Published in final edited form as:
Int J Colorectal Dis. 2013 December ; 28(12): . doi:10.1007/s00384-013-1715-8.

Descriptive profile of *PIK3CA*-mutated colorectal cancer in postmenopausal women

Amanda I. Phipps,
Public Health Sciences Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N., M4-C308, Seattle, WA 98109, USA

Karen W. Makar, and
Public Health Sciences Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N., M4-B402, Seattle, WA 98109, USA

Polly A. Newcomb
Public Health Sciences Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N., M4-B402, Seattle, WA 98109, USA

Amanda I. Phipps: aphipps@fhcrc.org

Abstract

Purpose—Approximately 10–30 % of colorectal cancers exhibit somatic mutations in the phosphoinositide-3-kinase, catalytic, alpha polypeptide gene (*PIK3CA*). We evaluated the relationship between *PIK3CA* mutation status and demographic factors, lifestyle factors, and other tumor characteristics and the relationship between *PIK3CA* mutation status and colorectal cancer survival.

Methods—The population-based study included postmenopausal women with invasive colorectal cancer diagnosed between 1998 and 2002 in Western Washington State. Participants were interviewed, and tumor specimens were tested for *PIK3CA* mutations in exons 9 and 20 hotspots, *KRAS* exon 2 mutations, *BRAF* p.V600E mutation, and microsatellite instability. We used Cox regression to evaluate the association between *PIK3CA* mutation status and disease-specific and overall survival. Stratified analyses were conducted by *KRAS* mutation status.

Results—*PIK3CA* mutations were evident in approximately 13 % of cases (N=35). Women with *PIK3CA*-mutated colorectal cancer were significantly more likely than those with *PIK3CA* wild-type disease to be non-white, to have proximal colon cancer, and to have *KRAS*-mutated tumors (p<0.05). In Cox proportional hazards regression analyses, overall survival was poorer, although not statistically significantly so, for women with *PIK3CA*-mutated versus wild-type colorectal cancer (hazard ratio=1.74, 95 % confidence interval 0.86–3.50). This association between *PIK3CA* mutation status and survival was evident only when analyses were restricted to cases without somatic *KRAS* mutations (hazard ratio=2.94, 95 % confidence interval 1.12–7.73).

Conclusions—*PIK3CA*-mutated colorectal cancer appears to have a distinct epidemiologic profile that is of clinical significance. Women with *PIK3CA*-mutated colorectal cancer experience a poorer prognosis than those with *PIK3CA* wild-type disease.

Correspondence to: Amanda I. Phipps, aphipps@fhcrc.org.

Conflict of interest The authors declare that they have no conflicts of interest.

Keywords

Colorectal cancer; PIK3CA; Survival; KRAS

Introduction

The phosphoinositide-3-kinase, catalytic, alpha polypeptide gene (*PIK3CA*) is mutated in approximately 10–30 % of all colorectal cancers [1-7]. Activating mutations in *PIK3CA* result in stimulation of the Akt pathway which, in turn, contributes to increased proliferation and tumor invasion [8, 9]. Although the PI3K/Akt pathway is likely to play a critical role in colorectal tumorigenesis and colorectal cancer progression, the prognostic significance of *PIK3CA* mutation status, and the descriptive epidemiology of *PIK3CA*-mutated colorectal cancer, has not yet been well characterized. Previous studies have suggested a possible relationship in colorectal cancer between somatic mutations in the PI3K/Akt and RAS/RAF/MAPK pathways, both of which are EGFR-dependent. In particular, *PIK3CA* mutations appear to be more common in *KRAS*-mutated than *KRAS* wild-type colorectal cancers [1-5]. The relationship between *PIK3CA* mutation status and other clinically relevant tumor characteristics, however, remains to be elucidated.

Using data from a population-based case–control study of incident invasive postmenopausal colorectal cancer [10], we evaluated differences in tumor characteristics, including *KRAS* mutation, *BRAF* mutation, microsatellite instability (MSI), and CpG island methylator phenotype (CIMP) status, as well as differences in patient characteristics and survival after diagnosis in women with *PIK3CA*-mutated versus *PIK3CA* wild-type colorectal cancer.

