Influence of Prediagnostic Dietary Patterns and Cigarette Smoking on Colorectal Cancer Survival: A Cohort Study

by

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Abstract

Cigarette smoking and dietary patterns are associated with colorectal cancer (CRC) incidence; however, little is known about their influence on survival after CRC diagnosis. This study was designed to: 1) investigate the association of dietary patterns with all-cause (overall survival; OS) and disease-free survival (DFS) among CRC patients; 2) examine the association of smoking with OS and DFS among CRC patients.

A cohort of 750 CRC patients diagnosed from 1999 to 2003 in the Canadian province of Newfoundland and Labrador was followed for mortality and recurrence until April 2010. Participants reported their smoking history and dietary intakes using a personal history questionnaire and a food frequency questionnaire. Dietary patterns were identified with factor analysis. Multivariate hazard ratios (HRs) and 95% confidence intervals (*CI*s) were calculated with Cox proportional hazards regression, controlling for major known prognostic factors.

Results from this study are presented in two parts: 1) Disease-free survival among CRC patients was significantly worsened among patients with a high dietary intake of processed meat (the highest versus the lowest quartile HR: 1.82, 95%*CI*: 1.07-3.09). No associations were observed with the prudent vegetable or the high sugar patterns and DFS. The association between the processed meat pattern and survival in CRC patients was restricted to patients diagnosed with colon cancer (the highest versus the lowest quartile: DFS: HR: 2.29, 95%*CI*: 1.19-4.40; OS: HR: 2.13, 95%*CI*: 1.03-4.43). Potential effect modification was noted for sex (DFS: P=0.04, HR: 3.85 for women and 1.22 for men). 2) Compared with never smoking, current (HR: 1.78; 95%*CI*: 1.04-3.06), but not former (HR: 1.06; 95%*CI*: 0.71-1.59), smoking was associated with decreased OS, although this association was limited to tumors in the colon. The

associations of cigarette smoking with the study outcomes were higher among patients with >40 pack-years of smoking (OS: HR: 1.72; 95%*CI*: 1.03-2.85; DFS: HR: 1.99; 95%*CI*: 1.25-3.19). Potential interaction was noted for sex (DFS: *P*=0.04, HR: 1.68 for men and 1.01 for women) and age at diagnosis (OS: *P*=0.03, HR: 1.11 for patients aged < 60 and 1.69 for patients aged ≥60).

In summary, 1) processed meat dietary pattern prior to diagnosis is associated with higher risk of tumor recurrence, metastasis, or death from any cause among colon cancer patients; 2) pre-diagnosis cigarette smoking is associated with worsened prognosis among patients with colon cancer.

Key Words: Colorectal cancer, smoking, dietary pattern, cancer survival, interaction, MSI, *BRAF V600E*

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Candidate's Contribution to the Work

This project was part of the Canadian Institute of Health Research (CIHR) in Interdisciplinary Research on Colorectal Cancer, to which numerous people contributed. Specifically, the CIHR Colorectal Cancer Research Team members contributed to this study by recruiting eligible subjects and gathering personal and dietary information from consenting patients. Dr. Roger Green and Dr. Mike Woods' laboratories preformed all relevant genetic analyses. Dr. Peizhong Peter Wang and the candidate conceptualized and designed the specific research project presented in this thesis. The candidate conducted the literature review and compiled the predominantly secondary data obtained from the Newfoundland Familial Colorectal Cancer Registry (NFCCR). Finally, the candidate was responsible for all statistical analyses using the SAS program and the subsequent presentation and interpretation of findings from this thesis.

Abbreviations

BMI	Body Mass Index
CI	Confidence Interval
CIHR	Canadian Institute of Health Research
CRC	Colorectal Cancer
CTCC	Canadian Institutes of Health Research Team in Colorectal Caner
DFS	Disease-free Survival
EFA	Exploratory Factor Analysis
FFQ	Food Frequency Questionnaire
FHQ	Family History Questionnaire
FL	Factor Loading
HCA	Heterocyclic Amines
HR	Hazard Ratio
IARC	International Agency for Research on Cancer
ICD	International Classification of Disease
MMR	DNA Mismatch Repair System
MSI	Microsatellite Instability
NCTRF	Newfoundland Cancer Treatment and Research Foundation
NFCCR	Newfoundland Familial Colorectal Cancer Registry
NL	Newfoundland and Labrador
NSAID	Nonsteroidal Anti-inflammatory Drug
OFCCR	Ontario Familial Colorectal Cancer Registry
OS	Overall Survival
PAHs	Polycylic Aromatic Hydrocarbons
PCA	Principal Component Analysis

PHQ	Personal History Questionnaire
SNPs	Single-Nucleotide Polymorphisms
STRs	Short Tandem Repeats

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Manuscripts

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Chapter 1 Introduction

1.1 Background

Colorectal cancer (CRC) is a major health problem in Canada, with an estimated 22,200 new cases and 8,900 deaths in 2011 [1]. CRC incidence and mortality rates vary by geographic region. In particular, Newfoundland and Labrador (NL), according to Canadian Cancer Statistics, has the highest age-standardized incidence and mortality rates of CRC in Canada. It is estimated that in the province of NL alone 530 people will be diagnosed with CRC and 240 will die from it in 2013 [2].

It seems likely that the high rates of the disease in NL are due in part to the remarkable hereditary predisposition to CRC in this population (recent research has observed a very high prevalence of indicators of familial risk among CRC patients in NL [3]); however, environmental factors have been shown to be important causes of CRC as well [4-8], which have been supported by evidence from migrant studies. Such studies report that when people migrate from low-risk areas of CRC such as Japan or Polish to high-risk areas such as United States or Australia, they approach the risk profile of the host country gradually [4, 5, 7]. To date, epidemiological research has identified a variety of environmental and lifestyle factors that appear to be known or putative risk factors for CRC. These include smoking, alcohol consumption and physical activity levels; however, the results have often been conflicting.

It is generally believed that diet would strongly influence CRC risk, and up to 70% of this cancer burden could be reduced by alterations in dietary habits [9]. Accumulating evidence has indicated that high consumptions of red meat products and low intakes of fibre and dietary calcium are associated with increased CRC risk

[10-12]; however, there has been minimal research examining the association between dietary factors and survival after CRC diagnosis [13, 14]. Furthermore, most existing nutrition studies have focused primarily on individual foods or nutrients. Since foods and nutrients act synergistically rather than in isolation [15-18], any assessment of single effect of individual food is likely to be confounded by other foods in the diet [19, 20]. In addition, individuals eat freely; the amount and type of single food they consumed may frequently change, but the dietary patterns are relatively entrenched. Thus, a growing body of research has utilized dietary patterns to investigate the role of diet in cancer carcinogenesis. Dietary patterns identified in prior research often include the "Western" and "prudent" patterns. Adherence to the Western diet pattern, characterized by high intakes of meat, fat, sweets and desserts, is often associated with increased risk of CRC [17, 18, 21-23]. Whereas strong adherence to the prudent pattern, characterized by high intakes of fruit, vegetable, fish and poultry, often shows an inverse [21, 22] or null [17, 18, 24] association with CRC risk. To our best knowledge, however, there is only one study that specifically investigated the relationship between dietary patterns and survival among CRC patients. That prospective cohort study of 1009 stage III colon cancer patients [23] reported a deleterious disease-free colon cancer prognosis for patients reporting high levels of the Western dietary intake. Conversely, the authors observed no significant association between the prudent dietary pattern and cancer recurrence or mortality.

Newfoundland and Labrador (NL) is the most eastern province in Canada. Geographically isolated in the Atlantic Ocean, NL has long maintained a Western-style diet consisting of a large proportion of processed meat, red meat and insufficient amount of vegetables [12]. Several studies have partially attributed the high CRC incidence rate in NL to its unique diet [12, 25, 26], but no study has

explored the association between the NL diet and its impact on survival among CRC patients.

Cigarette smoking is another modifiable lifestyle factor associated with CRC tumorigenesis. In 2011, cigarette smoking was estimated to account for more than 20% of all deaths in Canada and 6 million deaths worldwide [27]. Moreover, more than 16% of Canadians over the age of 15 still smoke [28]. Smoking is clearly associated with malignancies in lung and larynx as well as chronic respiratory diseases [29]. In its 2012 monograph, the International Agency for Research on Cancer (IARC) listed the colorectum among the tumor sites for which there is sufficient evidence of carcinogenesis by tobacco smoking [30]. Carcinogens in cigarettes, such as polycyclic aromatic hydrocarbons (PAHs) and aromatic amines [31-33], may reach the bowel mucosa through direct ingestion [34] and through the circulatory system [35], thus inducing DNA damage in colorectum mucosa cells, thereby increasing CRC incidence. Recent data suggest that the association of smoking and CRC incidence may differ by tumor molecular phenotype, although this evidence base is still emerging [36, 37]. The influence of smoking on CRC survival is unclear. Some studies [37, 38] have suggested that cigarette-use is strongly associated with reduced survival among CRC patients, whereas other studies have reported no significant differences in survival rates between smokers and never smokers with CRC [39-41]. The apparent discrepancy between studies is attributed to the long induction period of CRC [29], as well as the potential for modulating effects of important prognostic variables [42-44], many of which were not accounted for in the previous studies. For example, the microsatellite instability-high (MSI-H) phenotype and the somatic *p.V600E BRAF* mutation are strongly associated with both smoking status [45] and cancer prognosis [43, 46, 47], thus a potential interaction between smoking and these tumor phenotypes

should be appreciated. To date, only one study has explored the potential interaction between smoking and molecular tumor phenotype on mortality among CRC patients; this study showed a prominent association between smoking and CRC-specific mortality among patients whose tumors exhibited the MSI-H phenotype [37].

Together, the influence of dietary patterns and cigarette smoking on patients' survival after CRC diagnosis has been inadequately examined. Novel research is clearly needed to explore these associations, if any.

1.2 Study Purpose and Hypotheses

Using the data of more than 700 colorectal cancer patients in Newfoundland and Labrador, the study presented in this dissertation aims to:

1) Investigate the associations of dietary patterns and colorectal cancer all-cause (overall survival; OS) and disease-free survival (DFS).
2) Explore if the relationship between dietary pattern and colorectal cancer survival is modified by sex, physical activity levels and *BRAF V600E* mutation.
3) Examine the association of smoking with colorectal cancer all-cause and disease-free survival.
4) Assess potential interactions of smoking with sex, age at diagnosis, tumor stage at diagnosis, MSI status, and *BRAF V600E* mutation status on survival among colorectal cancer patients.

We hypothesize that prediagnostic "Western" dietary pattern and cigarette smoking are associated with poor survival among CRC patients.

1.3 Organization of the Thesis

The thesis is organized as follows:

Chapter 1 is an overall introduction to this study. Chapter 2 reviews the literature and briefly summarizes the epidemiology of CRC, molecular pathways of CRC, as well as relevant risk and prognostic factors associated with CRC. Chapter 3 and Chapter 4 are two distinct but related subprojects, each including its own *Methods*, *Results*, and *Discussion* section. Chapter 5 summaries the key findings, discusses the implications and limitation of the study, and finally makes suggestions for further research directions.

Chapter 2 Literature Review

2.1 Overview

Colorectal cancer (CRC) is the third most frequent cancer and the fourth leading cause of cancer death, with approximately 1,230,000 new cases and 608,000 deaths worldwide per year [48]. The impact of CRC is huge, not only because of its adverse influence on individual's overall quality of life [49] but also because of the loss in life years caused by the disease, which is only exceeded by lung and breast cancer in North America (932,000 years in total in 2012) [50]. Therefore, there is a compelling need for novel research to explore potential modifiable factors associated with CRC risk and survival, so as to inform prioritization of control strategies to reduce cancer risk in susceptible populations and to maximize survival of cancer patients [51].

2.2 Epidemiology of CRC

2.2.1 CRC Incidence and Trends

Colorectal cancer has a ten-fold variation in international incidence, with the highest rates being observed in Australia, New Zealand, and parts of Europe, followed by North America, and the lowest in Asia, Africa and most of Latin America, ranging from more than 60/100,000 to less than 5/100,000 people [48, 52]. Indeed, the developed regions account for almost 60% of all CRC incidence [48]. The geographic variation suggests environmental causes of CRC, which has also been supported by the fact that immigrants rapidly reach the incidence rates of the adopted country [5, 7]. In Canada, the 2010 age-standardized incidence rate of CRC was 62/100,000 among men and 41/100,000 among women, with the highest

incidence rate being observed in NL [53]. More than half of new CRC cases occurred among people aged 70 years or older [53]. Meanwhile, CRC incidence was found substantially higher in men than in women.

More recently, Center and his colleagues in American Cancer Society led a new study [54] that comprehensively reviewed CRC incidence data from 51 cancer registries worldwide from the Cancer Incidence in Five Continents (CI5) databases. They analyzed the change in incidence rates over the past 20 years: 1983-1987 through 1998-2002, and found that CRC incidence increased significantly, especially for males, for 27 out of 51 cancer registries included in the analysis. The greatest increases were seen in economically transitioning countries including most of Asia, and select countries of Eastern Europe and South America. In particular, in Miyagi, Japan, rates increased by 92% in men and 47% in women. The author ascribed the substantial increase in CRC in economically transitioning countries to the progressive "westernization" of lifestyles in these populations. Many changes in modifiable lifestyle habits due to "westernization" are suspected to increase CRC risk, including high consumption of red/processed meats, physical inactivity, and increased prevalence of smoking behavior [54]. However, this increase in CRC incidence may also be attributable, at least partly, to improved reporting and diagnostic techniques in these countries. The only country where CRC incidence gradually decreased was the United States [54].

However, the long-term data cannot capture short-term fluctuation of rate change. According to the 2011 Canadian Cancer Statistics, the incidence of CRC in Canada increased substantially between 1982 and 1985, then declined through the mid-1990s, and rose slightly to 2000; thereafter, the incidence of CRC declined slightly [1]. A

major reason for recent declines in CRC incidence might be the high screening rates.

2.2.2 CRC Mortality and Trends

About 608,000 deaths from CRC are recorded worldwide in 2008, that is, nearly 8% of all cancer deaths, making CRC the fourth most common cause of cancer-related death [48]. The highest CRC mortality rates in both sexes are seen in Central and Eastern Europe, while the lowest rates are observed in Middle Africa [48]. In recent years, the mortality rate of CRC has declined significantly in regions such as United States, New Zealand, Australia, and Western Europe [55-57], but increased at a crude rate of 5-15% per year in some parts of Eastern Europe [52].

In Canada, the age-standardized mortality rate was 25/100,000 among men and 15/100,000 among women in 2011, with the highest mortality rate seen in NL, which is almost twice of that in British Columbia for males [1]. According to the 2011 Canadian Cancer Statistics [1], over the past sixteen years, mortality due to CRC in Canada has been decreasing by approximately 1.5% every year in males and 1.8% in females. This decline in mortality highlights the improvements made in screening, early detection, training program, and treatment for colorectal cancer, for example, including new chemotherapeutic agents [1].

2.2.3 CRC Survival and Prognosis

Colorectal cancer survival is closely correlated with stage and histological grade of disease at diagnosis. Generally, the earlier the stage at diagnosis, the longer the time of survival. It has been estimated that the 5-year survival rate for CRC is over 90%

for patients with tumors detected at the localized stage; 70% for regional cancers, and only 10% for people diagnosed with distant metastatic CRC [58].

There is enormous variability in CRC survival worldwide. The geographical differences in survival is likely due to global/regional inequalities in prompt cancer detection and treatment of disease [52]. However, survival for CRC at all stages has been increasing substantially on a world scale over the past decades [52, 58]. Recent progress in cancer control and treatment are definitely one of the reasons that can explain such increasing trend in CRC survival.

2.3 Molecular Pathways of Colorectal Carcinogenesis

Genomic instability is a major driving force for the transformation of normal colorectal mucosa to carcinoma, and three distinct molecular pathways have been implicated in CRC development [59]; these are the chromosomal instability (CIN) pathway, the microsatellite instability (MSI) pathway, and the serrated pathway, which are largely distinct from each other but are not mutually exclusive [59].

2.3.1 The Chromosomal Instability Pathway

The majority (approximately 65%-70%) of sporadic CRCs arise through the chromosomal instability pathway, which is induced by defects in chromosome segregation, telomere dysfunction, or defects in the DNA damage response system [60]. The CIN pathway is characterized by aneuploidy, chromosomal genomic amplifications, and loss of heterozygosity [59]. Allele gains related to the CIN pathway are commonly found on chromosomes 7, 8q, 13q, 20 and X, while allele

losses are often detected on 1, 4, 5, 8p, 14q, 15q, 17p, 18, 20p, and 22q [60]. These insertions or deletions frequently cause mutations affecting some critical oncogenes and tumor suppressor genes, such as adenomatous phlyposis coli (*APC*) gene, *K-ras*, *p53* and *SMAD* genes [61], and mutations in the *APC* and *K-ras* genes are the commonest genetic alternations [60].

