Statin use and colorectal cancer risk according to molecular subtypes in two large prospective cohort studies

Jung Eun Lee[1], **Yoshifumi Baba**[2], **Kimmie Ng**[2], **Edward Giovannucci**[3,4], **Charles S. Fuchs**[2,3], **Shuji Ogino**[2,5], and **Andrew T. Chan**[3,6]

[1] Department of Food and Nutrition, Sookmyung Women's University, Seoul, Korea

[2] Department of Medical Oncology, Dana-Farber Cancer Institute, Boston

[3] Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts

[4] Departments of Nutrition and Epidemiology, Harvard School of Public Health, Boston, Massachusetts

[5] Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts

[6] Gastrointestinal Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

Abstract

Use of statins is hypothesized to reduce colorectal cancer risk, but the evidence remains inconsistent. This may be partly explained by differential associations according to tumor location or molecular subtypes of colorectal cancer. We examined the association between statin use and colorectal cancer risk according to tumor location, *KRAS* mutation status, microsatellite instability (MSI) status, PTGS2 (cyclooxygenase-2, COX-2) expression, or CpG island methylator phenotype (CIMP) status in two large prospective cohort studies, the Nurses' Health Study and Health Professionals Follow-up Study. We applied Cox regression to a competing-risks analysis. We identified 1818 colorectal cancers during 1990-2006. Compared to non-users, current statin use was not associated with colorectal cancer (Relative Risk [RR] = 0.99, 95% CI = 0.86 to 1.14) or colon cancer (RR = 1.10, 95% CI = 0.94 to 1.29), but was inversely associated with rectal cancer (RR = 0.59, 95% CI = 0.41 to 0.84, P for heterogeneity <0.001). When we examined the association within strata of *KRAS* mutation status, we found no association with *KRAS*-mutated cancers (RR = 1.20, 95% CI = 0.87 to 1.67), but did observe a possible inverse association among *KRAS*-wildtype cancers (RR = 0.80, 95% CI = 0.60 to 1.06, P for heterogeneity=0.06). The association did not substantially differ by PTGS2 expression, microsatellite instability (MSI) status or CIMP status. Current statin use was not associated with risk of overall colorectal cancer. The possibility that statin use may be associated with lower risk of rectal cancer or *KRAS*-wild type colorectal cancer requires further confirmation.

Corresponding Author: Andrew T. Chan, MD, MPH, Gastrointestinal Unit, Massachusetts General Hospital, 55 Fruit Street, GRJ 728A, Boston, Massachusetts 02114. achan@partners.org; fax: (617) 724-6832.
Jung Eun Lee and Yoshifumi Baba contributed equally. Shuji Ogino and Andrew T. Chan contributed equally.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Introduction

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, commonly known as statins, are widely used in the treatment of hypercholesterolemia. In experimental studies, statins inhibit colorectal carcinogenesis. However, human studies have been inconsistent. In some case-control studies, statins are associated with a reduction in the risk of colorectal cancer, but most cohort studies and secondary analyses of randomized clinical trials have not shown a benefit (1-3).

These conflicting data may be in part related to the molecular heterogeneity of colorectal cancer. Cancers have distinct molecular alterations that may predict variation in clinical behavior or responsiveness to therapeutic agents as well as reflect differences in etiopathogenic mechanism. Statins inhibit generation of mevalonate, a precursor for synthesis of isoprenoids used in prenylation, a necessary post-translational step for functional *KRAS*. Similarly, statins have been hypothesized to be anti-inflammatory and in some studies and function synergistically with drugs with cyclooxygenase-2 (PTGS2, COX-2) activity such as aspirin and non-steroidal anti-inflammatory drugs (NSAIDs)(4-7). In addition, clinical features and risk factors appear to vary according to microsatellite instability (MSI) status, CpG island methylator phenotype (CIMP) status, or tumor location (8-13). We therefore prospectively examined whether the association of statin use and colorectal cancer risk differed by *KRAS* mutation status, PTGS2 (COX-2) overexpression, MSI, or CIMP status. To our knowledge, no prior prospective studies have examined the association between statin use and colorectal cancer risk in relation to these tumor subtypes.

Materials and Methods

Study population

The Nurses' Health Study (NHS) was established in 1976, when 121,700 female registered nurses who were 30 to 55 years of age returned a mailed questionnaire. The Health Professionals Follow-up Study (HPFS) was initiated in 1986 when 51,529 male health professionals aged 40 to 75 years returned a mailed questionnaire. Participants provided detailed information about their medical history, lifestyle, and risk factors for chronic diseases on biennial follow-up questionnaires. Because retrospective information on statin use was collected in 2000 to define statin use from 1994 forward in the NHS and from 1990 forward in the HPFS, we started follow-up in 1994 in the NHS and 1990 in the HPFS. This approach is consistent with prior analyses in these cohorts (14-15).

We excluded participants with missing data on statin use, had been previously diagnosed with cancer (except non-melanoma skin cancer), or had ulcerative colitis. As a result, 91,155 women in the NHS and 40,767 men in the HPFS were available for the analysis. Because there was no heterogeneity for the association of statin use and colorectal cancer between NHS and HPFS (P for heterogeneity >0.33 for each category of statin duration), we combined data from the two cohorts. The institutional review boards at Brigham and Women's Hospital and the Harvard School of Public Health approved this study.

