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Prognostic utility of molecular factors by age at diagnosis of colorectal cancer

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Abstract

PURPOSE—We hypothesized that adverse prognostic associations of specific tumor molecular factors vary by patient age at colorectal cancer (CRC) diagnosis.

EXPERIMENTAL DESIGN—We examined the prognostic associations and interactions by age at CRC diagnosis (<60 vs. 60–74 vs. ≥75 years old) of key molecular factors – CpG island methylator phenotype (CIMP), microsatellite instability (MSI), *KRAS*, *BRAF*, and *PIK3CA* mutations, and nuclear CTNNB1 expression status – on CRC-specific survival and overall survival, utilizing 1280 incident CRC cases (median age 69 years, range 38–91 years) within the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) cohorts.

RESULTS—MSI-high was associated with better survival while *BRAF* mutation was associated with worse survival, but these associations did not appreciably differ by age group. Status of

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CIMP, *KRAS* mutation, or *PIK3CA* mutation was not associated with prognosis regardless of age. Nuclear CTNNB1 expression was associated with a trend toward worse prognosis among older adults (age ≥75) [multivariate hazard ratio (HR), 1.67; 95% confidence interval (CI) 0.89 to 3.13 (for CRC-specific survival); multivariate HR 1.44; 95% CI 0.93 to 2.24 (for overall survival)] but not among younger patients, and there was a statistically significant interaction by age (p-interaction=0.03 for CRC-specific survival; p-interaction=0.007 for overall survival).

CONCLUSIONS—Tumor nuclear CTNNB1 expression may be associated with higher mortality among older CRC patients but not among younger patients. Our findings need to be confirmed in independent datasets. Detailed exploration of tumor molecular signatures in older CRC patients in large populations is warranted.

INTRODUCTION

Colorectal cancer (CRC) is the 4th leading cancer diagnosis and 2nd cause of cancer death in the United States, with an estimated 140,000 diagnoses and 50,000 deaths annually.([1]) Despite 36% of CRC patients being age 75 or older at diagnosis, guidance on how to approach the treatment of this population is limited. Among those selected to receive chemotherapy, survival outcomes are similar to younger patients in most,([2–4]) but not all studies.([5]) Yet, only one-third of older adults receive indicated chemotherapy.([6]) There is a paucity of research concerning which factors should determine patient selection for treatment and the resulting survival outcomes. Most of this limited research focuses on age alone as a predictor of treatment and survival outcomes.

Advancing age has long been recognized as a potent risk factor for the development of cancer, further underscored by the fact that nearly 60% of CRC is diagnosed in those age ≥65. Several investigators have postulated mechanisms by which aging impacts CRC carcinogenesis, including accumulation of somatic mutations over time and epigenetic silencing.([7]) Baseline rates of detectable somatic mosaicism in the general population are low, but generally higher in older adults (~2% in people with age ≥75) than in younger adults (< 0.5% in people with age <50).([7, 8]) However, the degree to which CRC carcinogenesis differs by age at diagnosis, as driven by somatic mutations and epigenetic changes, is not well known. We hypothesized that adverse prognostic associations of key molecular factors would be disproportionately higher in older adults than younger adults at diagnosis of CRC.

To test this hypothesis, we examined the prognostic associations of CpG island methylator phenotype (CIMP), microsatellite instability (MSI), *KRAS*, *BRAF*, and *PIK3CA* mutations, and CTNNB1 (ß-catenin) nuclear expression status according to age group (at CRC diagnosis) among 1280 cases of CRC within two large prospective longitudinal cohorts. We tested the statistical interaction of age at CRC diagnosis with each molecular factor on CRC-specific survival and overall survival. The combined prospective cohorts used for analysis provide the unique advantage of a large age distribution of incident, previously untreated CRC cases with well-annotated tumor molecular data to address the hypothesis.

MATERIALS AND METHODS

Study Population

Initiated in 1976, the Nurses' Health Study (NHS)([9]) is a prospective U.S. nationwide cohort of 121,700 female registered nurses age 30 to 55 years at the time of enrollment, who responded to a mailed questionnaire regarding cancer and cardiovascular risk. The Health Professionals Follow-up Study (HPFS) subsequently enrolled 51,529 male health professionals, age 40 to 75 years beginning in 1986. Both cohorts continue to complete biennial follow-up questionnaires updating information on medical history and potential risk factors. The studies were approved by the Human Subjects Committees at Harvard T.H. Chan School of Public Health and Brigham and Women's Hospital (both Boston, MA). All participants signed informed consent permitting questionnaire, blood and tumor data to be used in research studies.

The study population consists of NHS and HPFS subjects with pathologically confirmed colon or rectal carcinoma diagnosed up to June 1, 2010 for NHS and January 1, 2010 for HPFS with available CRC tumor specimen for analysis (Figure 1). Subjects with other cancer occurring within 3 years before colorectal cancer diagnosis (except non-melanoma skin cancer) were excluded from the analysis (n=33). The final cohort includes 1280 subjects including 690 from NHS and 590 from HPFS.

Identification of Colorectal Cancer

For respondents reporting a diagnosis of CRC within the prior 2 years, we requested permission to review all hospital and pathology records pertaining to CRC. Once obtained, study physicians blinded to patient outcomes extracted information on American Joint Committee on Cancer (AJCC) stage, histology, tumor location and date of diagnosis. Cause and date of death were obtained from the National Death Index (NDI) for non-respondents. ([10]) Nearly 96% of all incident CRC cases were identified by either of these two methods. ([11]) For deceased participants with known or suspected cancer for which we have not been able to obtain medical records, we contacted the state tumor registry to confirm and classify the cancer. CRC treatment data are not available in these databases.

