in younger and older patients (2, 3, 4, 5, 31).

Materials and Methods

Patient inclusion and exclusion criteria

The Medical University of South Carolina (MUSC) Institutional Review Board approved all study activities. The study was conducted in accordance with the Federal Policy for the Protection of Human Subjects, or the U.S. Common Rule (Department of Health and Human Services regulation 45 CFR part 46.110). Our study was approved as an expedited review (46.110); no written informed consent was obtained because materials were collected previously for nonresearch purposes. All data was deidentified. The Cancer Registry at Hollings Cancer Center (HCC) at MUSC (Charleston, SC) was used to identify all cases of colorectal cancer. The registry is part of a state-mandated data system that ascertains all incident cancer cases in South Carolina. The study population was comprised of a convenience sample of histologically confirmed cases diagnosed between January 1, 2000, and June 30, 2013. Patients were of either AA or CA descent and we used self-identified race to classify patients. Patients were excluded if too little tumor tissue was available for analysis (<5 mm) or there existed a known familial hereditary of colorectal cancer, such as Lynch syndrome or familial adenomatous polyposis.

Data collection

We abstracted data on demographic characteristics, clinical and pathologic variables at diagnosis, treatment received, and patient outcome from the HCC cancer registry. Independent variables obtained included sociodemographic characteristics (age at diagnosis, sex, and race). Tumor-related variables obtained from the registry included tumor grade (well-differentiated, moderately differentiated, or poorly differentiated/undifferentiated), anatomic location of the primary tumor (proximal colon, distal colon, and rectum), TNM stage (I, II, III, and IV), and all first-line therapies (chemotherapy, surgery, radiation, and/or other).

Pathologic assessment

For each case, we obtained a representative 5- μ m-thick H&E slide. At the start of the study, all nontumor portions of the slide were covered so that the pathologist was unaware of any patient or clinical information. Each slide was numbered with a study identification number. For each case, the study pathologist documented the histologic type of primary carcinoma (mucinous, mucinous component, not otherwise specified (NOS) adenocarcinoma, etc.), tumor border type (infiltrating and pushing), and, when available, the adjacent adenoma type (tubular, tubulovillous, villous, sessile serrated, etc.). In addition, the pathologist evaluated histopathologic features including patterns and degrees of lymphocytic reaction within and around tumor areas using an established methodology (28). The four types of lymphocytic

reactions include: number of intratumoral-infiltrating lymphocytes per high-powered field (HPF; 0, 1–2, 3–10, 10+), intratumoral aggregate reactions (no, yes), peritumoral (i.e., Crohn-like) aggregate lymphoid reaction (no, yes), and peritumoral (border) lymphocytic reaction (no, yes; ref. 28). For the intratumoral lymphocyte count, the pathologist counted the number of lymphocytes per HPF; a representative region was chosen by the pathologist. Using the presence or absence of each of these types of reactions, each tumor was also classified according to a summary lymphocyte score: none (no reaction present), low (1 reaction type present), medium (2 reaction types present), or high (3+ reaction types present).

Statistical analysis

We compared clinical and pathologic factors at diagnosis by race using *t* tests and χ^2 tests. In addition, we compared lymphocytic reaction score components of colorectal cancers by race. The primary endpoint was overall survival, defined as the time from diagnosis to death from any cause. In the univariate analysis, we used Cox proportional hazards regression to model the hazard of death as a function of lymphocyte score (none, low, medium, and high; none=reference) and race (CA and AA; CA = reference) using a product interaction term in the Cox proportional hazards regression model (see example below). We computed the HRs (95% CIs) for each lymphocyte score category (relative to “none”) for each race using the orthogonal linear contrasts. *P* values for linear trends and for interactions were derived from appropriate orthogonal linear contrasts. We generated five additional Cox regression models to examine the association of the lymphocyte reaction score for each race while adjusting for standard clinical prognostic markers (age, sex, stage, and chemotherapy treatment) alone (model 2) or with the addition of clinicopathologic prognostic markers (i.e., MSI status, anatomic colonic location, tumor grade, and histologic type) in models 3–6. An example of our fitted model is provided in the Supplementary Table S1.

First-line chemotherapy treatment was coded as a dummy variable (yes and no). For the purposes of analysis, TNM stage was separated into two groups because we had too few stage IV cases: early (I and II) and late (III and IV). A similar approach was taken for tumor grade (low and high).

Because we observed a significant interaction between race and age (as a continuous variable) on overall survival (*P* = 0.03), we performed an age and race stratified analysis. We also examined the racial difference in risk of death (AAs vs. CAs) in categories of lymphocyte reaction score (none, low, medium, and high) or into two groups (none-low and medium-high). We defined younger aged patients as those in the lowest tertile (<59 years) and older age patients as those in the upper tertile (70 years and older). All tests were two-sided and the comparison-wise type I error rate was controlled at level 0.05.

