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Statin Use After Diagnosis of Colon Cancer and Patient Survival

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BACKGROUND & AIMS: Statin use has been associated with a reduced incidence of colorectal cancer and might also affect survival of patients diagnosed with colon cancer. Statins are believed to inhibit Ras signaling and may also activate the bone morphogenetic protein (BMP) signaling pathway in colorectal cancer cells. We investigated the effects of statins on overall survival of patients with a diagnosis of colon cancer, and whether their effects were associated with changes in KRAS or the BMP signaling pathways. **METHODS:** Data were derived from the PHARMO database network (Netherlands) and linked to patients diagnosed with colon cancer from 2002 through 2007, listed in the Eindhoven Cancer Registry. We obtained information on causes of death from statistics Netherlands. We constructed a tissue microarray of 999 colon cancer specimens from patients who underwent surgical resection from 2002 through 2008. Survival was analyzed with statin user status after diagnosis as a time-dependent covariate. Multivariable Poisson regression survival models and Cox analyses were used to study the effect of statins on survival. Tumor tissues were analyzed by immunohistochemistry for levels of SMAD4, BMPR1A, BMPR1B, and BMPR2 proteins. Tumor tissues were considered to have intact BMP signaling if they contained SMAD4 plus BMPR1A, BMPR1B, or BMPR2. DNA was isolated from tumor tissues and analyzed by quantitative polymerase chain reaction to detect mutations in KRAS. The primary outcome measures were overall mortality and cancer-specific mortality. **RESULTS:** In this cohort, 21.0% of the patients (210/999) were defined as statin users after diagnosis of colon cancer. Statin use after diagnosis was significantly associated with reduced risk of death from any cause (adjusted relative risk [RR], 0.67; 95% confidence interval [CI], 0.51–0.87; $P = .003$) and death from cancer (adjusted RR, 0.66; 95% CI, 0.49–0.89; $P = .007$). Statin use after diagnosis was associated with reduced risk of death from any cause or from cancer for patients whose tumors had intact BMP signaling (adjusted RR, 0.39; 95% CI, 0.22–0.68; $P = .001$), but not for patients whose tumors did not have BMP signaling (adjusted RR, 0.81; 95% CI, 0.55–1.21; $P = .106$; $P < .0001$ for the interaction). Statin use after diagnosis was not associated with reduced risk of death from any cause or from cancer for patients whose tumors did not contain KRAS mutations (adjusted RR, 0.81; 95% CI, 0.56–1.18; $P = .273$) or whose tumors did have KRAS mutations (adjusted RR, 0.59; 95% CI 0.35–1.03; $P = .062$; $P = .90$ for the interaction). **CONCLUSIONS:** In an analysis of 999 patients with a diagnosis of colon cancer, we associated statin with reduced risk of death from any cause or from cancer. The benefit of statin use is greater for patients whose tumors have intact BMP signaling, independent of KRAS mutation status. Randomized controlled trials are required to confirm these results.

Keywords: Colorectal cancer; Signal Transduction; Patient Selection; Cholesterol-lowering Drug.

Although colorectal cancer survival has doubled in the last 40 years, 5-year survival remains low at only 65%.[1] Current chemotherapy treatment for colorectal cancer results in significant toxicity, limiting its use in early stage disease, in the elderly, and in patients with comorbidity, so that there is a clear unmet clinical need for new, less toxic treatment options. Previously, aspirin has been shown to increase survival when used after diagnosis, thus providing a potential new minimally toxic adjuvant treatment option for colorectal cancer.[2,3] Statins may represent such a treatment option as well, either alone or in combination with aspirin. Aside from their proven efficacy in primary and secondary prevention of cardiovascular morbidity and mortality,[4] statins have been shown, in several but not all studies, to reduce the risk of developing colorectal cancer.[5] In vitro and in vivo studies indicate that statins inhibit proliferation and induce apoptosis in colorectal cancer cells.[6,7] However, the exact molecular mechanism by which statins influence colorectal cancer remains under debate. Cancer therapy is increasingly focused on personalized therapy via pharmacologic modulation of specific molecular pathways targeted to sensitive tumors identified by molecular subtyping; statins could potentially be more effective in a specific subgroup of cancers.

There are several potential molecular mechanisms of action to explain the therapeutic effects of statins in

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Abbreviations used in this paper: BMP, bone morphogenetic protein; CI, confidence interval; RR, rate ratio.

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EDITOR'S NOTES

BACKGROUND AND CONTEXT

The long-term use of statins has been linked to improved survival after a diagnosis of colorectal cancer but the mechanism for this protective effect is unclear.

NEW FINDINGS

The researchers show that statin use after a diagnosis of colorectal cancer is associated with improved cancer specific survival. This effect was strongest in cancers with an intact BMP cell signaling pathway and independent of cancer KRAS mutations.

LIMITATIONS

This is a retrospective molecular pathological epidemiological study.

IMPACT

BMP pathway analysis of colorectal cancers could help identify patients that benefit most from statin use.

colorectal cancer. Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase; an enzyme that plays an essential role in mevalonate synthesis. Inhibition of the mevalonate pathway not only disrupts cholesterol synthesis, but also farnesyl pyrophosphate synthesis, which is essential for the prenylation of GTPases like *KRAS*.[8] It is therefore thought that statins might act on colorectal cancer by inhibiting *KRAS*. *KRAS* mutations are prevalent in 40% of colorectal cancers and result in constitutively active form of *KRAS*.[9]

Another theory hypothesizes that statins act through activation of the bone morphogenetic protein signaling (BMP) pathway. Statins activate the BMP pathway in bone,[10] and we have previously shown that they also do this in colorectal cancer.[11] Interestingly, statins are only effective in colorectal cancer cells where BMP signaling is intact.[7] BMP signaling is frequently disrupted in colorectal cancer through loss of SMAD4, the central component of the signaling cascade, or reduced BMP receptor expression.[12,13]

The aim of our study was to evaluate whether statins might be effective as adjuvant therapy in colon cancer by correlating post-diagnosis statin use with patient survival in a cohort in which we have previously observed a survival benefit with aspirin use after diagnosis. Secondly, we tried to uncover the molecular background in which statins are able to execute their tumor-suppressive function, thereby considering the *KRAS* mutational status and the BMP signaling pathway functionality in relation to statin use and patient survival.