The Wnt signaling pathway plays a principal role in both CRC initiation and progression [60]. This pathway is triggered by β-catenin through binding to T-cell factor (TCF)/lymphoid enhancer factor (LEF), which initiates the transcription of Wnt target genes [62]. Normally, the *APC* functions to modulate the Wnt signaling pathway through negative regulation of β-catenin. However, loss of *APC* gene function caused by specific mutations may lead to accumulation of cytosolic β-catenin, thereby resulting in nuclear translocation and over-activation of Wnt signaling pathway [60].

K-ras mutation is observed in 30-60% of CRCs and most adenomas [63, 64]. The *K-ras* oncogene produces a 21 kDa membrane bound protein that functions to transduce cellular signals. The *K-ras* protein is normally hydrolyzed by GTPase. However, specific mutations can result in decreased intrinsic GTPase activity. Its inability to deactivate *K-ras* allows *K-ras* to maintain an activated form. The activated *K-ras* oncogene promotes the transition from adenoma to carcinoma by activating downstream targets such as *BCL-2*, *H2AFZ*, *RAP1B*, *TBX19*, and *MMP1* [60, 63].

2.3.2 The Mismatch Repair deficient, Microsatellite Instability Pathway

Microsatellites are “short tandem repeats” (STRs) in DNA sequences that spread over the whole genome [60]. Microsatellite DNA is intrinsically unstable and error

prone. The DNA mismatch repair (MMR) system normally recognizes and corrects mismatch errors that are generated during DNA replication [65]. Several genes function in MMR, such as *MLH1*, *MSH2*, *MSH6* and *PMS2*. Inadequate MMR function would lead to frameshift mutations in the microsatellite repeats and therefore gene inactivation, known as instability of microsatellites [66]. It is estimated that about 15% of all CRCs evolve through the MSI pathway; of these, approximately 3% are correlated with Lynch syndrome due to the inherited mutations in MMR genes, and the remaining 12% are sporadic, caused by promoter hypermethylation of the *MLH1* [67]. MSI tumors have a distinct profile, for example, including a tendency to occur in the proximal colon, to have lymphocytic infiltrate, and to be mucinous and poorly differentiated [67, 68]. Furthermore, microsatellite instability-high (MSI-H) tumors are often associated with better survival than microsatellite stable (MSS) tumors [65].

2.3.3 Serrated Pathways

The serrated pathway, accounting for approximately 10%-20% of CRCs, is highly correlated with both the CpG island methylator phenotype (CIMP) and the *BRAF V600E* mutation [69].

2.3.3.1 CpG Island Methylator Phenotype

CpG Island Methylator Phenotype refers to the presence of concordant methylation in multiple tumorigenesis-related genes [60]. There are generally two types of CIMP in sporadic cancers: CIMP high and CIMP low. Weisenberger *et al*. [70] proposed a robust panel of markers to distinguish CIMP high from CIMP low status

including markers in *CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3*, and *SOCS1* genes. DNA methylation of at least three out of these five markers is defined as CIMP positive, and approximately 15-20% of sporadic CRC exhibit CIMP-high [60]. Most sporadic MSI colon tumors are CIMP positive, but CIMP is rarely detected in MSI-H tumors arising from Lynch syndrome, suggestive of distinct underlying molecular mechanisms [70, 71]. CIMP and the clinicopathologic features of CRC have been widely investigated. Specifically, CIMP is significantly more frequent in females, senior age groups, and tumors of the proximal colon [71-73]. Moreover, CIMP-high cancers are associated with poor tumor differentiation, microsatellite unstable tumor phenotype, *BRAF V600E* mutations, *K-ras* mutations, and wildtype *p53* [72-75]. Finally, Ogino *et al*. [73] reported a significant relationship between CIMP-high and a low CRC-specific mortality that is independent of other molecular phenotypes.

2.3.3.2 *BRAF V600E* Mutant Phenotype

BRAF V600E is a somatic mutation observed in colorectal tumors. This mutation results in a substitution of a valine amino acid with a glutamic acid amino acid at position 600 in the *BRAF* protein [76]. The *BRAF V600E* mutation is found to be tightly correlated with the CIMP and the MSI pathways [70, 74], whereas it is uncommon in Lynch syndrome-related cancers that exhibit MSI-H [77]. *BRAF V600E* mutant CRC accounts for 10-18% of all CRCs and normally has distinct features. It is more common in females, smokers, and proximal colon location (right colon) [78-80]. Pathologically, tumors harboring the *BRAF V600E* mutation are often poorly differentiated, and hence they are associated with a poor prognosis [81].

2.4 Factors Associated with CRC Incidence

The causative etiology of CRC is complex and multi-factorial [82]. Approximately 15% to 20% of all CRCs are familial, and several heritable risk factors and CRC susceptibility genes are implicated [83]. On the other hand, the majority of CRC cases are sporadic; they arise through cumulative effects of environmental factors and the complex interaction between heritable genetic and environmental components [84].

2.4.1 Family History

Family history is one of the most consistent risk factors for CRC. People with a positive family history of CRC have a two- to four-fold increased risk of developing CRC than those without it [85]. The risk is higher among people whose relatives have early-onset CRC (age at diagnosis <50) than those whose relatives are diagnosed later [86]. Hence, the joint guideline for early detection of CRC developed by multiple organizations—the American Cancer Society, the US Multi Society Task Force on CRC, and the American College of Radiology—recommends early screening for individuals who have a family history of CRC [87].

Part of the familial risk can be attributed to several inherited cancer syndromes, and two major CRC predisposition syndromes are familial adenomatous polyposis (FAP, 1% of all CRC cases) and hereditary non-polyposis colorectal cancer (HNPCC, 5-7% of all CRC cases) [88]. Both are inherited in an autosomal dominant fashion. FAP is caused by inactivating mutation of a tumor suppressor gene, the adenomatous polyposis coli (*APC*) gene. Patients affected by FAP usually present with hundreds of adenomatous polyps in the colon, which will inevitability transform to carcinoma if

left untreated [89]; HNPCC results from mutations in mismatch repair genes, commonly *MLH1*, *MSH2* and *MSH6* [89]. More than 90% of tumors arising in HNPCC patients shows microsatellite instability [89].

In recent genome wide association (GWA) studies, there have been links identified between at least ten common low-penetrance variations and the occurrence of CRC [84]. Tenesa *et al*. [84] analyzed Scottish GWA data and reported that up to 170 common independent variants were contributing to inherited susceptibility to CRC. To date, CRC susceptibility has been shown to be related to a variety of genes linked to chromosome segments 8q23.3, 8q24, 10p14, 11q23, 15q13, and 18q21 *et al.* [69, 90-93].

2.4.2 Modifiable Environmental Factors

A wide range of environmental factors has been studied both experimentally and epidemiologically as potential risk factors for CRC; these include dietary factors, physical activity levels, and smoking. Each of these potential risk factors will be described individually in more detail.

2.4.2.1 Diet Factors

It is generally believed that diet would strongly influence the CRC risk through either physical interaction with the intestinal mucosa [69] or alterations in intestinal microbiota, which are strongly associated with colonic polyp formation and with the risk of developing CRC [94]. According to statistics, up to 70% of this cancer burden could be reduced by alterations in dietary habits [9].

2.4.2.1.1 Total Energy and Macronutrients

Although the 2007 WCRF/AICR expert report indicated that there is no simple association between total energy and CRC risk [95, 96], much evidence in the literature would suggest that intakes of total energy are positively associated with risk of CRC [97-99]. The underlying biological mechanisms are not fully understood, but it is suggested that high energy intake could induce glycemic overload and compensatory increase of serum insulin and insulin-like growth factor-1 (IGF-1), which may subsequently promote cell proliferation, inhibit apoptosis, and eventually elevate the risk of colorectal tumorigenesis [100-103].

Furthermore, evidence on relationships between CRC risk and intakes of macronutrients (e.g., carbohydrates, proteins, and fats) remains limited and sometimes inconsistent. For example, a 1990 report from the Nurses' Health Study linked the consumption of animal fat to an increased risk of CRC after the adjustment of total energy intake, but the authors found no correlation between vegetable fat and cancer initiation [104]. Conversely, several studies observed no effect of animal fat on CRC risk [105, 106]. Simultaneously, non-red meat sources of animal protein have been typically correlated with a reduced risk [100, 105], whereas total carbohydrate consumption has been linked with increased risk in some studies [107], but not others [95, 108].

2.4.2.1.2 Fruit, Vegetables and Fibre

The majority of over 20 case-control (reviewed in [109]) and 7 cohort studies (reviewed in [110]) have suggested that high consumption of fruits and vegetables may be associated with a reduction of CRC risk. However, which fruits or vegetables account most for this reduction remains elusive. In the 9.6-year follow up of the

Swedish Mammography Screening Cohort Study [110], a protective effect was found for total fruit and vegetable consumption. However, little relation was seen between CRC risk and the intake of cereal fibre in this study.

A meta-analysis of 13 case-control studies [111] suggested that dietary fibre may protect against CRC, although results from prospective cohort studies [112-114] have been essentially negative. Admittedly speculative, one possibility is that the protective effect of dietary fibre needs a much longer response latency than the follow-up time frame of the research that has failed to support such association [115].

2.4.2.1.3 Red and Processed Meat

"Red meat" is generally defined as flesh from domesticated mammals, which is mainly comprised of red muscle fibres, such as beef, pork, lamb and goat; "processed meat" refers to meats preserved by smoking, curing, or salting, to which nitrites/nitrates or other preservatives are artificially added, such as ham, bacon, and salami [116].

To date, the evidence that links red and processed meat consumption to increased CRC risk is quite convincing—specifically, all of the 16 cohort studies and most of the 71 case-control studies that examine red meat and CRC risk (reviewed by the 2007 WCRF/AICR expert panel [116]) have reported an increased risk for individuals in the highest intake group relative to those in the lowest, and an apparent dose-response effect has been observed. These observations are further supported by a recent meta-analysis of 15 prospective studies, which indicated that a daily augment in red meat consumption of 120 gram is associated with a 28% (95%CI: 18%-39%) increase in CRC risk [117]. As well, twelve out of fourteen cohort studies (reviewed in [116]) have shown a positive association between processed meat intake and CRC

risk, with the association being statistically significant in four [104, 106, 118, 119]. A meta-analysis of five studies conducted by the panel gave a summary RR (relative risk) of 1.21 (95% CI 1.04-1.42) per 50 g/day augment of processed meat intake [116].

There are several mechanisms that may rationalize the associations observed between red/processed meat and CRC. First, fried or high-temperature cooked meat may contain carcinogenic heterocyclic amines and polycyclic aromatic hydrocarbons [116]. Many processed meats are also high in salt, nitrite, and nitrate that may promote the subsequent formation of endogenous N-nitroso compounds; these compounds are suspected human carcinogens and have been suggested as significant contributors for CRC development [120, 121]. Moreover, high consumption of red meat has been associated with increased iron level, which may catalytically stimulate the production of reactive oxygen species, and hence result in DNA damage [122, 123].

2.4.2.1.4 Minerals and Vitamins

Several antioxidants, for example, beta carotene, riboflavin, folate, and vitamins A, C and D have long been regarded as natural inhibitors that influence cancer initiation or progression [26, 124-127]. Since these antioxidants can provide antioxidant and anti-inflammatory effects, they are suggested to protect against CRC. In addition, dietary calcium and selenium have been consistently associated with a lower risk of CRC, whereas dietary iron has increased the risk because of its catalytic activity on the production of oxygen radicals [26, 128].

2.4.2.1.5 Dietary Patterns

Most nutrition studies have focused primarily on individual food or nutrients.

Since food and nutrients act synergistically rather than in isolation [15-18], recent research has investigated the role of dietary patterns on CRC incidence. Dietary patterns identified in prior research often include the "Western" and "prudent" patterns. Adherence to the Western diet pattern, characterized by high intakes of meat, fat, sweets and desserts, is often associated with increased risk of CRC [17, 18, 21-23]. Whereas strong adherence to the prudent pattern, characterized by high intakes of fruit, vegetable, fish and poultry, often shows an inverse [21, 22] or null [17, 18, 24] association with CRC risk. A recent review of data from existing cohort studies (2000-2011) [129] concluded that risk factors for CRC include Western diet, processed pork meat, traditional meat eating, potatoes, and refined grain pattern, while protective dietary patterns for CRC include healthy and prudent diet.

It is not possible that the relationship between dietary patterns and CRC risk is attributable to a causal effect of any specific food, because each pattern contains a complex mixture of diverse foods, all of which may contribute to an effect [116]. Instead, it seems more likely that individual food and nutrient interact with each other and work together to give rise to CRC. Unfortunately, the existing investigations on dietary patterns and diseases are limited in number, and definitions for specific dietary patterns are rather different across studies.

2.4.2.2 Cigarette Smoking

Smoking has been widely investigated as a risk factor for incident CRC [130-139]. Most studies have reported that chronic smoking is associated with an increased risk of CRC incidence [130-137]. CRC risk also increases with an increasing number of pack-years and cigarettes per day in a dose-dependent manner [130, 131, 133, 134].

Furthermore, the smoking-CRC association has been observed to be stronger for women than for men [136, 137] and for rectal cancer [140, 141]/proximal colon cancer [29, 142] than for the distal colon cancer. A comprehensive meta-analysis conducted by Botteri *et al*. [29] suggested that ever smoking was associated with an almost 20% increase in CRC risk compared with never smoking (RR: 1.18; 95%*CI*: 1.11-1.25), while the risk estimates were higher for cancer of the rectum than of the colon in current smokers (*P*=0.02). Tsoi *et al*. [140] examined a total of 1,463,796 participants in 28 prospective cohort studies and concluded that current smokers carried a modestly higher risk of CRC relative to never smokers (RR: 1.20; 95%*CI*: 1.10-1.30), and the increased risk of CRC was dose-related to higher levels of daily cigarette consumption/pack-years, and longer years of smoking. When stratified by tumor molecular subtypes, however, smoking appeared to be most strongly correlated with the risk of CRC that exhibited MSI-high [138, 139]. Among studies that assessed the effect of smoking cessation on CRC risk, three [137, 142, 143] reported no consistent trend in risk whereas two [136, 144] reported significantly increased risk with prolonged cessation time.

Nevertheless, some investigators have reported inconsistent results. Nyren *et al*. [145] examined a cohort of 135,000 Swedish construction workers and found no significant association between current smoking status, number of daily cigarette consumption or number of years smoking, and the risk of CRC. Even among heavy smokers with a long smoking duration (≥30 years at the beginning of follow-up), the age-adjusted RRs were not statistically significant. The reasons for the discrepancies in comparison with recent data might be that Swedish men have a tradition of snuff suction, which might have led to contamination of negative control, resulting in the hazardous effects of smoking not yet being detected. Another hospital-based

case-control study on CRC from India [146] also reported non-significant association between ever smoking and CRC (smokers vs. non-smokers: OR: 0.7; 95%*CI*: 0.4-1.1). For that analysis, smoking history was classified into categories of ever and never smoking; regrettably, effects of gradients of smoking duration or intensity on CRC were not examined.

There are several biologic mechanisms that may explain the higher risk of CRC among smokers. First, carcinogens in cigarettes, such as polycyclic aromatic hydrocarbons and aromatic amines [31-33], may reach the bowel mucosa through direct ingestion [34] or through the circulatory system [35], thus exerting growth promoting effects on cancer cells in the colorectum and increasing CRC incidence. Second, tobacco smoking may mutate the *GSTM1* gene, resulting in further impaired detoxification of these carcinogens [147]. Moreover, smoking may also induce aberrant promoter DNA methylation, thus silencing regulatory genes (e.g., *ECAD*, *p16*, *MGMT*, and *DAPK*) in tumor initiation and promotion [148].

2.4.2.3 Physical Activity Levels

Physical inactivity is a well-accepted risk factor for CRC that is independent of other confounders including obesity [69]. Physical activity is suggested to improve body's metabolic efficiency and to reduce blood pressure and insulin resistance. Nearly all of the existing cohort and case-control studies investigating physical activity and CRC demonstrated a decreased risk of developing the disease with increased habitual levels of physical activity, except for one early study that reported a positive but non-significant association between physical activity and CRC among men who aged <45 years (RR: 2.23; 95%*CI*: 0.88-5.66) [149]. It is estimated that high

habitual levels of physical activity could reduce up to 50% of all incident CRC [150], and engaging in two hours or more of physical activity per week could significantly reduce CRC risk in most people [151].

