DIABETES, METFORMIN USE, AND COLORECTAL CANCER SURVIVAL IN WOMEN - A RETROSPECTIVE COHORT STUDY

A thesis

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ABSTRACT

Background: Observational studies have associated Metformin use with lower colorectal cancer incidence but few studies have examined survival impact. We examined the relationship between Metformin use, diabetes, and survival in postmenopausal women diagnosed with colorectal cancer while participating in the Women's Health Initiative (WHI) Clinical Trial or Observational Study.

Methods: 2,066 postmenopausal women with colorectal cancer diagnosed while enrolled in the WHI were followed for a median of 4.1 years, with 589 deaths overall and 414 colorectal cancer deaths. In this cohort, overall and colorectal cancer-specific survival were compared among women with diabetes who reported ever using Metformin (n=84); those with no reported use of Metformin (n=128); and women without diabetes (n=1854). Cox proportional hazard models were used to estimate the associations between Metformin use, diabetes and survival outcomes from the time of colorectal cancer diagnosis. Two strategies were used to adjust for potential confounders: multivariate adjustment with known predictors of colorectal cancer survival and construction of a propensity score for the likelihood of receiving Metformin, with model stratification by propensity score quintile.

Results: After adjusting for age and stage at diagnosis, women with diabetes on Metformin had no significant difference in colorectal-cancer specific survival compared to Metformin non-users (HR 0.75; 95% CI 0.40 -1.38, p=0.67) or compared to women without diabetes (HR 1.00; 95% CI 0.61 - 1.66, p=0.99). With propensity score

adjustment, the HR for colorectal cancer-specific survival in women with diabetes on Metformin compared to non-users was 0.90 (95% CI 0.48 – 1.69, p=0.75). Women with diabetes reporting Metformin use had no significant difference in overall survival compared to non-users (HR 0.84; 95% CI 0.51 – 1.37; p=0.48) or non-diabetics (HR 1.20; 95% CI 0.80 - 1.79; p=0.39).

Conclusions: In postmenopausal women with colorectal cancer and diabetes, no statistically significant difference was seen in colon-cancer specific survival in those who used Metformin. Analyses in larger populations of colorectal cancer patients are warranted.

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INTRODUCTION

There is an emerging body of evidence supporting the hypothesis that type 2 diabetes mellitus (DM) is a risk factor for colorectal cancer (CRC). In several large population studies, DM or abnormal glucose metabolism was associated with an increased risk of CRC as well as several other neoplasms(1–4). While the biological relationship between the two diagnoses is unclear, altered glucose metabolism, hyperinsulinemia and insulin-like growth factor (IGF-I) may play roles in tumorigenesis(4–6).

DM is also associated with an inferior prognosis among CRC patients. In a recent retrospective study, patients with DM and colon cancer had significantly worse disease free survival and overall survival as compared to colon cancer patients without DM, even after adjustment for predictors of colon cancer survival(7). Another study reported that both men and women with DM had a roughly 25% significantly increased risk of fatal colon cancer as compared to those without DM(1).

Based on these associations, investigators explored the relationship between anti-diabetic therapy and survival outcomes for CRC. Many patients with DM are treated with the oral hypoglycemic agent Metformin, which is hypothesized to also have anti-neoplastic activity. Downstream effects of Metformin cause activation of AMP-Kinase, which suppresses activity of the mammalian target of rapamycin (mTOR) pathway, resulting in decreased protein synthesis and reduced cellular proliferation(8). In addition, epidemiologic data have indicated a decreased incidence of cancer in users of Metformin as compared to sulfonylurea or insulin users, as well as a decreased risk of nonspecific

cancer-related mortality(9–12). However, few studies have examined Metformin use in relation to CRC-specific survival.

In this study, we examined the associations between Metformin use, DM and CRC-specific and overall survival among postmenopausal women diagnosed with CRC while enrolled in the Women's Health Initiative (WHI), a national, multi-center prospective clinical trial and observational study that collected adjudicated health outcomes on over 160,000 women over the course of 15 years, including data on heart disease, osteoporosis, and cancer. Given the decrease in overall cancer-related mortality in persons with DM who use Metformin, we hypothesized that Metformin use would be associated with improved survival as compared to non-use among women with CRC and DM.

METHODS

Study population

The Women's Health Initiative (WHI) was a long-term national health study that contained clinical trial (CT) and observational study (OS) components that focused on strategies to prevent or control heart disease, cancer, and osteoporotic fractures in postmenopausal women. The original WHI study included 161,808 postmenopausal women aged 50-79 years, enrolled at one of 40 WHI clinical centers across the United States between 1993 and 1998. The first component was a randomized controlled clinical trial (CT) that enrolled 68,132 women into trials testing three prevention strategies: hormone therapy, dietary modification, and calcium with vitamin D supplementation. If eligible, women could choose to enroll in one, two, or all three of the trial components.

The CT cohort was followed until March 2005, after which participants were invited to enroll in the WHI Extension Study for collection of health outcomes data without intervention through 2010. The Observational Study tracked the medical history and health habits of 93,676 women who were ineligible or unwilling to join the CT, and examined relationships between lifestyle, health, risk factors, and specific disease outcomes through 2010. All participants provided written informed consent and the study was approved by each of the clinical centers' institutional review boards. The Fred Hutchinson Cancer Research Center in Seattle, WA serves as the WHI Clinical Coordinating Center for data collection, management, and analysis of the WHI. Further details on scientific rationale, eligibility requirements, and other design aspects of the WHI have been previously published(13).

Eligibility criteria for this analysis included any patient who reported a diagnosis of CRC after enrollment in the CT or OS arm of the WHI. Women who reported a history of CRC prior to WHI enrollment and cases of CRC that were identified only at the time of death were excluded. The definition of type 2 diabetes (DM) was a positive answer to the question "did a doctor ever say that you had sugar diabetes or high blood sugar when you were not pregnant?" or the reported use of any anti-diabetic medication at any time. Individuals diagnosed with diabetes before age 20 or who were ever hospitalized for diabetic coma were considered to be type 1 diabetics and excluded from the analysis(14). The study population was then divided into the following exposure cohorts to examine the association between Metformin use, DM and survival outcomes: (1) Patients with a diagnosis of CRC who fit the criteria for DM and reported the use of Metformin or a Metformin-containing medication at any time; (2) Patients with a diagnosis of CRC who

fit the above criteria for DM and had no reported use of Metformin; and (3) Patients with a diagnosis of CRC and no evidence of DM.