Methods

Study population

Details of the study population have been published elsewhere [10]. Briefly, eligible participants included women diagnosed with invasive colorectal cancer between January 1998 and June 2002 who, at the time of diagnosis, were aged 50–74 years and resided in Clallam, Grays Harbor, Island, Jefferson, Kitsap, Mason, San Juan, Skagit, Thurston, or Whatcom counties in Western Washington State. Women from three large additional counties (King, Pierce, and Snohomish) were also eligible for participation but were not included in the present analysis. All cases were identified through the population-based Surveillance, Epidemiology, and End Results (SEER) cancer registry serving Western Washington State. Study eligibility was limited to English speakers with a publicly available telephone number. Of 439 individuals contacted and identified as eligible, 44 (10 %) were deceased, 37 (8 %) were lost prior to interview, 3 (0.7 %) refused participation, and 2 (0.5 %) completed only a partial interview. In total, 80 % of eligible cases provided informed consent and were enrolled in the study (N=353). The present analysis was limited to enrolled cases for whom diagnostic tumor specimens could be obtained (N=279, 79 %), excluding two (0.7 %) cases for whom collected tumor specimens were not adequate for *PIK3CA* mutation testing.

At an average 15.9 months after diagnosis (median 2.5 months), participants completed a structured telephone interview in which they were asked to provide detailed information on a number of potential risk factors, including smoking history, body mass index, and use of selected medications, including nonsteroidal anti-inflammatory drugs (NSAIDs).

This study was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center in accordance with assurances filed with and approved by the U.S. Department of Health and Human Services.

PIK3CA mutation testing and additional tumor characterization

DNA was extracted from paraffin-embedded formalin-fixed (FFPE) tumor tissue using the QIAamp DNA FFPE Tissue kit (QIAGEN, Germantown, MD, USA). For cases for whom tumor DNA was successfully extracted (N=277), pyrosequencing was used to detect mutations in *PIK3CA* in three hotspots: codons 542 and 545 in exon 9 and codon 1047 in exon 20. These hotspots account for approximately 80 % of all *PIK3CA* mutations [11, 12]. Pyrosequencing was performed using the PyroMark Q96-MD and Q24 systems (QIAGEN), with an optimized dispensation order to maximize the detection of known variants in the exons 9 and 20 hotspots. For quality control purposes, pyrosequencing was also conducted on three cell lines known to have mutations in these hotspot regions and any failed samples were repeated at least once. A subset of cases (N=20) were tested for *PIK3CA* mutations using both pyrosequencing and Sanger sequencing of hotspot regions for further assay validation; Sanger sequencing was performed using the BigDyeTerminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Life Technologies, Grand Island, NY, USA) and was run on a 3130xl DNA sequencer (Applied Biosystems, Foster City, CA, USA). Cases for whom testing repeatedly failed or test results were equivocal for mutations in any of these regions were classified as having unknown *PIK3CA* mutation status (N=2).

Extracted tumor DNA was also tested for mutations in *KRAS* and *BRAF*, CIMP, and MSI. With respect to *KRAS*, the coding sequence of *KRAS* exon 2 was amplified [13], and mutations in exon 2 were identified via forward and reverse sequencing of amplified tumor DNA [14]. Testing for the c.1799T>A (p.V600E) *BRAF* mutation was conducted using a fluorescent allele-specific PCR assay as described previously [15]. With respect to MSI status, testing was based on a 10-gene panel assayed in tumor DNA and in DNA extracted from normal surrounding tissue (BAT25, BAT26, BAT40, MYCL, D5S346, D17S250, ACTC, D18S55, D10S197, and BAT34C4) using a 3130xl DNA sequencer (Applied Biosystems) [10, 16]; tumors were classified as MSI-H if instability was observed in ≥30 % of markers and as MSS if instability was observed in <30 % of markers. CIMP status was determined using the MethyLight assay on a 7900HT sequence detection system (Applied Biosystems) for a five-gene panel (*CACNA1G, IGF2, NEUROG1, RUNX3*, and *SOCS1*) [17]. Based on this assay, cases were classified as CIMP high if at least three of the five markers had a percent methylated of reference (i.e., percent methylated DNA in tumor relative to a control reference sample) greater than 10; otherwise, they were classified as CIMP-negative [17]. Primers for all assays were obtained from Invitrogen (Life Technologies), and all qPCR probes and PCR reagents were obtained from Applied Biosystems (Life Technologies).