2.5 Factors Associated with CRC Survival

2.5.1 Clinicopathologic Factors

Most known factors that influence CRC survival are clinicopathologic features which are regrettably unmodifiable, including tumor stage, histological grade, treatments received, and tumor molecular phenotype.

In particular, the tumor stage and histological grade of CRC have been well documented in the literature as indicators of severity of disease and chance of survival. The 5-year survival rate was estimated at over 90% for tumors detected at the localized stage; 70% for regional, and only 10% for people diagnosed for distant metastatic CRC [58]. For those metastatic CRCs, receiving an accepted form of systemic treatment appears to largely lengthen the median survival time of CRC patients (from less than 9 months without any treatment to about 24 month in Wolpin *et al*.'s [152]).

Furthermore, there is some evidence that associations exist between selected tumor molecular features and survival from CRC. For instance, MSI-H CRCs has been linked to an excellent 5-year survival, while *BRAF*-mutant tumors have portended a poor survival [46, 47, 153].

Besides, FLT2 and KDR are primary receptors for the vascular endothelial growth factor-A (VEGFA); they may influence tumor progression via promoting

angiogenesis [154]. Slattery *et al*. [154] analyzed data from a case-control study of more than 5,000 participants and found that four single-nucleotide polymorphisms (SNPs) in FLT1 were predictive of colon cancer survival while three SNPs in KDR were predictive of rectal cancer survival.

2.5.2 Modifiable Environmental Factors

2.5.2.1 Diet Factors

2.5.2.1.1 Total Energy and Macronutrients

Epidemiological evidence on the association between intake of total energy and CRC survival remains controversial. Dray *et al*. [14] analyzed 10-year survival data on 148 patients who received resection of the tumor and concluded that high energy intake, resulting from high intakes of carbohydrate, protein, and lipid, was strongly correlated with better 5-year survival, the HR for the highest tertile of energy intake being 0.18 (95%*CI*: 0.07-0.44) in comparison to the two lowest tertiles. Likewise, Slattery *et al*. [13] examined the influence of diet in 411 colon cancer cases in Utah. After the adjustment of major known prognostic factors, the highest quartile of intake for total calories, fat and protein was associated with increased cancer survival, HRs being 0.60, 0.80, and 0.66 respectively. Nevertheless, some recent data somewhat contradicted the intriguing results from Dray's and Slattery's. For example, one study [155] showed an adverse impact of a diet high in carbohydrates on disease-free survival, recurrence-free survival, and overall survival for colon cancer patients. Sichieri *et al*. [156] found that total fat intake was possitively associated with CRC mortality. The contradictory results are likely due in part to adverse causal or residual confounding by other factors such as disease stage at diagnosis that had not been

adjusted for in earlier studies.

2.5.2.1.2 Fruit, Vegetables and Fibre

Ever since Burkitt [157] proposed the hypothesis that the lower CRC mortality in blacks than that in whites was attributable to high dietary fibre consumption, the protective effects of fruit, vegetables and fibre against cancer mortality have aroused strong research interest. Diets higher in fruit and vegetables tend to be associated with a lower risk of CRC mortality [158]. The favorable roles of fruit and vegetables on CRC progression may be attributable not only to the antioxidant vitamins but also to their fibre content. This is supported by the findings from an ecological analysis of the Seven Countries Study [159] showing a strong inverse association between dietary fibre and CRC mortality. Intriguingly however, the Cancer Prevention Study II conducted by researchers from American Cancer Society observed some inverse associations between certain fruits rich in fibre and the risk of fatal CRC [160, 161]. Nor in the study by Dray *et al*. [14], a significant association has been observed between fruit, vegetables, and CRC survival.

2.5.2.1.3 Red and Processed Meat

Although red and processed meat has been confirmed as a moderate risk factor for CRC [162], minimal research has specifically examined its role in survival after CRC diagnosis. In the CRC gene-environment study supported by the National Cancer Institute [163], Zell *et al*. compared the overall survival rates for 511 CRC patients by quartile levels of prediagnostic meat consumption and found them to be significantly different among familial CRC patients (10-year OS: the highest quartile: 42%, the lowest quartile: 65%; P=0.017), but not different among sporadic CRC

patients, indicating potential gene-environmental interactions. Likewise, McCullough *et al*. [164] examined 2,315 patients with CRC who reported both pre- and post-diagnosis diets in the Cancer Prevention Study II Nutrition Cohort. Results showed that neither pre- nor post-diagnosis red/processed meat consumption was significantly associated with CRC mortality risk. However, individuals with consistently high intakes of red/processed meat before and after diagnosis were 1.79 (95%*CI*: 1.11-2.89) times more likely to die from CRC than those with consistently low intakes. In line with Zell *et al*.'s [163], a borderline interaction between family history and red/processed meat was detected in McCullough's study, with a higher risk of all-cause mortality observed among patients who have a positive family history (*P* for interaction=0.05). The authors ascribed the observed interaction to genetic polymorphisms in the detoxification of carcinogenic mutagens (e.g., Heterocyclic Amines and Polycyclic Aromatic Hydrocarbons) that were often found in smoked, fried or high-temperature cooked meat [164].

2.5.2.1.4 Minerals and Vitamins

Significant associations have been identified between selected minerals/vitamins and survival from CRC, for example, including protective effects of high premorbid levels of selenium, vitamin B6, retinol, β–carotene, lycopene, total carotene, and provitamin A [124, 165-167]. However, no consistent trend in risk for death as a result of CRC has been observed for any of the aforementioned nutrients in an ecological survey in 49 Chinese rural counties [158] nor in a study of 16 cohorts of the Seven Countries Study [168].

2.5.2.1.5 Dietary Patterns

Research that has examined the relationship between dietary patterns and survival among CRC patients are limited in number. The only study identified by our literature review investigated a cohort of 1,009 stage III colon cancer patients across North America from a randomized adjuvant chemotherapy trial [23]. In that study, two major dietary patterns—prudent and Western—were identified by factor analysis. The authors reported a significant detriment in colon cancer survival—defined as disease-free survival (HR: 3.25; 95%*CI*: 2.04-5.19; *P* for trend <0.001), recurrence-free survival (HR: 2.85; 95%*CI*: 1.75-4.63) and overall survival (HR: 2.32; 95%*CI*: 1.36-3.96) —for patients in the highest quintile of Western dietary pattern intake, compared to those in the lowest quintile group. However, a prudent diet was not associated with either CRC recurrence or mortality.

2.5.2.2 Cigarette Smoking

Numerous studies have investigated the role of smoking in CRC survival with conflicting results. Most studies [37, 38, 169-171], but not all [39-41], have indicated that cigarette smoking is associated with greater risk of CRC mortality and worse survival, specifically for tumors located in the rectum than in the colon; however, the associations vary in both direction and magnitude. There have been four main studies that assessed the effect of smoking cessation on the risk of CRC mortality, two of which have reported protective effects of smoking cessation on CRC mortality risk [170, 171]. The other two [41, 172] have found no significant association.

Based upon this, Botteri *et al.* [29] undertook a complete review of 106 observational studies on cigarette smoking in relation to both CRC incidence and mortality. They reported that ever smoking relative to never smoking status was

linked with a 1.25-fold increased risk (95%*CI*: 1.14-1.37) [29]. The dose-response analyses suggested that the risk of CRC mortality increased linearly with increasing number of cigarettes smoked per day and prolonged duration of smoking. Specifically, an increase of 10 cigarettes/day (0.5 pack/day) and 10 years of smoking led to a 7.4% (95%*CI*: 5.7%-9.2%) and a 9.5% (95%*CI*: 5.5%-13.7%) increase in RRs for CRC mortality, respectively. Liang *et al.* [141] also examined 36 prospective studies and observed an approximately 40% (95%*CI*: 1.06-1.84) augment in RR for CRC mortality in current smokers compared to never smokers. For every additional 20 cigarettes per day, the risk of CRC mortality increased by 40.7%.

Nevertheless, other studies have reported no significant differences in survival rates between smokers and never smokers with CRC [39-41]. The apparent discrepancy between studies is attributed to the long induction period of CRC [29], as well as the potential for modulating effects of important prognostic variables [42-44], many of which have not been accounted for in the previous studies. To date, only one study has explored the potential interaction between smoking and molecular tumor phenotype on mortality among CRC patients; this study showed a prominent association between smoking and CRC-specific mortality among patients whose tumors exhibited the MSI-H phenotype [37].

2.5.2.3 Physical Activity Levels

Higher levels of physical activity have been associated with a lower risk of CRC mortality in previous epidemiological studies [173-175]. For example, in a study of CRC survival, Campbell *et al*. [175] examined 184,194 participants in the Cancer Prevention Study-II (CPS-II) Nutrition Cohort, and reported protective effects of

being physically active both before and after cancer diagnosis. The RR of all-cause mortality was 0.72 (95%*CI*: 0.58-0.89), comparing highest to lowest category of prediagnostic physical activity, and 0.58 (95%*CI*: 0.47-0.71) when the highest category of postdiagnostic physical activity was compared to the lowest. In the same study, prediagnostic and postdiagnostic sitting time ≥6 hours/day was correlated with increased all-cause mortality (prediagnostic RR: 1.36, 95%*CI*: 1.10-1.68; postdiagnostic RR: 1.27, 95%*CI*: 0.99-1.64).

In a comprehensive review of current evidence from prospective cohort studies [173], patients who participated in any amount of physical activity before and after diagnosis were found to be at lower risk of CRC-specific mortality compared with patients who did not participate in any physical activity. (prediagnostic RR: 0.75, 95%*CI*: 0.65-0.87, $P < 0.001$; postdiagnostic RR: 0.74, 95%*CI*: 0.58-0.95, $P = 0.02$).

2.5.2.4 Socio-Economic Status (SES)

Socio-economic status, normally measured by surrogates such as income, marital status, and education attainment, is a major prognostic factor for CRC. A review of 42 studies conducted throughout North America and Western Europe suggested that SES differences might cause considerably inequalities in access to diagnostic and treatment services, leading to SES variation in cancer survival [176]. Findings from five studies suggested that CRC patients in high social class had better survival than those in low social class [177-181], although the difference was only significant among men in Vagero's study [181]. Nevertheless, two other investigations showed non-significant socioeconomic differences in CRC survival [182, 183].

Chapter 3 Project 1. Dietary Patterns and Colorectal Cancer Recurrence and Survival

This part of work has been published as a peer-reviewed article in *BMJ Open* [184] and can also be viewed on the journal's website at www.bmjopen.bmj.com.

Authorship Statement

The candidate is the first author of the published paper. All authors have contributed substantially to this work. Specifically, the candidate and Peizhong Peter Wang conceptualized and designed the manuscript. The candidate conducted all relevant data analyses and drafted the first version of the manuscript with Hao Wu. Peizhong Peter Wang, Sevtap Savas, Jennifer Woodrow, Tyler Wish, Rong Jin, and Elizabeth Dicks contributed to the subsequent revision of this article. Roger Green, Sevtap Savas, Michael Woods, Barbara Roebothan, Sharon Buehler, John R Mclaughlin, Peter T Campbell, and Patrick S Parfrey were responsible for the data collection.

3.1 Method and Materials

3.1.1 Study Design

This is a prospective cohort study based on data from a follow-up of 529 newly diagnosed CRC patients from NL, Canada. The prospective nature of design provides stronger observational evidence than do retrospective studies in support of the hypotheses. This type of study offers preliminary evidence on the roles of environmental determinants in cancer etiology and serves as a basis for hypotheses

formulation for future experimental studies, such as randomized controlled clinical trial [185].

3.1.2 Study Population

Patients in this prospective cohort study were enrolled through the Newfoundland Familial Colorectal Cancer Registry (NFCCR), described in detail elsewhere [3, 186]. Patients were eligible for inclusion if they were:

1) Newly diagnosed with pathologically confirmed CRC (ICD-9 codes: 153.0-153.9, 154.0-154.3, and 154.8 or ICD-10 codes: 18.0-18.9, 19.9, and 20.9);
2) Diagnosed between January 1999 and December 2003;
3) Diagnosed at the ages of 20 to 75 years;
4) Residents of NL who had lived in NL for at least two years at the time of diagnosis.

Eligible patients were inquired about their willingness to participate. If patients died before they could give consent (the median time from date of diagnosis to date of consent was 1.8 years), a close relative/proxy, who has lived with the patient, was invited to participate. Enrolling deceased cases through proxies could remove the potential bias of eliminating patients at a late distant stage [186]. Written, informed consent was required from each study participant/proxy to access their archived tumor tissue and medical records. As a result, the inception cohort consisted of 750 eligible patients (64%).

Consenting participants completed and returned a detailed personal history

questionnaire (PHQ, see appendix 1), a food frequency questionnaire (FFQ, see appendix 2), and a family history questionnaire (FHQ). All questionnaires were self-completed. Assistance from study staff was available to help with understanding items on the questionnaires. Those who did not respond were sent a reminder card, and a follow-up call was made if needed. To capture additional cancer diagnosis or recurrence in the family after enrollment, the FHQ was distributed to participants for the second time midway through the follow-up.

To be included in this analysis, patients had to have completed at least the FFQ, provided informative lifestyle and medical data from the PHQ, and had known vital status information by the end of the follow-up period (April, 2010). For patients who died prior to enrollment, the designated relative/proxy completed the aforementioned questionnaires. The final analytical cohort comprised 529 eligible participants (45%). The study protocol was approved by the Human Investigation Committee of Memorial University of Newfoundland.

3.1.3 Measurement of Exposure and Relevant Variables

3.1.3.1 Dietary Assessment and Food Grouping

Diet was assessed using a semi-quantitative FFQ, which was developed from the well-known Hawaii FFQ [187] on the basis of a validated instrument adapted for the Canadian population [188-190]; and for this study, it was modified slightly to include foods indigenous to the Newfoundland population (e.g., salted/pickled meat and smoked/pickled fish). The FFQ included 170 food items, beverages, and vitamin- and dietary-supplements [191]. For each food item or beverage, participants were asked to estimate their frequency of consumption and usual portion size as ‘Small’, ‘Regular’

or ‘Large’ from one year prior to cancer diagnosis. Portion sizes for specific food were specified and showed to participants by photographs. Nutrient and total energy intakes were calculated by multiplying the frequency of consumption of each food by the nutrient content of the portion size based on the composition values from the 2005 Canadian Nutrient file.

Taking a similar grouping scheme to that used elsewhere [15], we collapsed individual food items on the FFQ into 39 predefined food groups based on the roles of food in diet and cancer etiology. Distinct food items were reserved as individual categories if it was deemed inappropriate to combine them (e.g., jam, pies, beer, and wine). As a result, the 39 predefined food groups included milk, yogurt, sugar, tea, coffee, soft drinks, cheese, egg, mixed dishes, cured/processed red meat, cured/processed meat, game, poultry, fish, processed fish, fruit juice, root vegetables, cruciferous vegetables, other fruit, other greens, tomato sauce, other vegetables, beans/peas, pickled vegetables, total cereals/grains, whole grains, citrus, berries, dried fruit, vegetable juice, beer, white wine, red wine, liquor, desserts/sweets, pies/tarts, canned fruit, and jam/jelly.

3.1.3.2 Covariates

Sociodemographic data, such as age, sex, marital status, and education attainment, were gathered by the self-administered PHQ. The PHQ also included items regarding medical history, bowel screening history, physical activity, reproductive factors (female only), and alcohol and tobacco use. Family history of cancer was assessed by the FHQ.

3.1.3.3 Study Outcomes

Study outcomes were ascertained from follow-up questionnaires, local newspapers (e.g., death notices), death certificates, autopsy, pathology, radiology, surgical reports, as well as physician's notes. Additional data were gathered from the Dr. H. Bliss Murphy Cancer Care Foundation and Statistics Canada [192]. The cause of death was obtained for 93 of 168 deceased patients in this cohort, classified according to the International Classification of Disease (ICD) codes for underlying or contributing cause of death [193]; the majority (91%) of these had died from CRC. Since specific cause of death was not available for all deceased participants, all-cause mortality was used for analysis. In this study, two end points were considered: the first was disease-free survival, defined as time from cancer diagnosis to the first confirmed tumor recurrence, metastasis, or death from all causes occurring up to April, 2010; the second end point was overall survival, measured from the date of cancer diagnosis to the date of death from all causes. Patients who did not have an event by the end of the follow-up were censored at the date of last contact.