Materials and Methods

Retrospective Study Cohort

All patients diagnosed with colon cancer between 2002 and 2007 were selected from the Eindhoven Cancer Registry. This southern region of the Netherlands is served by 10 hospitals each serving a population between 150,000 and 250,000 people. Data on statin use (simvastatin, pravastatin, cerivastatin, fluvastatin, atorvastatin, and rosuvastatin) was derived from the PHARMO database network (PHARMO, Netherlands). The central patient database of the PHARMO record linkage system has recently been linked to the Eindhoven Cancer Registry database; this is described in detail by Van Herk-Sukel et al.[14] Information about cause of death was obtained from Statistics Netherlands. Formalin-fixed paraffin-embedded tissue blocks were retrieved from 1026 patients with colon cancer who had a surgical resection between 2002 and 2008.[15] Rectal cancers were not included because many of these tissue specimens will have been exposed to preoperative radiotherapy, which may influence tumor molecular characteristics. Twenty-seven patients with more than 1 colon tumor at the time of diagnosis were excluded for this study; thus the total cohort consisted of 999 patients.

TMA Production

Three 1.0-mm diameter cores were obtained from formalin-fixed paraffin-embedded tumor blocks and transferred into a receiver paraffin block using the TMA Master (3D Histech, Budapest, Hungary) as previously described.[15] Representative tumor sites were identified by 2 independent researchers using H&E-stained sections (with a qualified pathologist confirming the identification of the tumor).

Immunohistochemistry and TMA Scoring System

Determination of microsatellite stability status by immunohistochemical analysis has been previously described.[15] SMAD4 and BMP receptors were stained according to previously described methods.[16] Examples of tumor core stainings are shown in Supplementary Figure 1. Scoring was performed in a blinded fashion by 2 investigators (P.V. and J.H.) independently according to previously described methods.[16] Three cores per tumor were analyzed and an average score per tumor was calculated. The concordance between investigators was 87% (κ = 0.70, 95% confidence interval [CI] 0.604–0.796). Final scoring was reached by consensus. The tumors were divided in "intact BMP signaling" and "non-intact BMP signaling" based on the expression of SMAD4, BMPR1a, BMPR1b, and BMPR2. The tumor was designated as having "non-intact BMP signaling" if either SMAD4 or one of the BMP receptors scored negative.

KRAS Mutation Analysis

DNA was extracted from 2.0-mm diameter cores taken randomly from 663 of the 999 blocks, as previously described.[15] For determination of *KRAS* mutations status, hydrolysis probe assays were performed for the major known mutations (hotspots) in codon 12 and 13 for *KRAS*; c.34G>A; p.G12S, c.34G>C; p.G12R, c.34G>T; p.G12C, c.35G>A; p.G12D, c.35G>C; p.G12A, c.35G>T; p.G12V, c.38G>A; p.G13D and c.37G>T; p.G13C, as previously described.[15] Hydrolysis probe assays were analyzed using quantitative polymerase chain reaction analysis software (CFX manager version 3/0, Bio-Rad, Hercules, CA). Mutation detection was performed blindly by 2 independent observers (M.S.R. and R.E.).

Statistics

Definition of statin user. Statistical analyses were performed using the statistical packages SPSS (version 20.0 for

Windows, IBM SPSS statistics, Armonk, NY) and STATA/SE (version 12 for windows, StataCorp LLC, College Station, TX). A P value of $<.05$ was considered statistically significant. The vital status of patients (alive/dead) was identified via medical records or through linking the Eindhoven Cancer registry data with the municipal population registries that have information on the vital status (alive or deceased). Follow-up started 30 days from diagnosis of colon cancer (T0), and was ended January 2012 or at the date of death. Patients who died within 30 days after diagnosis were excluded from the survival analyses (2.4%). Statin non-users were classified as those who never had a prescription for statin or had a prescription for less than 14 consecutive days after diagnosis of colon cancer. Statin users were defined as those who had been given a prescription for statins for 14 days or more after a colon cancer diagnosis.

Time-dependent survival analyses. A time-dependent exposure survival analysis for Overall Mortality (deaths from any cause) and Cancer-Specific Mortality (deaths from cancer) was performed in which patients were defined as non-users from T0 to the first use and users from first use to the end of the follow-up. A parametric survival model with an exponential (Poisson) distribution was used. Secondly, a Cox proportional hazard model was used with statin use as a time-varying covariate to confirm the analyses. Adjustments for potential confounders were made for sex, age (continuous), stage (pathologic stage or clinical stage when pathologic stage was unknown), adjuvant chemotherapy (yes/no), grade, year of incidence, microsatellite status, and comorbidity (yes/no). Comorbidities included respiratory disease, cardiovascular disease, digestive disease, musculoskeletal disease, neurologic disease, or endocrine disease. Subgroup analyses were performed for sex, age, stage, grade, chemotherapy, aspirin use after diagnosis, and frequent use of statin ($\geq$3, $\geq$5, or $\geq$7 prescriptions). Frequent users were defined as patients who had a given number (or more) consecutive repeat prescriptions for statins. User time started after the completion of the 3, 5, or 7 prescriptions. Survival time of patients who had $<$3, 5, or 7 refills and time before the prescription requirements of the frequent users was classified as non-user time in this analysis.

Sensitivity analyses for ACE inhibitors and benzodiazepines. A sensitivity analyses was performed to assess the association of 2 other groups of medicine, benzodiazepines and ACE inhibitors, and survival. For this, the same methodology as for statin use was followed and a time-dependent parametric survival model with an exponential (Poisson) distribution was used, in which patients were defined as non-users from T0 to the first use and users from first use to the end of the follow-up. Adjustments for potential confounders were made for sex, age (continuous), stage (pathologic stage or clinical stage when pathologic stage was unknown), adjuvant chemotherapy (yes/no), grade, year of incidence, microsatellite status, and comorbidity (yes/no). An extra analysis was performed excluding statin users from the group of post-diagnosis ACE inhibitors users.

A lag of 6 months. In another analysis, a lag of 6 months after diagnosis was introduced to reduce any bias in prescribing that may arise because of impending death, although our definition of a statin user was specifically chosen to minimize this sort of bias caused by disease progression.

Molecular subtypes. Finally, differential associations of statin use with cancer-specific mortality by tumor molecular subtype were investigated by subgroup analyses for intact BMP signaling status and *KRAS* mutation status followed by an interaction analysis.