Data Collection

Upon enrollment, women completed baseline questionnaires followed by an interview, physical exam, and blood sample collection. Information on demographics, exposures, lifestyle, dietary habits, family history and medical history were obtained through the use of standardized questionnaires. Physical measurements including pulse, blood pressure, height, weight, and waist and hip circumference were taken by certified staff at the initial clinical visit. Details regarding medication information were obtained via interviewer-administrated questionnaires at baseline for all participants. Medication information was collected again at years 1, 3, 6, and 9 for CT participants and at year 3 for OS participants. For all diabetic medications, the data collected included product and generic name, dosage form, strength, and duration of use. Medical history updates were obtained by mail or telephone questionnaires bi-annually for CT participants and annually for OS participants. The clinical outcomes obtained by questionnaire and telephone interview were reviewed by local, trained physician adjudicators who verified cases of identified CRC by reviewing medical records and pathology reports. CRC cases were confirmed by blinded adjudication at the clinical coordinating center and coded using the Surveillance, Epidemiology, and End Results system.

Outcome measures

In this analysis, survival outcomes in female CRC cancer patients with DM who used Metformin, female CRC cancer patients with DM who did *not* use Metformin, and female CRC cancer patients without DM were compared. CRC-specific survival was the primary outcome, measured as the number of days from the date of diagnosis of CRC to date of death due to CRC, or last known date alive. Patients who were not deceased or who died of causes other than CRC were censored at the last known date alive or date of death, respectively. Overall survival was analyzed as a secondary outcome, and defined as the number of days from the date of diagnosis of CRC to the date of death or last known date alive. For this secondary outcome, only those patients who were not deceased were censored at the last known date alive.

Statistical analyses

Baseline patient characteristics were compared between the three exposure cohorts as described above. For continuous variables that were normally distributed, the student's t-test was used; for non-normally distributed variables, the Wilcoxon rank sum was used. For categorical variables the Chi-Square test was used, however Fisher's exact test was used when comparing groups with fewer than 5 subjects. Kaplan-Meier survival analyses stratified by exposure group were used to generate median survival curves for both CRC-specific and overall survival. Univariate Cox proportional hazard models were used to estimate hazard ratios and their 95% confidence intervals to compare CRC-specific and overall survival differences by known prognostic factors. These included age at diagnosis (ages 50-59 years, 60-69 years, or ≥70 years), race (black, white, or

other), body-mass index (BMI) (by BMI category: <18.5-24.9, 25.0-29.9, 30.0 - 34.9, and 35.0 - ≥40), stage at diagnosis (localized vs. regional and distant), smoking status (never, past, or current), alcohol use (Nondrinker, Past drinker, <7 drinks/week,7+ drinks/week), dietary history (use of diabetic or high-fiber diet), activity level measured in total MET-hours per week (kcal/wk/kg as a continuous variable), insulin use (yes/no), and total number of diabetic medications per patient (0, 1, 2, 3, or ≥4). Two strategies were used to address confounding of the association between Metformin use, DM and survival outcomes. First, multivariate Cox proportional hazard models for CRC-specific and overall survival were built that included any prognostic factor that had a significant impact on survival as determined by a univariate hazard ratio (HR) with a p-value <0.2. Patients with unknown stage at diagnosis were excluded from these multivariate models (n=26). As a second strategy, given the small number of outcome events, a propensity score model for the probability of reported Metformin use was developed. A propensity score was generated from a logistic regression model for the reported use of Metformin (ever/never) that included all of the prognostic factors listed above (age at diagnosis, race, BMI, stage at diagnosis, smoking status, alcohol use, dietary history, activity level, insulin use, and total number of diabetic medications per patient). The likelihood of reported Metformin use based on the identified predictors was computed for each patient and the area under the curve (AUC) was used to quantify the predictive strength of the model. Propensity score quintiles were then used as a stratification variable in Cox proportional hazards models (see Appendix part 2 for more detail on propensity score).

All analyses were performed using the SAS System for Windows, version 9.2 (SAS Institute, Cary, NC), and all reported p-values were 2-sided.

RESULTS

Study population and demographic characteristics

Of the 161,808 women initially enrolled in the WHI, a total of 2,167 women were diagnosed with CRC after enrollment. After excluding those who were diagnosed at the time of death (N=101), and those who had only a self-reported history of CRC at enrollment but no adjudicated case of CRC while on study (N=916), there were 2,066 women who met eligibility criteria for this analysis. 212 of these women met criteria for a diagnosis of DM, and the remaining 1,854 were classified as non-diabetics. Of women with DM, 84 women reported the use of Metformin or a Metformin-containing medication at any time while on study.

Detailed demographic, lifestyle, and staging information for the three exposure cohorts is presented in Table 1. The median age at diagnosis was slightly lower in women without DM (median 60, range 50-79) and higher in women with DM who did not use Metformin (66.5, 50-78) than Metformin users (62.5, 50-77) (p=0.01). The cohort of women without DM had fewer African Americans and more whites than the cohorts of women with DM. Not surprisingly, women without DM had a lower median BMI than either of the other cohorts. The women without DM were also more active than women with DM (total median MET-hours per week 7.5 kcal/wk/kg in non-diabetics vs. 7 for women with DM on Metformin and 4.8 for women with DM not on Metformin). There were no significant differences in family history of CRC, smoking status, stage of disease at diagnosis, or use of a high-fiber diet between groups. As expected, more women with DM were on a diabetic diet as compared to those without DM. Among women with DM,

those on Metformin reported a higher total number of diabetic medications than those not on Metformin, however there was no difference in insulin use by Metformin status.

Metformin and survival outcomes

Median follow up for all patients with CRC was 4.1 years (range, 3 days – 14.4 years). No significant differences in follow-up time between groups were found. In the entire study population, there were a total of 589 deaths (28.5%), with 414 (20.0%) deaths attributable to CRC. Of 1854 women without DM, there were 516 deaths (27.8%) overall and 365 deaths (19.7%) due to CRC. In women with DM on Metformin, there were 26 deaths overall (31%) and 17 (20.2%) due to CRC. In women with DM not on Metformin, there were 47 deaths overall (36.7%) and 32 (25%) due to CRC. Median time to death in those without DM, women with DM on Metformin, and women with DM not on Metformin was 1.7 years, 2.1 years, and 1.7 years, respectively, with no significant difference noted between groups (p= 0.64). Similarly, median time to CRC-related death in the same cohorts was 1.3, 1.9, and 1.1 years, respectively (p=0.60).

In the unadjusted Kaplan-Meier survival analysis, there was no significant difference in overall survival or CRC-specific survival between groups (Figures 1 & 2). Univariate analysis with Cox proportional hazards models were used to determine if age, race, BMI, smoking habits, family history, alcohol use, stage of disease at diagnosis, use of a diabetic or high-fiber diet, activity level (measured in MET-hours per week), insulin use, or number of diabetic medications used had an effect on overall survival or CRC-specific survival in the study population (Appendix Table 1). Stage at diagnosis was a significant predictor of overall survival for the entire study population, with age and

activity level (Total MET-hours/week) significant prognostic factors in the cohort of women without DM.