3.1.3.4 Molecular Assessment

The *p.V600E BRAF* mutation and MSI status for the tumor DNA have been determined in previous studies using standard protocols [194, 195]. Briefly, the mutational hotspot c.1799T>A. (p.Val600Glu) in the *BRAF* gene was detected using *BRAF V600E* allele-specific primers. Positive mutations were then verified by direct automatic sequencing. For MSI analyses, a panel of 10 microsatellite repeats (BAT25, BAT26, BAT40, BAT34C4, D5S346, D17S250, ACTC, D18S55, D10S197, and MYCL) were used to amplify both tumor and normal DNA [194, 195]. MSI status

was defined as MSI-High if 30% or more of the markers were unstable and MS-Stable/MSI-Low if less than 30% of the markers showed instability [37, 196]. The primer sequences and PCR conditions are provided in detail in earlier studies from this cohort [186, 194, 195].

3.1.4 Statistical Analysis

3.1.4.1 Descriptive Statistics

Comparisons for baseline characteristics across quartiles of dietary patterns were performed using ANOVA test for continuous variables and Chi-Square test for categorical variables. Statistical significance was accepted for two-sided $P<0.05$. All data management and analyses were performed with SAS software version 9.2 (SAS Institute Inc, Cary, NC).

3.1.4.2 Principal Component Factor Analysis

Exploratory principal component factor analysis [197] was used to identify major dietary patterns based on 39 predefined food groups from the FFQ. A varimax rotation (orthogonal) procedure was applied to rotate these factors, meaning that it produces uncorrelated, easily interpreted components that explain the greatest amount of variance in the original food groups [198]. We determined the number of factors to retain for interpretation on the basis of criteria as follows: factor eigenvalue greater than 1.5, the scree plot, the proportion variance explained, and factor interpretability [23]. Patterns were labeled based on food groups with absolute rotated factor loading matrix greater than or equal to 0.50 on that factor. Each participant was assigned a

factor score for each pattern (factor) by summing the intakes from each food group multiplied by optimal weights (factor loadings) [17]. Individuals with a higher factor score had a closer adherence to that pattern [17].

3.1.4.3 Multivariate Cox Proportional Hazard Analysis

Cox proportional hazards models, each adjusting for energy intake and critical covariates, were used to evaluate the association between individual dietary pattern and CRC recurrence and mortality, represented by hazard ratios (HR) and 95% confidence intervals (CI). Potential confounders were assessed by the log-rank test in a univariate setting; those with the p-value less than 0.1 were considered for inclusion. The final models only retained the items that entered the models at $P<0.1$ or altered the effect estimates by 10% or more; these include sex (male, female), age at diagnosis (18-49, 50-59, 60-69, and 70+ years), stage at diagnosis (I, II, III, IV), body mass index (BMI<25.0, 25.0-29.9, and $\geq 30 kg/m^2$), marital status (single, married or living as married), family history (yes, no), reported screening procedure (yes, no), reported chemoradiotherapy (yes, no), and MSI status (MSI-high, MSS/MSI-low). All models were run with the adjustment for total energy intake by including total calories in the model. The assumption of proportional hazard rates was verified by checking the parallelism of the Kaplan-Meier curves and by including time-dependent covariates in the models to test for statistical significance [199].

3.1.4.4 Other Analyses

Statistical linear trend was examined by modeling the median value of each quartile as an ordinal variable in the regression model [17]. Potential interactions were

evaluated by comparing estimates from stratified analyses and testing significance of multiplicative interaction terms with the Wald test [17]. A sensitivity analysis was implemented by eliminating stage-advanced patients enrolled through proxies and re-calculating survival time from the completion of the first questionnaire to a predefined event, in order to determine whether associations might vary with the exclusion of stage-advanced cancer.

3.2 Results

The cohort was followed for a median of 6.4 years (minimum: 1.3 years; maximum: 10.9 years). A total of 168 patients died from all causes and 30 had a cancer recurrence or metastasis by the end of study follow-up (April, 2010).

3.2.1 Dietary Patterns

Three distinct dietary patterns, labeled “processed meat pattern”, “prudent vegetable pattern” and “high sugar pattern”, were extracted using the aforementioned factor analysis procedure. These patterns explained 73.82% of total variance in the original 39 food groups (**Table 3.4.1**). A higher factor loading matrix of a given food group is representative of a greater contribution of that food group on that specific pattern. Therefore, the first pattern, termed “processed meat”, was characterized by higher loadings and thus higher consumption of cured/processed meat, cured/processed red meat, red meat, fish, and processed fish; the second pattern, labeled “prudent vegetable”, displayed higher loadings on other greens, other fruit, other vegetables, and tomato sauce; and the third pattern, named “high sugar”, showed higher loadings on desserts and sweets, pies and tarts.

Table 3.4.1 Factor Loadings and Explained Variances (VAR) for the Three Major Dietary Patterns Identified from the Food Frequency Questionnaire at baseline using a Principal Component Factor Analysis

Food Groups	Processed Meat Pattern	Prudent Vegetable Pattern	High Sugar Pattern
Milk	-	0.19	-
Yogurt	-	0.31	-
Sugar	-	-0.19	0.20
Tea	-	-	0.17
Coffee	0.17	-	-
Soft drinks	0.19	-	-
Cheese	0.15	0.21	-
Egg	0.21	-	0.16
Mixed dishes	0.31	0.17	0.23
Red meat	**0.69**	-	0.17
Cured/processed red meat	**0.73**	-	0.21
Cured/processed meat	**0.93**	-	-
Game	0.23	-	-
Poultry	0.22	0.27	-
Fish	**0.58**	0.32	-0.22
Processed fish	**0.50**	0.25	-
Fruit juice	-	0.24	0.23
Root vegetables	0.28	-	0.15
Cruciferous vegetables	-	**0.54**	-
Other fruit	-	**0.59**	-
Other greens	-	**0.60**	-0.22
Tomato sauce	-	**0.50**	-
Other vegetables	0.22	**0.54**	-
Beans, peas	0.15	0.25	-
Pickled vegetables	0.15	0.26	0.15
Total cereals and grains	0.23	0.38	0.28
Whole grains	-	0.33	-
Citrus	-	0.34	-
Berries	-	0.45	-
Dried fruit	-	0.39	-
Vegetable juice	-	0.17	-
Beer	0.19	-	-
White wine	-	-	-
Red wine	-	-	-
Liquor	-	-	-
Desserts and sweets	0.31	-	**0.63**
Pies, tarts	0.15	-	**0.54**
Canned fruit	-	0.21	0.23
Jam, jelly	-	-	0.26
Proportion of VAR explained (%)	39.79	22.93	11.10
Cumulative VAR explained (%)	39.79	62.72	73.82

Absolute loading values <0.15 were not listed for simplicity.
Those with loadings of 0.50 or greater are in bold.

3.2.2 Baseline Characteristics by Quartiles of Dietary Patterns

Higher processed meat pattern scores at baseline were detected in men, ever smokers, patients who were single and individuals who had higher BMI at the time of diagnosis (**Table 3.4.2**). Higher prudent vegetable pattern scores were observed in women, never smokers, those with a slightly later age of diagnosis and with patients who had a tumor harboring the *p.V600E BRAF* mutation. None of these characteristics varied significantly by quartiles of high sugar pattern scores.

Table 3.4.2 Baseline Characteristics of 529 Colorectal Cancer Patients by Quartiles of the Three Major Dietary Patterns [a]

	Processed Meat Pattern				*P* Value[c]	Prudent Vegetable Pattern				*P* Value[c]	High Sugar Pattern				*P* Value[c]
	Q1 (n=132)	Q2 (n=132)	Q3 (n=133)	Q4 (n=132)		Q1 (n=132)	Q2 (n=132)	Q3 (n=133)	Q4 (n=132)		Q1 (n=132)	Q2 (n=132)	Q3 (n=133)	Q4 (n=132)	
Age at diagnosis[b]	61.4±8.7	60.6±9.0	60.2±8.8	59.3±9.3	0.29	57.4±10.3	60.1±7.9	61.0±9.0	62.1±8.0	<.0001	59.5±9.3	60.2±9.1	60.0±8.8	61.7±8.6	0.21
Sex[b]															
Female	67(50.8)	66(50.0)	39(29.3)	39(29.6)		38(28.8)	39(29.5)	58(43.6)	76(57.6)		60(45.5)	49(37.1)	51(38.3)	51(38.6)	
Male	65(49.2)	66(50.0)	94(70.7)	93(70.5)	<.0001	94(71.2)	93(70.5)	75(56.4)	56(42.4)	<.0001	72(54.5)	83(62.9)	82(61.7)	81(61.4)	0.50
Stage at diagnosis															
I/II	87(65.9)	81(61.4)	70(52.6)	71(53.8)		72(54.5)	71(53.8)	83(62.4)	83(62.9)		79(59.8)	77(58.3)	77(57.9)	76(57.6)	
III/IV	45(34.1)	51(38.6)	63(47.4)	61(46.2)	0.09	60(45.5)	61(46.2)	50(37.6)	49(37.1)	0.27	53(40.2)	55(41.7)	56(42.1)	56(42.4)	0.98
BMI (kg/m^2)															
<25.0	38(30.6)	47(36.1)	35(26.5)	27(21.1)		42(33.6)	32(24.8)	34(26.4)	38(29.7)		33(25.6)	40(31.0)	36(28.1)	38(29.7)	
25.0-29.9	57(46.0)	52(40.0)	53(40.2)	53(41.4)		45(35.2)	57(44.2)	55(42.6)	58(45.3)		55(42.6)	47(36.4)	58(45.3)	55(43.0)	
≥30	29(23.4)	31(23.9)	44(33.3)	48(37.5)	0.03	40(31.2)	40(31.0)	40(31.0)	32(25.0)	0.78	41(31.8)	42(32.6)	34(26.6)	35(27.3)	0.63
Physical activity (MET h/wk)															
<24.9	73(55.3)	71(53.4)	56(42.1)	65(49.2)		68(51.5)	60(45.4)	69(51.9)	68(51.5)		68(51.5)	71(53.8)	69(51.9)	57(43.2)	
≥24.9	59(44.7)	61(46.6)	77(57.9)	67(50.8)	0.13	64(48.5)	72(54.6)	64(48.1)	64(48.5)	0.67	64(48.5)	61(46.2)	64(48.1)	75(56.8)	0.32
Marital status															
Single	31(23.5)	29(22.0)	18(13.5)	37(28.0)		26(19.7)	27(20.4)	27(20.3)	35(26.5)		26(19.7)	30(22.7)	30(22.6)	29(22.0)	
Married or living as married	101(76.5)	103(78.0)	115(86.5)	95(72.0)	0.04	106(80.3)	105(79.6)	106(79.7)	97(73.5)	0.50	106(80.3)	102(77.3)	103(77.4)	103(78.0)	0.93
Smoking status															
Ever	77(58.3)	94(71.2)	113(85.0)	104(78.8)		108(81.8)	97(73.5)	100(75.2)	83(62.9)		101(76.5)	95(72.0)	95(71.4)	97(73.5)	
Never	55(41.7)	38(28.8)	20(15.0)	28(21.2)	<.0001	24(18.2)	35(26.5)	33(24.8)	49(37.1)	0.006	31(23.5)	37(28.0)	38(28.6)	35(26.5)	0.79
Tumor location															
Colon	91(69.5)	90(68.2)	85(63.9)	79(59.9)		75(56.8)	91(69.5)	87(65.4)	92(69.7)		82(62.1)	85(64.9)	87(65.4)	91(68.9)	
Rectum	40(30.5)	42(31.8)	48(36.1)	53(40.1)	0.34	57(43.2)	40(30.5)	46(34.6)	40(30.3)	0.10	50(37.9)	46(35.1)	46(34.6)	41(31.1)	0.71
Reported chemoradiotherapy															
Yes	36(27.3)	31(23.5)	20(15.0)	21(15.9)		24(18.2)	23(17.4)	24(18.1)	37(28.0)		30(22.7)	28(21.2)	25(18.8)	25(18.9)	
No	96(72.7)	101(76.5)	113(85.0)	111(84.1)	0.04	108(81.8)	109(82.6)	109(81.9)	95(72.0)	0.10	102(77.3)	104(78.8)	108(81.2)	107(81.1)	0.83
MSI status															
MSS /MSI-L	108(86.4)	110(86.6)	113(91.9)	106(86.9)		107(85.6)	104(86.7)	113(91.1)	113(88.3)		107(84.9)	106(87.6)	110(88.0)	114(91.2)	
MSI-H	17(13.6)	17(13.4)	10(8.1)	16(13.1)	0.49	18(14.4)	16(13.3)	11(8.9)	15(11.7)	0.57	19(15.1)	15(12.4)	15(12.0)	11(8.8)	0.50
BRAF mutation status															
Wild type	104(85.2)	107(89.9)	109(90.8)	106(93.0)		108(91.5)	103(87.3)	112(95.7)	103(84.4)		103(88.8)	110(91.7)	106(89.1)	107(89.2)	
V600E mutant	18(14.8)	12(10.1)	11(9.2)	8(7.0)	0.25	10(8.5)	15(12.7)	5(4.3)	19(15.6)	0.02	13(11.2)	10(8.3)	13(10.9)	13(10.8)	0.88

[a] Abbreviations are as follows: BMI, body mass index; CRC, colorectal cancer; MSI, microsatellite instability; MSS/MSI-L, microsatellite stable/microsatellite instability-low; MSI-H, microsatellite instability-high; Q1-Q4, quartile of dietary pattern scores

[b] Continuous variables presented as mean±SD (standard deviation); categorical variables presented as number

[c] *P* values are for the significance of the ANOVA test for continuous variables and of the Chi-Square test for categorical variables

3.2.3 Dietary Patterns and Cancer Recurrence or Death

The highest quartile of processed meat pattern was significantly associated with poorer DFS after the adjustment for other predictors of CRC recurrence and death (HR: 1.82, 95%*CI*: 1.07-3.09), although no overall trend was observed in the HRs across the whole distribution of factor scores (*P* for trend=0.09) (**Table 3.4.3**). Nevertheless, neither the prudent vegetable pattern nor the high sugar pattern was observed to be significantly associated with patient outcomes (i.e., DFS and OS).

When stratified by tumor site, however, the association between processed meat pattern and DFS remained statistically significant only for patients who had tumors located in the colon (the highest versus the lowest quartile, HR: 2.29, 95%*CI*: 1.19-4.40) but not the rectum (HR: 0.97, 95%*CI*: 0.38-2.45). Similarly, when OS was the outcome, the positive association between increasing consumption of the processed meat pattern and mortality was restricted to patients whose tumors were diagnosed in the colon (the forth versus first quartiles: HR: 2.13, 95%*CI*: 1.03-4.43).

Table 3.4.3 Hazard Ratios Associated with Disease-Free and Overall Survival in Colorectal Cancer Patients for Quartiles of Dietary Patterns [a]

	Disease-Free Survival				Overall Survival			
	No. of Events[b] /No. at Risk	Overall CRC HR (95%*CI*) [c]	Colon cancer HR (95%*CI*) [c]	Rectal cancer HR (95%*CI*) [c]	No. of Events[b] /No. at Risk	Overall CRC HR (95%*CI*) [c]	Colon cancer HR (95%*CI*) [c]	Rectal cancer HR (95%*CI*) [c]
Processed meat pattern								
Q1	38/132	1.00	1.00	1.00	33/132	1.00	1.00	1.00
Q2	45/132	1.51(0.95-2.41)	1.69(0.97-2.96)	0.91(0.39-2.14)	40/132	1.47(0.89-2.44)	2.18*(1.16-4.09)	0.75(0.28-2.03)
Q3	58/132	1.56(0.97-2.49)	1.37(0.76-2.48)	1.72(0.85-3.95)	49/133	1.32(0.78-2.22)	1.44(0.74-2.79)	1.54(0.57-4.13)
Q4	57/132	1.82*(1.07-3.09)	2.29*(1.19-4.40)	0.97(0.38-2.45)	46/132	1.53(0.85-2.74)	2.13*(1.03-4.43)	1.17(0.41-3.36)
P for trend [d]		0.09	0.12	0.91		0.25	0.40	0.59
Prudent vegetable pattern								
Q1	46/132	1.00	1.00	1.00	41/132	1.00	1.00	1.00
Q2	54/132	1.21(0.79-1.85)	1.35(0.78-2.34)	0.97(0.47-2.01)	45/132	1.09(0.69-1.73)	1.18(0.65-2.14)	0.90(0.41-1.98)
Q3	50/133	1.18(0.75-1.86)	1.16(0.63-2.13)	1.30(0.65-2.60)	40/133	0.82(0.49-1.36)	1.04(0.55-1.97)	0.59(0.25-1.42)
Q4	48/131	1.12(0.69-1.84)	1.02(0.52-1.99)	1.28(0.58-2.83)	42/132	1.03(0.61-1.75)	0.96(0.47-1.96)	1.00(0.42-2.40)
P for trend [d]		0.62	0.83	0.19		0.90	0.60	0.92
High sugar pattern								
Q1	42/131	1.00	1.00	1.00	30.132	1.00	1.00	1.00
Q2	54/132	1.07(0.70-1.63)	0.96(0.54-1.68)	1.30(0.64-2.65)	48/132	1.25(0.77-2.04)	1.21(0.62-2.36)	2.12(0.87-5.14)
Q3	54/133	1.09(0.69-1.73)	0.94(0.51-1.73)	1.44(0.67-3.07)	50/133	1.64(0.98-2.75)	1.35(0.66-2.78)	2.49*(1.02-6.10)
Q4	48/132	1.02(0.62-1.69)	0.99(0.52-1.89)	1.49(0.61-3.63)	40/132	1.27(0.72-2.25)	1.16(0.54-2.47)	1.68(0.55-5.08)
P for trend [d]		0.89	0.90	0.11		0.52	0.56	0.64

[a] Abbreviations are as follows: CRC, colorectal cancer; HR, hazard rate ratios; *CI*, confidence interval; Q1-Q4, quartile of dietary pattern scores.