Colorectal cancer cases and their molecular markers

Self-reported information on new diagnoses of colorectal cancers was obtained on each questionnaire; participants (or next-of-kin for those who died) who reported a diagnosis of colorectal cancer were asked for permission to access medical records related to the diagnosis. The National Death Index was also used to identify fatalities (16). Investigators blinded to participants' risk factor status reviewed medical records to confirm the diagnosis, and classify cancers according to anatomic location, and histologic types. A total of 1818 (952 in the NHS and 866 in the HPFS) cases were included in these analyses.

We retrieved, from the pathology departments of treating hospitals, available pathological specimens from participants diagnosed with colorectal cancer; 438 cases (51%) diagnosed in the HPFS and 449 cases (47%) in the NHS through 2006. The baseline characteristics of participants with colorectal cancer whose tumors we analyzed were similar to those of participants whose tumors we did not analyze (17). We extracted DNA from paraffin-embedded tumor tissue sections and performed PCR and pyrosequencing targeted for *KRAS* codons 12 and 13 as previously described (18). MSI status was assayed using 10 microsatellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67, and D18S487)(19). If instability of markers was 30% or more, tumors were classified as MSI-high. We quantified DNA methylation in eight CIMP-specific promoters [*CACNA1G, CDKN2A* (p16), *CRABP1, IGF2, MLH1, NEUROG1, RUNX3,* and *SOCS1*](20-22). We defined CIMP-high if six or more of eight promoters were methylated, CIMP-low if one to five of eight promoters were methylated or CIMP-0 if none of eight were methylated. PTGS2 (COX-2) immunohistochemical assays were performed from incubating deparaffinized tissue sections as previously described (19, 23). Compared to adjacent normal colonic mucosa, PTGS2 overexpression in tumor cells was classified using a standardized grading system (absent, weak, moderate, or strong). The pathologist classified staining of tumor cells as "absent" if PTGS2 expression was at the same level of intensity as adjacent normal colonic epithelium; weak, moderate, or strong staining indicated progressively increasing degrees of overexpression (17, 23). Consistent with our prior analyses, absent or weak overexpression was categorized as PTGS2-negative, and moderate or strong was categorized as PTGS2-positive. A pathologist (S.O.) blinded to statin use or any other data interpreted tumor marker status. A random sample of 108 cancers was reread by a second pathologist unaware of the data on the participants; the concordance between the two pathologists was 0.92 (K=0.62; P<.0001)(17).

Assessment of statin use

In 2000, women in the NHS were asked whether they regularly used statins and their duration of statin use in categories (0-2, 3-5, and 6+ years). Similarly, men in the HPFS were asked whether they regularly used statins and their duration of statin use in categories (1-2, 3-5, 6-9 and 10+ years). We did not specifically query participants about the dose of statin use. Consistent with prior studies (14-15), we retrospectively defined statin use from 1994 forward in the NHS and from 1990 forward in the HPFS using the 2000 questionnaire. Participants updated their current use of statins on each biennial questionnaire after 2000. Duration of statin use was calculated by using the retrospective responses on the 2000 questionnaires and updating the subsequent responses to current use on the biennial questionnaires from 2002. Our assessment of covariates for our analyses has been previously reported (24).

Statistical analysis

We calculated the relative risks (RRs) and 95% confidence intervals (95% CIs) associated with current statin use for each study using the Cox proportional hazards model (25) with SAS PROC PHREG (26). Person-years of follow-up were calculated from the date of return

of the baseline questionnaire to the date of colorectal cancer diagnosis, date of death, or end of follow-up (May 31, 2006, for women, and January 31, 2006, for men), whichever came first. We stratified the data by study, age, and calendar year of the current questionnaire cycle. Because participants may have varied their use of statins over the study period, we utilized time-varying covariates such that each individual participant contributed person-time according to the statin data they provided on each biennial questionnaire. Consistent with secular patterns in the chronic use of these drugs, very few individuals who reported current use subsequently discontinued use (e.g. only 4% of those who used statins in 2000 did not continue using them through 2004). Thus, we did not have a sufficiently large enough number of former users to conduct a statistically robust analysis of former use; if a participant subsequently reported discontinuing use, he/she then contributed person-time to the category of nonusers. In the multivariate models, we adjusted for time-varying covariates listed in the footnotes of the Tables.

We used a competing-risk analysis using duplication-method Cox regression to examine whether the association between statin use and colorectal cancer differed by tumor sites or molecular subtypes (27-28). To test for the difference between tumor sites or molecular subtype, we compared the model fit that produces separate associations of statin use with different tumor sites or molecular markers to the model fit that assumed a common association using the likelihood ratio test. For the test for trend, participants were assigned the median value of statin duration and this variable was entered into the model as a continuous term. To examine whether the association for statin use varied by BMI, age, aspirin use, and alcohol intake, a likelihood ratio test was used to compare the model fit including the cross-product term of statin use and the specific modifier with the model fit without the cross-product term. All statistical tests were two-sided, and P values less than .05 were considered statistically significant.