Results

A total of 211 patients were included in the analysis (159 CA and 52 AA). Univariate associations of demographic and clinical characteristics with race are shown in Table 1. Overall, AAs were younger at diagnosis (60.5 years vs. 66 years, *P* = 0.05) had more proximal tumors (60% vs. 42%, *P* = 0.03), and were less likely to present with MSI

colorectal cancer (6% vs. 16%, $P=0.08$). AAs were more likely to have colorectal cancers with higher intratumoral lymphocyte density ($P=0.02$) and intratumoral aggregates ($P=0.003$). We did not observe differences in peritumoral aggregate or peritumoral lymphocytes. Overall, the lymphocyte summary score was broadly similar in AAs and CAs (Table 1).

Factors associated with survival

Table 2 shows the association of the lymphocytic reaction score and survival by race in the unadjusted and adjusted models for age, sex, stage, treatment, and several clinicopathologic features (MSI, location, grade, and histologic type). The association between lymphocytic reaction score components, race and survival are shown in Supplementary Table S2. The lymphocytic reaction score strongly impacted survival, even after adjusting for the aforementioned factors, especially in AA patients. As the lymphocyte reaction increased in tumor, the risk of death decreased for both AAs and CAs but the test of trend was only statistically significant in the AAs (Table 2). For example, among AAs in the highest category of lymphocyte reaction (compared with those with no reaction present), the risk of death was decreased in AAs (HR 0.14; 95% CI, 0.03–0.80) and CAs (HR 0.38; 95% CI, 0.11–1.26; Table 2). The results for the adjusted models were broadly similar to the base model (Table 2). The strong inverse association observed between lymphocyte score and death in AAs is partly due to the very high risk of death in AAs that have no lymphocyte reaction in their tumors. For example, in the comparison of those with no lymphocyte reaction to those with low, medium, or high reactions, the HR was markedly increased in AAs (HR 4.48; 95% CI, 1.58–12.7) but not significantly increased in CAs (HR 1.14; 95% CI, 0.49–2.63; Table 2).

Association of race and survival by age group and lymphocyte score level

Next, we evaluated the risk of death in AAs versus CAs overall and in younger and older patients (Table 3). AAs had a significantly higher mortality in both the univariate (HR = 1.63; 95% CI, 1.00–2.66) and age, sex, stage, treatment, and lymphocyte score adjusted multivariable model (HR = 1.82; 95% CI, 1.10–3.01). We observed a significant interaction between race and age ($P=0.03$) on survival. Younger AA patients (lowest third, <59 years of age) compared with younger CAs had a significantly higher risk of death (HR= 3.45; 95% CI, 1.17–10.17). The HR was weaker in the same comparison (AAs vs. CAs) among older patients (those in the highest tertile, 70 years of age or greater): HR = 1.76; 95% CI, 0.70–4.37.

We next evaluated the racial difference in death (AAs vs. CAs) in the four lymphocytic reaction score categories: none, low, medium, and high (Table 3). The risk of death in AAs versus CAs was most pronounced in the category of no lymphocytic reaction: HR 6.16; 95% CI, 1.94–19.53. For younger patients, the HRs for AAs (vs. CA) were higher among those with low lymphocytes (none low) in their colorectal cancers HR 2.30 (95% CI, 0.64–8.29) and high (medium-high score) HR 3.57; 95% CI, 0.66–19.19. Although not statistically significant, the point estimates for the risk of death among the younger aged AAs compared with CAs are similar after adjustment for several prognostic variables (Table 3). Among older patients (highest tertile), the risk of death for AAs (vs. CAs) in those low lymphocyte categories was of a similar magnitude to younger patients: HR 2.81; 95% CI, 0.78–10.06.

However, there was no racial difference in survival among older patients with medium-high lymphocytes scores (HR for AA race 1.33; 95% CI, 0.42–4.19).

Discussion

Our analysis indicates increasing lymphocyte reaction scores were strongly associated with a decreased risk of death. The overall lymphocyte score was similar by race but two of the components (the intratumoral aggregates and intratumoral lymphocytes counts per HPF) were higher in AAs compared with CAs, a pattern consistent with better outcomes in patients with greater lymphocytic reaction in their colorectal cancers. However, a subset of AAs fared poorly, especially those with low lymphocytic reactions within their tumors. In younger patients, the racial disparity in survival was also evident in those with a high lymphocytic reaction. Our results point to possible differences in the lymphocyte reaction score at diagnosis by race and its impact on differences by race in colorectal cancer survival.

The importance of the lymphocytic immune response within established colorectal cancers has been recognized for over 30 years (19–24). Our results are broadly consistent with several previous studies (20, 21, 25, 32) which have reported that densities of lymphoid cells in the TME are associated with good colorectal cancer prognosis. However, less is known about how the lymphocyte reaction impacts prognosis in colorectal cancers in different phenotypes, colonic locations, histology, tumor grade, and personal characteristics such as age and race. Our study results suggest that the clinicopathologic prognostic factors did not strongly influence the relationship between race and lymphocyte score on survival. We did not have the statistical power to examine interactions between various clinicopathologic prognostic factors and the race-lymphocyte score risk of death but could be important in future studies. For example, cytotoxic effector T cells have been found to be more prevalent in proximal colorectal cancers. On the other hand, regulatory T cells, which act to suppress inflammation and repress effector T cells, are positive prognostic indicators for rectal cancer (12), but do not appear to influence treatment response (33, 34).