Analysis of Tumor Molecular Factors

The term *molecular factors* is used in this study to denote the accumulated somatic mutations associated with promotion of CRC. Archival CRC tumor specimens were collected from the hospitals at which subjects underwent resection or biopsy of CRC. All genomic DNA extraction from paraffin-embedded tissue and whole genome amplification by polymerase chain reaction (PCR) were performed as previously described.([9]) All CRC tumor block specimens and hematoxylin and eosin (H&E)-stained tissue sections were reviewed by a pathologist (S.O.) with established quality control measures consistent with the strategy used in prior NHS/HPFS analyses.([9]) We use HUGO (Human Genome Organisation)-approved official symbols for genes and gene products – including *BRAF*, CTNNB1 [catenin (cadherin-associated protein), beta 1, 88kDa; so-called β-catenin], *KRAS*, and *PIK3CA*.([12])

Analysis of *PIK3CA, KRAS, BRAF* and CIMP

PCR and pyrosequencing of PIK3CA (exons 9 and 20),([11], [13]) KRAS (codons 12, 13, 61 and 146),([14], [15]) and BRAF (codon 600) were performed as previously described.([16]) Using MethyLight assay,([17]) DNA methylation was quantified in 8 CIMP-specific promoters [*CACNA1G, CDKN2A (p16), CRABP1, IGF2, MLH1, NEUROG1, RUNX3*, and *SOCS1*].([18], [19]) Using these 8 markers, CIMP-high was defined as ≥6 methylated markers, CIMP-negative as 0 methylated markers, and the remainder as CIMP-low, as the previously established criteria.([20])

Analysis of MSI

MSI status was quantified using a 10-marker panel using D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67, and D18S487.([21]) MSI-high was defined as presence of instability in ≥30% of the markers, microsatellite instability low (MSI-low as 1–29% unstable markers, and microsatellite stability (MSS) as no unstable marker. Given no difference in prognosis between MSI-low and MSS tumors in prior analysis,([9]) they were combined in the present study.

Analysis of CTNNB1 (β-catenin)

Immunohistochemistry for CTNNB1 nuclear expression was performed as previously described, and interpreted as negative (weak or no expression) or positive (moderate or strong expression) by a pathologist (T.M.).([22]) A subset of cases (n=292) were independently interpreted by a second pathologist (S.O.); agreement between the two pathologists was 0.90 for CTNNB1 nuclear expression (κ=0.80; $p<0.0001$), indicating good to substantial agreement.

Definition of Age

The age at diagnosis was classified into three age groups (<60, 60–74 and ≥75). Older adults are defined as those age ≥75 years at diagnosis. Sensitivity analyses were conducted to examine other definitions of age to allow comparisons consistent with prior studies in the epigenetic,([9]) CRC and geriatric oncology([5], [23]) literature.

Outcome Measurement

Patients are observed until death and censored at last questionnaire prior to data analysis as of January 1, 2011. The two primary outcomes are CRC-specific survival and overall survival. For NHS/HPFS, follow-up began from date of CRC diagnosis. CRC-specific survival is defined as the time from CRC diagnosis to CRC-specific death; deaths from other causes are censored at the time of death. Overall survival is defined as the time from CRC diagnosis to death due to any cause. Date of death was obtained by report from family, postal authority or confirmation via the NDI.([10]) Cause of death was assigned by study physicians blinded to questionnaire responses. Nearly 98% of deaths were confirmed by these methods.([9])

Covariate Assessment

Known and potential prognostic factors affecting CRC-specific and overall survival were extracted from the hospital and pathology records, including AJCC stage, grade of tumor differentiation, histology, date and age of diagnosis. Body mass index (BMI), prediagnosis activity level and diagnosis of type 2 diabetes mellitus (DM), myocardial infarction (MI)/ congestive heart failure (CHF) and cerebrovascular accident (CVA) were taken from the biennial questionnaire preceding date of diagnosis as previously reported.([22, 24–28])

Statistical Analysis

Cox proportional hazards models were used to calculate hazard ratios (HRs) of death or death resulting from CRC according to molecular factors, adjusted using the stepwise variable selection method including BMI (<30, 30+, missing), prediagnosis activity (<18, 18+, missing), tumor location, tumor differentiation, and other markers (MSI, CIMP, *KRAS*, *BRAF*, CTNNB1 and *PIK3CA*). Gender, regular aspirin use and comorbidity (DM, MI/CHF, CVA) were forced in the model. Regular aspirin use is defined as at least 2 tablets/week in NHS and at least 2 times/week in HPFS. Disease stage was used as a stratifying variable. The analysis results are from Cox regression models where all patient data is censored at 5 years or 10 years for the respective CRC-specific and overall survival. We tested interaction impact of age on the association between each molecular factor and survival by including the cross-product of age as a continuous variable and each molecular factor in the model. We evaluated the distribution of molecular tumor factors using the X^2 test (categorical variables) and analysis of variance (ANOVA, continuous variables) across patient and disease factors. We considered multiple hypothesis testing adjusting the p for significance level to p=0.01 given 5 molecular factors evaluated. All analyses used SAS software, version 9.3 (SAS Institute, Inc, Cary, NC). Smoothing splines of log hazard were used to visualize the relationship between CRC-specific and overall survival at 5 years by molecular factor status.