[b] Events are defined as death, recurrence, or metastasis (whichever occurred earliest) for disease-free survival and deaths for overall survival.

[c] Cox proportional hazard model adjusted for total energy intake, sex, age at diagnosis, stage at diagnosis, marital status, family history, reported screening procedure, reported chemoradiotherapy and MSI status, where appropriate.

[d] Two-sided *P* value for test of linear trend was calculated by modeling median values for each quartile of dietary pattern scores as an ordinal variable.

* Significantly different from reference category, $P<0.05$.

In the stratified analyses for dietary patterns, there was evidence for effect modification by sex (P =0.04) for the association of processed meat pattern with DFS (HR: 3.85 for women and 1.22 for men) (**Table 3.4.4**). However, no evidence was observed to suggest that the effects of other dietary patterns on cancer recurrence or death were modified by physical activity, *BRAF V600E* mutation status and MSI (data not shown).

In the sensitivity analysis, when advanced-stage patients who died before admittance were excluded, the association between processed meat pattern and survival among CRC patients remained significant.

Table 3.4.4 Disease-Free Colorectal Cancer Survival in Relation to Quartiles of Dietary Patterns by Selected Lifestyle and Tumor Characteristics [a]

	No. of Events / No. at Risk	Quartiles HR (95%*CI*)[c]				*P* for Trend[d]	*P* for Interaction[e]
		Q1	Q2	Q3	Q4		
Processed meat pattern							
Sex							
Female	65/210	1.00	2.20(0.99-4.91)	2.38(0.97-5.85)	3.85*(1.49-9.99)	0.03	
Male	133/318	1.00	1.20(0.66-2.18)	1.23(0.69-2.17)	1.22(0.64-2.32)	0.27	0.04
Physical activity							
<24.9 MET h/wk	97/263	1.00	1.96*(1.05-3.67)	2.13*(1.11-4.11)	2.03(0.96-4.30)	0.42	
≥24.9 MET h/wk	101/264	1.00	1.22(0.59-2.55)	1.27(0.62-2.62)	1.64(0.74-3.62)	0.01	0.64
BRAF mutation status							
Wild type	163/425	1.00	1.28(0.77-2.12)	1.41(0.80-2.34)	1.80*(1.01-3.21)	0.009	
V600E mutant	17/49	1.00	1.82(0.40-8.34)	0.54(0.10-2.83)	0.79(0.09-7.01)	0.50	0.80
Prudent vegetables pattern							
Sex							
Female	65/210	1.00	1.57(0.59-4.20)	1.55(0.63-3.85)	1.22(0.46-3.24)	0.71	
Male	133/318	1.00	1.25(0.76-2.04)	1.08(0.62-1.88)	1.14(0.62-2.09)	0.67	0.65
Physical activity							
<24.9 MET h/wk	97/263	1.00	1.48(0.80-2.76)	1.52(0.81-2.87)	1.22(0.56-2.69)	0.66	
≥24.9 MET h/wk	101/264	1.00	1.02(0.55-1.89)	1.02(0.53-1.96)	1.05(0.55-2.04)	0.03	0.83
BRAF mutation status							
Wild type	163/425	1.00	1.32(0.83-2.10)	1.29(0.80-2.08)	1.19(0.70-2.02)	0.58	
V600E mutant	17/49	1.00	2.50(0.38-16.59)	0.88(0.06-12.99)	1.24(0.12-13.20)	0.73	0.80
High sugar pattern							
Sex							
Female	65/210	1.00	1.41(0.63-3.16)	0.88(0.36-2.15)	0.82(0.30-2.27)	0.42	
Male	133/318	1.00	1.14(0.67-1.97)	1.34(0.75-2.39)	1.39(0.73-2.66)	0.06	0.72
Physical activity							
<24.9 MET h/wk	97/263	1.00	1.01(0.55-1.86)	1.10(0.56-2.16)	1.19(0.56-2.54)	0.06	
≥24.9 MET h/wk	101/264	1.00	1.36(0.70-2.65)	1.21(0.60-2.45)	1.04(0.49-2.22)	0.86	0.26
BRAF mutation status							
Wild type	163/425	1.00	0.99(0.61-1.59)	1.20(0.71-2.01)	1.03(0.59-1.82)	0.70	
V600E mutant	17/49	1.00	0.53(0.07-4.25)	0.27(0.04-1.66)	0.32(0.04-2.64)	0.09	0.33

[a] Abbreviations are as follows: *CI*, confidence interval; METs/week, metabolic equivalent hours per week; Q1-Q4, quartile of dietary pattern scores

[b] Events are defined as death, recurrence, or metastasis (whichever occurred earliest) for disease-free survival and deaths for overall survival.

[c] Cox proportional hazard model adjusted for total energy intake, sex, age at diagnosis, stage at diagnosis, BMI, marital status, family history, reported screening procedure, reported chemoradiotherapy, and MSI status, where appropriate.

[d] Two-sided *P* value for test of linear trend was calculated by modeling median values for each quartile of dietary pattern scores as an ordinal variable.

[e] *P* for interaction is the significance of interaction term between smoking and respective stratification variable, calculated by the Wald test.

* Significantly different from reference category, $P<0.05$.

3.3 Discussion

Three dietary patterns, termed “processed meat pattern”, “prudent vegetable pattern” and “high sugar pattern”, were generated in this cohort study. We found that high conformity with the processed meat pattern, characterized by high intakes of processed meat, red meat, fish, and processed fish, is associated with decreased DFS of CRC, specifically of colon cancer. The differential associations by subsite indicate disease heterogeneity. On the contrary, increasing consumption of the prudent vegetable pattern and the high sugar pattern displayed no clear relationships with mortality or recurrence.

The processed meat pattern in the present study shares most characteristics of the Western diet referred to in previous studies on CRC risk, which indicates a positive association between the Western dietary pattern and CRC risk [21, 23]. However, there has been minimal research examining the association between dietary factors (e.g., nutrient, carbohydrate, protein and lipid intake) and survival of CRC patients [13, 14]; moreover, our literature review identified only one study that investigated the relationship between dietary patterns and survival among CRC patients. Consistent with our results, that prospective cohort study of 1009 stage III colon cancer patients [23] reported a deleterious disease-free colon cancer prognosis for patients reporting high levels of the Western dietary pattern intake. A prudent dietary pattern was not related to cancer recurrence or mortality in that study.

The mechanisms explaining the impact of red and processed meat on CRC mortality are still unclear; however, some biologic mechanisms that link diet factors to CRC risk may continue after diagnosis and subsequently impact cancer progression and survival [200]. For example, strong carcinogens such as *N*-nitroso compounds

(NOCs) and probable carcinogenic mutagens like heterocyclic amines (HCA) and polycylic aromatic hydrocarbons (PAH), which have been suggested as significant contributors for CRC development [120, 121], are found in smoked, fried or high-temperature cooked meat. Sandhu *et al.* [201] reported a Western dietary pattern is related to high levels of serum insulin and insulin-like growth factors (IGF), and these hormones are found to be associated with tumor growth and the inhibition of apoptosis. In addition, a growing body of evidence suggests that disruption of the normal gut microflora is associated with human disease, including the pathogenesis of the intestinal tract (e.g. inflammatory bowel disease) and other diseases such as obesity, cardiovascular disease, and autoimmune conditions [202, 203]. Alterations in intestinal microbiota are also strongly associated with colonic polyp formation and with the risk of developing CRC [94]. Given the major role of diet on the intestinal microbiome [204], our findings between dietary patterns and CRC survival may also be explained by the impact of dietary patterns on gut microflora and health outcomes. We did not observe any significant association between a prudent vegetable pattern and CRC survival. Given that more than 90% of adults in NL do not consume 5 servings of fruit and vegetables per day, let alone the 7-10 servings that are recommended [205], it is possible that the total intake of fruits and vegetables was not sufficient to exert a protective effect in this cohort.

The influence of processed meat pattern on survival was evident among women rather than men in our study. Previous studies revealed that the higher colon pH and longer intestine transit time in women compared to men can influence the production of secondary bile acid or NOCs [206], resulting in gender differences in the CRC development. This is the first study that considered effect modifications between dietary patterns and tumor molecular phenotype (i.e. *BRAF* mutation) on CRC

survival. *BRAF V600E* mutation is found to be significantly associated with poor CRC survival in some studies [207]; however, whether it can modify the impacts of dietary factors on CRC survival is not known. Although stratified analyses in our study demonstrated a processed meat diet to significantly decrease survival time only in patients with *BRAF* wild type tumor, no evident interactions were detected. Further research is clearly warranted to verify these findings and to determine the biologic pathways that rationalize the underlying interactions between diet and tumor molecular features.

A reasonably large sample size with detailed information of patients is a merit of our study. These data not only include demographic and personal lifestyle information, but also some molecular characteristics obtained from genetic testing. The ample information enables us to perform stratification analysis to control and assess effect modifiers and confounders. Several limitations of this study should be recognized. Firstly, the results may be skewed by recall bias since the participants recalled their food consumption from one year prior to CRC diagnosis; however, this non-differential misclassification is only expected to bias the results towards the null. Secondly, dietary patterns in this study only reflect food consumption before diagnosis; it is unknown whether participants modified their diet post diagnosis. Since previous research has shown minimal change in diet between pre- and post- diagnosis among cancer patients [14], the current study did not examine dietary changes before and after diagnosis. Additionally, although we have controlled for many known prognostic factors, possible residual confounding might still exist. Besides, immortal person-time bias might impact our results; however, this is minimized by using proxies to enroll deceased patients.

In summary, we found that high conformity to the processed meat pattern is

significantly associated with an increased risk of all-cause mortality and recurrence of CRC. Though our study did not find a difference in effect by tumor molecular phenotype, larger molecular studies should be conducted to examine if such differences exist. Ultimately, confirmation of these findings and the underlying mechanisms await further studies. Our observation not only adds to the mounting evidence that encourages people to engage in a healthy diet, but also provides some guidance to efficacious dietary interventions [22]; that is, people may lower their risk of CRC mortality by the avoidance of a processed meat pattern diet.

Chapter 4 Project 2. Influence of Prediagnostic Cigarette Smoking on Colorectal Cancer Survival: Overall and by Tumor Molecular Phenotype

4.1 Subjects and Methods

4.1.1 Study Participants

A detailed description of the study cohort has been published elsewhere [186]. In brief, participants were incident CRC patients identified through the population-based Newfoundland and Labrador Colorectal Cancer Registry (NFCCR). Eligibility criteria included patients who were:

1) Newly diagnosed with pathologically confirmed, invasive CRC (ICD-9 codes: 153.0-153.9, 154.0-154.3, and 154.8 or ICD-10 codes: 18.0-18.9, 19.9, and 20.9);
2) Diagnosed between January 1999 and December 2003;
3) Diagnosed at the ages of 20 to 75 years;
4) Residents of NL who had lived in NL for at least two years at the time of diagnosis.

Seven hundred and fifty consenting patients (64%) completed and returned detailed epidemiologic questionnaires: a Personal History Questionnaire (PHQ, see appendix 1), a Food Frequency Questionnaire (FFQ, see appendix 2), and a Family History Questionnaire (FHQ). Patients were also asked to donate a blood sample and for permission to access their archived tumor tissue and medical records. Exclusions from this analysis were made if patients had unknown clinical outcome or smoking status (n=41), or provided insufficient information on other critical prognostic factors

(n=3). Thus, the final cohort consisted of 706 eligible participants. Ethics approval for this study was received from the Human Investigation Committee of Memorial University of Newfoundland.

4.1.2 Measurement of Exposure and Relevant Variables

4.1.2.1 Assessment of Smoking Exposure and Baseline Information Collection

The epidemiologic questionnaire included items regarding age, sex, marital status, education attainment, medical history, bowel screening history, medication use, physical activity, reproductive factors (female only), and alcohol and tobacco use. Participants were asked whether they have smoked at least one cigarette a day for 3 months or longer in their life. Participants who responded 'yes' were then asked about the age at which they started smoking, their usual number of cigarettes smoked per day, the duration (years/months) during which they smoked and, where applicable, the dates when they quit smoking. For this analysis, cigarette smoking was represented by categories of smoking status (never, current, or former), years of smoking (none, <20, 20-29, and ≥30), cigarettes daily (none, <20 , 20-29, and ≥30), years of abstention (non-smoker, <10 , 10-29, and ≥30), and lifetime cigarette pack-years (none, 20, 20-39, and ≥40; calculated as the average number of cigarettes smoked per day divided by 20 and multiplied by the number of years smoked) [133]. BMI was calculated as weight in kilograms divided by the square of height in meters. Clinical and pathologic data (e.g., tumor stage at diagnosis) were abstracted from pathology reports and medical records.

4.1.2.2 Study Outcomes

The cohort was followed up for mortality and recurrence from diagnosis to April, 2010. During this period, the FHQ was distributed to participants for the second time to collect information on additional cancer diagnosis and recurrence in their family. If a patient was deceased, a close proxy was asked to participate [186]. Information on vital status (i.e., death, recurrence, and metastasis) was gathered from follow-up questionnaires, local newspapers, death certificates, autopsy, pathology, radiology, surgical reports, as well as physician's notes. Additional data were collected from the Dr. H. Bliss Murphy Cancer Care Foundation [192]. For the purposes of this analysis, OS was the primary outcome, defined as time from CRC diagnosis to death from all causes. The secondary outcome, DFS, was measured from the date of cancer diagnosis to the date of death, recurrence, or metastasis (whichever came first). Patients who were still alive or who did not have a recurrence or metastasis by the end of the follow-up period were censored at the time of last contact.

4.1.2.3 Molecular Assessment

Molecular analyses for MSI and *BRAF V600E* mutation were performed using standard protocols as described previously [194, 195, 208]. Briefly, for MSI analyses, both tumor DNA and normal DNA were amplified by polymerase chain reaction (PCR) with a panel of 10 microsatellite markers: BAT25, BAT26, BAT34C4, BAT40, ACTC, D5S346, D17S250, D18S55, D10S197, and MYCL [194, 195]. The appearance of a discordant number of bands between tumor and normal DNA was interpreted as instability [194]. Tumors were classified as MSI-high if 30% or more of the repeats were unstable and MS-stable/MSI-Low if less than 30% of the repeats

demonstrated instability [37]. Exon 15 of the *BRAF* gene, spanning the mutational hotspot c.1799T>A. (p.Val600Glu), was amplified by PCR using *BRAF V600E* allele-specific primers, followed by direct automatic sequencing to verify the mutations [208]. Detailed descriptions of each assay, including the primer sequences and PCR conditions, are provided in earlier studies from this cohort [186, 194, 195, 208].

4.1.3 Statistical Analysis

4.1.3.1 Descriptive Statistics

Group comparisons were performed with Dunnett's tests for continuous variables and Pearson's Chi-square tests of independence for categorical variables [147]. The Kaplan-Meier technique was applied to graphically delineate overall and stratified survival distributions. Statistical significance was conducted at two-sided $P<0.05$. All calculations were performed with SAS software (version 9.2).