Individuals of African ancestry exhibit higher levels of immune activation to antigens and a stronger proinflammatory response than CA (14–18). Our study is the first to examine the impact of the lymphocytic score and its influence on survival by race. A few previous reports have examined the relationship between self-identified race and immune infiltrates in colorectal cancers. For example, two studies contrasted cytotoxic immune-cell density in the colorectal cancers using standard IHC analyses (29, 30). The first identified nonsignificantly lower levels of CD8+ cytotoxic T-cell responses in the TME in AAs compared with CAs (30), but no difference in CD8+ density was found in another study. In the latter study, however, AAs had a significantly lower granzyme B infiltrate, a classic marker of effector immune-cell cytotoxicity (29). The only study (35) to compare gene expression profiles of colorectal cancer by race identified cytotoxic and inflammatory immune-related genes that differed between AAs and CAs. Together, these studies are suggestive of a reduced cytotoxic response, and in the gene expression analysis, a higher inflammatory burden in AAs versus CAs.

In our data, we found evidence of a higher lymphocytic response in AAs compared with CAs. One of the reasons for this may be due to the higher lymphocyte count in colorectal cancers in proximal cancers compared with rectal cancers (13, 28). Many have reported a higher prevalence of proximal neoplasia in AAs compared with CAs (30, 36–39). However, the few number of rectal cancer cases in our study precluded a thorough examination of these issues. Alternatively, subsets of AAs with higher immune infiltrates had better survival than CAs. For example, of the few patients with intratumoral aggregates ($n = 8$), 7 of these were AAs and none of these patients died. Our data also revealed poor prognosis among AAs without a strong lymphocyte reaction within the tumor environment. One possibility is these lymphocyte poor tumors may contain abundance of myeloid derived suppressor cells (MDSC). These are a heterogeneous grouping of innate immune cells, associated with higher cancer stages, metastasis, and poorer outcomes (40). MDSC density is also correlated with a lower cytotoxic T-cell response and a higher inflammatory Th17 cell density (41–46). To our knowledge, MDSCs have not been investigated in colorectal cancers by race but could explain the poor prognosis in AAs lacking a strong reaction. Our study results suggest that a detailed immunologic profiling of the colorectal cancer tumors will be an important next step to understand the contributions of different immune cell subsets in colorectal cancer risk and prognosis.

Several studies have reported that younger AAs have poorer prognosis than younger CAs (2, 3, 5, 31) and that the racial disparity is less pronounced in older patients. Our findings suggest a differential impact of lymphocyte score on survival in younger and older AA patients. That is, in older patients (70 years old or greater) with medium to high lymphocyte counts in their tumors at diagnosis, faced no significant disparity in survival. While both older and younger AAs with lower lymphocyte scores had poorer survival, higher lymphocyte score in younger AAs was also associated with an increased risk of death when compared with their CA counterparts. The reasons why younger AAs patients die more than younger CAs is not known but a higher lymphocyte score can be indicative of poor prognosis in certain circumstances. For example, cytotoxic T cells may be present in the tumor but have decreased killing capacity as a result of T-cell exhaustion. Exhausted tumors often express PD-L1, which has been associated with poor prognosis in several cancers (47, 48). Importantly, PD-L1 inhibitors are now a mainstay of immunotherapeutic treatment for metastatic colon cancer (49). These are thought to be effective by restoring effector T-cell functioning (50, 51). Another possible contributing factor for the poorer prognoses in patients with higher lymphocytes is that the lymphocytic lineage (i.e., Th17 vs. Th1) is actually more proinflammatory (Th17) than cytotoxic. African ancestry is associated with a stronger proinflammatory Th17 response (antibacterial and antifungal; refs. 16, 52), which is associated with poorer prognosis in colorectal cancer (27).

The differences in immune response by race could also influence the efficacy of treatment and impact survival. Two large studies (53, 54) have found that even when treatment is administered at the same rates, AAs had lower response rates to therapy. A lower response rate may stem from differences in the immune response to tumor. For example, bevacizumab not only inhibits angiogenesis, but may also inhibit the immunosuppressive signaling within the TME (55). Although we did not observe large differences in treatment by race, we were limited in the number of patients receiving chemotherapy. Moreover, the racial disparity in

younger aged patients is also observed in early stage colorectal cancer (stages 0–2), which do not typically receive chemotherapy. Additional studies with a larger number of patients will be needed to fully evaluate the intersection of the immune response on treatment and outcomes.