RESULTS

Patient characteristics

The incident CRC cases with tumor sample availability in the combined NHS/HPFS cohort included 1280 patients (n=224 age <60, 756 age 60–74 and 300 age ≥75). We assessed patient and disease characteristics according to age at diagnosis (Table 1). Older patients were more likely to be male (65% vs. 30% age <60, 43% age 60–74), have a lower body mass index (93% BMI <30 vs. 86% age <60, 83% age 60–74), present with lower rate of stage IV disease at diagnosis (8% vs. 16% age <60, 14% age 60–74) and have tumors in the proximal colon (52% vs. 36% age <60, 49% age 60–74) (all p<0.001). Of those with reporting presence of comorbid medical conditions, older adults had the highest rate of prior cerebrovascular accident (56% vs. 1% age <60, 43% age 60–74) but second highest rate of DM (35% vs. 7% age <60, 57% age 60–74) and MI or CHF (45% vs. 2% age <60, 54% age 60–74) (Table 1). We adjusted all analyses for the presence of cerebrovascular accident, diabetes mellitus and myocardial infarction/congestive heart failure. There were no appreciable differences in race, year of diagnosis, tumor differentiation, number of lymph nodes examined, and number of lymph nodes positive.

Prevalence of molecular factors by age

We examined the distribution of potentially prognostic and/or predictive molecular changes in CRC tumor samples by age (Table 2). Fifteen percent of the overall cohort had MSI-high tumors; there was a statistically significant difference in the rate of MSI-high across age groups (8% age < 60, 17% age 60–74, 16% age ≥75; p=0.006) (Table 2). Similarly, 16% of the overall cohort has CIMP-high tumors and the distribution of CIMP-high was statistically significantly increased in older adults (5% age < 60; 18% age 60–74, and 18% age ≥75; p<0.0001). There were no differences in rates of *KRAS* mutation (p=0.53), *BRAF* mutation (p=0.14), *PIK3CA* mutation (p=0.50), and CTNNB1 nuclear expression positive (p=0.69) among the three age groups.

Prognostic utility of molecular factors by age

At 5 years following diagnosis, 297 (23%) patients died of CRC (22% <60; 23% age 60–74, 24% ≥75; p=0.90) and 372 (29%) patients died of CRC or other causes (23% <60; 28% age 60–74, 35% ≥75; p=0.008). Similar differences in CRC-specific and overall survival were noted at 10 years (p=0.98 and <0.001, respectively). Despite the observed similar rates of events at each time point, older age was associated with inferior CRC-specific and overall survival at 5 (CRC-specific survival p=0.003, overall survival p<0.0001) and 10 years (CRC-specific survival p=0.0002, overall survival p<0.0001), adjusting for gender, regular aspirin use, comorbid medical conditions (DM, MI/CHF, CVA), BMI, prediagnosis physical activity, tumor location, and tumor differentiation, stratifying by disease stage (Table 3), possibly reflecting difference in treatment receipt and tolerance.

The associations of molecular factors on CRC-specific and overall survival by age are depicted in Table 4 and Table 5. Adjusting for the afore-mentioned covariates as well as other molecular factors (e.g. for analysis of MSI, adjusting for CIMP, *KRAS, BRAF, PIK3CA* and CTNNB1), we examined the CRC-specific and overall survival at 10 years among the three age groups by each molecular factor. For the overall cohort, MSI-high was associated with improved CRC-specific and overall survival (data not shown) but there was no statistically significant interaction by age (p=0.17 for CRC-specific survival and p=0.94 for overall survival). In contrast, CIMP-high, *KRAS* mutation and *PIK3CA* was not associated with CRC-specific and overall survival (data not shown) and not associated with a statistically significant interaction by age (CRC-specific survival: p=0.92, 0.89, and 0.24, respectively; overall survival: p=0.53, 0.57, and 0.09, respectively). In contrast, *BRAF* mutation was associated with inferior CSS and OS within age group 60–74 years (CRC-specific survival: p=0.002; overall survival: p=0.02) but not in the other age groups [(age <60 – CRC-specific survival: p=0.65; overall survival: p=0.73), (age ≥75 – CRC-specific survival: p=0.83; overall survival: p=0.94)]. There was no statistically significant interaction of *BRAF* and CRC-specific and overall survival by age (p=0.25, 0.71 respectively). Although statistical power was limited in subgroup analyses, among those patients whose tumors are both MSI-high and CIMP-high, *BRAF* mutation might be prognostic of inferior survival [HR 1.48 (95% CI 0.97 to 2.24) for CRC-specific survival at 10 years; HR 1.28 (95% CI 0.91 to 1.80) for overall survival at 10 years]. However, there was no statistically significant interaction of age and *BRAF* mutation, among MSI-high/CIMP-high (p-within age strata = 0.07 for CRC-specific survival, p-within age strata = 0.15 for overall survival).

Positive CTNNB1 nuclear expression was associated with a trend toward inferior survival at 10 years and the adverse prognostic impact of positive CTNNB1 nuclear expression was significantly greater among older patients (p-interaction=0.03 for CRC-specific survival and 0.007 for overall survival). As depicted in Supplemental Figure 1, splines show the association of CTNNB1 nuclear expression status with CRC-specific and overall survival. The inflections within the splines for hazard ratios greater than 1 were observed at older age, as suggested in the trend toward inferior survival noted in Cox proportional hazards analyses. This association was not consistently modified by tumor location or presence of comorbid medical conditions. Older age was associated with nuclear CTNNB1 expression in the proximal colon (8% age <60, 15% age 60–75, 22% age ≥75; p=0.03). However, CTTNB1 expression in the distal colon or rectum was not appreciably different by age (data not shown; p=0.66 and p=0.30, respectively). Further, there was not a statistically significant difference in positive nuclear CTNNB1 expression by presence of diabetes mellitus (3% age <75 vs. 7% age ≥75; p=0.67), myocardial infarction/congestive heart failure (3% age <75 vs. 10% age ≥75; p=0.39) or cerebrovascular accident (2% age <75 vs. 7% age ≥75; p=0.07).