Results

Table 1 shows the characteristics of 91,155 eligible women and 40,767 eligible men according to duration of statin use reported in 2000. Women and men who used statins were more likely to be older, to use aspirin and multivitamins, to have ever had endoscopy, and to smoke in the past than those who did not use statins. Women who used statins were more likely to have higher BMI and less likely to drink alcohol. Compared to non-users, the prevalence of hypercholesterolemia (>=240 mg/dl) was higher among those men and women who began to use statins recently. In contrast, the prevalence of hypercholesterolemia was generally lower for men and women who had been on long-term statin treatment.

We documented 1818 colorectal cancers (952 in women and 866 in men) over 1,688,745 person-years of follow-up. We observed no significant association between the duration of statin use and overall risk of colorectal cancer (Table 2). The age-adjusted RR of colorectal cancer was 0.85 (95% CI = 0.66 to 1.10) comparing 6 or more years of statin use to nonusers. When we additionally adjusted for use of aspirin and history of endoscopy, the RR was attenuated (multivariate RR = 0.97, 95% CI = 0.75 to 1.25). Further adjustment for other covariates did not alter the association (multivariate RR = 0.97, 95% CI = 0.75 to 1.25).

We examined whether the association between statin use and colorectal cancer varied according to the anatomic site of the tumor (Table 3). Compared to non-users, statin users did not have a lower risk of colon cancer (multivariate RR = 1.10, 95% CI = 0.94 to 1.29); similarly, there was no association with cancer of the distal (multivariate RR = 1.03, 95% CI = 0.79 to 1.34) or proximal (multivariate RR = 1.14, 95% CI = 0.93 to 1.39) colon. In contrast, the effect of statins appeared to differ for rectal cancer compared with colon cancer

(P for heterogeneity<0.001). Compared to non-users, statin users had a significantly lower risk of rectal cancer (multivariate RR = 0.59, 95% CI = 0.41 to 0.84).

We confirmed that the lack of association between statin use and risk of colorectal cancer was consistent for cases with tissue available for analysis of molecular subtypes (multivariate RR = 0.92, 95% CI = 0.74 to 1.13) (Table 4) compared with cases without available tissue (multivariate RR = 1.07, 95% CI = 0.89 to 1.29). We evaluated the influence of statins on risk of colorectal cancer according to *KRAS* mutation status. Compared with non-users, we found a lower, but non-significant, risk of *KRAS*-wildtype cancer in statin users (multivariate RR = 0.80, 95% CI = 0.60 to 1.06). In contrast, statin use was not associated with the risk of *KRAS*-mutated colorectal cancer (multivariate RR = 1.20, 95% CI = 0.87 to 1.67). A formal test for heterogeneity for the association of statin use with *KRAS*-mutated or *KRAS*-wildtype tumors approached statistical significance (P for heterogeneity = 0.06). When we confined the analysis to rectal cancers, we also observed a non-significant inverse association between statin use and risk of *KRAS*-wildtype rectal cancers (multivariate RR = 0.65, 95% CI = 0.32 to 1.32) but not *KRAS*-mutated rectal cancer (multivariate RR = 0.90, 95% CI = 0.42 to 1.94).

We also examined the effect of statins according to PTGS2 (COX-2) overexpression, MSI, or CIMP status. There did not appear to be any differential association between statin use and risk of PTGS2 positive tumors compared with PTGS2 negative tumors (P for heterogeneity = 0.40), tumors defined as MSI-high compared with MSS or MSI-low (P for heterogeneity = 0.50) or tumors defined as CIMP-high compared with CIMP-low/CIMP-0 (P for heterogeneity = 0.83).

Finally, we examined whether the associations between statin use and colorectal cancer varied by subgroups defined by other colorectal cancer risk factors, including BMI (<25, 25-<30, ≥30 kg/m^2), age (<65, ≥65 years), aspirin use (none, ≤5, ≥6 tablets/wk), and alcohol intake (none, <15, ≥15 g/d). There did not appear to be any significant associations according to any of these strata (all P for interaction > 0.5).

Discussion

In this large, prospective study of two well-characterized, population-based cohorts, we found that statin use was not significantly associated with overall risk of colorectal cancer. However, we did observe a potential inverse association with risk of rectal cancer. In addition, our results suggest a possible differential association according to *KRAS* mutation status. To our knowledge, our study is the first to examine statin use and colorectal cancer according to molecular features of the tumor.

Substantial experimental data support a potential anti-cancer effect of statins. Statins induce apoptosis, inhibit cell proliferation, attenuate angiogenesis, and delay the metastatic process in *in vitro* and *in vivo* studies (29-33). However, the results from human studies have not been consistent. Two systematic reviews observed modest reductions in colorectal cancer with statin use in case-control studies, but no clear association in randomized clinical trials or cohort studies (1-3, 34). The lack of association between statin use and overall colorectal cancer we observed in this study is largely consistent with prior cohort studies. Generally, cohort studies may be less prone to the biases of case-control studies, which include differential sampling of cases and controls and differential recall of statin use or other confounding factors by cases compared with controls.