We recognize strengths of our study, including a racially diverse population of patients with careful characterization of lymphocyte reaction and pathologic characteristics as well as vital status. However, we also recognize the study's limitations. First, we had no data on immune-cell-level factors (such as type of infiltrate or phase of differentiation) or detailed treatment regimen data, which could have confounded or modified the association between race and colorectal cancer survival. Second, we did not have access to important clinical and lifestyle data such as obesity, diabetes, or smoking status. Third, we lacked information on the genetic ancestry of patients making it difficult to understand the biological contribution to differences in immune reaction by race. Fourth, our results on the differences in survival by race and age, and lymphocyte score should be considered exploratory and will need to be validated in a larger cohort. Overall, our results point to the need for detailed studies identifying the immune prognostic signatures and how they differ by race, age, and anatomic location, and their potential impact on treatment to help advance understanding of the racial disparity in colorectal cancer survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, et al. Colorectal cancer statistics, 2017. *CA Cancer J Clin* 2017;67: 177–193. [PubMed: 28248415]
2. Andaya AA, Enewold L, Zahm SH, Shriver CD, Stojadinovic A, McGlynn KA, et al. Race and colon cancer survival in an equal-access health care system. *Cancer Epidemiol Biomarkers Prev* 2013;22:1030–6. [PubMed: 23576691]
3. Wallace K, DeToma A, Lewin DN, Sun S, Rockey D, Britten CD, et al. Racial differences in stage IV colorectal cancer survival in younger and older patients. *Clin Colorectal Cancer*. 2017;16:178–86 [PubMed: 28065664]
4. Holowatyj AN, Ruterbusch JJ, Rozek LS, Cote ML, Stoffel EM. Racial/ethnic disparities in survival among patients with young-onset colorectal cancer. *J Clin Oncol* 2016;34:2148–56. [PubMed: 27138583]
5. Yoon HH, Shi Q, Alberts SR, Goldberg RM, Thibodeau SN, Sargent DJ, et al. Racial differences in BRAF/KRAS mutation rates and survival in stage III colon cancer patients. *J Natl Cancer Inst* 2015;107 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5758035/pdf/djv186.pdf>.
6. Du XL, Meyer TE, Franzini L. Meta-analysis of racial disparities in survival in association with socioeconomic status among men and women with colon cancer. *Cancer* 2007;109:2161–70. [PubMed: 17455219]
7. Robbins AS, Siegel RL, Jemal A. Racial disparities in stage-specific colorectal cancer mortality rates from 1985 to 2008. *J Clin Oncol* 2012;30:401–5. [PubMed: 22184373]

8. Yan B, Noone AM, Yee C, Banerjee M, Schwartz K, Simon MS. Racial differences in colorectal cancer survival in the detroit metropolitan area. *Cancer* 2009;115:3791–800. [PubMed: 19598220]
9. Byers TE, Wolf HJ, Bauer KR, Bolick-Aldrich S, Chen VW, Finch JL, et al. The impact of socioeconomic status on survival after cancer in the United States: findings from the national program of cancer registries patterns of care study. *Cancer* 2008;113:582–91. [PubMed: 18613122]
10. Breslin TM, Morris AM, Gu N, Wong SL, Finlayson EV, Banerjee M, et al. Hospital factors and racial disparities in mortality after surgery for breast and colon cancer. *J Clin Oncol* 2009;27:3945–50. [PubMed: 19470926]
11. Boissiere-Michot F, Lazennec G, Frugier H, Jarlier M, Roca L, Duffour J, et al. Characterization of an adaptive immune response in microsatellite-unstable colorectal cancer. *Oncoimmunology* 2014;3:e29256. [PubMed: 25101223]
12. Berntsson J, Nodin B, Eberhard J, Micke P, Jirstrom K. Prognostic impact of tumour-infiltrating B cells and plasma cells in colorectal cancer. *Int J Cancer* 2016;139:1129–39. [PubMed: 27074317]
13. Prizment AE, Vierkant RA, Smyrk TC, Tillmans LS, Nelson HH, Lynch CF, et al. Cytotoxic T cells and granzyme B associated with improved colorectal cancer survival in a prospective cohort of older women. *Cancer Epidemiol Biomarkers Prev* 2017;26:622–631. [PubMed: 27979806]
14. Mbow M, de Jong SE, Meurs L, Mboup S, Dieye TN, Polman K, et al. Changes in immunological profile as a function of urbanization and lifestyle. *Immunology* 2014;143:569–77. [PubMed: 24924958]
15. Roetynck S, Olotu A, Simam J, Marsh K, Stockinger B, Urban B, et al. Phenotypic and functional profiling of CD4 T cell compartment in distinct populations of healthy adults with different antigenic exposure. *PLoS One* 2013;8:e55195. [PubMed: 23383106]
16. Ye CJ, Feng T, Kwon HK, Raj T, Wilson MT, Asinowski N, et al. Intersection of population variation and autoimmunity genetics in human T cell activation. *Science* 2014;345:1254665. [PubMed: 25214635]
17. Labuda LA, de Jong SE, Meurs L, Amoah AS, Mbow M, Ateba-Ngoa U, et al. Differences in innate cytokine responses between European and African children. *PLoS One* 2014;9:e95241. [PubMed: 24743542]
18. Russell SB, Smith JC, Huang M, Trupin JS, Williams SM. Pleiotropic effects of immune responses explain variation in the prevalence of fibroproliferative diseases. *PLoS Genet* 2015;11:e1005568. [PubMed: 26540410]
19. Halvorsen TB, Seim E. Association between invasiveness, inflammatory reaction, desmoplasia and survival in colorectal cancer. *J Clin Pathol* 1989;42:162–6. [PubMed: 2921357]
20. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 2006;313: 1960–4. [PubMed: 17008531]
21. Jass JR. Lymphocytic infiltration and survival in rectal cancer. *J Clin Pathol* 1986;39:585–9. [PubMed: 3722412]
22. Menon AG, Janssen-van Rhijn CM, Morreau H, Putter H, Tollenaar RA, van de Velde CJ, et al. Immune system and prognosis in colorectal cancer: a detailed immunohistochemical analysis. *Lab Invest* 2004;84:493–501. [PubMed: 14968119]
23. Galon J, Pages F, Marincola FM, Thurin M, Trinchieri G, Fox BA, et al. The immune score as a new possible approach for the classification of cancer. *J Transl Med* 2012;10:1. [PubMed: 22214470]
24. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity* 2013;39:782–95. [PubMed: 24138885]
25. Sinicrope FA, Rego RL, Ansell SM, Knutson KL, Foster NR, Sargent DJ. Intraepithelial effector (CD3+)/regulatory (FoxP3+) T-cell ratio predicts a clinical outcome of human colon carcinoma. *Gastroenterology* 2009;137: 1270–9. [PubMed: 19577568]
26. Maby P, Tougeron D, Hamieh M, Mlecnik B, Kora H, Bindea G, et al. Correlation between density of CD8+ T-cell infiltrate in microsatellite unstable colorectal cancers and frameshift mutations: a rationale for personalized immunotherapy. *Cancer Res* 2015;75:3446–55. [PubMed: 26060019]