After adjustment for age and stage at diagnosis using multivariate Cox proportional hazards models, there was no difference in CRC-specific survival between women with DM on Metformin compared to those not on Metformin (HR 0.75; 95% CI 0.40 -1.38; p=0.67) (Table 2). In addition, no significant CRC-specific survival difference was found in women with DM on Metformin as compared to those women without DM (HR 1.00; 95% CI 0.61 - 1.66; p=0.99) (Table 4). In terms of overall survival, there was no difference in overall survival between women with DM taking Metformin vs. not taking Metformin (HR 0.84; 95% CI 0.51 – 1.37; p=0.48) (Table 3), or between Metformin-using women with DM compared to women without DM (HR 1.20; 95% CI 0.80 - 1.79; p=0.39) (Table 5).

The propensity score model generated to predict likelihood of Metformin use had an AUC of 0.7. After using quintiles of propensity score as a stratification variable in the Cox model, the hazard ratio for colorectal-cancer specific survival in women with DM on Metformin compared to women with DM not using Metformin was 0.90 (95% CI 0.48 – 1.69).

DISCUSSION

In this cohort of postmenopausal women with CRC and DM, no statistically significant association was observed between Metformin use and CRC-specific or overall survival. Though our findings were not statistically significant, our effect estimates are similar in direction and magnitude to those reported in recent studies(15–17). We

observed a hazard ratio of less than one for the association between Metformin and CRC-specific survival in the analysis of women with DM. In addition, we observed that women with DM on Metformin had a similar CRC specific survival as women without DM.

In several recent studies, statistically significant improvements in the survival of patients with CRC who took Metformin have been reported. In a retrospective analysis of 595 Korean patients with newly diagnosed colorectal cancer and type 2 diabetes, Metformin use was associated with a lower risk of CRC-specific (HR, 0.66; 95% CI 0.45–0.975; p=0.037) and overall mortality (HR, 0.66; 95% CI 0.476–0.923; p= 0.015)(15). , While the rates of CRC-specific death in our study in Metformin users vs. non-users were similar to those in the Korean cohort, the number of patients in our cohort with both diabetes and colon cancer was well less than half the size of theirs. Thus, the null result in our study could well be due to a lack of statistical power. Lastly, this study used multivariate Cox proportional hazards models to adjust for confounders in the Metformin vs. non-Metformin groups, however one could argue that this method does not adequately adjust for the confounding by indication that exists between Metformin-treated and untreated groups. It is possible that a propensity score analysis would have better accounted for confounding by indication and could have changed their study findings.

Other studies have looked at overall but not CRC-specific survival in patients with DM taking Metformin. Researchers from MD Anderson Cancer Center performed a retrospective analysis of 397 patients with CRC and type II noninsulin-dependent diabetes mellitus (NIDDM), and found that overall survival in the group that used

Metformin was 76.9 months (95% CI, 61.4-102.4) vs. 56.9 months in non-users of Metformin (95% CI, 44.8-68.8) ($p = 0.048$)(16). A second group used the VA Cancer Registry to evaluate the effect of Metformin on overall survival in 1,708 CRC patients. They found a statistically significant improvement in overall survival in Metformin users compared to non-users, however the comparison cohort of non-Metformin users included both persons with diabetes and non-diabetics(17). Several differences exist between the above two studies and our analysis. First, because neither of these groups report on CRC-specific survival, observed survival benefits may not be cancer-specific. Confounding by severity of DM and competing risks of death in persons with DM could in part explain differences in survival between Metformin users and non-users. In our analysis, we evaluated both overall survival and CRC-specific survival as endpoints. In contrast to the second analysis, we categorized women with DM not on Metformin as a distinct comparator cohort to attempt to address baseline differences in survival that may exist between women with and without DM. Another important difference to note is that the sample sizes of these studies were larger than that of the WHI analysis, allowing for higher statistical power to detect a difference between groups. Finally, these analyses and the Korean study mentioned above included men.

Our investigation was limited by several factors. First, our analyses were hampered by a lack of statistical power. Despite the large number of patients enrolled in the WHI, the cohort of women with DM and CRC was small (N=212), and fewer than 50 CRC deaths occurred in this group, resulting in only 70% power to detect even a large HR of 0.5 assuming alpha=0.05. A second limitation to the study was the potential for selection bias, since Metformin may be associated with a reduced risk of CRC and this

analysis includes only women with a known diagnosis of CRC (Appendix Figure 1: DAG, (18,19)). Therefore it is possible that we preferentially selected those women who may have "Metformin-resistant" disease, which would bias effect estimates towards the null value. Third, though the method we used to establish a diagnosis of DM has been validated(14), we were unable to calculate either the duration of Metformin use or the timing of Metformin use in relation to CRC diagnosis. Thus, in our analysis, use of Metformin was configured as a simple binary variable (Metformin use: ever vs. never) and did not incorporate timing, duration or dosage of exposure. Since this exposure misclassification is expected to be non-differential with respect to the outcome, we anticipate this would again bias hazard ratios towards the null. Fourth, we were also missing detailed information on patients' CRC regarding tumor subtype, disease stage, treatment modalities utilized, date of progression, and laboratory values as they were not collected as part of the WHI. Fifth, we lacked indicators of diabetic disease severity (ie Hemoglobin A1c) and contraindications to Metformin use (ie serum creatinine), both potential key confounders of the Metformin – survival association. Nonetheless, we attempted to account for confounding by creating a propensity score for likelihood of being on Metformin using available predictors of diabetic severity and CRC prognosis. Regardless of how we applied the propensity score analytically, we found that the propensity score-adjusted hazard ratios were closer to the null as compared to the age and stage adjusted models, indicating negative confounding by factors captured in the propensity score. The threat of residual confounding by unmeasured or imperfectly measured factors remains. Finally, as mentioned previously we were restricted to the

population of the WHI, which included only postmenopausal women, limiting our ability to generalize findings to male CRC patients.

Despite these limitations, several strengths are notable in our analysis. First, the WHI was a large, prospective cohort study of a homogeneous population of postmenopausal women in which all cancer outcomes were verified by review of medical records and pathology reports. Secondly, women with DM were identified using a previously validated method that showed a high concordance rate (>75%) between self-reported incidence rates of DM and medication inventories(14). Also, because information on diabetes medication use was updated throughout the study, our method of capturing Metformin users included not only those taking the medication at baseline but also women who initiated Metformin at any point after enrollment. Finally, our study results, though non-significant, are similar in magnitude to recently published studies on Metformin and CRC survival in patients with DM (15–17).