We did observe a potential inverse association between statin use and rectal cancer. Because this finding is based on a relatively limited number of rectal cancer cases, these results should be interpreted cautiously. However, a test for heterogeneity of the association of

statin use with colon or rectal cancer was statistically significant and a differential effect by site is biologically plausible. There are significant differences in risk factors, prevalence of specific molecular alterations, and gene expression levels that have been described according to cancer site (10, 35-36). A few studies have similarly observed inverse associations between statin use and rectal cancer (37-39). Statin use was associated with 30-62% lower risk of rectal cancer in case-control studies that included 136 cases in US (39) and 344 cases Israel (38). Using information from a pharmacy database linked to hospital records in the Netherlands, a prospective cohort study observed a RR of 0.48 (0.95% CI = 0.16-1.48) of rectal cancer associated with statin use. However, others have not found inverse associaions (40-44). Further investigation of the effect of statins on rectal cancer compared with colon cancer is warranted.

We examined the effect of statin use according to risk of cancers defined by *KRAS* mutation status. Although the specific mechanism by which statins are chemopreventative is not known, statins are hypothesized, at least in part, to modulate *KRAS*, which mediates downstream signaling of the epidermal growth factor receptor (EGFR). Statins inhibit the mevalonate pathway thereby suppressing biosynthesis of cholesterol as well as isoprenoids utilized for the post-translational prenylation of RHO and RAS proteins, including *KRAS*. Prenylation is a requisite step for functional *KRAS* in its signaling and transforming activities (6). Mutations in *KRAS*, found in 40% of colorectal cancers, lead to a constitutively active state of cell growth and proliferation independent of EGFR signaling. Previous work has demonstrated that EGFR inhibitors are effective in treatment of *KRAS*-wildtype colorectal cancer but not *KRAS*-mutant colorectal cancer (45). Our data similarly suggest that a potential inverse association between statin use and colorectal cancer may be limited to *KRAS*-wildtype tumors. This highlights the possibility that statins have a more important effect on *KRAS* signaling driven by EGFR activation than *KRAS* that is permanently activated through mutation. Nonetheless, these findings should be interpreted with caution since the p value for interaction was not statistically significant, particularly in view of the multiple hypotheses tested.

Beyond mevalonate pathways, statins have been proposed to inhibit cancer through its anti-inflammatory mechanisms (46). Although experimental studies suggest that statins may act synergistically with NSAIDs (5-7), most, but not all (4) human studies have not seen significant modification of the effect of statins by concurrent use of NSAIDs (38-39, 42, 47-48). In our study, we also did not observe a differential effect of statin use according to use of aspirin or expression of the pro-inflammatory enzyme PTGS2 (COX-2). However, it remains possible that statins may have anti-inflammatory effects that are independent of aspirin or COX-2 related pathways.

Finally, we also considered the effect of statins according to tumor subtypes defined by MSI or CIMP status. About 15% of sporadic colorectal cancers are MSI-H, arising primarily through epigenetic silencing of DNA mismatch repair proteins (49). In contrast, the vast majority of sporadic colorectal cancers are MSS or MSI-L, developing through traditional chromosomal instability pathways. Colorectal cancer with CIMP-high status was associated with MSI and is well characterized as a major epigenetic marker in colorectal cancer (13, 21-22). However, in the present analysis, we did not observe a differential effect of statins on cancers subtyped by MSI or CIMP status.

Our study has several important strengths. First, our prospective cohort design minimized potential biases introduced by differential selection and recall found in case-control studies. Second, we collected detailed and updated information on statin use and other potential important confounding factors in a cohort with a high follow-up rate. Third, because our participants were all health professionals, the accuracy of self-reported statin use is likely to

be high and is more likely to reflect their actual use than prescription records. Finally, we examined the effect of statins on molecularly-defined subtypes of colorectal cancer, an example of the emerging interdisciplinary field of "*Molecular Pathologic Epidemiology* (MPE)"(8, 50), Through molecular pathologic epidemiology studies, a known or suspected etiologic or modifying lifestyle factor, such as statin use, can be related to a specific somatic molecular change to gain insight into mechanism, provide evidence for causality, and potentially lead to more targeted approaches to prevention or therapy (8, 50).

There are several limitations to our study. First, our study is observational. Thus, we cannot rule out the possibility of residual confounding. However, our overall findings are consistent with most prospective cohort studies. Moreover, our results suggesting an inverse association with *KRAS*-wildtype tumors has biological plausibility given our understanding of the effect of statins on the mevalonate pathway. Second, the prevalence of statin use, especially in the longer duration categories, was relatively low. However, as a prospective cohort study, our prevalence reflects secular trends in statin use (15% of current use) in the U.S. over the time period of the study (1994-2006) and is consistent with the prevalence of statin use described in another U.S. population-based cohort (20% of current use) conducted in an overlapping time period (1997-2001)(47). Nonetheless, as the number of individuals prescribed statins increases over time, further studies with more extended follow-up are needed. Third, we did not specifically examine different types of statins. Although it has been suggested that specific statins may have distinct effects depending on whether they are hydrophilic or lipophilic, no studies that have directly compared various statins in the same population have observed significant differences in associations. Finally, we did not have tumor tissue available from all cases of confirmed colorectal cancer ascertained in the two cohorts. However, the characteristics of cases without available tumor tissue did not appreciably differ from those of cases with available tumor tissue (17).

In summary, our study does not support an overall effect of statin use on risk of colorectal cancer. However, a possible inverse association with risk of rectal cancer or *KRAS*-wildtype colorectal cancer requires further investigation. If confirmed, our results would provide mechanistic insight into the anti-cancer effects of statins and support the potential use of molecular markers to tailor chemoprevention.