The observed association of examined molecular factors on CRC-specific and overall survival was not altered when alternative modeling of age is used (data not shown). For example, we examined age as a continuous variable as well as divided into two categories (age <70, ≥70) consistent with prior oncologic and geriatric literature,([2], [5], [29–32]) noting no change in associations of CTNNB1 on CRC-specific and overall survival.

DISCUSSION

In this prospective cohort of men and women diagnosed with colorectal cancer (CRC), we hypothesized that adverse prognostic associations of key molecular factors would be disproportionately higher in older adults than younger adults at diagnosis of CRC. We noted a higher prevalence of MSI-high and CIMP-high as well as a similar prevalence of *KRAS* mutation, *BRAF* mutation, positive CTNNB1 (β-catenin) nuclear expression and *PIK3CA* mutation in older adults compared to younger counterparts. Regardless of age, MSI-high was associated with better prognosis and *BRAF* mutation was associated with worse survival (consistent with earlier analysis([9], [33])), whereas neither CIMP, *KRAS*, nor *PIK3CA* status was associated with prognosis. Positive CTNNB1 nuclear expression in CRC tumors was associated with a trend toward worse prognosis among older adults with a statistically significant interaction by age, making CTNNB1 an interesting molecular factor of interest for older adults diagnosed with CRC.

Analyses of tumor molecular features of CRC have become important in clinical practice and research.([34–38]) Prognostic associations of tumor molecular features according to age at diagnosis of CRC have not been adequately studied. Given a recent trend of increasing age at CRC diagnosis, our prospective cohort studies could provide us with an unique opportunity to address this critical unmet need.

CTNNB1 (the β-catenin gene) is a mediator of the canonical WNT signaling pathway regulating key genes, including those involved in CRC carcinogenesis and tumor progression.([39]) CTNNB1 nuclear expression has been associated inversely with CIMP-

high, independent of MSI status.([40]) Obesity and physical activity have been associated with higher risk of CTNNB1 nuclear-negative CRC,([28]) and with higher CRC mortality in CTNNB1 nuclear-negative subtype.([22]) There is no known age-specific data in cancer but it has been associated with other aging-related disease. WNT pathway activation triggers accelerated cellular senescence in klotho mouse model of accelerated aging,([41]) failure of vascular cell proliferation necessary for vascular repair,([42]) Alzheimer's disease,([39]) and has been implicated in the pathogenesis of osteoarthritis.([39], [43]) In CRC, the tumor suppressor gene APC is implicated in hereditary CRC, Familial Adenomatous Polyposis, and most of sporadic CRCs have somatic APC mutations. APC is a known negative regulator of the WNT pathway.

Inhibitors of the WNT pathway have yet to be tested in CRC or specifically in older adults with cancer; however, evidence suggests that NSAIDs, such as aspirin, may act as modifiers of CTNNB1-associated CRC carcinogenesis and progression via modification of the WNT/CTNNB1 pathway.([44]) Evaluation of the impact of regular aspirin use on the observed age-related difference in CTNNB1 and CRC-specific and overall survival among older adults with CRC and unaffected counterparts is needed to understand how aspirin use may modify survival among CRC tumors lacking CTNNB1 nuclear expression. Finally, additional research is needed to determine the correlation of MSI/CIMP status with rates of somatic mutations that may have downstream implications for prognosis, response to therapy, and potentially for treatment resistance. Investigators of The Cancer Genome Atlas Network performed exome capture DNA sequencing on 224 tumor and normal pairs of human CRC cases.([45]) Age-specific analysis within this cohort is forthcoming with validation within the NHS/HPFS cohort, linked to Medicare claims data on chemotherapy treatment, to determine the impact of explored somatic mutations on CRC-specific and overall survival. Such analysis will provide additional insights because the current analysis lacks information on treatment or bench-marking against normal cases of older adults without CRC, particularly given the association of CTNNB1 with other age-related conditions.

The combined NHS/HPFS database provided the unique advantage of integrative molecular pathological epidemiology([46]) data within a large age distribution of incident previously untreated CRC cases. Nonetheless, there are limitations to this analysis. We lack available treatment data for the cohort. While the yield and quality of CRC tumor specimens in NHS/HPFS were high, there were some incident CRC cases for which tumor specimens were not available. However, there were no substantial differences in patient or tumor characteristics between incident CRC patients with and without available tumor specimens.([9]) In addition, residual confounding might be an issue in any observational study; however, one of the advantages of the NHS/HPFS cohorts is availability of data on potential confounders including comorbidities and detailed clinical and tumor characteristics. Lastly, the majority of the younger cohort age <60 were diagnosed with CRC before 2002. This is a consequence of the age at which patients were enrolled in the NHS and HPFS cohorts, potentially negatively impacting the overall cancer specific survival within this age group.