Tumor site and stage at diagnosis information were obtained from SEER. Tumors located in the cecum through the splenic flexure were grouped together as proximal colon cancers (ICD-O-3 codes C180, C182, C183, C184, and C185) [18]. Tumors in the descending (C186) and sigmoid colon (C187) were classified as distal colon cancer, and tumors in the rectosigmoid junction (C199) and rectum (C209) were grouped together as rectal cancer. Stage at diagnosis was classified according to the American Joint Committee on Cancer staging conventions (I, II, III, and IV) [19].

Survival information

Vital status was determined periodically via linkage to SEER and the National Death Index. For cases who died during study follow-up, information was obtained on the date and cause

of death, classified according to ICD-10 conventions [20]. Deaths with an underlying cause attributed to ICD-10 codes C18.0-C20.0 or C26.0 (i.e., colorectal cancer) were classified as disease-specific mortality events.

Statistical analysis

We compared *PIK3CA*-mutated and *PIK3CA* wild-type colorectal cancer cases with respect to the distribution of demographic and lifestyle factors and additional tumor characteristics using chi-square tests. We also used Cox proportional hazards regression to evaluate the association between *PIK3CA* mutation status and survival after colorectal cancer diagnosis. The time axis for analysis was defined as days since diagnosis; participants were left-censored until the date of study enrollment. We conducted separate analyses using an outcome of all-cause mortality (i.e., overall survival) and death due to colorectal cancer (i.e., disease-specific survival). In analyses of disease-specific survival, persons who died due to causes other than colorectal cancer were censored at the time of death. All regression models were adjusted for age (5-year categories). We also assessed potential confounding by stage at diagnosis (I, II, and III–IV). Proportional hazards assumptions were assessed by testing for a nonzero slope of the scaled Schoenfeld residuals on ranked failure times [21]. All analyses were conducted in Stata SE version 12.0 (College Park, TX, USA).

Results

PIK3CA mutations were detected in approximately 13 % of cases (N=35). Characteristics of the study population are presented in Table 1 by *PIK3CA* mutation status. Compared to *PIK3CA* wild-type cases, cases with *PIK3CA*-mutated colorectal cancer were significantly more likely to be non-white (23 versus 4 %, p<0.001) and were slightly younger at diagnosis. There were no differences in the distribution of smoking history, body mass index, or NSAID use by *PIK3CA* mutation status. With respect to tumor characteristics, there were also no significant differences in the distribution of stage at diagnosis, CIMP, MSI, or *BRAF* mutation status by *PIK3CA* mutation status. However, we did find that *PIK3CA*-mutated colorectal cancer was significantly more likely to be *KRAS*-mutated (51 versus 30 %, p=0.01) and exhibited a right-sided shift: compared to *PIK3CA* wild-type colorectal cancer, *PIK3CA*-mutated tumors were more likely to be located in the proximal colon and less likely to be located in the rectum (p=0.03).

Over the course of study follow-up (mean=2 years, range 6 months–11 years), 35 % (N=97) of participants died (Fig. 1). Among those who died, 64 % (N=62) died due to CRC. Small numbers limited survival analyses; however, nonsignificant associations were suggestive of poorer survival in those with *PIK3CA*-mutated colorectal cancer, particularly with respect to overall survival [hazard ratio (HR)= 1.74, 95 % confidence interval (CI) 0.86–3.50 for overall survival and HR=1.25, 95 % CI 0.49–3.16 for disease-specific survival] (Table 2). Adjusting for stage at diagnosis, in addition to age at diagnosis, had an attenuating effect on point estimates. Further adjustment for all patient and tumor characteristics in Table 1 resulted in a stronger, but not statistically significant association between *PIK3CA* mutation status and overall survival (HR=1.82, 95 % CI 0.50–6.64), but attenuated the association with disease-specific survival (HR=1.08, 95 % CI 0.19–6.11). Adjustment for lifestyle factors and MSI status had the greatest impact on point estimates (results not shown). Although case numbers were limited, we found the association between *PIK3CA* mutation status and survival to be limited to women with *KRAS* wild-type disease: after excluding cases with *KRAS*-mutated colorectal cancer, age-adjusted point estimates for overall and disease-specific survival were elevated to 2.94 (95 % CI 1.12–7.73) and 2.13 (95 % CI 0.63–7.18), respectively.