Acknowledgments

References

1. Bonovas S, Filioussi K, Flordellis CS, Sitaras NM. Statins and the risk of colorectal cancer: a meta-analysis of 18 studies involving more than 1.5 million patients. J Clin Oncol. 2007; 25:3462–8. [PubMed: 17687150]

2. Bardou M, Barkun A, Martel M. Effect of statin therapy on colorectal cancer. Gut. 2010; 59:1572–85. [PubMed: 20660702]

3. Robertson DJ, Riis AH, Friis S, Pedersen L, Baron JA, Sorensen HT. Neither Long-Term Statin Use nor Atherosclerotic Disease Is Associated With Risk of Colorectal Cancer. Clin Gastroenterol Hepatol. 2010; 8:1056–61. [PubMed: 20816860]

4. Hoffmeister M, Chang-Claude J, Brenner H. Individual and joint use of statins and low-dose aspirin and risk of colorectal cancer: a population-based case-control study. Int J Cancer. 2007; 121:1325–30. [PubMed: 17487832]

5. Agarwal B, Rao CV, Bhendwal S, Ramey WR, Shirin H, Reddy BS, et al. Lovastatin augments sulindac-induced apoptosis in colon cancer cells and potentiates chemopreventive effects of sulindac. Gastroenterology. 1999; 117:838–47. [PubMed: 10500066]

6. Swamy MV, Patlolla JM, Steele VE, Kopelovich L, Reddy BS, Rao CV. Chemoprevention of familial adenomatous polyposis by low doses of atorvastatin and celecoxib given individually and in combination to APCMin mice. Cancer Res. 2006; 66:7370–7. [PubMed: 16849589]

7. Reddy BS, Wang CX, Kong AN, Khor TO, Zheng X, Steele VE, et al. Prevention of azoxymethane-induced colon cancer by combination of low doses of atorvastatin, aspirin, and celecoxib in F 344 rats. Cancer Res. 2006; 66:4542–6. [PubMed: 16618783]

8. Ogino S, Chan AT, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. Gut. 2010

9. Limsui D, Vierkant RA, Tillmans LS, Wang AH, Weisenberger DJ, Laird PW, et al. Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. J Natl Cancer Inst. 2010; 102:1012–22. [PubMed: 20587792]

10. Wei EK, Giovannucci E, Wu K, Rosner B, Fuchs CS, Willett WC, et al. Comparison of risk factors for colon and rectal cancer. Int J Cancer. 2004; 108:433–42. [PubMed: 14648711]

11. Campbell PT, Jacobs ET, Ulrich CM, Figueiredo JC, Poynter JN, McLaughlin JR, et al. Case-control study of overweight, obesity, and colorectal cancer risk, overall and by tumor microsatellite instability status. J Natl Cancer Inst. 2010; 102:391–400. [PubMed: 20208017]

12. Nosho K, Irahara N, Shima K, Kure S, Kirkner GJ, Schernhammer ES, et al. Comprehensive biostatistical analysis of CpG island methylator phenotype in colorectal cancer using a large population-based sample. PLoS ONE. 2008; 3:e3698. [PubMed: 19002263]

13. Samowitz WS, Albertsen H, Herrick J, Levin TR, Sweeney C, Murtaugh MA, et al. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. Gastroenterology. 2005; 129:837–45. [PubMed: 16143123]

14. Eliassen AH, Colditz GA, Rosner B, Willett WC, Hankinson SE. Serum lipids, lipid-lowering drugs, and the risk of breast cancer. Arch Intern Med. 2005; 165:2264–71. [PubMed: 16246993]

15. Tsai CJ, Leitzmann MF, Willett WC, Giovannucci EL. Statin use and the risk of cholecystectomy in women. Gastroenterology. 2009; 136:1593–600. [PubMed: 19208351]

16. Stampfer MJ, Willett WC, Speizer FE, Dysert DC, Lipnick R, Rosner B, et al. Test of the National Death Index. Am J Epidemiol. 1984; 119:837–9. [PubMed: 6720679]

17. Chan AT, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. N Engl J Med. 2007; 356:2131–42. [PubMed: 17522398]

18. Ogino S, Kawasaki T, Brahmandam M, Yan L, Cantor M, Namgyal C, et al. Sensitive sequencing method for KRAS mutation detection by Pyrosequencing. J Mol Diagn. 2005; 7:413–21. [PubMed: 16049314]

19. Ogino S, Brahmandam M, Cantor M, Namgyal C, Kawasaki T, Kirkner G, et al. Distinct molecular features of colorectal carcinoma with signet ring cell component and colorectal carcinoma with mucinous component. Mod Pathol. 2006; 19:59–68. [PubMed: 16118624]

20. Ogino S, Kawasaki T, Kirkner GJ, Kraft P, Loda M, Fuchs CS. Evaluation of markers for CpG island methylator phenotype (CIMP) in colorectal cancer by a large population-based sample. J Mol Diagn. 2007; 9:305–14. [PubMed: 17591929]

21. Ogino S, Cantor M, Kawasaki T, Brahmandam M, Kirkner GJ, Weisenberger DJ, et al. CpG island methylator phenotype (CIMP) of colorectal cancer is best characterised by quantitative DNA methylation analysis and prospective cohort studies. Gut. 2006; 55:1000–6. [PubMed: 16407376]