27. Tosolini M, Kirilovsky A, Mlecnik B, Fredriksen T, Mauger S, Bindea G, et al. Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, th2, treg, th17) in patients with colorectal cancer. *Cancer Res* 2011;71:1263–71. [PubMed: 21303976]
28. Ogino S, Nosho K, Irahara N, Meyerhardt JA, Baba Y, Shima K, et al. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. *Clin Cancer Res* 2009;15:6412–20. [PubMed: 19825961]
29. Basa RC, Davies V, Li X, Murali B, Shah J, Yang B, et al. Decreased anti-tumor cytotoxic immunity among microsatellite-stable colon cancers from African Americans. *PLoS One* 2016;11:e0156660. [PubMed: 27310868]
30. Carethers JM, Murali B, Yang B, Doctolero RT, Tajima A, Basa R, et al. Influence of race on microsatellite instability and CD8+ T cell infiltration in colon cancer. *PLoS One* 2014;9:e100461. [PubMed: 24956473]
31. Wallace K, Hill EG, Lewin DN, Williamson G, Oppenheimer S, Ford ME, et al. Racial disparities in advanced-stage colorectal cancer survival. *Cancer Causes Control* 2013;24:463–71. [PubMed: 23296454]
32. Ogino S, Nosho K, Kirkner GJ, Kawasaki T, Meyerhardt JA, Loda M, et al. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. *Gut* 2009;58:90–6. [PubMed: 18832519]
33. McCoy AN, Araujo-Perez F, Azcarate-Peril A, Yeh JJ, Sandler RS, Keku TO. *Fusobacterium* is associated with colorectal adenomas. *PLoS One* 2013;8: e53653. [PubMed: 23335968]
34. Park NJ, Kang DH. Inflammatory cytokine levels and breast cancer risk factors: racial differences of healthy Caucasian and African American women. *Oncol Nurs Forum* 2013;40:490–500. [PubMed: 23975184]
35. Jovov B, Araujo-Perez F, Sigel CS, Stratford JK, McCoy AN, Yeh JJ, et al. Differential gene expression between African American and European American colorectal cancer patients. *PLoS One* 2012;7:e30168. [PubMed: 22276153]
36. Corley DA, Jensen CD, Marks AR, Zhao WK, de Boer J, Levin TR, et al. Variation of adenoma prevalence by age, sex, race, and colon location in a large population: implications for screening and quality programs. *Clin Gastroenterol Hepatol* 2013;11:172–80. [PubMed: 22985608]
37. Lieberman DA, Williams JL, Holub JL, Morris CD, Logan JR, Eisen GM, et al. Race, ethnicity, and sex affect risk for polyps >9 mm in average-risk individuals. *Gastroenterology* 2014;147:351–8. [PubMed: 24786894]
38. Friedenbergh FK, Singh M, George NS, Sankineni A, Shah S. Prevalence and distribution of adenomas in black Americans undergoing colorectal cancer screening. *Dig Dis Sci* 2012;57:489–95. [PubMed: 22052446]
39. Xicola RM, Gagnon M, Clark JR, Carroll T, Gao W, Fernandez C, et al. Excess of proximal microsatellite-stable colorectal cancer in African Americans from a multiethnic study. *Clin Cancer Res* 2014;20:4962–70. [PubMed: 25013126]
40. Diaz-Montero CM, Finke J, Montero AJ. Myeloid-derived suppressor cells in cancer: therapeutic, predictive, and prognostic implications. *Semin Oncol* 2014;41:174–84. [PubMed: 24787291]
41. Akazawa Y, Kubo M, Zhang R, Matsumoto K, Yan F, Setiawan H, et al. Inhibition of arginase ameliorates experimental ulcerative colitis in mice. *Free Radic Res* 2013;47:137–45. [PubMed: 23215832]
42. OuYang LY, Wu XJ, Ye SB, Zhang RX, Li ZL, Liao W, et al. Tumor-induced myeloid-derived suppressor cells promote tumor progression through oxidative metabolism in human colorectal cancer. *J Transl Med* 2015;13:47. [PubMed: 25638150]
43. Solito S, Marigo I, Pinton L, Damuzzo V, Mandruzzato S, Bronte V. Myeloid-derived suppressor cell heterogeneity in human cancers. *Ann N Y Acad Sci* 2014;1319:47–65. [PubMed: 24965257]
44. Ortiz ML, Kumar V, Martner A, Mony S, Donthireddy L, Condamine T, et al. Immature myeloid cells directly contribute to skin tumor development by recruiting IL-17-producing CD4+ T cells. *J Exp Med* 2015;212:351–67. [PubMed: 25667306]