Discussion

In this population-based cohort of women with incident invasive colorectal cancer, we found that *PIK3CA*-mutated tumors were distinct from *PIK3CA* wild-type tumors with respect to demographic and tumor characteristics and with respect to survival after diagnosis. In particular, we found that *PIK3CA*-mutated colorectal cancers were significantly more likely to be *KRAS*-mutated and that, among women with *KRAS* wild-type disease, the presence of a *PIK3CA* mutation was associated with significantly poorer survival. Unlike some previous studies, we found no association between *PIK3CA* mutation status and CIMP [1, 3] or *BRAF* mutation status [5].

Our findings regarding the relationship between *PIK3CA* mutation status and *KRAS* mutation status, and the impact of this relationship on colorectal cancer survival, is consistent with at least two previous analyses [4, 7]. Ogino et al. [4] reported that the presence of a *PIK3CA* mutation was associated with significantly poorer disease-specific survival in cases with *KRAS* wild-type colon cancer (HR=3.8, 95 % CI 1.6–9.3) but reported no such association in cases with *KRAS*-mutated disease. In a cohort of patients with chemotherapy–refractory metastatic colorectal cancer, De Roock et al. [7] found that *KRAS* wild-type patients with a *PIK3CA* mutation in exon 20 had significantly poorer overall survival (HR=3.3, 95 % CI 1.5–7.5) and progression-free survival (HR=2.3, 95 % CI 4.7) than those without a *PIK3CA* exon 20 mutation; however, associations between *PIK3CA* mutation and survival outcomes were not significant when including patients with *KRAS*-mutated disease.

The basis for this suggestive pattern of interaction in the association between *PIK3CA* mutation status and colorectal cancer survival by *KRAS* mutation status is unclear. However, it is known that KRAS can activate PI3K signaling [22]; thus, it is plausible that the relative impact of a *PIK3CA* mutation on activation of the PI3K/Akt pathway could be lower in *KRAS*-mutated colorectal cancer with constitutively active KRAS. Thus, the prognostic significance of a *PIK3CA* mutation may be limited to the setting where the *KRAS* oncogene, and the downstream PI3K/AKT signaling pathway, has not been constitutively activated by a *KRAS* mutation.

The results presented here should be interpreted in the context of study limitations. In particular, due to small numbers, we were not able to conduct analyses distinguishing between cases with *PIK3CA* mutations in exon 9 (helical domain) versus exon 20 (catalytic domain). These mutations may have differing functional consequences [23] and have been suggested to be associated with slightly different molecular profiles [3] and prognosis [7]. Small numbers also precluded more detailed stratified analyses for survival outcomes. Additionally, despite the population-based nature of this study, the fact that the study was limited to postmenopausal women may impact upon the broader generalizability of findings presented here; however, our findings with respect to survival and the relationship between *PIK3CA* and *KRAS* mutation status are consistent with previous studies not limited to postmenopausal women [4, 7]. Lastly, in the absence of detailed treatment information, we were not able to adjust for received treatment in our survival analyses; however, at present, *PIK3CA* mutations are rarely tested for in clinical settings and, as such, it is unlikely that treatment would have differed according to *PIK3CA* mutation status.

Inhibition of the PI3K/Akt pathway is being explored as a possible therapeutic approach for colorectal cancer [24], such that testing for the presence of a somatic *PIK3CA* mutation may become more clinically relevant. The presence of a *PIK3CA* mutation has also been suggested to predict low response to widely used targeted anti-EGFR therapy, primarily in the absence of a somatic *KRAS* mutation [7]. All cases enrolled in the present study were

diagnosed before anti-EGFR therapy was approved for the treatment of colorectal cancer; therefore, our results demonstrate that the relationship between *PIK3CA* and *KRAS* mutation status is significant beyond the setting of anti-EGFR therapy.