In summary, we did not find evidence of a statistically significant association between Metformin use and CRC-specific survival in this cohort of postmenopausal women with diabetes and CRC. Continued research on this topic should be pursued given the promising basic science data and results from similar analyses done on larger cohorts. In particular, analysis of large cohorts with detailed information on the timing and duration of Metformin use is warranted.

Table 1 – Baseline Demographic and Treatment Characteristics

	No DM N=1854	DM + Metformin N=84	DM – Metformin N=128	p-values[a]
Age at screening				
Mean (range)	66 (50-79)	64 (50-77)	66 (50-78)	0.01
50-59(N, %)	344, 19%	26, 31%	16, 12%	
60-69(N, %)	872, 47%	38, 45%	70, 55%	
70-79+(N,%)	638, 34%	20, 24%	42, 33%	
Ethnicity - N (%)				
American Indian/Alaskan Native	8 (0%)	0 (0%)	1 (1%)	
Asian/Pacific Islander	38 (2%)	3 (4%)	1 (1%)	<0.0001
Black/African American	151 (8%)	18 (21%)	31 (24%)	
Hispanic/ Latino	42 (2%)	5 (6%)	3 (2%)	
White	1590 (85%)	56 (67%)	90 (70%)	
Other	21 (1%)	2 (2%)	2 (2%)	
BMI (kg/m^2)				
Median	27.1	31.9	31.9	<0.0001
Range	15.5 – 66.6	20.1 – 49.8	19.0 – 65.3	
Category (N, %)				
Underweight (<18.5)	7 (0%)	0 (0%)	0 (0%)	
Normal (18.5-24.9)	605 (33%)	4 (5%)	18 (14%)	
Overweight (25.0-29.9)	665 (36%)	26 (31%)	30 (23%)	
Obesity I (30.0 - 34.9)	365 (20%)	23 (27%)	33 (26%)	<0.0001
Obesity II (35.0 - 39.9)	131 (7%)	22 (26%)	30 (23%)	
Extreme Obesity (≥40)	66 (4%)	9 (11%)	16 (13%)	
Missing	15 (1%)	0 (0%)	1 (1%)	
Family history of colon cancer (N, %)				
Yes	353 (19%)	15 (17%)	25 (20%)	0.99
No	1346 (73%)	59 (70%)	95 (74%)	
Missing	155 (8%)	10 (12%)	8 (6%)	
Smoking status				
Never smoked	884 (48%)	41 (49%)	72 (56%)	0.36
Past smoker	805 (43%)	36 (42%)	44 (34%)	
Current smoker	137 (7%)	6 (7%)	11 (9%)	
Missing	28 (2%)	1 (1%)	1 (1%)	
Stage[b]				
Localized	851 (46%)	38 (45%)	56(44%)	0.37
Regional/Distant	978 (53%)	44 (52%)	72(56%)	
Unknown	24 (1%)	2 (2%)	0(0%)	
Diabetic diet				
Yes	17 (1%)	59 (70%)	95(74%)	<0.0001
No	1837 (99%)	25 (30%)	33(26%)	
High-fiber diet				
Yes	353 (19%)	12 (14%)	31(24%)	0.2
No	1462 (79%)	68 (81%)	94(73%)	
Missing	39 (2%)	4 (5%)	3(2%)	
Total MET-hours per week (kcal/wk/kg)				
Mean	11.5	9.1	8.8	0.02
Median (IQR)	7.5 (2 – 16.7)	7 (0.6 – 13.6)	4.8 (0.5 – 11.8)	
Range	0 – 90.8	0 – 69.0	0 – 53.5	
Patients using insulin				
Yes	N/A	18 (21%)	30 (23%)	0.73‡
No		66 (79%)	98 (77%)	
Total number of diabetic meds				
0		0 (0%)	39 (30%)	
1		14 (17%)	66 (51%)	<0.0001‡
2	N/A	54 (64%)	18 (14%)	
3		11 (13%)	3 (2%)	
4-5		5 (6%)	2 (2%)	

[a]P-value is for comparison between the three cohorts except where indicated

[b]Localized disease includes tumors staged as in situ or localized; regional/distant includes tumors staged as regional or distant.

‡P-value is for comparison between diabetic Metformin users and non-users

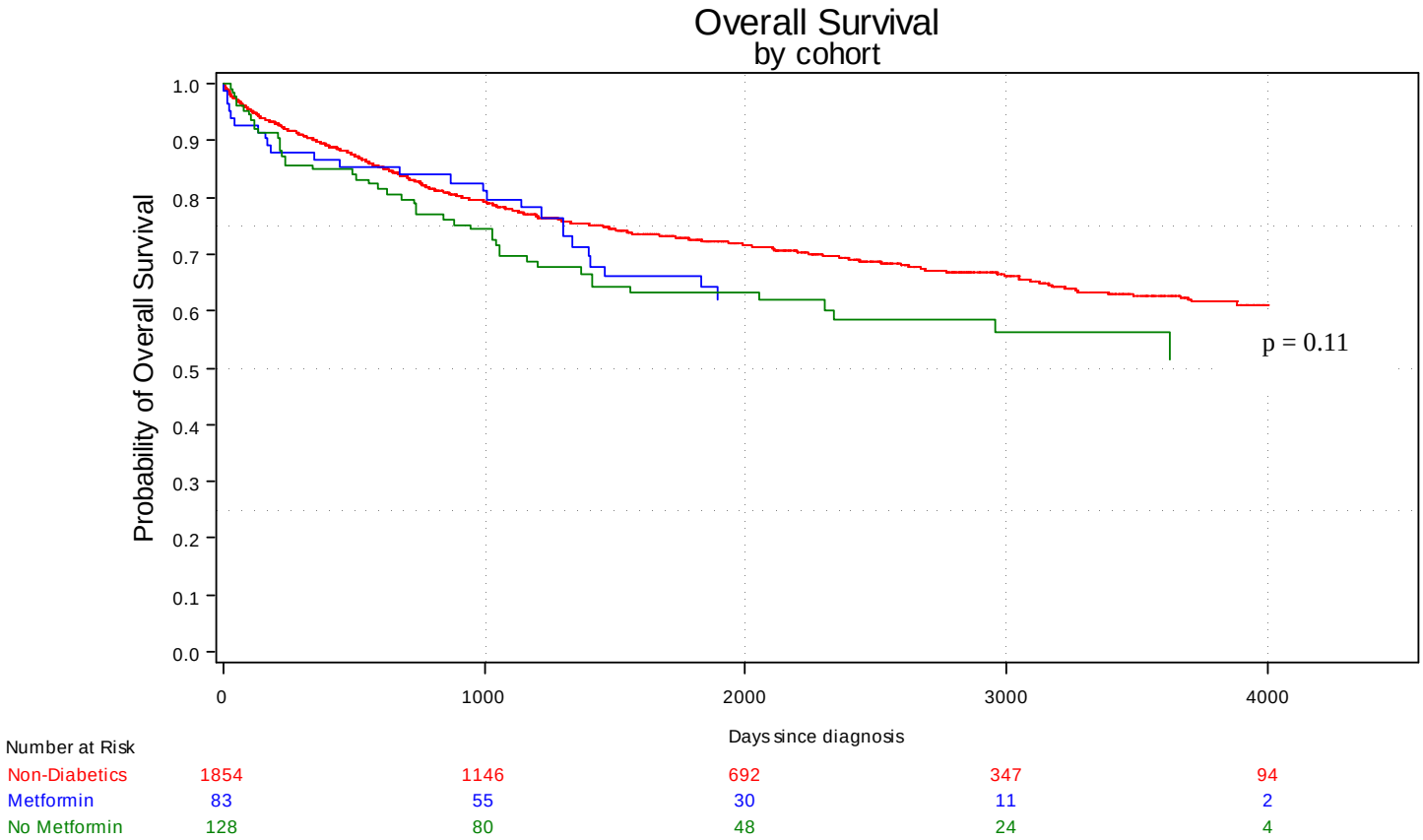
Table 2. Metformin Treatment and Colorectal Cancer-Specific Survival in Diabetics (N=212)			
	Unadjusted HR (95% CI)	**Age-adjusted HR (95% CI)**	**Multivariate-Adjusted* HR (95% CI)**
No Metformin	1.00	1.00	1.00
Metformin	0.77 (0.42 – 1.39)	0.76 (0.41 – 1.41)	0.75 (0.40 -1.38)
p-values	0.38	0.39	0.67
*Adjusted for age (by deciles) and stage at diagnosis (localized and regional/distant), excluding those with unknown stage at diagnosis (N=2 in diabetics on Metformin, 0 in diabetics not on Metformin)			