Results

Statin use After Colon Cancer Diagnosis and Survival

In total, 999 colon cancer patients were included in this study. Table 1 shows the characteristics of the population according to statin use or non-use after diagnosis and Figure 1 shows a flow diagram of the patient inclusion in the various analyses. In this cohort, 21.0% (210/999) were defined as statin users after diagnosis. During follow-up until January 2012, 465 deaths were recorded, of whom 69 were statin users (32.9% of statin users) and 396 were nonusers (50.2% of nonusers). Statin users were predominately male, older age, and had more comorbidities. Furthermore, tumors found in statin users have a lower stage than tumors found in non-users ($P = .005$). The mean and median duration of prescriptions was 76.6 and 90 days, respectively, and the mean number of prescriptions per patient was 23 (range, 1–215). Overall, 51.7% of the

Table 1. Baseline Characteristics of the Colon Cancer Patients According to Use of Statin After Diagnosis

	All patients (N=999)	Statin non-users (N=789)	Statin users (N=210)	*P* value
Sex				
Male	505 (50.6)	379 (48.0)	126 (60.0)	.002
Female	494 (49.4)	410 (52.0)	84 (40.0)	
Age				
<65	342 (34.2)	282 (35.7)	60 (28.6)	<.001
66–74	304 (30.4)	211 (26.7)	93 (44.3)	
≥75	353 (35.4)	296 (37.5)	57 (27.1)	
Year of diagnosis				
2002–2004	451 (45.2)	354 (44.9)	97 (46.2)	.732
2005–2007	548 (54.8)	435 (55.1)	113 (53.8)	
Disease stage				
I	138 (13.8)	99 (12.6)	39 (18.6)	.005
II	402 (40.2)	308 (39.0)	94 (44.8)	
III	287 (28.7)	231 (29.3)	56 (26.7)	
IV	169 (16.9)	149 (18.9)	20 (9.5)	
Unknown	3 (0.3)	2 (0.3)	1 (0.5)	
Comorbidity				
No	443 (44.3)	401 (50.8)	42 (20.0)	<.001
Yes	556 (55.7)	388 (49.2)	168 (80.0)	
Microsatellite status				
MSI	90 (9.0)	70 (8.9)	20 (9.5)	.727
MSS	870 (87.1)	690 (87.5)	180 (85.7)	
Unknown	39 (3.9)	29 (3.7)	10 (4.8)	
Chemotherapy				
No	691 (69.2)	542 (68.7)	149 (71.0)	.529
Yes	308 (30.8)	247 (31.3)	61 (29.0)	

MSI, microsatellite instable tumors; MSS, microsatellite stable tumors.

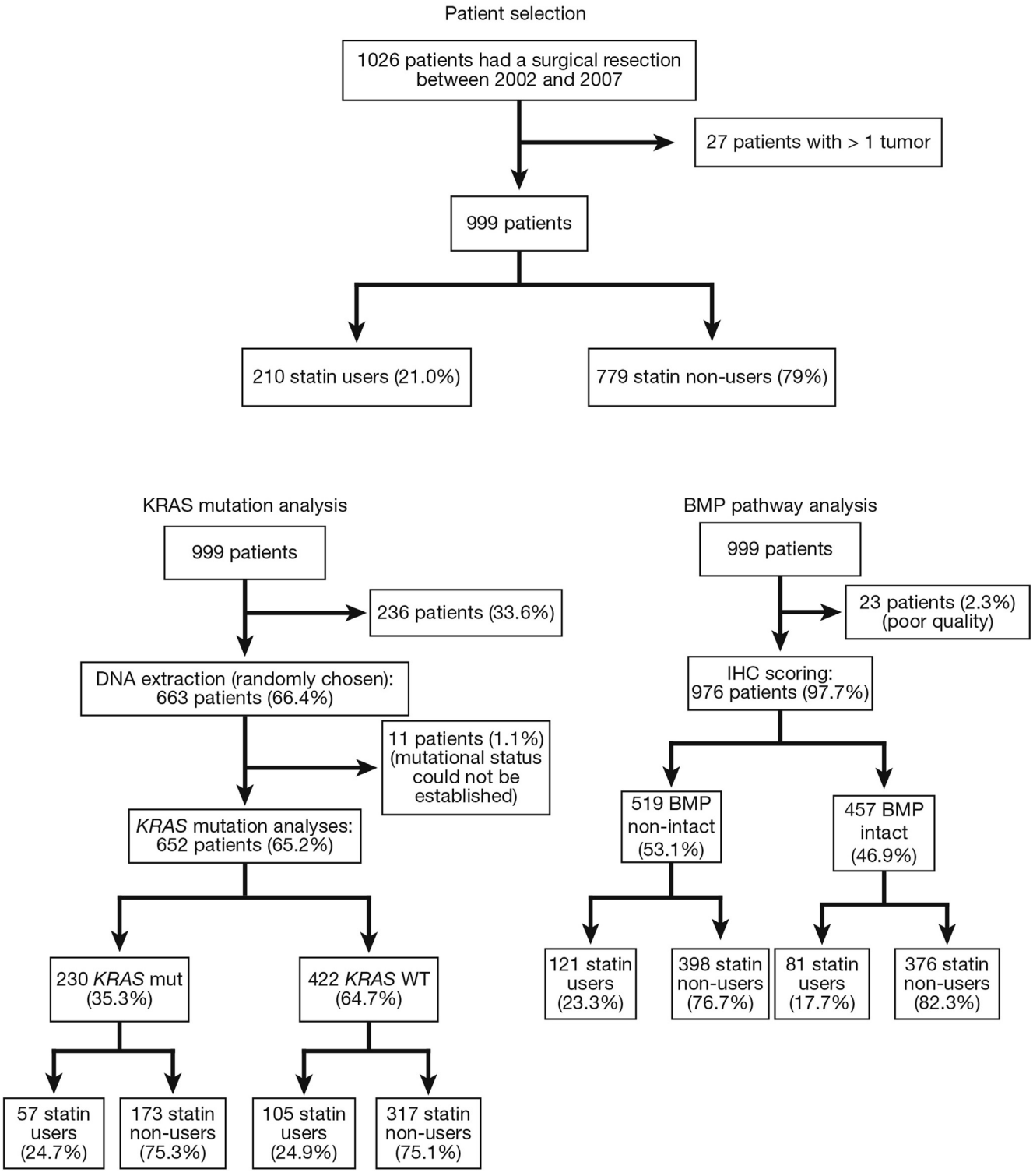

Figure 1. Flow diagram of the patient inclusion in the various analyses.

patients showed no discontinuation over the study period and 36.6% stopped for 30 days or less, which is not considered as discontinuation. Overall, 11.7% of the patients discontinued statin use for more than 30 days and 7% more than 90 days. Median follow-up of the cohort was 3.3 years (range, 0.01–8.27 years), with a median follow-up of 4.1 years (range, 0.001–7.94 years) for patients who were alive during the study period. Median first start of post-diagnosis statin use was at 1.9 years (range, 0.005–6.31 years). There were 396 deaths in 789 non-users of statins and 69 deaths in 210 patients who used statins.