4.1.3.2 Multivariate Cox Proportional Hazard Analysis

Proportional hazards models were used to estimate the impact of smoking on mortality among CRC patients, while adjusting for co-variates. Hazard ratios (HR) and corresponding 95% confidence intervals (*CI*) were calculated for approximate quartiles of exposure, using never smokers as the reference group. Subjects with missing data on any smoking exposure variables were excluded but for specific smoking-related variable analysis only. In the selection approach of the multivariate models, we assessed an extensive list of potential confounders, including

demographic variables, diet, lifestyle factors, treatment, clinicopathologic, and a series of molecular predictors. Factors were considered for inclusion in the multivariable Cox model if the log-rank test had a *P*-value of 0.2 or less in the univariate setting [209]. Only terms that entered the model at $P<0.1$, altered the effect estimates by 10% or more, or improved the fit of the models were retained for the final models [210]. The final models in this analysis included sex (male, female), age at diagnosis (18-49, 50-59, 60-69, and 70+ years), BMI (<25.0, 25.0-29.9, and $\geq 30 kg/m^2$), stage at diagnosis (I, II, III, IV), marital status (single, married or living as married), alcohol drinking (yes, no), fruit intake (0-6, 6-7, 7-14, and >14 servings/week), family history of CRC (yes, no), reported chemoradiotherapy (yes, no), and MSI status (MSI-high, MSS/MSI-low). The assumption of proportional hazard rates was verified by checking the parallelism of the Kaplan-Meier curves and by testing the statistical significance of time-dependent covariates when included in the model [199].

4.1.3.3 Other Analyses

Evidence of linear trends was tested by modeling ordinal variables of exposure as a continuous variable in the regression model [133, 211]. Potential interactions were evaluated by including interaction terms between smoking and respective stratification variable in the model with the Wald test.

4.2 Results

By the end of the follow-up period, during a maximum of 10.9 years of observation, there were 338 deaths from all causes. At baseline, 506 patients were

ever smokers and 200 patients were never smokers.

4.2.1 Baseline Characteristics by Smoking Status

Current smokers were slightly younger, leaner, drank more alcohol, more likely to be men, and showed a greater proportion of MSI-H tumors (21.5%) compared to never smokers (MSI-H tumors: 10.9%, *P*=0.03) (**Table 4.2.1**). Similarly, most former smokers were male, married or living as married, and reported greater alcohol consumption and less chemoradiotherapy use relative to never smokers.

Table 4.2.1 Selected Demographic and Clinicopathologic Characteristics of Study Population, by Smoking Status at Baseline [a]

	Never Smoker	Former Smoker		Current Smoker	
	No. (%)	No. (%)	*P*-value	No. (%)	*P*-value
Age at diagnosis (years)	60.7±9.6	61.1±8.5	0.84	56.2±9.4	0.0004
Sex					
Women	120 (60.0)	110(29.9)		27 (36.5)	
Men	80 (40.0)	258(70.1)	<0.0001	47 (63.5)	0.001
BMI (kg/m^2)					
<25.0	62 (32.6)	89 (24.8)		27 (39.1)	
25.0-29.9	83 (43.7)	150 (41.8)		26 (37.7)	
≥30	45 (23.7)	120 (33.4)	0.03	16 (23.2)	0.003
Marital status					
Single	57 (28.6)	63 (17.2)		27 (36.5)	
Married or living as married	142 (71.4)	303 (82.8)	0.002	47 (63.5)	0.21
Tumor site					
Colon	137 (68.8)	244 (66.3)		43 (58.1)	
Rectum	62 (31.2)	124 (33.7)	0.54	31 (41.9)	0.10
Tumor stage at diagnosis					
I/II	100 (50.0)	186 (50.5)		46 (62.2)	
III/IV	100 (50.0)	182 (49.5)	0.90	28 (37.8)	0.07
Alcohol drinking					
No	130 (65.0)	96 (26.1)		14 (18.9)	
Yes	70 (35.0)	272 (73.9)	<0.0001	60 (81.1)	<0.001
Family history of CRC					
No	178 (89.0)	327 (88.9)		66(89.2)	
Yes	22 (11.0)	41 (11.1)	0.96	8 (10.8)	0.96
Reported screening procedure					
No	176 (88.0)	316 (85.9)		69 (93.2)	
Yes	24 (12.0)	52 (14.1)	0.48	5 (6.8)	0.21
Reported chemoradiotherapy					
No	142 (71.0)	306 (83.2)		60 (81.1)	
Yes	58 (29.0)	62 (16.8)	0.001	14 (18.9)	0.09
MSI status					
MSS /MSI-L	171 (89.1)	312 (90.2)		51 (78.5)	
MSI-H	21 (10.9)	34 (9.8)	0.68	14 (21.5)	0.03
BRAF mutation status					
Wide type	164 (88.2)	289 (88.7)		58 (90.6)	
V600E mutant	22 (11.8)	37 (11.3)	0.87	6 (9.4)	0.59

[a] Abbreviations are as follows: BMI, body mass index; CRC, colorectal cancer; MSI, microsatellite instability; MSS/MSI-L, microsatellite stable/ microsatellite instability-low; MSI-H, microsatellite instability-high.
[b] *P* values are for the significance of the Dunnett's test for continuous variables and of the Chi-square test for categorical variables.
[c] Continuous variables presented as mean±SD (standard deviation).

4.2.2 Prediagnostic Smoking and Mortality

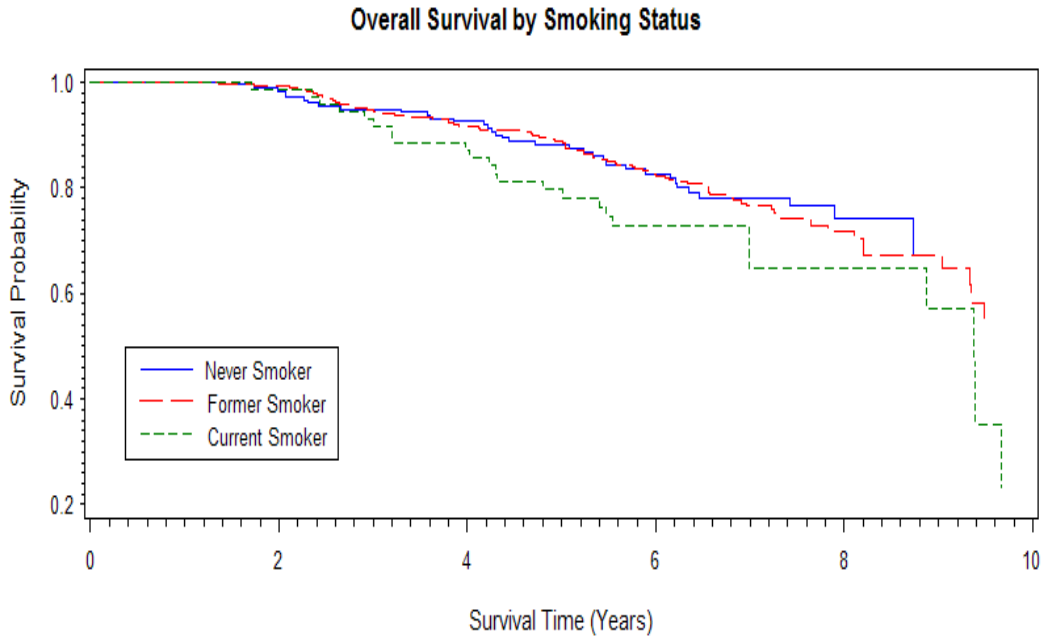
Current smoking (HR: 1.78, 95%*CI*: 1.04-3.06) was significantly associated with higher risk of all-cause mortality in multivariable models (**Table 4.2.2, Figure 4.2.1**). The higher risks of mortality from smoking persisted in more detailed definitions of the exposure, including pack-years (≥40 pack-years, HR: 1.72, 95%*CI*: 1.03-2.85), number of cigarettes smoked daily (≥30 cigarettes per day, HR: 1.41, 95%*CI*: 0.79-2.54), and years of smoking (≥30 years, HR: 1.28, 95%*CI*: 0.83-1.97), although, for the latter two variables, the risk estimates of being in the highest quartile of exposure did not quite attain statistical significance at the 0.05 level. Moreover, there was a stepwise gradient of decreasing risk of mortality with increasing years of abstention for former smokers (*P* for trend=0.03). Similarly, when DFS was the outcome, the HRs were elevated in the groups who had a smoking history of ≥30 years (HR: 1.53, 95%*CI*: 1.01-2.34), individuals who smoked ≥30 cigarettes per day (HR: 1.80, 95%*CI*: 1.22-2.67), and those with ≥40 pack-years of smoking (HR: 1.99, 95%*CI*: 1.25-3.19). When the data were stratified by tumor site, marked differences in multivariate HRs were noted (**Table 4.2.2**); that is, smoking was associated with decreased survival for colon cancer, but not for rectal cancer. Specifically, rates of all-cause death among colon cancer patients elevated in current smoking (HR: 2.34, 95%*CI*: 1.01-5.45) and greater pack-years of smoking (≥40 pack-years, HR: 2.08, 95%*CI*: 1.11-3.87), while incremental effects on DFS were found across categories of cigarette years (≥30 years, HR: 1.73, 95%*CI*: 1.03-2.93, *P* for trend=0.02), number of cigarettes smoked daily (≥30 cigarettes per day, HR: 2.12, 95%*CI*: 1.26-3.57, *P* for trend=0.15), and pack-years of smoking (≥40 pack-years, HR: 2.45, 95%*CI*: 1.34-4.46, *P* for trend=0.03).

Table 4.2.2 Hazard Ratios Associated with Overall and Disease-Free Survival in Colorectal Cancer Patients for Cigarette Smoking Exposures [a]

	Overall Survival				Disease-Free Survival			
	No. of Events[b] /No. at Risk [c]	Overall CRC HR (95%*CI*) [d]	Colon Cancer HR (95%*CI*) [d]	Rectal Cancer HR (95%*CI*) [d]	No. of Events[b] /No. at Risk[c]	Overall CRC HR (95%*CI*) [d]	Colon Cancer HR (95%*CI*) [d]	Rectal Cancer HR (95%*CI*) [c]
Cigarette status								
Non-smoker	90/200	1.00	1.00	1.00	97/200	1.00	1.00	1.00
Ever-smoker	248/506	1.25(0.84-1.88)	1.52(0.91-2.54)	0.90(0.45-1.82)	272/505	1.30(0.90-1.88)	1.57(0.97-2.50)	0.87(0.46-1.63)
Former	151/368	1.06(0.71-1.59)	1.46(0.87-2.45)	0.80(0.38-1.67)	172/367	1.21(0.83-1.77)	1.50(0.93-2.43)	0.82(0.43-1.57)
Current	33/74	1.78*(1.04-3.06)	2.34*(1.01-5.45)	1.23(0.52-2.93)	36/74	1.69(0.99-2.84)	2.03(0.95-4.33)	1.11(0.48-2.55)
Cigarette years								
<20	52/117	1.11(0.66-1.87)	1.11(0.56-2.18)	1.17(0.50-2.72)	58/117	1.26(0.77-2.03)	1.29(0.67-2.45)	1.02(0.49-2.16)
20-29	53/107	1.13(0.67-1.89)	1.50(0.79-2.83)	0.73(0.29-1.81)	57/107	1.22(0.75-2.00)	1.61(0.87-2.99)	0.63(0.28-1.43)
≥30	110/226	1.28(0.83-1.97)	1.45(0.85-2.48)	0.97(0.45-2.10)	122/225	1.53*(1.01-2.34)	1.73*(1.03-2.93)	1.01(0.52-1.99)
P trend [e]		0.05	0.09	0.62		0.08	0.02	0.76
Cigarettes daily								
<20	75/186	0.95(0.60-1.52)	1.16(0.66-2.05)	0.70(0.30-1.62)	83/185	0.88(0.64-1.22)	1.03(0.70-1.52)	0.65(0.31-1.38)
20-29	99/193	1.38(0.88-2.17)	1.47(0.82-2.63)	1.13(0.53-2.41)	105/193	1.05(0.77-1.44)	1.17(0.79-1.72)	0.85(0.43-1.70)
≥30	45/77	1.41(0.79-2.54)	1.93(0.92-4.06)	0.83(0.31-2.19)	53/77	1.80*(1.22-2.67)	2.12*(1.26-3.57)	1.66(0.73-3.77)
P trend [e]		0.12	0.02	0.95		0.21	0.15	0.36
Pack-years of smoking								
<20	117/257	0.96(0.61-1.50)	1.06(0.60-1.87)	0.86(0.40-1.87)	126/257	1.03(0.67-1.57)	1.20(0.70-2.08)	0.72(0.36-1.43)
20-39	67/135	1.30(0.81-2.07)	1.43(0.79-2.57)	1.02(0.45-2.31)	73/134	1.37(0.88-2.13)	1.67(0.95-2.92)	0.87(0.41-1.83)
≥40	64/114	1.72*(1.03-2.85)	2.08*(1.11-3.87)	1.07(0.43-2.66)	73/114	1.99*(1.25-3.19)	2.45*(1.34-4.46)	1.26(0.57-2.75)
P trend [e]		0.08	0.06	0.47		0.07	0.03	0.48
Years of abstention [f]								
≥30	28/69	1.10(0.63-1.95)	1.13(0.56-2.29)	1.08(0.41-2.87)	31/69	1.21(0.71-2.05)	1.21(0.62-2.37)	1.27(0.53-3.03)
10-29	66/162	1.26(0.79-2.00)	1.49(0.86-2.58)	0.67(0.27-1.64)	75/162	1.36(0.88-2.10)	1.76*(1.02-3.04)	0.75(0.36-1.55)
<10	32/77	1.28(0.74-2.20)	1.49(0.76-2.93)	0.87(0.33-2.34)	37/76	1.42(0.85-2.36)	1.77(0.92-3.41)	0.87(0.40-1.91)
P trend [e]		0.03	0.06	0.42		0.03	0.05	0.47

[a] Abbreviations are as follows: HR, hazard rate ratios; *CI*, confidence interval.
[b] Events are defined as deaths for overall survival and death, recurrence, or metastasis (whichever occurred earliest) for disease-free survival.
[c] Subjects with missing data on any smoking exposure variables are excluded but for specific smoking-related variable analysis only.
[d] Cox proportional hazard model adjusted for sex, age at diagnosis, stage at diagnosis, BMI, marital status, alcohol consumption, intake of fruits, family history, reported screening procedure, reported chemoradiotherapy, and MSI status, where appropriate.
[e] Linear trend tested by modeling the ordinal variables of exposure as a continuous variable.
[f] Excludes current smokers.
* Significantly different from reference category, $P<0.05$.

A.



B.

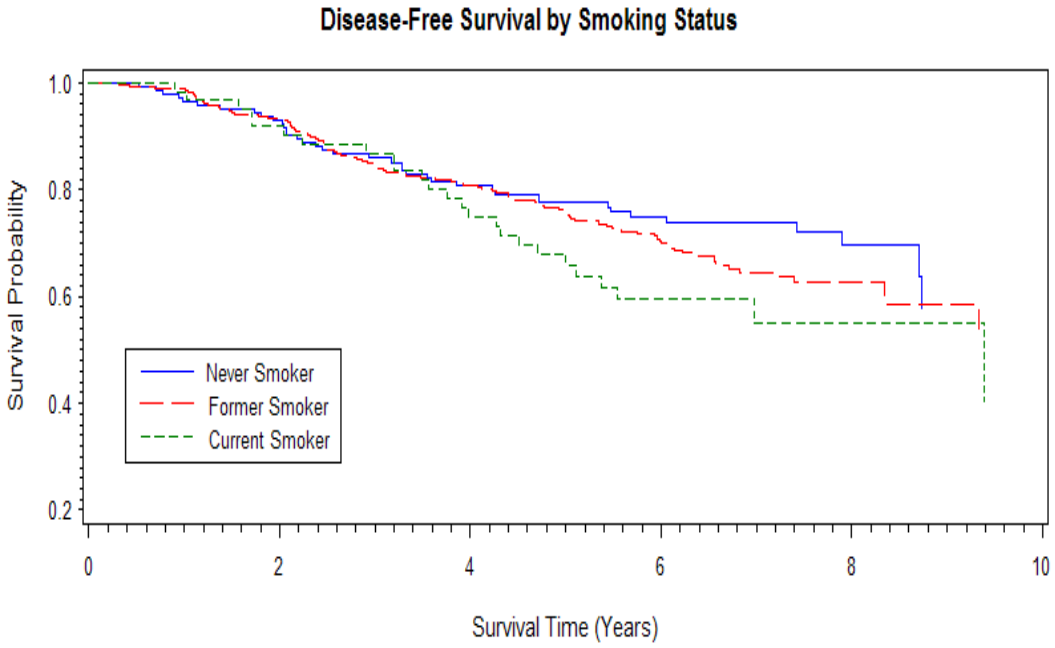


Figure 4.2.1 Survival Curves for A. Overall Survival and B. Disease-Free Survival by Smoking Status. Adjusted for sex, age at diagnosis, stage at diagnosis, BMI, marital status, alcohol consumption, family history, reported chemoradiotherapy, and MSI status .