Table 3. Colorectal Cancer Specific Survival in Cox Proportional Hazards Models by Cohort (N=2066)				
	Unadjusted HR (95% CI)	**p-value**	**AHR† (95% CI)**	**p-value**
Non-Diabetic	1.00	Ref	1.00	Ref
Diabetics on Metformin	1.01 (0.61 - 1.66)	0.98	1.00 (0.61 - 1.66)	0.99
Diabetics not on Metformin	1.32 (0.92 - 1.89)	0.13	1.23 (0.86 - 1.77)	0.26
† Adjusted for age and stage at diagnosis *Subjects with unknown stage at diagnosis excluded from analysis (n=24 in non-diabetics, 2 in diabetics on Metformin)				

Table 4. Metformin Treatment and Overall Survival in Diabetics (N=212)			
	Unadjusted HR (95% CI)	**Age-adjusted HR (95% CI)**	**Multivariate-Adjusted* HR (95% CI)**
No Metformin	1.00	1.00	1.00
Metformin	0.82 (0.50 – 1.33)	0.85 (0.52 – 1.39)	0.84 (0.51 – 1.37)
p-values	0.41	0.52	0.48
*Adjusted for age (by deciles) and stage at diagnosis (localized and regional/distant), excluding those with unknown stage at diagnosis (N=2 in diabetics on Metformin, 0 in diabetics not on Metformin)			

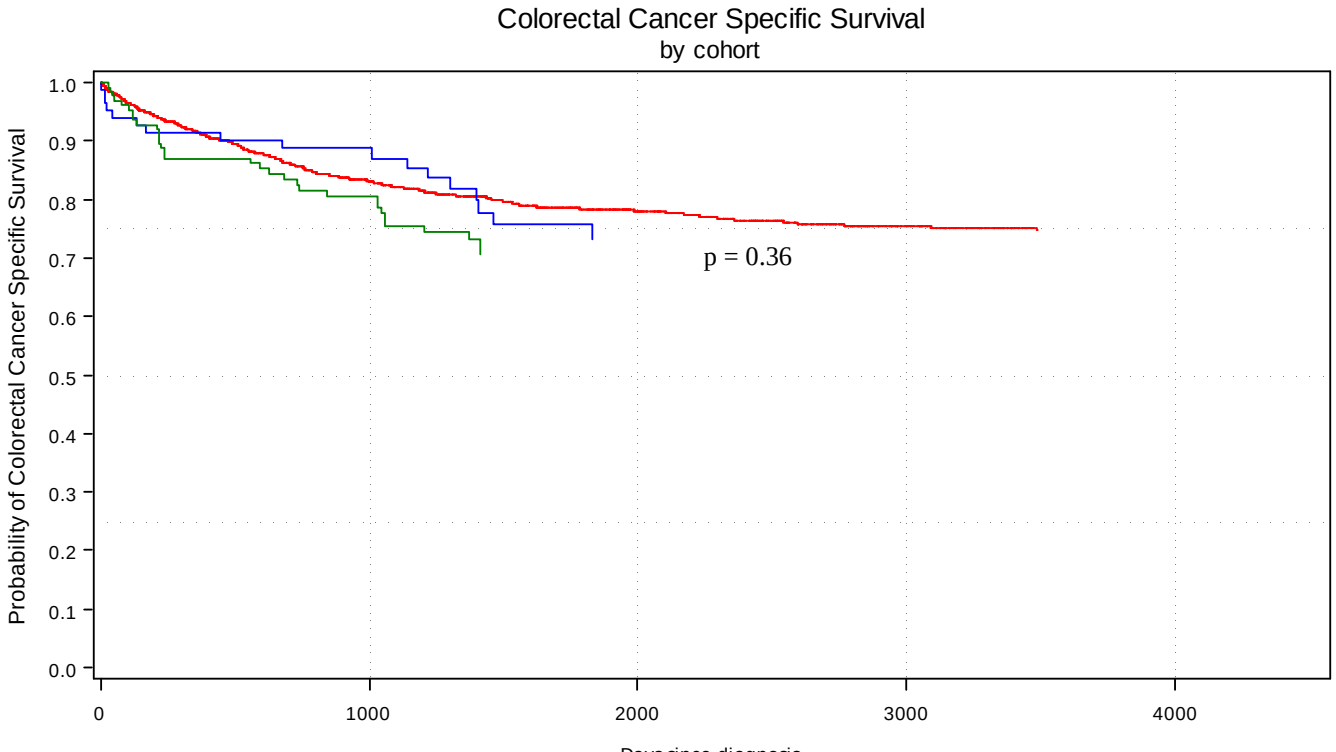
Table 5. Overall Survival in Cox Proportional Hazards Models by Cohort (N=2066)				
	Unadjusted HR (95% CI)	**p-value**	**AHR† (95% CI)**	**p-value**
Non-Diabetic	1.00	ref	1.00	ref
Diabetics on Metformin	1.13 (0.76 - 1.67)	0.54	1.20 (0.80 - 1.79)	0.39
Diabetics not on Metformin	1.38 (1.03 - 1.87)	0.03	1.32 (0.98 - 1.78)	0.07
† Adjusted for age (by deciles) and stage at diagnosis (localized and regional/distant) *Subjects with unknown stage at diagnosis excluded from analysis (n=24 in non-diabetics, 2 in diabetics on Metformin)				

Figure 1.