The 5-year overall survival for non-users was 54.6% (95% CI 50.8–58.1) and 65.7% (95% CI 57.8–72.4) for statin users. Statin use after diagnosis was significantly

associated with a reduced risk of death from any cause with a rate ratio (RR) of 0.65 (95% CI 0.50–0.84, $P = .001$) and death from cancer; RR of 0.64 (95% CI 0.47–0.85, $P = .002$). When adjusted for potential confounders, this effect remained with an adjusted RR of 0.67 for overall survival (95% CI 0.51–0.87, $P = .003$) and an adjusted RR of 0.66 for CSS (95% CI 0.49–0.89, $P = .007$) (Table 2). Supplementary Table 1 shows the RRs for variables used in the multivariable analyses other than statin use. Subgroup analyses by stage at diagnosis showed no association for mortality in stage I (adjusted RR 1.42; 95% CI 0.50–4.02, $P = .90$) and stage III (adjusted RR 1.10; 95% CI 0.65–1.88, $P = .71$), but showed an association between statin use and mortality for stage II (adjusted RR 0.45; 95% CI 0.24–0.87, $P = .02$) and stage IV (adjusted RR 0.43; 95% CI 0.25–0.76, $P = .004$). The interaction test for mortality and stage is $P < .01$. Interestingly, frequent use of statins, especially more than 7 refills, further reduced risk of death, with an adjusted RR of 0.63 (95% CI 0.44–0.89, $P = .009$).

We have previously shown that low-dose aspirin use after diagnosis was associated with a survival benefit in this cohort.[15] Therefore, we performed a further analysis in patients that only used statins or only used aspirin or a combination of both (Table 3). Compared with patients who used neither aspirin or statins after diagnosis, isolated statin use and isolated aspirin use were both significantly associated with a reduced risk of death from cancer, with an adjusted RR of 0.48 (95% CI 0.31–0.73) for isolated statin use and 0.54 (95% CI 0.35–0.85) for isolated aspirin use. A combination of both statin and aspirin use was not significantly associated with mortality; adjusted RR of 0.69 (95% CI 0.46–1.03). The notion that statins act independently of aspirin use is further confirmed by the fact that statin use was significantly associated with a reduced risk of death in patients who did not use aspirin after diagnosis (adjusted RR 0.51, 95% CI 0.33–0.79, $P = .003$).

Including a lag of 6 months was associated with a reduced risk of death from cancer with a RR of 0.64 (95% CI 0.47–0.85, $P = .002$) and an adjusted RR of 0.66 (95% CI 0.48–0.89, $P = .007$).

Table 2. Rate Ratio for Death (Time-Dependent Analysis Overall Mortality and Cancer-Specific Mortality), According to Use or Nonuse of Statin after Diagnosis

Statin (all prescriptions)	Statin non-users	Statin users[a]	*P* value
Patients	789	210	
Deaths from any cause	396	69	
Rate ratio (95% CI)	1.0 (reference)	0.65 (0.50–0.84)	.001
Adjusted rate ratio (95% CI)[b]	1.0 (reference)	0.67 (0.51–0.87)	.003
Deaths from cancer	311	53	
Rate ratio (95% CI)	1.0 (reference)	0.64 (0.47–0.85)	.002
Adjusted rate ratio (95% CI)[b]	1.0 (reference)	0.66 (0.49–0.89)	.007
Stage subgroups (CSS)			
Stage I	1.0 (reference)	1.42 (0.50–4.02)	.51
Stage II	1.0 (reference)	0.45 (0.24–0.87)	.02
Stage III	1.0 (reference)	1.10 (0.65–1.88)	.71
Stage IV	1.0 (reference)	0.43 (0.25–0.76)	.004
Statin frequent use ≥3 prescriptions			
Patients	802	197	
Deaths from cancer	313	51	
Rate ratio (95% CI)	1.0 (reference)	0.63 (0.47–0.85)	.002
Adjusted rate ratio (95% CI)[b]	1.0 (reference)	0.72 (0.52–1.00)	.049
Statin frequent use (≥5 prescriptions)			
Patients	815	184	
Deaths from cancer	319	45	
Rate ratio (95% CI)	1.0 (reference)	0.57 (0.42–0.78)	<.001
Adjusted rate ratio (95% CI)[b]	1.0 (reference)	0.65 (0.46–0.91)	.012
Statin frequent use (≥7 prescriptions)			
Patients	826	173	
Deaths from cancer	322	42	
Rate ratio (95% CI)	1.0 (reference)	0.56 (0.41–0.78)	<.001
Adjusted rate ratio (95% CI)[b]	1.0 (reference)	0.63 (0.44–0.89)	.009

[a]Statin use after diagnosis.
[b]Adjusted for sex, age, comorbidity, year of incidence, histologic grade, stage, microsatellite status, chemotherapy, and aspirin use.

ACE Inhibitors and Benzodiazepines

We performed sensitivity analyses with post-diagnosis ACE inhibitor, a group of cardiovascular medicines not associated with colon cancer survival, and observed a significant RR of 0.75 (95% CI 0.59–0.96, $P = .024$), but a non-significant adjusted RR of 0.81 (95% CI 0.62–1.05, $P = .114$). When excluding statin users, ACE inhibitor use has a RR of 0.98 (95% CI 0.70–1.38, $P = .93$). We also analyzed a completely different class of drugs; benzodiazepines. These were not associated with improved survival ($P = .03$).

Statin use, Survival, and KRAS Mutations

KRAS mutation status (wild-type/mutation) was established in 98% (652/663) of the tumor cores that were randomly taken from the 999 original tumor cores. There were no differences in baseline characteristics between

Table 3. Rate Ratio for Cancer-Specific Mortality (Time-Dependent Analysis), According to Aspirin or Statin Use

	N	Deaths from cancer	Adjusted rate ratio (95% CI)	*P* value
Aspirin or statin use				
No aspirin or statin use	711	288	1.0 (reference)	.0002
Statin use, no aspirin	106	23	0.48 (0.31–0.73)	
Aspirin use, no statin	78	23	0.54 (0.35–0.85)	
Both aspirin and statin use	104	30	0.69 (0.46–1.03)	
Aspirin non-users				
Statin non-users	711	288	1.0 (reference)	.003
Statin users	106	23	0.51 (0.33–0.79)	

patients of whom the DNA was extracted and the KRAS mutation status was established and those in which this was not successful. A *KRAS* mutation was found in 35.3% (230/652) of the samples, which shows a prevalence that is in accord with other studies.[17] Supplementary Table 2 summarizes the clinical characteristics of the patients based on the KRAS mutation status and statin use. Statin use was not significantly different in patients with *KRAS* wild-type tumors (24.9%) and *KRAS* mutated tumors (24.8%). The effect of *KRAS* mutation status on the survival benefit associated with statin use after diagnosis was analyzed. Statin use after diagnosis was not associated with a reduced risk of death from cancer in *KRAS* wild-type tumors (adjusted RR 0.82; 95% CI 0.54–1.25, $P = .35$) or *KRAS* mutated-tumors (adjusted RR 0.59; 95% CI 0.33–1.08, $P = .086$); test for interaction $P = .4566$ (Table 4). A Cox proportional hazard model confirmed the results of the Poisson model (adjusted RR for KRAS mutant cancers, 0.64; 95% CI 0.35–1.17, $P = .148$). An interaction analysis showed no statistically significant difference between the 2 molecular tumor subtypes (P value for interaction = .457). Supplementary Table 4 shows the results based on overall mortality.