22. Weisenberger DJ, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet. 2006; 38:787–93. [PubMed: 16804544]

23. Ogino S, Brahmandam M, Kawasaki T, Kirkner GJ, Loda M, Fuchs CS. Combined analysis of COX-2 and p53 expressions reveals synergistic inverse correlations with microsatellite instability and CpG island methylator phenotype in colorectal cancer. Neoplasia. 2006; 8:458–64. [PubMed: 16820091]

24. Lee JE, Willett WC, Fuchs CS, Smith-Warner SA, Wu K, Ma J, et al. Folate intake and the risk of colorectal cancer and adenoma: modification by time. Am J Clin Nutr. 2011; 93:817–25. [PubMed: 21270374]

25. Cox DR. Regression models and life-tables. J Royal Stat Soc. 1972; 34:187–220.

26. SAS Institute Inc.. SAS/STAT User's Guide, Release 91. Cary, NC: SAS Institute Inc.; 2004. The PHREG Procedure.

27. Lunn M, McNeil D. Applying Cox regression to competing risks. Biometrics. 1995; 51:524–32. [PubMed: 7662841]

28. Glynn RJ, Rosner B. Comparison of risk factors for the competing risks of coronary heart disease, stroke, and venous thromboembolism. Am J Epidemiol. 2005; 162:975–82. [PubMed: 16207808]

29. Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. Nat Rev Cancer. 2005; 5:930–42. [PubMed: 16341084]

30. Denoyelle C, Vasse M, Korner M, Mishal Z, Ganne F, Vannier JP, et al. Cerivastatin, an inhibitor of HMG-CoA reductase, inhibits the signaling pathways involved in the invasiveness and metastatic properties of highly invasive breast cancer cell lines: an in vitro study. Carcinogenesis. 2001; 22:1139–48. [PubMed: 11470741]

31. Calleros L, Sanchez-Hernandez I, Baquero P, Toro MJ, Chiloeches A. Oncogenic Ras, but not (V600E)B-RAF, protects from cholesterol depletion-induced apoptosis through the PI3K/AKT pathway in colorectal cancer cells. Carcinogenesis. 2009; 30:1670–7. [PubMed: 19700418]

32. Yasui Y, Suzuki R, Miyamoto S, Tsukamoto T, Sugie S, Kohno H, et al. A lipophilic statin, pitavastatin, suppresses inflammation-associated mouse colon carcinogenesis. Int J Cancer. 2007; 121:2331–9. [PubMed: 17657716]

33. Cho SJ, Kim JS, Kim JM, Lee JY, Jung HC, Song IS. Simvastatin induces apoptosis in human colon cancer cells and in tumor xenografts, and attenuates colitis-associated colon cancer in mice. Int J Cancer. 2008; 123:951–7. [PubMed: 18521906]

34. Singh H, Mahmud SM, Turner D, Xue L, Demers AA, Bernstein CN. Long-term use of statins and risk of colorectal cancer: a population-based study. Am J Gastroenterol. 2009; 104:3015–23. [PubMed: 19809413]

35. Iacopetta B. Are there two sides to colorectal cancer? Int J Cancer. 2002; 101:403–8. [PubMed: 12216066]

36. Dimberg J, Samuelsson A, Hugander A, Soderkvist P. Differential expression of cyclooxygenase 2 in human colorectal cancer. Gut. 1999; 45:730–2. [PubMed: 10517910]

37. Graaf MR, Beiderbeck AB, Egberts AC, Richel DJ, Guchelaar HJ. The risk of cancer in users of statins. J Clin Oncol. 2004; 22:2388–94. [PubMed: 15197200]

38. Poynter JN, Gruber SB, Higgins PD, Almog R, Bonner JD, Rennert HS, et al. Statins and the risk of colorectal cancer. N Engl J Med. 2005; 352:2184–92. [PubMed: 15917383]

39. Coogan PF, Smith J, Rosenberg L. Statin use and risk of colorectal cancer. J Natl Cancer Inst. 2007; 99:32–40. [PubMed: 17202111]

40. Kaye JA, Jick H. Statin use and cancer risk in the General Practice Research Database. Br J Cancer. 2004; 90:635–7. [PubMed: 14760377]

41. Boudreau DM, Koehler E, Rulyak SJ, Haneuse S, Harrison R, Mandelson MT. Cardiovascular medication use and risk for colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2008; 17:3076–80. [PubMed: 18957524]

42. Flick ED, Habel LA, Chan KA, Haque R, Quinn VP, Van Den Eeden SK, et al. Statin use and risk of colorectal cancer in a cohort of middle-aged men in the US: a prospective cohort study. Drugs. 2009; 69:1445–57. [PubMed: 19634923]

43. Haukka J, Sankila R, Klaukka T, Lonnqvist J, Niskanen L, Tanskanen A, et al. Incidence of cancer and statin usage--record linkage study. Int J Cancer. 2010; 126:279–84. [PubMed: 19739258]

44. Friedman GD, Flick ED, Udaltsova N, Chan J, Quesenberry CP Jr, Habel LA. Screening statins for possible carcinogenic risk: up to 9 years of follow-up of 361,859 recipients. Pharmacoepidemiol Drug Saf. 2008; 17:27–36. [PubMed: 17944002]