Number at Risk					
Non-Diabetics	1854	1146	692	347	94
Metformin	83	55	30	11	2
No Metformin	128	80	48	24	4

Figure 2.



Number at Risk					
Non-Diabetics	1854	1146	692	347	94
Metformin	83	55	30	11	2
No Metformin	128	80	48	24	4

Appendix Table 1. Baseline Characteristics and Overall- and Colorectal Cancer Specific Survival				
	Overall Survival		Colorectal Cancer-Specific Survival	
	HR (95% CI)	p-values	HR (95% CI)	p-values
Age				
50-59*	--	<0.0001	--	0.38
60-69	1.13 (0.78 - 1.62)		1.06 (0.71 - 1.58)	
70-79+	1.63 (1.15 - 2.31)		1.20 (0.81 - 1.78)	
Race/Ethnicity				
Black vs. White*	0.99 (0.75 - 1.29)	0.99	1.02 (0.74 - 1.41)	0.98
Other vs. White*	1.00 (0.71 - 1.41)		0.98 (0.65 - 1.48)	
Other vs. Black*	1.01 (0.67 - 1.54)		0.96 (0.58 - 1.58)	
BMI (kg/m^2)				
<18.5-24.9*	--	0.51	--	0.76
25.0-29.9	1.03 (0.84 - 1.27)		0.93 (0.73 - 1.18)	
30.0 - 34.9	1.12 (0.89 - 1.41)		1.05 (0.80 - 1.38)	
35.0 - ≥40	1.19 (0.92 - 1.55)		1.06 (0.78 - 1.45)	
Smoking status				
Never smoked*	--	0.05	--	0.15
Past smoker	1.16 (0.98 - 1.38)		1.20 (0.98 - 1.47)	
Current smoker	1.38 (1.02 - 1.86)		1.29 (0.89 - 1.86)	
Family history of colon cancer		0.33		0.25
No*	--		--	
Yes	0.90 (0.73 - 1.11)		0.86 (0.67 - 1.11)	
Alcohol use history				
Nondrinker*	--	0.24	--	0.48
Past drinker	1.07 (0.79 - 1.45)		1.01 (0.69 - 1.48)	
<7 drinks/week	0.96 (0.74 - 1.26)		1.16 (0.84 - 1.61)	
7+ drinks/week	1.23 (0.89 - 1.70)		1.27 (0.85 - 1.89)	
Stage‡				
Localized*	--	<0.0001	--	<0.0001
Regional/Distant	4.11 (3.36 - 5.01)		8.10 (5.99 - 10.95)	
Unknown	6.45 (3.71 - 11.21)		10.19 (5.00 - 20.77)	
Diabetic diet				
No*	--	0.13	--	0.74
Yes	1.23 (0.94 - 1.62)		1.06 (0.75 - 1.49)	
High-fiber diet				
No*	--	0.88	--	0.51
Yes	0.99 (0.80 - 1.21)		0.92 (0.72 - 1.18)	
Total MET-hours per week (kcal/wk/kg)	0.99 (0.98 – 1.00)	0.01	0.99 (0.98 – 1.00)	0.03
Insulin				
No	--	0.98	--	0.35
Yes*	1.01 (0.59 - 1.71)		0.70 (0.33 - 1.48)	
Number of diabetic medications				
0*	--		--	
1	1.30 (0.89 - 1.90)	0.29	1.23 (0.77 - 1.94)	0.64
2	1.37 (0.92 - 2.02)		1.23 (0.76 – 2.00)	
3	0.75 (0.24 - 2.34)		0.35 (0.05 - 2.47)	
4-5	0.45 (0.06 - 3.20)		(N=0 in this group)	
Metformin Use				
No Metformin*	--	0.41	--	0.38
Metformin	0.82 (0.50 – 1.33)		0.77 (0.42 – 1.39)	

*reference group

‡Localized disease includes tumors staged as in situ or localized; Regional/Distant includes tumors staged as regional or distant.

Appendix Table 2 – Diabetes Status and Colorectal Cancer-Specific Survival (N=2066)			
	Unadjusted HR (95% CI)	**Age-adjusted HR (95% CI)**	**Multivariate-Adjusted* HR (95% CI)**
No Diabetes	1.00	1.00	1.00
Diabetes	1.19 (0.88 – 1.61)	1.22 (0.90 – 1.65)	1.15 (0.85 -1.55)
p-value	0.25	0.19	0.38
*Adjusted for age (by deciles) and stage at diagnosis (localized and regional/distant), excluding those with unknown stage at diagnosis (N=24 in non-diabetics, 2 in diabetics)			

Appendix Table 3 – Diabetes Status and Overall Survival (N=2066)			
	Unadjusted HR (95% CI)	**Age-adjusted HR (95% CI)**	**Multivariate-Adjusted* HR (95% CI)**
No Diabetes	1.00	1.00	1.00
Diabetes	1.29 (1.00 – 1.65)	1.35 (1.05 – 1.72)	1.28 (1.00 -1.63)
p-value	0.05	0.05	0.05
*Adjusted for age (by deciles) and stage at diagnosis (localized and regional/distant), excluding those with unknown stage at diagnosis (N=24 in non-diabetics, 2 in diabetics)			

Appendix Table 4 –Metformin treatment and colorectal cancer-specific survival among diabetics by propensity score quintile		
Propensity Score Quintile (range, n)	**HR (95% CI)**	**p-value**
0 (0.02 – 0.25, n=42)	0.48 (0.06 – 3.75)	0.49
1 (0.25 – 0.34, n=43)	0.62 (0.13 – 2.89)	0.54
2 (0.35 – 0.45, n=42)	1.61 (0.46 – 5.56)	0.45
3 (0.45 – 0.51, n=43)	1.04 (0.23 – 4.65)	0.96
4 (0.53 – 0.86, n=42)	0.81 (0.22 – 3.03)	0.76

APPENDIX 1: EXPANDED INTRODUCTION

Despite an overall decrease in the incidence and death rate from colorectal cancer over the past ten years, it remains the third most common cancer and the third leading cause of cancer death in both men and women in the United States(20). It is estimated that 143,460 cases of colorectal cancer will be diagnosed in this country in 2012, with an estimated 51,690 deaths(20). Worldwide, colorectal cancer is the fourth leading cause of cancer death in men, and the third leading cause of cancer death in women(21). Given the burden of disease, improving the outcomes of patients with colorectal cancer is a priority. Despite significant advances in surgical techniques and adjuvant chemotherapy, currently it is estimated that more than 40% of patients who present with stage II or III disease will relapse following primary therapy. Further research is needed to clarify the risk factors contributing to the development of colorectal cancer and to find more effective ways to treat the disease.