4.2.3 Interactions between Smoking and Demographic or Tumor Characteristics

The multivariable models were repeated for smoking status between strata defined by demographic and tumor characteristics (**Table 4.2.3**). The *P*-values for interaction were statistically significant between strata of sex for DFS (*P*=0.04) and age at diagnosis for OS (*P*=0.03). More specifically, the risk of mortality associated with ever smoking seemed limited to males (DFS: HR: 1.68, 95%*CI*: 1.16-2.44) and to patients aged ≥60 years at CRC diagnosis (OS: HR: 1.69, 95%*CI*: 1.20-2.40). In regard to other variables, although the interaction terms were not statistically significant, the impacts of smoking on mortality were more marked for patients diagnosed at earlier stages (OS: HR: 1.83, 95%*CI*: 1.07-3.14; DFS: HR: 1.70, 95%*CI*: 1.04-2.78) than for those diagnosed at advanced stages (OS: HR: 1.19, 95%*CI*: 0.87-1.62; DFS: HR: 1.16, 95%*CI*: 0.86-1.57), and for individuals with microsatellite stable/microsatellite instability-low (MSS/MSI-L) tumors (OS: HR: 1.38, 95%*CI*: 1.04-1.82; DFS: HR: 1.32, 95%*CI*: 1.01-1.72) than for those with cancers exhibiting MSI-H (OS: HR: 1.04, 95%*CI*: 0.28-3.95; DFS: HR: 1.24, 95%*CI*: 0.37-4.14).

Table 4.2.3 Overall and Disease-Free Survival in Colorectal Cancer Patients in Relation to Cigarette Smoking by Sex, Age at Diagnosis, Stage at Diagnosis, MSI, and *BRAF V600E* Mutation Status [a]

	Never Smoker		Ever Smoker [b]		*P* for Interaction [d]
	Events [b]/ At Risk	HR [c] (95%*CI*)	Events [b]/ At Risk	HR [c] (95%*CI*) [b]	
Overall survival					
Sex					
Men	36/80	1.00	183/350	1.65*(1.12-2.44)	
Women	54/120	1.00	65/156	1.16(0.77-1.76)	0.12
Age at diagnosis					
<60	37/85	1.00	94/217	1.11(0.72-1.71)	
≥60	53/115	1.00	154/289	1.69*(1.20-2.40)	0.03
Stage at diagnosis					
I/II	21/100	1.00	79/241	1.83*(1.07-3.14)	
III/IV	69/100	1.00	169/265	1.19(0.87-1.62)	0.14
MSI status					
MSS /MSI-L	82/171	1.00	220/420	1.38*(1.04-1.82)	
MSI-H	5/21	1.00	11/51	1.04(0.28-3.95)	0.24
BRAF mutation status					
Wide type	72/164	1.00	197/397	1.15(0.75-1.77)	
V600E mutant	13/22	1.00	27/54	1.65(0.42-6.53)	0.42
Disease-free survival					
Sex					
Men	38/80	1.00	202/350	1.68*(1.16-2.44)	
Women	59/120	1.00	70/155	1.01(0.69-1.48)	0.04
Age at diagnosis					
<60	41/85	1.00	108/217	1.11(0.74-1.66)	
≥60	56/115	1.00	164/288	1.61*(1.15-2.26)	0.08
Stage at diagnosis					
I/II	25/100	1.00	92/240	1.70*(1.04-2.78)	
III/IV	72/100	1.00	180/265	1.16(0.86-1.57)	0.10
MSI status					
MSS /MSI-L	89/171	1.00	239/419	1.32*(1.01-1.72)	
MSI-H	5/21	1.00	16/51	1.24(0.37-4.14)	0.82
BRAF mutation status					
Wide type	78/164	1.00	216/396	1.23(0.83-1.82)	
V600E mutant	14/22	1.00	30/54	1.45(0.44-4.82)	0.52

[a] Abbreviations are as follows: HR, hazard rate ratios; *CI*, confidence interval.
[b] Events are defined as deaths for overall survival and death, recurrence, or metastasis (whichever occurred earliest) for disease-free survival; ever smokers are former and current smokers combined.
[c] Cox proportional hazard model adjusted for sex, age at diagnosis, stage at diagnosis, BMI, marital status, alcohol consumption, and MSI status, where appropriate.
[d] *P* for interaction is the significance of interaction term between smoking and respective stratification variable, calculated by the Wald test.
* Significantly different from reference category, $P<0.05$.

4.3 Discussion

We examined smoking status and gradients of smoking duration/intensity in relation to OS and DFS in a cohort of CRC patients. Pre-diagnostic smoking was associated with higher risk of mortality and poorer DFS. Evidence suggests that the

association between smoking and decreased survival was restricted to patients whose tumors were diagnosed in the colon only. Our work replicates the findings from a recent analysis in Washington State [37], in which, CRC patients who currently smoke have significantly higher disease-specific (HR: 1.30; 95%*CI*: 1.09-1.74) and all-cause mortality (HR: 1.51; 95%*CI*: 1.24-1.83) than non-smokers. These authors also reported higher associations of smoking on mortality for patients diagnosed with colon cancer than for rectal cancer. Likewise, another study in Tayside city [38], UK, with a cohort of CRC patients receiving curative surgery, found significantly worse cause-specific survival in active smokers compared with non-smokers (HR: 2.55; 95%*CI*: 1.40-4.64). Notably, current smoking, but not former smoking, was associated with worsened survival. This is important since it suggests that CRC patients who still smoke at the time of diagnosis could improve their survival by smoking cessation [38].

There are several biologic mechanisms that may explain the higher mortality among CRC patients who smoke. First, tobacco smoking may mutate the *GSTM1* gene, resulting in impaired detoxification of tobacco carcinogens [147]; these carcinogens may exert growth promoting effects to residual tumor cells, either through resistance to chemotherapy or through promotion of angiogenesis [38, 212]. Smoking may also induce aberrant promoter DNA methylation, thus silencing regulatory genes (e.g., *ECAD*, *p16*, *MGMT*, and *DAPK*) in tumor progression [148]. Possible explanations for the differential associations by subsite include the higher concentration of tobacco carcinogens in the colon and the longer contact time of tobacco constituent-carrying feces with the colon, where they are mainly stored, than the rectum [213].

In this study, cigarette smoking was associated with decreased survival only

among male patients. Plausible mechanisms that rationalize the sex differences in survival reduction attributable to tobacco exposure might include the protective effects of female sex hormones on cancer prognosis [214] and the reduced androgen concentration caused by smoking [215, 216] that may lead to stimulation of bowel carcinomas among men [217, 218].

Our findings suggest that smoking has a negligible impact on survival for those diagnosed with advanced-stage disease, perhaps because patient outcomes are inherently poor for this patient population irrespective of smoking status. Intriguingly however, smoking was observed to be significantly associated with poorer survival in patients diagnosed with early-stage disease, providing further support for the recommendation that newly diagnosed patients with less advanced CRC should consider immediate smoking cessation [219].

Smoking is strongly associated with specific somatic molecular alterations (e.g. microsatellite-instability phenotype, CpG island methylator phenotype (CIMP) and the *p.V600E BRAF* mutation [220]) that are linked with the MSI and serrated pathways of carcinogenesis [45]. As these alterations are also strongly related to overall survival, it is important to evaluate the influence of smoking on survival stratified by molecular features of the patients' tumors (e.g., MSI and *BRAF* mutation status). Our study is among the first to assess possible interactions between MSI, *BRAF V600E*, and smoking on both OS and DFS for CRC. We found that ever smoking was associated with higher risk of mortality among patients diagnosed with MSS/MSI-L tumors whereas smoking had little impact on patients diagnosed with MSI-H tumors (**Table 4.2.3**). To our knowledge, only one previous study on smoking and CRC survival has considered potential effect modification by molecular phenotypes of tumor (i.e., MSI status) [37]. Phipps *et al*. [37] reported a prominent

association between smoking and disease-specific mortality in CRC patients with MSI-H tumors (HR: 3.83; 95%*CI*: 1.32-11.11). Our data suggest that cigarette smoking per se does not universally confer poor survival to CRC patients, but rather that its effect diverges according to genetic background or, possibly, the oncogenic pathways of tumorigenesis [46]. More research is clearly needed to verify these findings and to understand the mechanisms that link smoking to decreased survival among colorectal cancer patients.

This study has both strengths and limitations. Firstly, the reasonably large sample size allowed us to perform stratified analyses. The availability of detailed information on personal, clinicopathologic, and molecular characteristics also allowed us to assess potential confounders, effect modifiers, and sources of potential heterogeneity. Moreover, a significant portion of patients in this study were chronic smokers; thus these data have the unique advantage of examining an association that is thought to have a long latent period [221, 222]. Limitations to this study include a lack of information on the cause of death for all deceased participants. The observed differences in OS and DFS could be deaths from causes other than CRC. However, the cause of death, classified according to the ICD codes, was obtained for 200 of 338 deceased patients in this cohort. Of these, the majority (86%) had died from CRC, which is in line with other studies [223, 224]. Secondly, smoking is self-reported by respondents from the distant past, and cigarette-use after diagnosis was not updated. Self-reported cigarette consumption has been shown to be accurate in current smokers but is misreported in approximately 20% of never smokers [147, 225]; however, such misclassification should be non-differential and therefore bias the study results toward the null [147].

In conclusion, pre-diagnosis cigarette smoking is independently predictive of

worse survival after CRC diagnosis. Results from this prospective population-based study underscore the importance of cultivating healthy lifestyle habits. This study presents preliminary results concerning potential interactions between smoking, clinicopathologic, and tumor molecular phenotype on CRC survival. Confirmation of these findings is needed in large replication studies and further analyses using tobacco-specific DNA adducts as quantitative measurements of exposure are warranted [147].

Chapter 5 Summary

The research project presented in this thesis comprises two components. The first mainly investigated the associations of dietary patterns and CRC all-cause (OS) and disease-free survival (DFS), and further explored if the relationship between dietary pattern and CRC survival was modified by sex, physical activity and *BRAF* mutation. Results showed that high conformity to the processed meat pattern was significantly associated with an increased risk of all-cause mortality and recurrence of CRC, whereas no associations were observed with the prudent vegetable or the high sugar patterns and DFS. The effect of processed meat pattern on cancer recurrence or death was modified by sex. This observation not only adds to the mounting evidence that encourages people to engage in a healthy diet, but also provides some guidance to efficacious dietary interventions; that is, people may lower their risk of CRC mortality by the avoidance of a processed meat pattern diet.

Nevertheless, this study is limited in self-reported dietary exposures from the past and dietary changes before and after diagnosis that have not been accounted for in the analyses. The choice of foods in the questionnaire is another source of bias. Some food items that are not listed on the questionnaire (e.g., some 'snacks') may comprise a large part of a typical diet and are also laden in sugars, salt and fat. Lack of such information may bias the dietary pattern analyses. Therefore, the initial findings need to be confirmed in a replicated. Further research should be more concerned with the development of more robust and discriminating dietary assessment methodologies for identifying dietary patterns that are valid and reproducible. Though our study did not find a difference in effect by tumor molecular phenotype (i.e., MSI status or *BRAF V600E* mutation), larger molecular studies should be conducted to examine if such differences exist.

The second component of this thesis examined the association of smoking with OS and DFS among CRC patients; and further assessed potential interactions of smoking with sex, age at diagnosis, tumor stage at diagnosis, MSI status, and BRAF mutation status on cancer mortality. Results obtained from this part of study demonstrated that pre-diagnosis cigarette smoking is independently predictive of worse survival after CRC diagnosis, and the relationship between smoking and CRC survival is modified by sex and age at diagnosis. Our results underscore the importance of cultivating healthy lifestyle habits. This study presents preliminary results concerning potential interactions between smoking, clinicopathologic, and tumor molecular phenotype on CRC survival.

Limitations to this study include a lack of information on the cause of death for all deceased participants. The observed differences in OS and DFS could be deaths from causes other than CRC. Secondly, smoking is self-reported by respondents from the distant past, and cigarette-use after diagnosis was not updated. Hence, confirmation of these findings is needed in large replication studies. Further directions would involve the use of tobacco-specific DNA adducts, which allows more precisely quantitative identification of smoking exposure values.

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Appendices

Appendix 1. Personal History Questionnaire

Please write in your answers where space is provided, or place tick marks in circles O

What date are you filling out this questionnaire? _____/_____/_____

Day Month Year

Identifying information

1. Are you male or female?
O male
O female

2. What is your date of birth?
___ ___ years
O don't know

2. What is your age?
day ___ ___
month ___ ___
year ___ ___
O don't know day
O don't know month
O don't know year

3. Are you a twin or triplet?
O yes, a twin
O yes, other multiple (triplet, quadruplet, etc.): _____

Please specify

O no
O don't know

If yes, please read the following statement and answer the question.

Non-identical twins are no more alike than ordinary brothers and sisters. Genetically identical twins, on the other hand, look so much alike *that is, they have a strong resemblance to each other in height, colouring, features of the face, etc.) that people often mistake one for the other, especially during their childhood.

Do you have a genetically identical twin or triplet?
O yes
O no
O don't know

5. What is your marital status?
O currently married or living as married
O separated
O divorced
O widowed
O single or never married
O don't know

6. Have you ever had a test for blood in your stool, called a smear test or a hemoccult? This test is frequently done as part of a routine physical examination, or it can be done at home.

O yes
O no → Please go to # 7
O don't know → Please go to # 7

6a. When did you first have this test?

age when first tested ___ ___
or
year of first test ___ ___ ___ ___
O don't know

6b. What were the reasons for your first test? Please tick all that apply.

O to investigate a new problem
O family history of colorectal cancer
O routine/yearly examination or check-up
O follow up of previous problem
O don't know

6c. How many times have you had a hemoccult test?

___ number of hemoccult tests
O don't know

6d. If you have had a hemoccult test more than once, when did you last have this test?

age when last tested ___ ___
or
year of last test ___ ___ ___ ___
O don't know

7. Have you ever had a sigmoidoscopy? sigmoidoscopy involves looking inside the lower bowel and rectum with a lighted instrument. This examination is usually done in a doctor's office without anesthesia.

O yes
O no → Please go to # 8
O don't know → Please go to # 8

7a. When did you first have this test?

age when first tested ___ ___
or
year of first test ___ ___ ___ ___
O don't know

7b. What were the reasons for your first sigmoidoscopy? Please tick all that apply.

O to investigate a new problem
O family history of colorectal cancer
O routine/yearly examination or check-up
O follow up of previous problem
O don't know

7c. How many times have you had a sigmoidoscopy?

___ number of sigmoidoscopies
O don't know

7d. If you have had a sigmoidoscopy more than once, when did you last have this test?

age when last tested ___ ___
or
year of last test ___ ___ ___ ___
O don't know

8. Have you ever had a colonoscopy? colonoscopy is an examination of the entire large bowel using a long flexible instrument. This examination is usually done under sedation.

O yes
O no → Please go to # 9
O don't know → Please go to # 9

8a. When did you first have this test?

age when first tested ___ ___
or
year of first test ___ ___ ___ ___
O don't know

8b. What were the reasons for your first

9. Has a doctor ever told you that you had polyps in your large bowel or colon or rectum? Polyps are growths in the lining of the colon which vary in size from a tiny dot several inches.

O yes
O no → Please go to # 10
O don't know → Please go to # 10

9a. When did your doctor first tell you that you have had polyps?

age when first tested ___ ___
or
year of first test ___ ___ ___ ___
O don't know

colonoscopy? Please tick all that apply.

O to investigate a new problem
O family history of colorectal cancer
O routine/yearly examination or check-up
O follow up of previous problem
O other: _____
Please specify
O don't know

8c. How many times have you had a colonoscopy?

___ number of colonoscopies
O don't know

8d. If you have had a colonoscopy more than once, when did you last have this test?

age when last tested ___ ___
or
year of last test ___ ___ ___ ___
O don't know

9b. Have you been told more than once that you had polyps?