Statin use, Survival, and BMP Signaling

Analysis of BMP signaling pathway components was performed on 976 tumor sections (97.7% of total). Loss of 2.3% of the tumor sections in this analysis was a consequence of staining artifacts and loss of material during the staining procedure. The tumors were categorized as having either "intact BMP signaling" or "non-intact BMP signaling" based on the expression levels of SMAD4 and the BMP receptors (see Materials and Methods section for a full description of the scoring system). We presumed that the BMP signaling pathway was intact when all components were expressed and that the BMP signaling pathway was "non-intact" when there was absence of expression of one of the components. In this cohort, 519 patients (53.2%) showed non-intact BMP signaling in their tumor sections and 457 patients (46.8%) showed intact BMP signaling. Supplementary Table 3 summarizes the clinical characteristics of the patients based on BMP signaling status and statin use; 23.3% of patients in whom we observed non-intact BMP signaling in the tumor sections were statin users and 17.7% of patients with intact BMP signaling were statin users. Statin users had more comorbidities in both groups. Within the "intact BMP signaling" group, low-stage tumors ($P = .012$) were more prevalent in statin users than in statin non-users.

To test our hypothesis that statins act via the BMP pathway in colon cancer prevention, we analyzed the effect of BMP signaling pathway status on statin-associated patient survival (Table 4). The significant reduction in risk of death associated with statin use after diagnosis was more prominent in the group of tumors that exhibited intact BMP component expression with an adjusted RR of 0.39 (95% CI 0.22–0.68, $P = .001$) than for patients with tumors in which we observed a non-intact BMP signaling pathway (adjusted RR 0.81; 95% CI 0.40–1.62, $P = .55$). A Cox proportional hazard model confirmed the results of the Poisson model (adjusted RR 0.42; 95% CI 0.24–0.74, $P = .003$). An interaction analysis showed a statistically significant difference between the 2 molecular tumor subtypes (P value for

Table 4. Rate Ratio for Cancer-Specific Mortality (Time-Dependent Analysis), According to Tumor KRAS Mutation Status[e], BMP Signaling Pathway Status[d] and Use or Non-use of Statin After Diagnosis

	Statin non-users	Statin users	*P* value
All cancers			
Patients	789	210	
Deaths from cancer	311	53	
Adjusted rate ratio (95% CI)[a,b]	1.0 (reference)	0.66 (0.49–0.89)	.007
Adjusted hazard ratio (95% CI)[a,c]	1.0 (reference)	0.71 (0.53–0.97)	.032
KRAS Wild-type cancers			
Patients	317	105	
Deaths from cancer	122	31	
Adjusted rate ratio (95% CI)[a,b]	1.0 (reference)	0.82 (0.54–1.25)	.35
Adjusted hazard ratio (95% CI)[a,c]	1.0 (reference)	0.86 (0.56–1.31)	.48
KRAS Mutant cancers			
Patients	173	57	
Deaths from cancer	74	15	
Adjusted rate ratio (95% CI)[a,b]	1.0 (reference)	0.59 (0.33–1.08)	.086
Adjusted hazard ratio (95% CI)[a,c]	1.0 (reference)	0.64 (0.35–1.17)	.148
Intact BMP-signaling			
Patients	376	81	
Deaths from cancer	124	18	
Adjusted rate ratio (95% CI)[a,b]	1.0 (reference)	0.39 (0.22–0.68)	.001
Adjusted hazard ratio (95% CI)[a,c]	1.0 (reference)	0.42 (0.24–0.74)	.003
Non-intact BMP-signaling			
Patients	398	121	
Deaths from cancer	179	33	
Adjusted rate ratio (95% CI)[a,b]	1.0 (reference)	0.81 (0.55–1.21)	.31
Adjusted hazard ratio (95% CI)[a,c]	1.0 (reference)	0.89 (0.60–1.33)	.58
Intact BMP-signaling and wild-type cancers			
Patients	150	44	
Deaths from cancer	43	12	
Adjusted rate ratio (95% CI)[a,b]	1.0 (reference)	0.81 (0.40–1.62)	.55
Intact BMP-signaling and mutant cancers			
Patients	89	18	
Deaths from cancer	34	6	
Adjusted rate ratio (95% CI)[a,b]	1.0 (reference)	0.59 (0.22–1.57)	.29

[a]Adjusted for sex, age, comorbidity, year of incidence, histologic grade, stage, microsatellite status, and chemotherapy.
[b]Poisson model.
[c]Cox model.
[d]KRAS analyses: 663 of 999 patients analysed; 652 of 663 samples included.
[e]BMP analyses: 976 of 999 samples included.

interaction <.001). Supplementary Table 4 shows the results based on overall mortality.

Discussion

In this large observational study, we show in our cohort that statin use initiated or continued after a diagnosis of colon cancer is associated with a significantly reduced risk of death from any cause with an RR of 0.65 and death from cancer with an RR of 0.64. To test for associations between the molecular tumor subtype and the effect of statins on colon cancer survival, we analyzed both the *KRAS* mutation status and the protein expression of multiple elements of the BMP signaling pathway in the tumor samples. We have previously performed similar analyses of the BMP pathway in both pancreatic ductal adenocarcinoma and colorectal cancer.[16,18] We found that the association between statins and survival did not differ in *KRAS* mutant versus KRAS wildtype tumor, but the survival benefit associated with statin use was stronger in tumors with an intact BMP signaling pathway. This is in accordance with findings from our previous in vitro and rodent studies showing that statins are only effective antitumor agents in tumors where the BMP signaling pathway is functional.[7,11]

Statins may be an attractive candidate for use as adjuvant therapy in colorectal cancer because they are already widely used and well tolerated.[19,20] Statins have previously been shown to reduce the incidence of colorectal cancer. A large retrospective study published in 2005 assessing the association between statin use and the incidence of colorectal cancer showed a 47% reduction in the risk of developing colorectal cancer.[5] Although several subsequent studies have failed to confirm this effect,[21,22] a recent meta-analysis combining 42 large observational studies, case-control studies, and randomized control trials showed an overall risk reduction of 10% for the development of colorectal cancer in statin users, providing more evidence for the antitumor potential of statins.[23]