Some well-known risk factors for colon cancer include age, genetic factors, family history, inflammatory bowel diseases, and a host of lifestyle factors such as obesity, physical inactivity, smoking, alcohol use, and an unhealthy diet. In a recent review the role of diet, lifestyle, and medication use in colorectal cancer risk was examined(22). The authors found strong evidence to support an increased risk of colon cancer in persons with a high intake of red and processed meats, high-fat dairy products, highly refined grains and starches, and sugars. Regarding lifestyle factors, they found that avoidance of smoking and heavy alcohol use, prevention of weight gain, and a reasonable level of physical activity can positively influence risk of colorectal cancer. The evidence supporting dietary supplements including vitamin D, folate, and B6 was uncertain, but

they did find that calcium supplementation was modestly beneficial. They also note that data in support of NSAID and aspirin use as chemoprevention for colorectal cancer is strong.

As the above-mentioned risk factors were identified, it became evident that there was significant overlap between these and the risk factors commonly known to cause type 2 diabetes mellitus. Based on this observation, researchers began to look for a biological link between diabetes and colorectal cancer. Several theories have emerged regarding a possible pathophysiologic link between diabetes and colorectal cancer. One theory suggests that insulin, via insulin-like growth factor-1 (IGF-1), IGF-receptors (IGF-R) and their downstream signaling pathways, may contribute to mutagenesis by mediating both proliferation and apoptosis in colonic epithelial cells(23,24). Insulin has been shown to stimulate cell growth in human colon cancer cell lines in vitro(25), and to have additional anti-apoptotic effects in breast cancer cell lines and hepatocytes(26),(27). The presence of insulin leads to increased bioavailablility of IGF-1, a known activator of the AKT signaling pathway, which subsequently leads to cell growth and proliferation and inhibition of apoptosis(23). Colon cancer cell lines have been shown to overexpress both IGF-1 and IGF-R(5), which can lead to activation of transcription factors that play a role in transformation(28–30), tumor invasion(31), metastasis(32), and protection from apoptosis(33–35). Another theory suggested that glucagon-like peptide-1 (GLP-1) may play a role in colorectal cancer development in persons with diabetes(24). GLP-1 is a peptide hormone that is released in the gut in response to nutrient intake, and it regulates glucose homeostasis by inhibiting glucagon and promoting insulin secretion(36),(37). GLP-1 also promotes direct resistance to apoptosis in cells that express GLP-1 receptors.

The research in this field is ongoing. A clear understanding of how this protein is involved with tumorigenesis is not yet known, but it provides more evidence linking the insulin pathway, type 2 diabetes, and colorectal cancer.

Epidemiologic studies have also explored the link between diabetes and colorectal cancer. In a meta-analysis published in 2005(38), the authors analyzed 15 studies including over 2.5 million participants. This included 6 case-control studies and 9 cohort studies evaluating the association between type 2 diabetes and the incidence of or mortality from colon, rectal, or colorectal cancer. In 8 of the 15 studies, a statistically significant positive association between diabetes and colorectal cancer incidence was found. In the pooled meta-analysis, a summary relative risk of 1.3 for the association between diabetes and colorectal cancer incidence was calculated. Once the association between diabetes and colon cancer incidence was established, further investigations explored the question of whether there was an increased risk of mortality from colorectal cancer in diabetic patients. Two recent studies reported an increased risk of colorectal cancer specific death in persons with diabetes(39,40). The first analysis was a retrospective study of 2,762 consecutive patients diagnosed with colon cancer from the Taipei General Veterans Hospital in Taipei, Taiwan(39). In this analysis, they subdivided the population into those with and without diabetes (N=469 patients with diabetes), and collected detailed information regarding staging, treatment, medication use, and cause of death. The authors compared overall survival and colon-cancer specific survival between cohorts. They found that patients with diabetes had worse overall survival (HR 1.31, p=0.001) and colon cancer specific survival (HR = 1.24, p=0.013) as compared to non-diabetic patients(39). A similar analysis was done on 2,278 patients

diagnosed with colon or rectal cancer while enrolled in the Cancer Prevention Study-II Nutrition Cohort(40). In this analysis, a small but statistically significant decrease in colorectal cancer specific survival was seen in patients with diabetes vs. those without diabetes (82% vs. 87%, p=0.04). In summary, there are multiple studies associating type 2 diabetes not only to an increased colorectal cancer incidence, but also to increased mortality from the disease.

The next step researchers took was to attempt to find associations between diabetic therapy and the incidence of and mortality from colorectal cancer. The majority of the data presented here comes from retrospective analyses of large, prospective cohort studies. These studies first showed that high levels of circulating endogenous insulin were associated with an increased risk of developing colon cancer(41,42). Next, therapeutic insulin use was associated with an increased risk of developing colon cancer in type 2 diabetes patients(43). Currie et al. found an increased incidence of colorectal cancer in diabetic patients on monotherapy with sulfonylurea or insulin as compared to those taking Metformin.(44) Libby et al, using a database from an observational cohort study in Tayside, Scotland including over 13,000 patients with diabetes, found lower incidence rates of cancer in users of Metformin as compared those who did not use Metformin(12). At this point, researchers began to investigate whether any of these therapies had an effect on cancer-related mortality. In a large retrospective study using an administrative database in Saskatchewan, Canada, investigators examined the association between insulin, Metformin or sulfonylurea use and cancer-related mortality in a subset of over 10,000 patients newly treated for type 2 diabetes(45). Diabetics exposed to sulfonylureas or exogenous insulin were significantly more likely to have a

cancer-related death than diabetics exposed to Metformin. Concurrent with these publications, research from basic science studies investigated the anti-neoplastic properties of Metformin.