In conclusion, our findings suggest an age-specific pattern of molecular factors associated with CRC-specific and overall survival among older adults diagnosed with CRC. Specifically, we observed trend toward an inferior survival among older adults by tumor

CTNNB1 nuclear expression status compared to younger counterparts. Given the call to integrate molecular, histopathologic and physiologic factors in the study of aging and cancer, ([7]) subsequent planned investigation includes determination of the molecular characterization of CRC by age and evaluation of interaction with chemotherapy treatment among older adults. Determination of the mechanisms underlying observed differences in survival and treatment response for older adults diagnosed with CRC may ultimately be translated from the laboratory to patient care to inform subsequent development of prevention strategies, targeted therapies and treatment selection for this population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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STATEMENT OF TRANSLATIONAL RELEVANCE

It is not known whether the prognostic associations of colorectal cancer (CRC) molecular factors vary by patient age at diagnosis. Advancing age has long been recognized as a potent risk factor for the development of cancer. Yet, whether and how CRC progression differs by age at diagnosis, influenced by tumor molecular features, remain poorly understood. We hypothesized that prognostic associations of key molecular factors would be disproportionately pronounced in older adults than younger adults at diagnosis of CRC. We found that positive CTNNB1 nuclear expression appeared to confer a greater adverse association among older patients. Evaluation of inhibitors of the WNT signaling pathway, such as aspirin, on the observed age-related association of CTNNB1 expression with CRC-specific and overall survival is warranted.

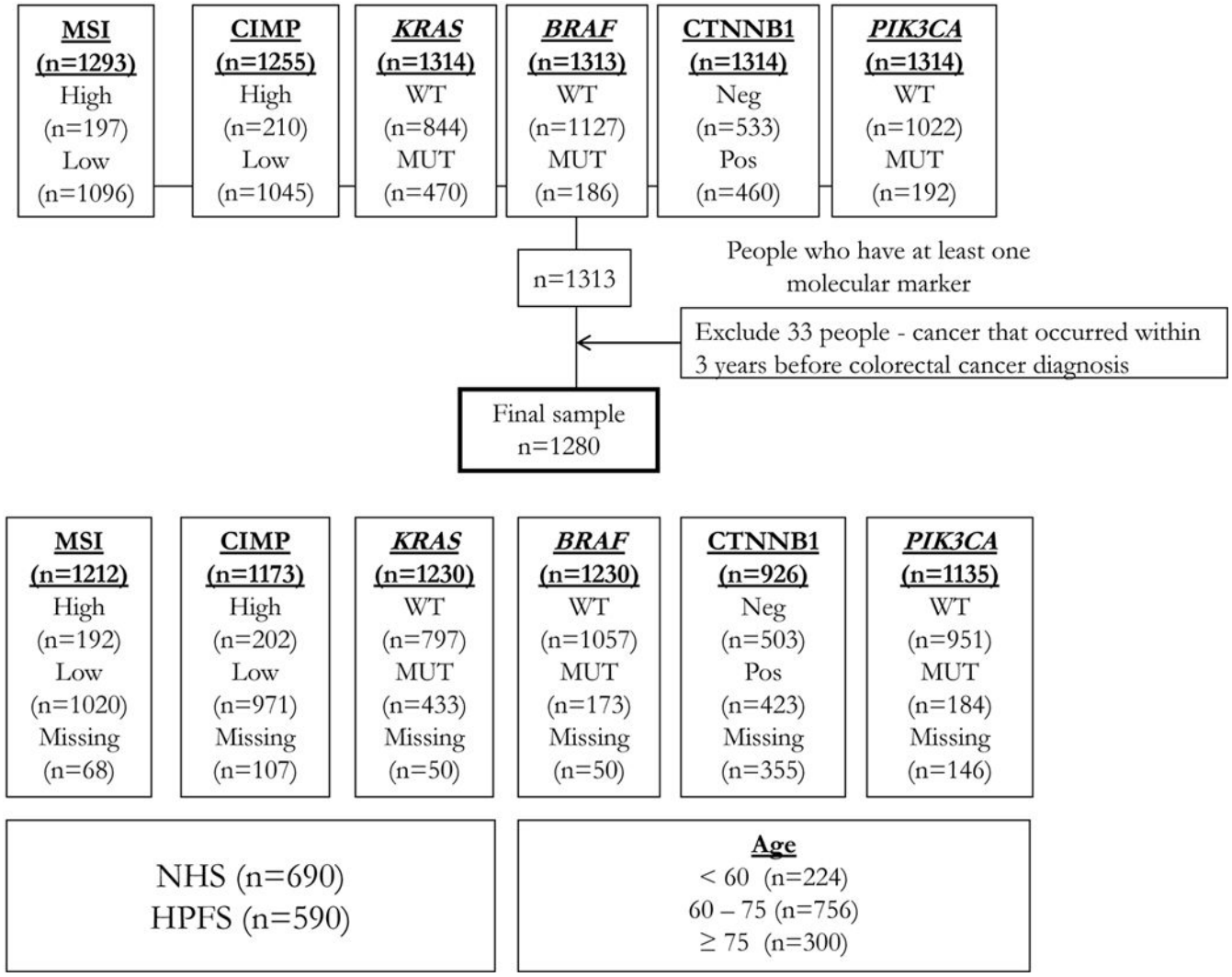

Figure 1.

Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) incident colorectal case cohort

Table 1

Baseline characteristics of colorectal cancer (CRC) cases by age group

Characteristic	Class		Age at CRC diagnosis		
		Overall N	<60 years	60–74 years	75+ years
Age	N	1280	224	756	300
	Mean		54.8	68.1	79.4
	SD		4.1	4.1	3.5
Gender	Female	690	156 (70%)	430 (57%)	104 (35%)
	Male	590	68 (30%)	326 (43%)	196 (65%)
Race	White	1214	218 (97%)	715 (95%)	281 (94%)
	Black	20	2 (0.9%)	13 (2%)	5 (2%)
	Asian	9	2 (0.9%)	5 (0.7%)	2 (0.7%)
Year of diagnosis	Before 2002	986	214 (96%)	610 (81%)	162 (54%)
	2002 or later	294	10 (5%)	146 (19%)	138 (46%)
BMI (kg/m^2)	<30	1098	192 (86%)	626 (83%)	280 (93%)
	30+	154	29 (13%)	108 (14%)	17 (6%)
Tumor location	Proximal colon	603	80 (36%)	367 (49%)	156 (52%)
	Distal colon	389	76 (34%)	242 (32%)	71 (24%)
	Rectum	282	66 (30%)	147 (19%)	69 (23%)
Disease stage	I	297	48 (21%)	173 (23%)	76 (25%)
	II	375	52 (23%)	238 (32%)	85 (28%)
	III	329	70 (31%)	185 (25%)	74 (25%)
	IV	161	35 (16%)	103 (14%)	23 (8%)
Tumor differentiation	Well/moderate	1144	201 (90%)	667 (88%)	276 (92%)
	Poor	125	21 (9%)	81 (11%)	23 (8%)
No. of lymph node examined	<12	556	103 (46%)	345 (46%)	108 (36%)
	12+	596	101 (45%)	338 (45%)	157 (52%)
No. of lymph node positive for metastasis	0	713	119 (53%)	429 (57%)	165 (55%)
	1–3	248	47 (21%)	150 (20%)	51 (17%)
	4+	249	53 (24%)	136 (18%)	60 (20%)

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Characteristic	Class	Overall N	Age at CRC diagnosis		
			<60 years	60–74 years	75+ years
Comorbid conditions	Diabetes mellitus	122	9 (4%)	70 (9%)	43 (14%)
	Myocardial Infarction (MI)	83	1 (0.4%)	47 (6%)	35 (12%)
	CVA	90	1 (0.4%)	39 (5%)	50 (17%)
	COPD	57	3 (1%)	35 (5%)	19 (6%)
	CHF	82	2 (0.9%)	39 (5%)	41 (14%)
	MI/CHF	125	2 (0.9%)	67 (9%)	56 (19%)

Abbreviations: BMI, body mass index; No., number; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CRC, colorectal cancer; CVA, cerebrovascular accident; MI, myocardial infarction; SD, standard deviation.

Table 2

Distribution of tumor molecular features according to age group at colorectal cancer (CRC) diagnosis

Characteristic	Class	Age Group <60 years (n=224)	60–74 years (n=756)	75+ years (n=300)	Overall N=1280	P-value
MSI						0.006
	Low/MSS	192 (86%)	590 (78%)	238 (79%)	1020 (80%)	
	High	18 (8%)	126 (17%)	48 (16%)	192 (15%)	
	Missing	14 (6%)	40 (5%)	14 (5%)	68 (5%)	
CIMP						<.0001
	Low/negative	202 (90%)	566 (75%)	203 (68%)	971 (76%)	
	High	11 (5%)	136 (18%)	55 (18%)	202 (16%)	
	Missing	11 (5%)	54 (7%)	42 (14%)	107 (8%)	
KRAS						0.53
	Wild-type	133 (59%)	446 (59%)	168 (56%)	747 (58%)	
	Mutant	81 (36%)	280 (37%)	122 (41%)	483 (38%)	
	Missing	10 (5%)	30 (4%)	10 (3%)	50 (4%)	
BRAF						0.14
	Wild-type	194 (87%)	615 (81%)	248 (83%)	1057 (83%)	
	Mutant	21 (9%)	108 (14%)	44 (15%)	173 (14%)	
	Missing	9 (4%)	33 (4%)	8 (3%)	50 (4%)	
CTNNB1 (nuclear expression)						0.69
	Negative	109 (49%)	308 (41%)	86 (29%)	503 (39%)	
	Positive	86 (38%)	256 (34%)	81 (27%)	423 (33%)	
	Missing	29 (13%)	192 (25%)	133 (44%)	354 (28%)	
PIK3CA						0.50

Characteristic	Class	Age Group			Overall N=1280	P-value
		<60 years (n=224)	60–74 years (n=756)	75+ years (n=300)		
	Wild-type	160 (71%)	563 (75%)	228 (76%)	951 (74%)	
	Mutant	27 (12%)	106 (14%)	51 (17%)	184 (14%)	
	Missing	37 (17%)	87 (12%)	21 (7%)	145 (11%)	

Abbreviations: CIMP, CpG island methylator phenotype; MSI, microsatellite instability; MSS, microsatellite stable.

Table 3

Colorectal cancer -specific and overall survival by age group

Survival Characteristic		Model	Age group <60 years (n=224)	Age group 60–74 years (n=756)	Age group 75+ years (n=300)	p-within age strata
CRC-specific survival	5yr	Event, N (%)	50 (22%)	175 (23%)	72 (24%)	
		Unadjusted, HR (95% CI)	1 (Referent)	1.05 (0.76 to 1.43)	1.14 (0.79 to 1.63)	0.57
		Adjusted, HR (95% CI)	1 (Referent)	1.46 (1.03 to 2.09)	2.13 (1.39 to 3.27)	0.003
	10yr	Event, N (%)	60 (27%)	207 (27%)	81 (27%)	
		Unadjusted, HR (95% CI)	1 (Referent)	1.06 (0.80 to 1.42)	1.15 (0.82 to 1.60)	0.38
		Adjusted, HR (95% CI)	1 (Referent)	1.45 (1.06 to 1.99)	2.15 (1.46 to 3.17)	0.0002
Overall survival	5yr	Event, N (%)	52 (23%)	214 (28%)	106 (35%)	
		Unadjusted, HR (95% CI)	1 (Referent)	1.24 (0.91 to 1.67)	1.63 (1.17 to 2.27)	0.002
		Adjusted, HR (95% CI)	1 (Referent)	1.67 (1.19 to 2.34)	2.68 (1.81 to 3.96)	<.0001
	10yr	Event, N (%)	63 (28%)	304 (40%)	152 (51%)	
		Unadjusted, HR (95% CI)	1 (Referent)	1.56 (1.19 to 2.05)	2.32 (1.73 to 3.12)	<.0001
		Adjusted, HR (95% CI)	1 (Referent)	1.91 (1.42 to 2.57)	3.20 (2.29 to 4.49)	<.0001