45. Allegra CJ, Jessup JM, Somerfield MR, Hamilton SR, Hammond EH, Hayes DF, et al. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in

patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol. 2009; 27:2091–6. [PubMed: 19188670]

46. Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. Nat Rev Drug Discov. 2005; 4:977–87. [PubMed: 16341063]

47. Jacobs EJ, Rodriguez C, Brady KA, Connell CJ, Thun MJ, Calle EE. Cholesterol-lowering drugs and colorectal cancer incidence in a large United States cohort. J Natl Cancer Inst. 2006; 98:69–72. [PubMed: 16391373]

48. Shadman M, Newcomb PA, Hampton JM, Wernli KJ, Trentham-Dietz A. Non-steroidal anti-inflammatory drugs and statins in relation to colorectal cancer risk. World J Gastroenterol. 2009; 15:2336–9. [PubMed: 19452574]

49. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res. 1998; 58:5248–57. [PubMed: 9823339]

50. Ogino S, Stampfer M. Lifestyle factors and microsatellite instability in colorectal cancer: the evolving field of molecular pathological epidemiology. J Natl Cancer Inst. 2010; 102:365–7. [PubMed: 20208016]

Abbreviations

CI	confidence interval
HMG-CoA	3-Hydroxy-3-methylglutaryl-coenzyme A
MSI	microsatellite instability
PTGS2	COX-2, cyclooxygenase-2
CIMP	CpG island methylator phenotype
NHS	Nurses' Health Study
HPFS	Health Professionals Follow-up Study
EGFR	epidermal growth factor receptor
NSAID	non-steroidal anti-inflammatory drugs

Table 1

Characteristics of participants in 2000 according to statin use in the Nurses' Health Study (NHS) and Health Professionals' Follow-up Study (HPFS) [*]

	Duration of statin use			
	Nonusers	<2 years	3-5 years	≥6 years
Nurses' Health Study				
No. of participants	75597	7672	5048	2838
Age (y)	66.4	67.7	68.3	69.2
Body mass index (kg/m^2)	23.0	27.3	27.5	26.9
Alcohol intake (g/d)	5.1	4.3	4.3	4.5
Red meat intake (servings/d)	0.9	0.9	0.9	0.9
Aspirin use, ≥2 tablets/wk (%)[†]	14.3	25.5	25.6	29.4
Ever had endoscopy (%)	52.5	59.0	60.6	63.0
Smoking status (%)				
Past	45.2	48.4	48.7	51.7
Current	9.5	8.7	9.6	9.2
Current multivitamin use (%)	53.6	67.7	66.6	67.2
Serum cholesterol levels, ≥240 mg/dL (%)[‡]	13.0	23.1	12.3	14.9
Health Professionals' Follow-up Study				
No. of participants	33685	3315	2299	1468
Age (y)	67.9	67.4	68.0	68.7
Body mass index (kg/m^2)	20.2	26.0	26.4	26.3
Alcohol intake (g/d)	10.8	11.0	11.1	11.2
Red meat intake (servings/d)	1.2	1.0	1.0	0.8
Aspirin use, ≥2 days/wk (%)[†]	28.9	62.3	66.5	67.1
Ever had endoscopy (%)	68.8	78.1	78.2	79.1
Smoking status (%)				
Past	48.6	52.8	55.2	56.5
Current	5.1	3.8	3.8	3.2
Current multivitamin use (%)	45.7	66.4	69.0	66.8
Serum cholesterol levels, ≥240 mg/dL (%)[‡]	6.3	7.8	2.7	4.1

[*] Values are standardized to the age distribution of the study except age. Mean values except those presented as percent

[†] Standard (326 mg) tablets

[‡] Self-reported total serum cholesterol level

Table 2

Relative risk (RR) of colorectal cancer according to statin use

	Nonusers	Duration of statin use			P for trend
		0-2	3-5 years	≥6 years	
Women					
No. of cases	804	68	44	36	
Multivariate-adjusted *	1.00	0.92 (0.72-1.18)	0.98 (0.72-1.34)	1.01 (0.71-1.43)	0.93
Men					
No. of cases	756	51	30	29	
Multivariate-adjusted *	1.00	1.12 (0.83-1.51)	1.05 (0.72-1.54)	0.95 (0.64-1.40)	0.96
Pooled					
Person-years	1,438,154	114,469	69,209	66,913	
Age-adjusted	1.00	0.94 (0.78 to 1.14)	0.93 (0.73 to 1.17)	0.85 (0.66 to 1.10)	0.17
Multivariate-adjusted*	1.00	0.99 (0.82 to 1.20)	1.01 (0.79 to 1.28)	0.97 (0.75 to 1.25)	0.85

*
Adjusted for age, calendar year, study, pack-years of smoking before age 30 (never smoker, 1-4 pack-years, 5-10 pack-years, and ≥ 11 pack-years of smoking), aspirin dose (never, past, current use 1-2, 3-5, 6-14, ≥15 tablets/wk), height (continuous), BMI (<23, 23-<25, 25-<28, 28-<30, ≥30 kg/m^2 in the NHS; <23, 23-<25, 25-<30, 30-<35, ≥35kg/m^2 in the HPFS), family history of colorectal cancer in parents and siblings (yes, no), history of endoscopy (yes, no), red meat intake (quintiles), alcohol intake (never, 0.1-9.9 g/d, 10-14.9 g/d, 15-29.9 g/d, ≥30 g/d), and total energy intake (continuous)