Despite the large number of trials that have investigated the association between the incidence of colorectal cancer and statin use, relatively few studies have investigated the effect of adjuvant statin use on patient survival in colorectal cancer. A study from Scotland that included 308 patients with colorectal cancer found a non-significant reduction in colorectal cancer-specific mortality in statin users.[24] An American study of 407 patients with rectal cancer who received chemo-radiotherapy also found a non-significant reduction in cancer-specific mortality in statin users before and after surgery.[25] Another American study in 842 patients with stage III colon cancer did not detect any association between patient-reported statin use after diagnosis and cancer recurrence[26]; and a Dutch study found no association between statin use, KRAS mutation status, and metastatic colorectal cancer progression-free survival after chemotherapy.[27] Some of these studies investigated specific colorectal cancer subgroups[25–27] or had limitations, such as relatively small size,[24–27] measurement of medication use at 1 time-point,[26] and potential for immortal time bias.[24] In the biggest study to date from the UK, a cohort of 7657 patients with colorectal cancer were analyzed using time-dependent Cox regression models. Statin use post-diagnosis was associated with a significant reduction in overall survival.[28] This is consistent with our findings of a strong association between statin use and overall patient survival. The strengths of our study are the use of registered drug prescriptions to ascertain drug exposure rather than patient questionnaires, a relatively large cohort of 999 patients, molecular analysis of tumor tissue, and analysis of the effect of combined exposure to aspirin and statins. This is particularly interesting in the light of conflicting evidence as to whether a combination of aspirin and statins may be more effective than one or the other alone.[29–32]

A recently published study with this cohort showed an association between low-dose aspirin use after diagnosis and improved survival.[15] Interestingly, isolated use of either statins or aspirin after diagnosis are both associated with an improved survival in patients, indicating that statins and aspirin improve patient outcome independently. Although there is considerable in vitro evidence that a combined therapy of statins and aspirin could be beneficial,[33] we could not find a synergistic or additive effect of statin combined with aspirin use. Separating this cohort based on statin and/or aspirin use results in a relative small number of patients per group; a larger cohort will be needed to assess the additive effect of combination therapy.

The molecular mechanisms responsible for the antitumor effects of statins on colorectal cancer are not fully understood. As mentioned in our introduction, several mechanisms have been proposed, 2 of which we have investigated in this study, namely that statins act on KRAS by influencing its prenylation and that statins act by activating the BMP signaling pathway.

Firstly, we investigated the influence of *KRAS* mutation status on the association between statin use and patient survival. There is evidence that statins act through the inhibition of the Ras/Raf pathway from studies performed in cancer cell line cells and xenograft mouse models. For example, it has been shown that lovastatin inhibits the Raf/MEK/ERK pathway in leukemia cells, resulting in apoptosis,[34] and that atorvastatin can disrupt *KRAS*/Raf complexes, leading to inhibition of AKT and ERK in non-small cell lung cancer cells.[35] The most clinically relevant data came from a study showing that simvastatin could overcome cetuximab resistance in colon cancer cell line cells harboring *KRAS* mutations and not in cells with *BRAF* mutations, implying that only *KRAS* mutant cancers would benefit from statin treatment.[36] We did not see a difference in the association between statin and survival comparing *KRAS* WT cancers and *KRAS* mutant cancers in our cohort. There was no significant reduction in risk of death associated with statin use in either group, although the results in *KRAS* mutant tumors nearly reached significance. The reason for the loss of a significant protective effect of statins when separating the cancers based on the *KRAS* mutation status is probably the low number of cancers in each group. A larger cohort is therefore necessary to adequately assess the influence of *KRAS* mutation status on the survival benefit associated with statin use. Our data is consistent with 2

studies that have found no association between the *KRAS* mutation status, statin use, and colorectal cancer survival.[26,27] However, in these studies, statin use after diagnosis was not associated with improved survival in colorectal cancer overall, in contrast to our study, making the studies difficult to compare.

Secondly, we investigated the influence of tumor expression of elements of the BMP signaling pathway on the association between statin use and patient survival. The BMP signaling pathway functions as a tumor suppressor in colorectal cancer, inducing cell differentiation and apoptosis of colonic epithelial cells and negatively regulating WNT signaling.[37,38] In a screen of 30,000 compounds, lovastatin and simvastatin were the 2 most potent enhancers of BMP2 expression in bone.[10] We have subsequently shown that statins inhibit colorectal cancer cell proliferation and induce apoptosis through increasing BMP2 expression, but only when the BMP pathway is fully functional.[7] BMP ligands bind to a complex of transmembrane serine threonine kinase receptors type 1 and 2, resulting in phosphorylation and activation of the BMP receptor type 2 (BMPR2). The activated BMPR2 activates BMP receptor type 1 (BMPR1a and BMPR1b), which phosphorylate the receptor-associated SMADS (SMAD1, 5, and 8) that subsequently complex with SMAD4 and translocate to the nucleus to regulate gene transcription. When expression of either SMAD4 or any one of the BMP receptors is lost, the canonical BMP signaling pathway cannot be activated. We assessed the expression levels of SMAD4, BMPR1a, BMPR1b, and BMPR2 using immunohistochemistry as we have performed previously. In this cohort, statins are associated with a reduced risk of death in cancers that express both SMAD4 and BMP receptors (described in the Results section as intact BMP signaling), suggesting that the anticancer benefit of statin may be BMP-dependent. Because this is the first study to investigate the role of the BMP signaling pathway in the association between statins and colon cancer mortality, comparison with other studies is not possible. These results require confirmation in further cohorts.

There are several limitations to the study. This is a retrospective study in which randomization was not possible. Immortal time bias was avoided by using a time-dependent model, but confounding by disease progression may still occur. Next to a parametric survival model with exponential (Poisson) distribution, a Cox proportional hazard model was used with statin use as a time varying covariate to confirm the analyses. Our methodology for defining user and non-user time will minimize confounding by disease progression. This can arise where poor prognosis or advanced disease influences statin usage, with statins being stopped in these patients potentially leading to a spuriously lower number of deaths in statin users. This depends on how "statin user" is defined and how statin user time is accrued. In our study, user time is accrued after the first prescription, even if a patient subsequently stopped using statins, effectively avoiding this bias.

Reverse causation was further addressed by applying a lag of exposure of 6 months, which also resulted in a significant difference.

Because reduced risk of death could potentially be explained by reductions in cardiovascular events in statin users, we also analyzed risk of death from any cause. Statin use can also be a sign of compliance. Consistent statin use could therefore be a surrogate marker for health consciousness, which can cause a "healthy user bias" in studies like ours. We observed similar effects of aspirin and statins on colon cancer mortality, implying that both medications are protective or that a hidden factor connecting aspirin and statins is responsible. However, analyses with ACE inhibitors and benzodiazepines proved that this was not the case.