Abbreviations: HR, hazard ratio; CI, confidence interval

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Table 4

Colorectal cancer (CRC)-specific survival at 10 years by age and molecular factor

Molecular Factor	Model	Age group	Class		P-within age strata	P-interaction
MSI			Low/MSS	High		
	Event/N	<60 years	56/192	2/18		
		60–74 years	179/590	17/126		
		75+ years	75/238	3/48		
	Unadjusted, HR (95% CI)	<60 years	1 (Referent)	0.36 (0.09 to 1.49)	0.16	0.11
		60–74 years	1 (Referent)	0.42 (0.26 to 0.69)	0.0007	
		75+ years	1 (Referent)	0.16 (0.05 to 0.52)	0.002	
	Adjusted, HR (95% CI)	<60 years	1 (Referent)	0.47 (0.11 to 1.97)	0.30	0.17
		60–74 years	1 (Referent)	0.42 (0.24 to 0.72)	0.002	
		75+ years	1 (Referent)	0.18 (0.06 to 0.58)	0.004	
CIMP			Low/negative	High		
	Event/N	<60 years	55/202	2/11		
		60–74 years	164/566	29/136		
		75+ years	64/203	10/55		
	Unadjusted, HR (95% CI)	<60 years	1 (Referent)	0.67 (0.16 to 2.74)	0.58	0.21
		60–74 years	1 (Referent)	0.75 (0.50 to 1.11)	0.15	
		75+ years	1 (Referent)	0.52 (0.27 to 1.01)	0.06	
	Adjusted, HR (95% CI)	<60 years	1 (Referent)	0.37 (0.09 to 1.59)	0.18	0.92
		60–74 years	1 (Referent)	0.89 (0.53 to 1.48)	0.65	
		75+ years	1 (Referent)	0.68 (0.32 to 1.44)	0.32	
KRAS			Wild-type	Mutant		
	Event/N	<60 years	34/133	24/81		
		60–74 years	109/446	88/280		
		75+ years	41/168	39/122		
	Unadjusted, HR (95% CI)	<60 years	1 (Referent)	1.21 (0.72 to 2.04)	0.47	0.80
		60–74 years	1 (Referent)	1.30 (0.98 to 1.73)	0.07	

Molecular Factor	Model	Age group		Class	P-within age strata	P-interaction
		75+ years	1 (Referent)	1.36 (0.88 to 2.11)	0.17	
	Adjusted, HR (95% CI)	<60 years	1 (Referent)	1.12 (0.64 to 1.94)	0.70	0.89
		60–74 years	1 (Referent)	0.96 (0.71 to 1.29)	0.78	
		75+ years	1 (Referent)	1.02 (0.64 to 1.61)	0.94	
BRAF			Wild-type	Mutant		
	Event/N	<60 years	53/194	5/21		
		60–74 years	162/615	34/108		
		75+ years	72/248	8/44		
	Unadjusted, HR (95% CI)	<60 years	1 (Referent)	0.92 (0.37 to 2.30)	0.86	0.52
		60–74 years	1 (Referent)	1.36 (0.94 to 1.97)	0.11	
		75+ years	1 (Referent)	0.58 (0.28 to 1.19)	0.14	
	Adjusted, HR (95% CI)	<60 years	1 (Referent)	0.80 (0.31 to 2.07)	0.65	0.25
		60–74 years	1 (Referent)	2.06 (1.31 to 3.23)	0.002	
		75+ years	1 (Referent)	1.09 (0.49 to 2.43)	0.83	
CTNNB1 (nuclear expression)			Negative	Positive		
	Event/N	<60 years	33/109	17/86		
		60–74 years	95/308	60/256		
		75+ years	18/ 86	25/81		
	Unadjusted, HR (95% CI)	<60 years	1 (Referent)	0.59 (0.33 to 1.06)	0.08	0.03
		60–74 years	1 (Referent)	0.72 (0.52 to 0.99)	0.04	
		75+ years	1 (Referent)	1.55 (0.85 to 2.85)	0.15	
	Adjusted, HR (95% CI)	<60 years	1 (Referent)	0.64 (0.35 to 1.16)	0.14	0.03
		60–74 years	1 (Referent)	0.69 (0.49 to 0.96)	0.03	
		75+ years	1 (Referent)	1.67 (0.89 to 3.13)	0.11	
PIK3CA			Wild-type	Mutant		
	Event/N	<60 years	44/160	9/27		
		60–74 years	152/563	28/106		
		75+ years	63/228	12/51		

Molecular Factor	Model	Age group		Class	P-within age strata	P-interaction
	Unadjusted, HR (95% CI)	<60 years	1 (Referent)	1.33 (0.65 to 2.71)	0.44	0.25
		60–74 years	1 (Referent)	0.98 (0.65 to 1.46)	0.92	
		75+ years	1 (Referent)	0.80 (0.43 to 1.48)	0.48	
	Adjusted, HR (95% CI)	<60 years	1 (Referent)	1.42 (0.68 to 2.95)	0.35	0.24
		60–74 years	1 (Referent)	0.88 (0.58 to 1.33)	0.54	
		75+ years	1 (Referent)	0.72 (0.38 to 1.36)	0.31	

Abbreviations: HR, hazard ratio; CI, confidence interval; CIMP, CpG island methylator phenotype; MSI, microsatellite instability; MSS, microsatellite stable.