Table 3
Relative risk (RR) of colon and rectal cancer according to statin use

Tumor site	Nonusers	Current use
Colon and Rectum [†]		
No. of cases	1446	234
Age-adjusted RR	1.00	0.90 (0.78 to 1.04)
Multivariate-adjusted RR[*]	1.00	0.97 (0.84 to 1.12)
	Nonuser	Current use
Colon		
No. of cases	1083	199
Age-adjusted RR	1.00	1.02 (0.88 to 1.20)
Multivariate-adjusted RR[*]	1.00	1.10 (0.94 to 1.29)[‡]
Rectum		
No. of cases	363	35
Age-adjusted RR	1.00	0.54 (0.38 to 0.77)
Multivariate-adjusted RR[*]	1.00	0.59 (0.41 to 0.84)[‡]

[*] Adjusted for age, calendar year, study, pack-years of smoking before age 30 (never smoker, 1-4 pack-years, 5-10 pack-years, and ≥ 11 pack-years of smoking), aspirin dose (never, past, current use 1-2, 3-5, 6-14, ≥15 tablets/wk), height (continuous), BMI (<23, 23-<25, 25-<28, 28-<30, ≥30 kg/m^2 in the NHS; <23, 23-<25, 25-<30, 30-<35, ≥35kg/m^2 in the HPFS), family history of colorectal cancer in parents and siblings (yes, no), history of endoscopy (yes, no), red meat intake (quintiles), alcohol intake (never, 0.1-9.9 g/d, 10-14.9 g/d, 15-29.9 g/d, ≥30 g/d), and total energy intake (continuous)

[†] Includes cases whose cases were confirmed as colon or rectal cancer.

[‡] P for heterogeneity for colon vs. rectum <0.001

Table 4
Relative risk (RR) of colorectal cancer by molecular features according to statin use

Molecular Marker (no. of cases)	Nonusers	Current use	P for heterogeneity[‡]
All[†]			
No. of cases	779	108	
Age-adjusted RR	1.00	0.87 (0.71 to 1.07)	
Multivariate-adjusted RR[*]	1.00	0.92 (0.74 to 1.13)	
KRAS			0.06
Mutated cancer			
No. of cases	276	47	
Age-adjusted RR	1.00	1.16 (0.84 to 1.60)	
Multivariate-adjusted RR[*]	1.00	1.20 (0.87 to 1.67)	
Wildtype cancer			
No. of cases	462	58	
Age-adjusted RR	1.00	0.76 (0.57 to 1.00)	
Multivariate-adjusted RR[*]	1.00	0.80 (0.60 to 1.06)	
PTGS2 (COX-2)			0.40
Positive			
No. of cases	343	40	
Age-adjusted RR	1.00	0.90 (0.64 to 1.26)	
Multivariate-adjusted RR[*]	1.00	0.94 (0.67 to 1.33)	
Negative			
No. of cases	211	17	
Age-adjusted RR	1.00	0.70 (0.42 to 1.16)	
Multivariate-adjusted RR[*]	1.00	0.73 (0.44 to 1.21)	
MSI			0.50
MSI-high			
No. of cases	109	15	
Age-adjusted RR	1.00	0.77 (0.44 to 1.33)	
Multivariate-adjusted RR[*]	1.00	0.80 (0.46 to 1.39)	
MSI-low/MSS			
No. of cases	607	85	
Age-adjusted RR	1.00	0.93 (0.74 to 1.18)	
Multivariate-adjusted RR[*]	1.00	0.98 (0.77 to 1.24)	
CIMP			0.83
CIMP-high			
No. of cases	119	22	
Age-adjusted RR	1.00	0.98 (0.62 to 1.57)	
Multivariate-adjusted RR	1.00	1.01 (0.63 to 1.62)	
CIMP-low/CIMP-0			
No. of cases	590	81	

Molecular Marker (no. of cases)	Nonusers	Current use	P for heterogeneity[‡]
Age-adjusted RR	1.00	0.91 (0.71 to 1.15)	
Multivariate-adjusted RR	1.00	0.95 (0.75 to 1.22)	

[*] Adjusted for age, calendar year, study, pack-years of smoking before age 30 (never smoker, 1-4 pack-years, 5-10 pack-years, and ≥ 11 pack-years of smoking), aspirin dose (never, past, current use 1-2, 3-5, 6-14, ≥15 tablets/wk), height (continuous), BMI (<23, 23-<25, 25-<28, 28-<30, ≥30 kg/m^2 in the NHS; <23, 23-<25, 25-<30, 30-<35, ≥35kg/m^2 in the HPFS), family history of colorectal cancer in parents and siblings (yes, no), history of endoscopy (yes, no), red meat intake (quintiles), alcohol intake (never, 0.1-9.9 g/d, 10-14.9 g/d, 15-29.9 g/d, ≥30 g/d), and total energy intake (continuous)

[†] Among cases with available tissue for analysis of molecular markers

[‡] For multivariate-adjusted RRs

MSI, microsatellite instability; MSS, microsatellite stability.