There were differences in baseline characteristics between statin users and statin non-users that could have confounded our results: statin users were more often male, were older, had more comorbidity, and had earlier stage tumors. However, when adjusting for these confounders, a clear association between statin use and a better prognosis remained. However, residual confounding by unknown factors could still influence the results. Lastly, the assessment of active BMP signaling is not perfect. The fact that all the components of BMP signaling are present or the fact 1 or more components are missing does not necessarily mean that there is or isn't active BMP signaling. Because of the complex matrix of downstream signaling targets, which can be activated or inhibited by canonical and/or noncanonical BMP signaling, it is difficult to find a suitable (surrogate marker) for BMP signaling activity. Phospho-SMAD1, 5, or 8 are commonly used in vitro to determine BMP signaling activity, but phospho-proteins are very sensitive to tissue processing and fixation, making the assessment of expression levels in human tissue inaccurate.[39] In another study using human colon cancer tissue, we found nuclear p-SMAD1, 5, or 8 without the presence of SMAD4, which is thought to be necessary for nuclear translocation of p-SMAD1, 5, or 8. We cannot evaluate whether this is the result of tissue processing or SMAD4-independent non-canonical BMP signaling. We do know that loss of SMAD4 in colon cancer is associated with a poor patient survival and negative nuclear p-SMAD1, 5, or 8 staining is not.[39]

In conclusion, in our cohort statin use after diagnosis is associated with a reduced risk of death in patients with colon cancer. More importantly, this reduction was most pronounced in patients whose tumors retain expression of BMP signaling pathway components. In the future, BMP signaling functionality may serve as a predictive biomarker to select patients for adjuvant statin therapy. However, our data are preliminary and other studies, preferably randomized clinical trials, are needed to confirm the beneficial effects of statins on colon cancer survival and BMP signaling as a predictive biomarker.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2017.05.011.

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Conflicts of interest
The authors disclose no conflicts.

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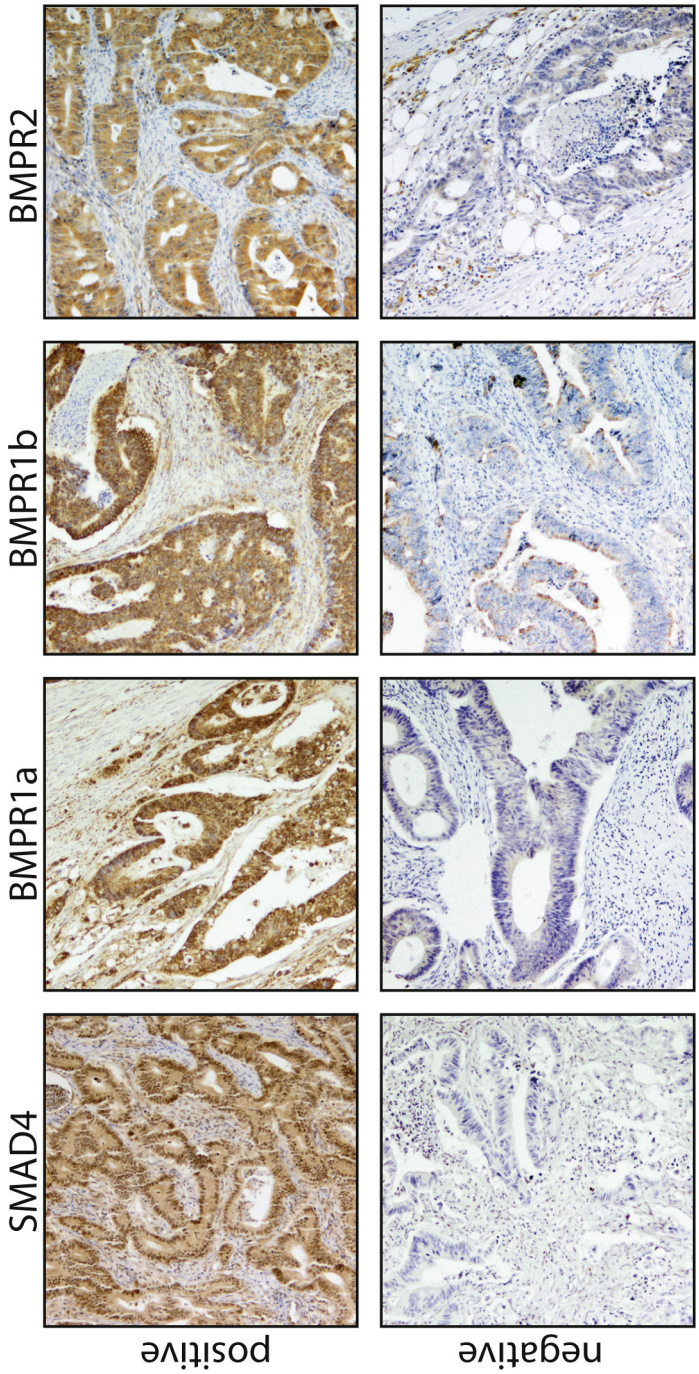

Supplementary Figure 1. Immunohistochemistry for SMAD4, BMPR1a, BMPR1b & BMPR2 in 567 human colon cancer. Representative cores that show positive or negative expression.

Supplementary Table 1. Rate Ratio for Cancer Specific Mortality (Time-Dependent Analysis) for Variables other than Statin Use

Multivariable model	Reference category	Rate ratio	*P* value
Sex	1.0 (male)	0.84 (0.67–1.04)	.10
Age	1.0 (continuous)	1.04 (1.03–1.06)	<.001
Comorbidity	1.0 (no comorbidity)	1.39 (1.10–1.77)	.007
Incidence year	1.0 (continuous)	1.02 (0.95–1.08)	.60
Grade	1.0 (grade I)	1.21 (0.80–1.84) grade II 2.29 (1.47–3.57) grade III 1.21 (0.70–2.08) unknown grade	<.001
Stage	1.0 (stage I)	1.53 (0.94–2.51) stage II 3.80 (2.31–6.27) stage III 22.93 (13.93–37.74) stage IV 3.15 (0.42–23.89) unknown stage	<.001
Microsatellite status	1.0 (negative)	0.93 (0.63–1.37)	.71
Chemotherapy	1.0 (no)	0.69 (0.52–0.90)	.007
Aspirin use	1.0 (no)	0.69 (0.50–0.95)	.02

Supplementary Table 2. Baseline Characteristics of the Colon Cancer Patients according to *KRAS* and Use of Statin After Diagnosis. Overall statin use: 210 patients (21.0%)