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Table 5

Overall survival at 10 years by age and molecular factor

Molecular Factor	Model	Age group	Class		P- within age strata	P-interaction
MSI			Low	High		
	Event/N	<60 years	57/192	3/18		
		60–74 years	251/590	38/126		
		75+ years	129/238	17/48		
	Unadjusted, HR (95% CI)	<60 years	1 (Referent)	0.53 (0.16 to 1.68)	0.28	0.57
		60–74 years	1 (Referent)	0.66 (0.47 to 0.93)	0.02	
		75+ years	1 (Referent)	0.53 (0.32 to 0.88)	0.01	
	Adjusted, HR (95% CI)	<60 years	1 (Referent)	0.53 (0.16 to 1.72)	0.29	0.94
		60–74 years	1 (Referent)	0.62 (0.40 to 0.95)	0.03	
		75+ years	1 (Referent)	0.53 (0.30 to 0.93)	0.03	
CIMP			Low	High		
	Event/N	<60 years	58/202	2/11		
		60–74 years	235/566	51/136		
		75+ years	110/203	27/55		
	Unadjusted, HR (95% CI)	<60 years	1 (Referent)	0.61 (0.15 to 2.50)	0.49	0.84
		60–74 years	1 (Referent)	0.90 (0.67 to 1.22)	0.51	
		75+ years	1 (Referent)	0.83 (0.54 to 1.26)	0.38	
	Adjusted, HR (95% CI)	<60 years	1 (Referent)	0.53 (0.13 to 2.20)	0.38	0.53
		60–74 years	1 (Referent)	1.11 (0.76 to 1.64)	0.59	
		75+ years	1 (Referent)	1.17 (0.73 to 1.87)	0.52	
KRAS			Wild-type	Mutant		
	Event/N	<60 years	36/133	24/81		
		60–74 years	171/446	119/280		
		75+ years	82/168	67/122		
	Unadjusted, HR (95% CI)	<60 years	1 (Referent)	1.16 (0.69 to 1.94)	0.58	0.87
		60–74 years	1 (Referent)	1.13 (0.89 to 1.42)	0.32	

Molecular Factor	Model	Age group	Class		P- within age strata	P-interaction
		75+ years	1 (Referent)	1.17 (0.85 to 1.62)	0.33	
	Adjusted, HR (95% CI)	<60 years	1 (Referent)	1.07 (0.63 to 1.81)	0.81	0.57
		60–74 years	1 (Referent)	0.88 (0.69 to 1.12)	0.29	
		75+ years	1 (Referent)	0.87 (0.62 to 1.21)	0.405	
BRAF			Wild-type	Mutant		
	Event/N	<60 years	55/194	5/21		
		60–74 years	241/615	48/108		
		75+ years	132/248	18/44		
	Unadjusted, HR (95% CI)	<60 years	1 (Referent)	0.86 (0.34 to 2.15)	0.75	0.38
		60–74 years	1 (Referent)	1.29 (0.95 to 1.76)	0.10	
		75+ years	1 (Referent)	0.69 (0.42 to 1.13)	0.14	
	Adjusted, HR (95% CI)	<60 years	1 (Referent)	0.84 (0.33 to 2.16)	0.73	0.71
		60–74 years	1 (Referent)	1.56 (1.07 to 2.27)	0.02	
		75+ years	1 (Referent)	0.98 (0.56 to 1.71)	0.94	
CTNNB1 (nuclear expression)			Negative	Positive		
	Event/N	<60 years	34/109	19/86		
		60–74 years	135/308	96/256		
		75+ years	39/ 86	47/81		
	Unadjusted, HR (95% CI)	<60 years	1 (Referent)	0.63 (0.36 to 1.10)	0.11	0.007
		60–74 years	1 (Referent)	0.80 (0.61 to 1.03)	0.09	
		75+ years	1 (Referent)	1.37 (0.89 to 2.09)	0.15	
	Adjusted, HR (95% CI)	<60 years	1 (Referent)	0.66 (0.38 to 1.17)	0.16	0.007
		60–74 years	1 (Referent)	0.78 (0.60 to 1.03)	0.08	
		75+ years	1 (Referent)	1.44 (0.93 to 2.24)	0.10	
PIK3CA			Wild-type	Mutant		
	Event/N	<60 years	44/160	10/27		
		60–74 years	228/563	37/106		
		75+ years	115/228	24/51		

Molecular Factor	Model	Age group		Class	P- within age strata	P-interaction
	Unadjusted, HR (95% CI)	<60 years	1 (Referent)	1.51 (0.76 to 3.00)	0.24	0.17
		60–74 years	1 (Referent)	0.85 (0.60 to 1.20)	0.35	
		75+ years	1 (Referent)	0.87 (0.56 to 1.36)	0.55	
	Adjusted, HR (95% CI)	<60 years	1 (Referent)	1.52 (0.76 to 3.05)	0.23	0.09
		60–74 years	1 (Referent)	0.77 (0.54 to 1.10)	0.15	
		75+ years	1 (Referent)	0.69 (0.44 to 1.09)	0.11	

Abbreviations: HR, hazard ratio; CI, confidence interval; CIMP, CpG island methylator phenotype; MSI, microsatellite instability; MSS, microsatellite stable.