45. Zhang B, Wang Z, Wu L, Zhang M, Li W, Ding J, et al. Circulating and tumor infiltrating myeloid-derived suppressor cells in patients with colorectal carcinoma. *PLoS One* 2013;8:e57114. [PubMed: 23437326]
46. Nagaraj S, Gabrilovich DI. Myeloid-derived suppressor cells in human cancer. *Cancer J* 2010;16:348–53. [PubMed: 20693846]
47. Guinney J, Dienstmann R, Wang X, de Reynies A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. *Nat Med* 2015;21:1350–6. [PubMed: 26457759]
48. Wang L, Ren F, Wang Q, Baldrige LA, Monn MF, Fisher KW, et al. Significance of programmed death ligand 1 (PD-L1) immunohistochemical expression in colorectal cancer. *Mol Diagn Ther* 2016;20: 175–81. [PubMed: 26891728]
49. BolandPM MaWW. Immunotherapy for colorectal cancer. *Cancers* 2017;9 <https://www.ncbi.nlm.nih.gov/pubmed/28492495>.
50. Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. *Trends Immunol* 2015;36:265–76. [PubMed: 25797516]
51. Zarour HM. Reversing T-cell dysfunction and exhaustion in cancer. *Clin Cancer Res* 2016;22:1856–64. [PubMed: 27084739]
52. Nedelec Y, Sanz J, Baharian G, Szpiech ZA, Pacis A, Dumaine A, et al. Genetic ancestry and natural selection drive population differences in immune responses to pathogens. *Cell* 2016;167: 657–69. [PubMed: 27768889]
53. Sanoff HK, Sargent DJ, Green EM, McLeod HL, Goldberg RM. Racial differences in advanced colorectal cancer outcomes and pharmacogenetics: a subgroup analysis of a large randomized clinical trial. *J Clin Oncol* 2009;27:4109–15. [PubMed: 19636001]
54. Polite BN, Sing A, Sargent DJ, Grothey A, Berlin J, Kozloff M, et al. Exploring racial differences in outcome and treatment for metastatic colorectal cancer: results from a large prospective observational cohort study (BRiTE). *Cancer* 2012;118:1083–90. [PubMed: 21800287]
55. Elamin YY, Rafee S, Toomey S, Hennessy BT. Immune effects of bevacizumab: killing two birds with one stone. *Cancer Microenviron* 2015;8:15–21. [PubMed: 25326055]

Table 1.