		KRAS wild-type (N=422)			*KRAS* mutation (N=230)		
	All patients (N=999)	No statin	Statin	*P* value	No statin	Statin	*P* value
Sex							
Male	505 (50.6)	151 (47.6)	67 (63.8)	.004	95 (54.9)	39 (68.4)	.073
Female	494 (49.4)	166 (52.4)	38 (36.2)		78 (45.1)	18 (31.6)	
Age							
<65	342 (34.2)	100 (31.6)	20 (19.1)	<.001	51 (29.5)	20 (35.1)	.190
66–74	304 (30.4)	82 (25.9)	53 (50.5)		47 (27.2)	20 (35.1)	
≥75	353 (35.4)	135 (42.6)	32 (30.5)		75 (43.4)	17 (29.8)	
Year of diagnosis							
2002–2004	451 (45.2)	159 (50.2)	49 (46.7)	.535	80 (46.2)	25 (43.9)	.754
2005–2007	548 (54.8)	158 (49.8)	56 (53.3)		93 (53.8)	32 (56.1)	
Disease stage							
I	138 (13.8)	41 (12.9)	25 (23.8)	.05	26 (15.0)	10 (17.5)	.291
II	402 (40.2)	132 (41.6)	44 (41.9)		63 (36.4)	22 (38.6)	
III	287 (28.7)	93 (29.3)	22 (21.0)		54 (31.2)	21 (36.8)	
IV	169 (16.9)	50 (15.8)	13 (12.4)		30 (17.3)	4 (7.0)	
Unknown	3 (0.3)	1 (0.3)	1 (1.0)				
Comorbidity							
No	443 (44.3)	153 (48.3)	16 (15.2)	<.001	82 (47.4)	16 (28.1)	.010
Yes	556 (55.7)	164 (51.7)	89 (84.8)		91 (52.6)	41 (71.9)	
Microsatellite status							
MSI	90 (9.0)	34 (10.7)	14 (13.3)	.750	11 (6.4)	4 (7.0)	.682
MSS	870 (87.1)	269 (84.9)	86 (81.9)		157 (90.8)	50 (87.7)	
Unknown	39 (3.9)	14 (4.4)	5 (4.8)		5 (2.9)	3 (5.3)	

MSI, microsatellite instable tumors; MSS, microsatellite stable tumors.

Supplementary Table 3. Baseline Characteristics of the Colon Cancer Patients According to Intact BMP Signaling and Use of Statin After Diagnosis

		Non-intact BMP signaling (N=519)			Intact BMP signaling (N=457)		
	All patients (N=999)	No statin	Statin	*P* value	No statin	Statin	*P* value
Sex							
Male	505 (50.6)	195 (49.0)	73 (60.3)	.029	178 (47.3)	48 (59.3)	.052
Female	494 (49.4)	203 (51.0)	48 (39.7)		198 (52.7)	33 (40.7)	
Age							
<65	342 (34.2)	137 (34.4)	41 (33.9)	.001	141 (37.5)	18 (22.2)	<.001
66-74	304 (30.4)	106 (29.6)	51 (42.2)		100 (26.6)	40 (49.4)	
≥75	353 (35.4)	155 (38.9)	29 (24.0)		135 (35.9)	23 (28.4)	
Year of diagnosis							
2002–2004	451 (45.2)	188 (47.2)	56 (46.3)	.854	155 (41.2)	36 (44.4)	.594
2005–2007	548 (54.8)	210 (52.8)	65 (53.7)		221 (58.8)	45 (55.6)	
Disease stage							
I	138 (13.8)	44 (11.1)	20 (16.5)	.055	52 (13.8)	17 (21.0)	.012
II	402 (40.2)	138 (34.7)	53 (43.8)		168 (44.7)	36 (44.4)	
III	287 (28.7)	128 (32.2)	32 (26.5)		96 (25.5)	23 (28.4)	
IV	169 (16.9)	86 (21.6)	16 (13.2)		60 (16.0)	4 (4.9)	
Unknown	3 (0.3)	2 (0.5)				1 (1.2)	
Comorbidity							
No	443 (44.3)	205 (51.5)	26 (21.5)	<.001	188 (50.0)	16 (19.8)	<.001
Yes	556 (55.7)	193 (48.5)	95 (78.5)		188 (50.0)	65 (80.3)	
Microsatellite status							
MSI	90 (9.0)	34 (8.5)	10 (8.3)	.425	35 (9.3)	10 (12.4)	.628
MSS	870 (87.1)	356 (89.5)	106 (87.6)		333 (88.6)	70 (86.4)	
Unknown	39 (3.9)	8 (2.0)	5 (4.1)		8 (2.1)	1 (1.2)	

MSI, microsatellite instable tumors; MSS, microsatellite stable tumors.

Supplementary Table 4. Time-Dependent Analysis Overall Mortality, According to Tumor KRAS Mutation Status, BMP Signaling Pathway Status, and Use or Non-use of Statin after Diagnosis

	Statin non-users	Statin users[a]	*P* value
All Cancers			
Patients	789	210	
Deaths	396	69	
Adjusted rate ratio (95% CI)[b,c]	1.0 (reference)	0.67 (0.51–0.87)	.003
Adjusted hazard ratio (95% CI)[b,d]	1.0 (reference)	0.68 (0.53–0.90)	.007
KRAS Wild-type cancers			
Patients	317	105	
Deaths	162	40	
Adjusted rate ratio (95% CI)[b,c]	1.0 (reference)	0.81 (0.56–1.18)	.273
Adjusted hazard ratio (95% CI)[b,d]	1.0 (reference)	0.85 (0.59–1.23)	.390
KRAS Mutant cancers			
Patients	173	57	
Deaths	89	18	
Adjusted rate ratio (95% CI)[b,c]	1.0 (reference)	0.59 (0.35–1.03)	.062
Adjusted hazard ratio (95% CI)[b,d]	1.0 (reference)	0.59 (0.34–1.03)	.065
Intact BMP signaling			
Patients	376	81	
Deaths	158	24	
Adjusted rate ratio (95% CI)[b,c]	1.0 (reference)	0.46 (0.29–0.74)	**.001**
Adjusted hazard ratio (95% CI)[b,d]	1.0 (reference)	0.49 (0.31–0.79)	**.003**
Non-intact BMP signaling			
Patients	398	121	
Deaths	229	42	
Adjusted rate ratio (95% CI)[b,c]	1.0 (reference)	0.75 (0.53–1.06)	.106
Adjusted hazard ratio (95% CI)[b,d]	1.0 (reference)	0.78 (0.55–1.11)	.169

[a]Statin use after diagnosis.
[b]Adjusted for sex, age, comorbidity, year of incidence, histologic grade, stage, microsatellite status, and chemotherapy.
[c]Poisson model.
[d]Cox model.