Point mutations in the helical (exon 9) and catalytic domains (exon 20) of *PIK3CA* have previously been hypothesized to contribute to colorectal tumorigenesis by activating the PI3K/Akt signaling pathway and promoting cellular proliferation [8, 9]. Despite small numbers, our results provide support for a role of *PIK3CA* mutations and, therefore, the PI3K/Akt pathway, in colorectal cancer survival as well. Our results also indicate a distinct clinicopathological profile of *PIK3CA*-mutated colorectal cancer, which includes an elevated prevalence of mutated *KRAS* and proximal location. We also observed a markedly higher prevalence of mutated *PIK3CA* in non-white study participants, which, to our knowledge, has not previously been reported and merits further investigation. Taken together with evidence from other small studies, our results provide support for the epidemiologic and clinical relevance of *PIK3CA* mutation status in colorectal cancer.

Acknowledgments

This work was supported by the National Cancer Institute, National Institutes of Health, United States Department of Health and Human Services (R01CA076366 to P.A.N.). A.I.P. was supported by a training grant from the National Cancer Institute (R25CA094880). The contribution of P.A.N. was also supported by the National Cancer Institute (K05CA152715 to P.A.N.). We wish to gratefully acknowledge the study participants and staff who made this research possible. We also thank Michelle A. Wurscher (Molecular Epidemiology Lab) and Cassie Sather (Genomics Shared Resource) at Fred Hutchinson Cancer Research Center for their technical expertise.

References

1. Nosho K, Kawasaki T, Ohnishi M, Suemoto Y, Kirkner GJ, Zepf D, et al. PIK3CA mutation in colorectal cancer: relationship with genetic and epigenetic alterations. Neoplasia. 2008; 10:534–541. [PubMed: 18516290]
2. Liao X, Morikawa T, Lochhead P, Imamura Y, Kuchiba A, Yamauchi M, et al. Prognostic role of PIK3CA mutation in colorectal cancer: cohort study and literature review. Clin Cancer Res. 2012; 18:2257–2268. [PubMed: 22357840]
3. Whitehall VL, Rickman C, Bond CE, Ramsnes I, Greco SA, Umapathy A, et al. Oncogenic PIK3CA mutations in colorectal cancers and polyps. Int J Cancer. 2012; 131:813–820. [PubMed: 21932420]
4. Ogino S, Nosho K, Kirkner GJ, Shima K, Irahara N, Kure S, et al. PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer. J Clin Oncol. 2009; 27:1477–1484. [PubMed: 19237633]
5. Velho S, Oliveira C, Ferreira A, Ferreira AC, Suriano G, Schwartz S Jr, et al. The prevalence of PIK3CA mutations in gastric and colon cancer. Eur J Cancer. 2005; 41:1649–1654. [PubMed: 15994075]
6. Li HT, Lu YY, An YX, Wang X, Zhao QC. KRAS, BRAF and PIK3CA mutations in human colorectal cancer: relationship with metastatic colorectal cancer. Oncol rep. 2011; 25:1691–1697. [PubMed: 21424126]
7. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol. 2010; 11:753–762. [PubMed: 20619739]
8. Samuels Y, Velculescu VE. Oncogenic mutations of PIK3CA in human cancers. Cell Cycle. 2004; 3:1221–1224. [PubMed: 15467468]
9. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell. 2007; 129:1261–1274. [PubMed: 17604717]
10. Newcomb PA, Zheng Y, Chia VM, Morimoto LM, Doria-Rose VP, Templeton A, Thibodeau SN, Potter JD. Estrogen plus progestin use, microsatellite instability, and the risk of colorectal cancer in women. Cancer Res. 2007; 67:7534–7539. [PubMed: 17671225]