A comparison of demographic, clinical, and tumor characteristics according to race

Variable	AA, % (n) n = 52	CA, % (n) n = 159	<i>P</i> ^a
Age (years)	60.5 (53.25–69.5)	66 (58–74)	0.05
Sex			0.5
Female (n = 105)	54 (28)	48 (77)	
Stage (n)			0.74
Early (n = 159)	63 (33)	66 (105)	
Late (n = 52)	37 (19)	34 (54)	
Colonic location			0.03
Proximal (n = 92)	60 (30)	42 (62)	
Distal (n = 45)	24 (12)	23 (33)	
Rectal (n = 60)	16 (8)	35 (52)	
Grade			0.7
Low (n = 176)	90 (44)	88 (132)	
High (n = 23)	10 (5)	12 (18)	
Histology ^b			0.7
NOS (n = 128)	65 (34)	59 (94)	
Mucinous <50% (n = 18)	8 (4)	9(14)	
Mucinous 50% (n = 39)	13 (7)	20 (32)	
Other (n = 26)	13 (7)	12 (19)	
Adjacent polyp			0.27
Tubular (n = 27)	21 (5)	33 (22)	
Villous (n = 61)	79 (19)	62 (42)	
SSA (n = 3)	0 (0)	5 (3)	
MSI status			0.08
MSS (n = 164)	94 (47)	84 (117)	
MSI (n = 25)	6 (3)	16 (22)	
Chemotherapy treatment			0.11
No (n = 120)	65 (34)	54 (86)	
Yes (n = 91)	35 (18)	46 (73)	
Intratumoral lymphocyte			0.02
None (n = 48)	19 (10)	23 (37)	
1–2 (n = 76)	23 (13)	40 (64)	
3+ (n = 87)	58 (29)	36 (58)	
Intratumoral aggregate			0.001
No (203)	88 (46)	99 (157)	
Yes (8)	12 (6)	1 (2)	
Peritumoral lymphocyte			0.73
No (n = 89)	44 (23)	42 (66)	
Yes (n = 122)	56 (29)	58 (93)	
Peritumoral aggregate (Crohns)			0.9

Variable	AA, % (n) n = 52	CA, % (n) n = 159	<i>P</i> ^a
No (n = 165)	79 (41)	78 (124)	0.75
Yes (n = 46)	21 (11)	22 (35)	
Overall lymphocyte score (n = 211)			
None (n = 25)	10 (5)	13 (20)	
Low (n = 69)	29 (15)	34 (54)	
Med (n = 81)	44 (23)	36 (58)	
High (n = 36)	17 (9)	17 (27)	

^a *P* values were determined using χ^2 tests (categorical variables) or *t* test (age).

^b Due to rounding, not all percentages add to 100%.

Table 2. The interaction between lymphocyte score and race on the risk of death in unadjusted and multivariable models

Lymphocyte score (n)	Model 1		Model 2		Model 3	
	CAs (n = 159), HR (95% CI)	AAs (n = 52), HR (95% CI)	CAs (n = 159), HR (95% CI)	AAs (n = 52), HR (95% CI)	CAs (n = 139), HR (95% CI)	AAs (n = 50), HR (95% CI)
None (25)	1.0	1.0	1.0	1.0	1.0	1.0
Low (69)	0.85 (0.37–1.96)	0.34 (0.11–1.09)	1.17 (0.48–2.48)	0.25 (0.07–0.24)	1.22 (0.38–3.97)	0.31 (0.08–1.13)
Medium (81)	0.78 (0.57–1.34)	0.32 (0.11–0.93)	0.86 (0.34–2.19)	0.18 (0.06–0.57)	0.88 (0.26–2.93)	0.22 (0.06–0.77)
High (36)	0.47 (0.15–1.45)	0.19 (0.04–0.99)	0.38 (0.11–1.26)	0.14 (0.03–0.80)	0.41 (0.10–1.69)	0.17 (0.03–1.03)
Pvalue for trend	0.34	0.02	0.47	0.003	0.64	0.02
Pvalue for interaction		0.17		0.05		0.14
None (25)	1.46(0.85–2.50)	3.29 (1.22–8.85)	1.14 (0.49–2.63)	4.48 (1.58–12.7)	1.14 (0.37–1.14)	3.72 (1.17–11.8)
High, med, and low (186)	1.0	1.0	1.0	1.0	1.0	1.0

Lymphocyte score	Model 4		Model 5		Model 6	
	CAs (n = 147), HR (95% CI)	AAs (n = 50), HR (95% CI)	CAs (n = 150), HR (95% CI)	AAs (n = 49), HR (95% CI)	CAs (n = 159), HR (95% CI)	AAs (n = 52), HR (95% CI)
None (25)	1.0	1.0	1.0	1.0	1.0	1.0
Low (69)	1.19 (0.48–2.97)	0.24 (0.06–0.86)	1.56 (0.59–4.13)	0.21 (0.06–0.72)	0.98 (0.38–2.51)	0.26 (0.08–0.89)
Medium (81)	1.03 (0.39–2.71)	0.19 (0.06–0.59)	1.03 (0.37–2.90)	0.17 (0.05–0.53)	0.82 (0.31–2.13)	0.19 (0.06–0.61)
High (36)	0.30 (0.08–1.31)	0.18 (0.03–1.18)	0.45 (0.13–1.61)	0.07 (0.01–0.64)	0.35 (0.10–1.22)	0.13 (0.02–0.77)
Pvalue for trend	0.47	0.006	0.83	0.001	0.36	0.004
Pvalue for interaction		0.06		0.02		0.08
None (25)	1.14 (0.48–2.70)	4.52 (1.58–12.95)	0.92 (0.36–2.32)	5.16 (1.78–14.90)	1.15 (0.50–2.67)	4.45 (1.57–12.63)
High, med, low (186)	1.0	1.0	1.0	1.0	1.0	1.0