Metformin is a biguanide oral hypoglycemic that decreases hepatic glucose production and intestinal absorption of glucose, and it improves insulin sensitivity by increasing peripheral glucose uptake. Metformin is also thought to partially inhibit oxidative phosphorylation via the mitochondrial respiratory chain, altering the ATP–AMP ratio(46). This in turn activates AMP-kinase, which suppresses activity of mammalian target of rapamycin (mTOR) and its effector proteins, which normally leads to an increase in protein synthesis and cell proliferation. By blocking this pathway, Metformin may play a role in the inhibition of tumor growth(47). Interestingly, the genetic basis of an autosomal dominant, inherited hamartomatous polyposis syndrome, Peutz-Jeghers syndrome, helps lend credence to the above theory. Peutz-Jehgers syndrome is caused by a mutation in the gene that encodes the serine/threonine-protein kinase 11, or STK11 (also known as LKB1), which in its normal state activates AMP-kinase and suppresses the mTOR pathway. Individuals with Peutz-Jehgers syndrome have mutations that inactivate this protein, leaving the mTOR pathway unsuppressed. These patients develop innumerable hamartomatous polyps in the small bowel and throughout the GI tract, and are at a significantly higher risk of developing gastrointestinal and pancreatic cancers. Therefore, if the effect of Metformin on the AMP-kinase pathway is similar to the effect of STK11, a known tumor suppressor, then Metformin may, indeed, have true anti-neoplastic capabilities.

In summary, while evidence suggests a physiologic link between diabetes, incident colorectal cancer, and colorectal cancer-specific mortality, the role of Metformin in colorectal cancer remains less clear. Epidemiologic studies have found an association between Metformin use and a decreased incidence of colorectal cancer in patients with diabetes, and laboratory studies have uncovered mechanisms by which Metformin may work as an anti-neoplastic agent. What remains to be determined is whether or not Metformin confers a protective effect once patients are diagnosed with colorectal cancer. In this retrospective analysis, we used data collected on postmenopausal women who developed colorectal cancer while enrolled in the Women's Health Initiative to compare colon-cancer specific and overall survival between diabetic users of Metformin, diabetic non-users of Metformin, and non-diabetics. Given the existing evidence suggesting a decrease in overall cancer-related mortality in diabetics who use Metformin, we hypothesized that use of Metformin would be associated with improved survival as compared to non-Metformin-using diabetics with colorectal cancer.

APPENDIX 2: EXPANDED DISCUSSION—PROPENSITY SCORES

In randomized controlled trials (RCTs), random treatment allocation ensures that treatment status will not be confounded with either measured or unmeasured baseline characteristics (48). In observational studies, since treatment allocation is not randomized, treated and non-treated groups may differ significantly in their baseline characteristics. If these baseline characteristics are associated not only with the decision to treat but also the study outcome, then treatment effects are subject to a type of bias called confounding by indication (49). This type of confounding is commonly encountered when analyzing observational data with the goal of estimating the effect of medical treatments or interventions on health outcomes.

Propensity scores have been developed as a technique to adjust for confounding by indication. The propensity score is defined as the probability of receiving a treatment conditional on observed baseline covariates. Though several other methods exist that attempt to account for confounding by indication, we found that the propensity score analysis had several advantages for our analysis. First, it allowed for inclusion of a greater number of measured confounders than a multivariate regression analysis given the small number of colorectal cancer deaths observed. In a multivariate regression model, it is recommended that there be at least 10 outcome events per covariate considered in the model (50), which can be a limiting parameter when working with a small dataset. However, when creating the propensity score estimate, all measured confounders thought to be associated with treatment assignment are included in a logistic regression model in which treatment status is regressed on observed baseline characteristics (48). This, in

effect, collapses all the characteristics included in the propensity model into one variable, the propensity score, which can be used to compare treated to untreated cohorts.

In our final analysis, we used propensity score quintile as a stratification variable in Cox proportional hazards models. We also tried adjusting the Cox proportional hazards model using quintiles of propensity score as covariates in the model and a single continuous covariate in the model. The HR for colorectal cancer-specific survival in diabetic Metformin users vs. non-users was 0.85 (95% CI 0.45 - 1.60, p=0.62) in the model using propensity score as a continuous variable, and 0.91 (95% CI 0.49 - 1.71, p=0.78) when using propensity score quintiles as covariates. These are variations of several different methods by which the propensity score can be used to account for confounding when estimating the effects of treatment on outcomes. Other methods include matching on propensity score and inverse probability of treatment weighting using the propensity score (48). While there is no consensus in the field as to which method is preferred, several studies have suggested that matching on propensity score eliminates a greater proportion of the systematic differences in baseline characteristics between treated and untreated subjects than stratification on or covariate adjustment using the propensity score (48,51–53). However, when matching on propensity score, treated patients without a matched control are excluded from the analysis, which can substantially decrease sample size. The cohort of diabetic Metformin users in our dataset included only 84 patients. Matching on propensity score would have further decreased the sample size, and thus further decreased the power of our already underpowered analysis. Alternatively, stratification on quintiles of propensity score, though not superior to matching, has been shown to reduce 90% of the imbalance of a confounder between

groups(53). Thus we preferred to stratify on propensity score, as this allowed for use of all available data.

A final and important point regarding use of propensity scores is the issue of how to assess whether the propensity score model had achieved its goal of eliminating bias due to measured confounders. The most commonly reported measure of "fit" of the propensity score is the c-statistic from the logistic regression model used to build the propensity score(54,55). The c-statistic, which is equivalent to the area under the receiver operating characteristic (ROC) curve, is a measure of the discriminatory power of a predictive model(56). While there is recent debate as to the appropriateness of the C statistic in evaluating propensity score fit(49,56,57), it was an attractive option for this project given that is not computationally intensive and has been commonly reported in the propensity score literature to date. In the context of the propensity score, the c-statistic would therefore represent the ability of the propensity score model to predict treatment status using observed covariates(56). A high c-statistic (>0.90) would indicate non-overlap in the distribution of propensity scores between treated and untreated subjects(57). This would call into question the subsequent evaluation of the treatment effect using the propensity score, because of the lack of comparability of the characteristics between treated and untreated subjects(49). Conversely, a c-statistic that is very low may indicate that important covariates are missing in the propensity model(49). Therefore Heinze and Jüni proposed that a c-statistic of 0.8 be used as a guideline for model fit(49). In our propensity model, the c-statistic was 0.7. This indicates that there may be missing confounders, however it also suggests that we are not over-fitting the propensity model.

In summary, we have attempted to adjust for confounding by indication for Metformin use and the association with colorectal cancer survival by using a propensity score approach. This technique was appealing given the small number of outcome events observed and the rich array of covariates available in the WHI dataset. While the WHI lacked information on anticipated indications and contraindications for Metformin use such as measures of diabetic severity and kidney function, we were able to build a propensity score model that adequately modeled the likelihood of receiving Metformin according to the C statistic.

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