11. Ligresti G, Militello L, Steelman LS, Cavallaro A, Basile F, Nicoletti F, Stivala F, McCubrey JA, Libra M. PIK3CA mutations in human solid tumors: role in sensitivity to various therapeutic approaches. Cell Cycle. 2009; 8:1352–1358. [PubMed: 19305151]
12. Baker CL, Vaughn CP, Samowitz WS. A PIK3CA pyrosequencing-based assay that excludes pseudogene interference. J Mol Diagn. 2012; 14:56–60. [PubMed: 22026957]
13. Oliner K, Juan T, Suggs S, Wolf M, Sarosi I, Freeman DJ, et al. A comparability study of 5 commercial KRAS tests. Diagn Pathol. 2010; 5:23. [PubMed: 20398393]
14. Alsop K, Mead L, Smith LD, Royce SG, Tesoriero AA, Young JP, et al. Low somatic K-ras mutation frequency in colorectal cancer diagnosed under the age of 45 years. Eur J Cancer. 2006; 42:1357–1361. [PubMed: 16765042]
15. Buchanan DD, Sweet K, Drini M, Jenkins MA, Win AK, English DR, et al. Risk factors for colorectal cancer in patients with multiple serrated polyps: a cross-sectional case series from genetics clinics. PLoS One. 2010; 5:e11636. [PubMed: 20661287]
16. 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; 58:5248–5257. [PubMed: 9823339]
17. 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; 38:787–793. [PubMed: 16804544]
18. World Health Organization. International classification of diseases for oncology. WHO; Geneva: 2000.
19. Greene, FL.; Page, DL.; Fleming, ID.; Fritz, AG.; Balch, CM.; Haller, DG.; Morrow, M. AJCC cancer staging handbook. 6. Springer; New York: 2002.
20. World Health Organization. International classification of diseases. WHO; Geneva: 2007.
21. Therneau, TM.; Grambsch, PM. Modeling survival data: extending the Cox model. Springer; New York: 2000.
22. Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nat Rev Cancer. 2007; 7:295–308. [PubMed: 17384584]
23. Zhao L, Vogt PK. Helical domain and kinase domain mutations in p110alpha of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. Proc Natl Acad Sci U S A. 2008; 105:2652–2657. [PubMed: 18268322]
24. Garrido-Laguna I, Hong DS, Janku F, Nguyen LM, Falchook GS, Fu S, et al. KRASness and PIK3CAness in patients with advanced colorectal cancer: outcome after treatment with early-phase trials with targeted pathway inhibitors. PLoS One. 2012; 7:e38033. [PubMed: 22675430]

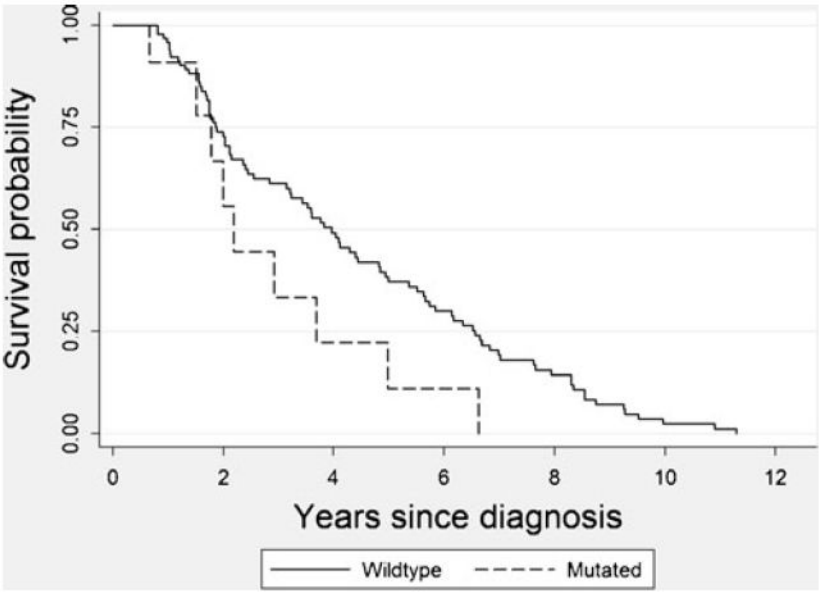

Fig. 1.