NOTE: Model 1 univariate model; Model 2 adjusted for age, sex, stage, and treatment; Model 3 adjusted for age, sex, stage, treatment, and MSI status (MSS, MSI); Model 4 adjusted for age, sex, stage, treatment, and anatomic location (proximal colon, distal colon, and rectum); Model 5 age, sex, stage, treatment, and grade (low and high); and Model 6 adjusted for age, sex, stage, treatment, and histologic type. HRs in bold are significant at $P < 0.05$.

Table 3.

Risk of death by race (AAs vs. CAs) and categories of lymphocyte score in younger and older patients

	Risk of death in AAs vs. CAs					
	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 3 HR (95% CI)	Model 4 HR (95% CI)	Model 5 HR (95% CI)	Model 6 HR (95% CI)
All ages						
Ages^a						
Race						
AAs (<i>n</i> = 52)	1.63 (1.00–2.66)	1.83 (1.11–3.03)	1.75 (1.02–2.98)	1.85 (1.09–3.12)	1.76 (1.02–3.02)	1.84 (1.11–3.05)
CAs (<i>n</i> = 159)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
Lymphocyte score						
None (<i>n</i> = 25)	3.61 (1.17–11.08)	6.16 (1.94–19.53)	5.30 (1.24–22.71)	6.22 (1.96–19.84)	8.71 (2.48–30.54)	6.03 (1.90–19.18)
Low (<i>n</i> = 69)	1.44 (0.61–3.44)	1.32 (0.60–3.21)	1.33 (0.54–3.35)	1.21 (0.46–3.18)	1.15 (0.44–2.98)	1.34 (0.55–3.27)
Medium (<i>n</i> = 81)	1.46 (0.67–3.16)	1.31 (0.60–2.88)	1.35 (0.59–3.08)	1.11 (0.49–2.53)	1.41 (0.61–3.14)	1.34 (0.61–2.94)
High (<i>n</i> = 36)	1.45 (0.28–7.47)	2.32 (0.44–12.19)	2.19 (0.42–11.51)	3.59 (0.63–20.40)	1.34 (0.15–12.08)	2.26 (0.43–11.86)
Younger-age patients						
Race						
AAs (<i>n</i> = 23)	2.92 (1.18–7.22)	3.45 (1.17–10.17)	4.23 (1.28–14.00)	5.49 (1.48–20.32)	2.15 (0.72–6.39)	4.04 (1.27–12.82)
CAs (<i>n</i> = 43)	1.0	1.0	1.0	1.0	1.0	1.0
Lymphocyte score						
None-low (<i>n</i> = 35)	1.95 (0.57–6.71)	2.30 (0.64–8.29)	2.61 (0.61–11.26)	2.88 (0.63–13.09)	1.69 (0.42–6.73)	2.24 (0.62–8.16)
Medium-high (<i>n</i> = 31)	5.49 (1.09–26.82)	3.57 (0.66–19.19)	7.58 (0.82–69.81)	7.08 (0.79–63.47)	2.66 (0.47–14.97)	3.68 (0.68–19.87)
Older-age patients						
Race						
AAs (<i>n</i> = 14)	1.20 (0.54–2.67)	1.76 (0.70–4.37)	1.84 (0.67–5.03)	1.50 (0.60–3.78)	2.08 (0.81–5.37)	1.75 (0.72–4.32)
CAs (<i>n</i> = 60)	1.0	1.0	1.0	1.0	1.0	1.0
Lymphocyte score						
None-low (<i>n</i> = 26)	1.55 (0.48–4.94)	2.81 (0.78–10.06)	3.17 (0.67–14.76)	2.82 (0.76–10.42)	2.49 (0.67–9.18)	2.70 (0.75–9.73)
Medium-high (<i>n</i> = 48)	0.98 (0.32–2.99)	1.33 (0.42–4.19)	1.46 (0.45–4.70)	1.18 (0.37–3.70)	1.91 (0.58–6.33)	1.34 (0.43–4.21)

NOTE: Model 1 univariate model; Model 2 adjusted for age, sex, stage, and treatment; Model 3 adjusted for age, sex, stage, treatment, and MSI status (MSS, MSI); Model 4 adjusted for age, sex, stage, treatment, and anatomic location (proximal, distal, and rectum); Model 5 age, sex, stage, treatment, and grade (low and high); and Model 6 adjusted for age, sex, stage, treatment, and histologic type. HRs in bold are significant at $P < 0.05$.

^aYounger-age patients defined as those in the lowest tertile (< 59 years). Older-age patients are defined as the highest tertile (> 70 years of age). The $P_{\text{interaction}}$ between race and age (continuous) on survival is $P = 0.04$.