Unadjusted Kaplan–Meier survival curve comparing survival from all causes of death in postmenopausal women with *PIK3CA* wild-type colorectal cancer (*N*=240) (*solid line*) versus *PIK3CA*-mutated colorectal cancer (*N*=35) (*dashed line*). Proportional hazards regression analyses, adjusted for age and stage, provide further support for a poorer prognosis in those with *PIK3CA*-mutated disease, although point estimates are not statistically significant

Table 1

Patient and tumor characteristics by *PIK3CA* mutation status

	***PIK3CA* wild type (*N*=240)**	***PIK3CA*-mutated (*N*=35)**	**Chi-square *p* value**
Age at diagnosis			
<60	69 (29)	10 (29)	0.11
60–69	99 (41)	20 (57)	
70–79	72 (30)	5 (14)	
Mean (SD)	64.0 (7.2)	63.3 (6.7)	
Race			
White	230 (96)	27 (77)	<0.001
Non-white	10 (4)	8 (23)	
Cigarette smoking history			
Never smoker	118 (49)	22 (63)	0.32
Former smoker	86 (36)	9 (26)	
Current smoker	36 (15)	4 (11)	
Body mass index (kg/m^2)			
<25.0	95 (40)	13 (37)	0.85
25.0–29.9	71 (30)	12 (34)	
≥30.0	74 (31)	10 (29)	
Ever regularly used nonsteroidal anti-inflammatory drugs			
No	107 (45)	20 (57)	0.19
Yes	129 (55)	15 (43)	
Missing	4	0	
Stage at diagnosis			
I	80 (33)	8 (24)	0.10
II	76 (32)	18 (53)	
III	61 (26)	9 (24)	
IV	21 (9)	0 (0)	
Missing	2	0	
Tumor site			
Proximal colon	109 (46)	21 (60)	0.03
Distal colon	56 (24)	11 (31)	
Rectum	71 (30)	3 (9)	
Missing	4	0	
CpG island methylator phenotype (CIMP) status			
CIMP-negative	153 (74)	20 (67)	0.43
CIMP-positive	55 (26)	10 (33)	
Missing	32	5	
Microsatellite instability (MSI)			
Microsatellite stable (MSS)	131 (77)	24 (89)	0.16
MSI-high	39 (23)	3 (11)	
Missing	70	8	

	***PIK3CA* wild type (*N*=240)**	***PIK3CA*-mutated (*N*=35)**	**Chi-square *p* value**
KRAS mutation status			
KRAS wild type	160 (70)	17 (49)	0.01
KRAS-mutated	70 (30)	18 (51)	
Missing	10	0	
BRAF mutation status			
BRAF wild type	188 (84)	28 (80)	0.60
BRAF-mutated	37 (16)	7 (20)	
Missing	15	0	

Table 2

Overall and disease-specific survival in relation to *PIK3CA* mutation status

	***PIK3CA* wild-type deaths/cases**	***PIK3CA*-mutated deaths/cases**	**HR (95 % CI)[a]**	**HR (95 % CI)[a,b]**
All cases:				
Overall survival	88/240	9/35	1.74 (0.86–3.50)	1.44 (0.70–2.98)
Disease-specific survival	57/240	5/35	1.25 (0.49–3.16)	0.96 (0.37–2.47)
Excluding *KRAS*-mutated cases				
Overall survival	60/170	5/17	2.94 (1.12–7.73)	2.43 (0.88–6.70)
Disease-specific survival	32/170	3/17	2.13 (0.63–7.18)	1.44 (0.40–5.16)
Excluding *KRAS* wild-type cases				
Overall survival	27/70	4/18	1.03 (0.35–3.04)	0.57 (0.15–2.18)
Disease-specific survival	23/70	2/18	0.64 (0.15–2.76)	0.40 (0.07–2.22)

[a] Hazard ratio (HR) and 95 % confidence interval (CI) for survival in *PIK3CA*-mutated versus *PIK3CA* wild-type cases, adjusted for age at diagnosis

[b] Also adjusted for stage at diagnosis (I, II, and III–IV)