O yes
O no
O don't know

9c. When did you your doctor last tell you that you had polyps?

age at last diagnosis ___ ___
or
year of last diagnosis ___ ___ ___ ___
O don't know

9d. Do you know what kind of polyps they were?

O benign
O adenomatous (pre-cancerous)
O hyperplastic
O other: _____
Please specify
O don't know

9e. Did you have the polyps removed (by a procedure called a polypectomy)? (This can be done during a sigmoidoscopy or colonoscopy.)

O yes
O no → Please go to # 10
O don't know → Please go to # 10

9f. When did you first have polyps removed?

age at first polypectomy ___ ___
or
year of first polypectomy ___ ___ ___ ___
O don't know

9g. Have you had polyps removed more than once?

O yes
O no
O don't know

9h. If you have had polyps removed more than once, when did you last have polyps removed?

age at first polypectomy ___ ___
or
year of first polypectomy ___ ___ ___ ___
O don't know

11. Has a doctor ever told you that you had Crohn's disease? This is where you have an inflammation that extends into the deeper layers of the intestinal wall. It may also affect other parts of the digestive tract, including the mouth, esophagus, stomach, and small intestine.

O yes
O no → Please go to # 12
O don't know → Please go to # 12

11a. When did your doctor first tell you that you had Crohn's disease?

age when first tested ___ ___
or
year of first test ___ ___ ___ ___
O don't know

12. Has a doctor ever told you that you had ulcerative colitis? This is an inflammation and ulceration of the lining of the bowel (colon) & rectum. It is not a stomach ulcer.

O yes
O no → Please go to # 13
O don't know → Please go to # 13

12a. When did your doctor first tell you that you had ulcerative colitis?

10. Has a doctor ever told you that you had familial adenmotaous polyposis, known also as FAP? This is a condition, sometimes occurring in families, in which numerous polyps line the inside of the large bowel or colon.

O yes
O no → Please go to # 11
O don't know → Please go to # 11

10a. When did your doctor first tell you that you had FAP?
age at first diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

13a. When did your doctor first tell you that you had irritable bowel syndrome?

age at first diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

14. Has a doctor ever told you that you had diverticular disease? This may also be called diverticulosis or diverticulitis. It's a condition in which the bowel may become infected, and can lead to pain and chronic problems with bowel habits. and small intestine.

O yes
O no → Please go to # 15
O don't know → Please go to # 15

14a. When did your doctor first tell you that you had diverticular disease?

age at first diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

15. Have you ever had any of your large bowel or colon removed?

O yes
O no → Please go to # 16
O don't know → Please go to # 16

Was it completed removed, or was only part of it removed?
O completed removed
O partly removed

age at first diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

13. Has a doctor ever told you that you had irritable bowel syndrome? This is a disorder of the bowels leading to cramping, gassiness, bloating and alternating diarrhea and constipation. It is sometimes called IBS, or spastic colon.

O yes
O no → Please go to # 14
O don't know → Please go to # 14

15b. Have you had more than one surgery to remove your bowel or colon?

O yes
O no → Please go to # 16
O don't know → Please go to # 16

15c. When did you last have any of your bowel or colon removed?

age at last operation ___ ___
or
year of last operation ___ ___ ___ ___
O don't know

16. Have you had your gallbladder removed?

O yes
O no → Please go to # 17
O don't know → Please go to # 17

16a. When did you have your gallbladder removed?

age at operation ___ ___
or
year of operation ___ ___ ___ ___
O don't know

17. Has a doctor ever told you that you had diabetes, also known as diabetes mellitus? Please do not include diabetes which you had only during pregnancy.

O yes
O no → Please go to # 14
O don't know → Please go to # 14

O don't know

15a. When did you first have any of your bowel or colon removed?

age at first operation ___ ___
Or
year of first operation ___ ___ ___ ___
O don't know

17a. When did your doctor first tell you that you had diabetes?

age at first diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

17b. Did you ever take medication to control your diabetes?

O yes
O no → Please go to # 18
O don't know → Please go to # 18

17c. What type of medication did you use, pill or insulin injections?

O pills
O insulin injections
O both
O don't know → Please go to # 18

17d. How often did you usually take it? Please choose the most appropriate category.

	Pills	Insulin
times per day or	___ ___	___ ___
times per week or	___ ___	___ ___
times per month or	___ ___	___ ___
times per year	___ ___	___ ___
don't know	O	O

17e. About one year before your recent cancer diagnosis, were you taking it?

	Pills	Insulin
O yes	O	O
O no	O	O
O don't know	O	O

17f. How long, in total, have you taken this medication?

	Pills	Insulin
number of moths or	___ ___	___ ___
number of years	___ ___	___ ___
don't know	O	O

18. Has a doctor ever told you that you had high cholesterol? If your doctor told you it borderline, please tick no.

O yes
O no → Please go to # 19
O don't know → Please go to # 19

18a. When did your doctor tell you that you had high cholesterol?

age at diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

18b. How you ever take medication to control your high cholesterol?

O yes
O no → Please go to # 19
O don't know → Please go to # 19

18c. How often did you usually take it? Please choose the most appropriate category.

___ ___ times per day or
___ ___ times per week or
___ ___ times per month or
___ ___ times per year or
O don't know

18d. About one year before your recent cancer diagnosis, were you taking it?

O yes
O no
O don't know

18e. How long, in total, have you taken this medication?

___ ___ number of months or
___ ___ number of years
O don't know

19. Has a doctor ever told you that you had high levels of fat (other than cholesterol) in your blood, also called high triglycerides? If your doctor told you it was borderline, Please tick no.

O yes
O no → Please go to # 20
O don't know → Please go to # 20

19a. What did your doctor first tell you that you had high triglycerides?

age at diagnosis
or
year of diagnosis
don't know

19b. Did you ever take medication to control the high levels of fat in your blood?

O yes
O no → Please go to # 20
O don't know → Please go to # 20

19c. How often did you usually take it? Please choose the most appropriate category.

___ ___ times per day or
___ ___ times per week or
___ ___ times per month or
___ ___ times per year or
O don't know

19d. About one year before your recent cancer diagnosis, were you taking it?

O yes
O no
O don't know

19e. How long, in total, have you taken this medication?

___ ___ number of months or
___ ___ number of years
O don't know

20. Has a doctor ever told you that you had any type of cancer?

O yes
O no → Please go to # 24
O don't know → Please go to # 24

20a. What type of cancer was it?
_____ cancer

20b. When did your doctor tell you that you had this type of cancer?

age at diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

20c. Were you treated with radiation therapy (radiotherapy) for this cancer?

O yes
O no
O don't know

21. Has a doctor ever told you that you had any other cancer?

O yes
O no → Please go to # 24
O don't know → Please go to # 24

21a. What type of cancer was it?
_____ cancer

21b. When did your doctor tell you that you had this type of cancer?

age at diagnosis ___ ___
or
year of diagnosis ___ ___ ___ ___
O don't know

21c. Were you treated with radiation therapy (radiotherapy) for this cancer?

O yes
O no
O don't know

19. Has a doctor ever told you that you had any cancer?

O yes

Medications

Have you ever taken any of the following medications regular (at least twice a week

O no → Please go to # 24
O don't know → Please go to # 24

22a. What type of cancer was it?

_____cancer

22b. When did your doctor first tell you that you had this type of cancer?

age at diagnosis
or
year of diagnosis
don't know

22c. Were you treated with radiation therapy (radiotherapy) for this cancer?

O yes
O no
O don't know

23. Has a doctor ever told you that you had any other cancer?

O yes
O no → Please go to # 24
O don't know → Please go to # 24

22a. What type of cancer was it?

_____cancer

23b. When did your doctor first tell you that you had this type of cancer?

age at diagnosis
or
year of diagnosis
don't know

23c. Were you treated with radiation therapy (radiotherapy) for this cancer?

O yes
O no
O don't know

for more than a month)?

24. Aspirin (such as Anacin, Bufferin, Bayer, Excedrin, Ecotrin)

O yes
O no → Please go to # 25
O don't know → Please go to # 25

24a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)? __ Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

24b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes
O no
O don't know

24c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

Have you ever taken any of the following medications regularly (at least twice a week for more than a month)? (continued)

25. Acetaminophen (such as Tylenod, Anacin-3, Panadol)

26. Ibuprofen medications (such as Advil, Motrin, Medipren, Indocid, Naprosyn, NSAIDS (NSAIDS are non-steroidal anti-inflammatory drugs)

O yes
O no → Please go to # 26
O don't know → Please go to # 26

25a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

25b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes
O no
O don't know

25c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

O yes
O no → Please go to # 27
O don't know → Please go to # 27

26a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

26b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes
O no
O don't know

26c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

Have you ever taken any of the following medications regularly (at least twice a week for more than a month)? (continued)

27. Bulk-forming laxatives (such as Metamucil, Citrucel, FibreCon. Serutan, psyllium)

O yes
O no → Please go to # 28
O don't know → Please go to # 28

27a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

28. Other laxatives (such as Ex-Lax, Correctol, Dulcolax, Senokot, Colace, castor, cod liver oil, mineral oil, milk of magnesia, lactulose, Epsom salts)

O yes
O no → Please go to # 29
O don't know → Please go to # 29

28a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

27b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes
O no
O don't know

27c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

___ ___ times per day or
___ ___ times per week
O don't know

28b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes
O no
O don't know

28c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

Have you ever taken any of the following medications regularly (at least twice a week for more than a month)? (continued)

29. Multivitamin supplements (such as One-A-Day, Theragram, Centrum, Unicap) (not individual vitamins)

O yes
O no → Please go to # 28
O don't know → Please go to # 28

29a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

29b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes

30. Folic acid or folate pills or tablets

O yes
O no → Please go to # 31
O don't know → Please go to # 31

30a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

30b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes

O no
O don't know

29c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

O no
O don't know

30c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

Have you ever taken any of the following medications regularly (at least twice a week for more than a month)? (continued)

31. Calcium pills or tablets

O yes
O no → Please go to # 32
O don't know → Please go to # 32

29a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

29b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes
O no
O don't know

29c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

32. Calcium-based antacids (such as Tums, Rolaids, Extra-strength Rolaids, Alka-Mints, Chooz Antacid gum)

O yes
O no → If female, Please go to # 33
If male. Please go to # 44
O don't know → If female, Please go to # 33
If male. Please go to # 44

32a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month? Please choose one of the following.

___ ___ times per day or
___ ___ times per week
O don't know

32b. About one year before your recent cancer diagnosis, were you taking it regularly?

O yes
O no
O don't know

32c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please _ count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

Men: please go to #44 on page 17
Women: please continue with #33 on page 13

Menstruation, Pregnancy, and Menopause

33. How old were you when you had your first menstrual period?

___ ___ years of age
O don't know
O never had a menstrual period

34. Have you ever been pregnant?

O yes
O no → Please go to # 35
O don't know → Please go to # 35
>How many times have you been pregnant? Please include miscarriages, stillbirths, tubal pregnancies and abortions.

___ ___ number of pregnancies
O don't know

34a. How many times were you pregnant with more than one baby (twins, triplets or ___ more)? If you are pregnant now, please do not include your current pregnancy. _

O never
___ ___ number of pregnancies with more than one baby
O don't know

34b. How many of your pregnancies lasted 6 months or longer? (Pregnancy usually lasts 9 months. Six months is about the earliest a baby could survive.) If you are pregnant now, please do not include your current _ pregnancy.

O never
___ ___ number of pregnancies lasting 6 months or longer
O don't know

34c. How many of your pregnancies resulted in live births?

O never
___ ___ number of pregnancies with live-born children
O don't know

34d. How old were you at the first live birth?

age at first birth ___ ___ or
year of first birth ___ ___ ___ ___
O don't know

34e. How old were you at the last live birth?

age at last birth ___ ___ or
year of last birth ___ ___ ___ ___
O don't know

35. Have you ever used birth control pills or other hormonal contraceptives (implants or injections) for at least one year?

O yes
O no → Please go to # 36
O don't know → Please go to # 36
>How old were you when you first used Any of these hormonal contraceptives?

age at first use ___ ___ or
year of first use ___ ___ ___ ___
O don't know

35a. Were you still using hormonal contraceptives about one year before your recent cancer diagnosis?

O yes
O no
O don't know

35b. In total, how long did you take these hormonal contraceptives? If you started and stopped and then started again, please count only the time you were taking these contraceptives.

___ ___ number of years
O don't know

36. Have you had a menstrual period in the last 12 months? Please include only menstrual bleeding, not bleeding that results from hormonal replacement therapy (HRT) or progesterones, progesttins or withdrawal bleeding.

O yes → Please go to #42
O no
O don't know → Please go to #42

Have your periods stopped permanently or only temporarily due to pregnancy, breast-feeding, or other conditions?

O permanently
O temporarily → Please go to #42

37. How old were you when your periods stopped permanently?

age they stopped ___ ___ or
year they stopped ___ ___ ___ ___
O don't know

38. Why did your menstrual periods stop permanently? Please tick all that apply.

O natural menopause
O surgery
O radiation or chemotherapy
O other reason
Please specify: _____
O Don't know

Please complete the next few questions which ask about surgeries you may have had.

39. Hysterectomy (only the uterus or womb Removed)

O yes
O no
O don't know
>age when removed ___ ___ or
years when removed ___ ___ ___ ___
O don't know

39a. Hysterectomy with one ovary or part of an Ovary removed)

O yes
O no
O don't know
>age when removed ___ ___ or
years when removed ___ ___ ___ ___
O don't know

39b. Hysterectomy with both ovaries removed

O yes
O no
O don't know
>age when removed ___ ___ or
years when removed ___ ___ ___ ___
O don't know

39c. One ovary removed, completely or partly, without hysterectomy

O yes
O no
O don't know
>age when removed ___ ___ or
years when removed ___ ___ ___ ___
O don't know

39d. Both ovaries removed without hysterectomy

O yes
O no
O don't know
>age when removed ___ ___ or
years when removed ___ ___ ___ ___
O don't know

40. If you had radiation or chemotherapy, when did you first have it?

42a. Were you still having menstrual periods when you first took these hormones?

O yes
O no
O don't know

42b. Were you prescribed either an estrogen-only pill or patch (such as Premarin) for

O had radiation or chemotherapy
>age when this was given ___ ___ or
year when this was given ___ ___ ___ _
O don't know
O never had radiation or chemotherapy

41. if your periods stopped permanently for any reason other than surgery, radiation or chemotherapy, when did you this occur?

O other reason
Please specify: _____
>age when occurred ___ ___ or
year when occurred ___ ___ ___ ___
O don't know
O not applicable

42. Doctors prescribe hormonal replacement therapy for many reasons, including menopausal symptoms, surgical removal of the ovaries, osteoporosis, and heart disease prevention. (Menopausal symptoms include hot flashes, sweating, and depression.)

Have you ever taken hormonal replacement therapy prescribed by a doctor and in the form of a pill or a patch?

Please do not include hormonal therapy that was prescribed for birth control, infertility, hormone therapy delivered by injections, vagina creams or vaginal suppositories, or herbal or soy products.

O yes
O no → Please go to #43
O don't know → Please go to #43

42e. Progesterone or progestin is frequently prescribed by doctors together with estrogen for hormone replacement therapy. One common brand name is Provera. Another one is Prometrium. Have you ever taken progesterone or progestin together with estrogens for hormone replacement therapy?

O yes
O no → Please go to #43
O don't know → Please go to #43
>How old were you when you first took progesterone or progestin together with estrogens?

age when first taken ___ ___ or
year when first taken ___ ___ ___ ___

hormone replacement therapy?

O yes
O no
O don't know
>How old were you when you first took estrogen-only medication?

age when first taken ___ ___ or
years when first taken _____ ___ _
O don't know

42c. Were you still using estrogen-only medication for hormone replacement therapy about one year before your recent cancer diagnosis?

O yes
O no
O don't know

42d. In total, how long did you take estrogen-only medication for hormone replacement therapy? If you started and stopped and then started again, please count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

43. Have you ever taken tamoxifen, raloxifene, or other anti-estrogen medication (such as Lupron or Depo-Provera)?

O yes
O no → Please go to #44
O possibly – I have participated in a clinical trial for tamoxifen or other anti-estrogen medication
O don't know
>What anti-estrogen medication did you take? Please tick all that apply.

O tamoxifen
O raloxifene
O other: _____

O don't know

42f. Were you still using progesterone or progestin medication about one year before your recent cancer diagnosis?

O yes
O no
O don't know

42g. In total, how long did you take progesterone or progestin together with estrogens? If you started and stopped and then started again, please count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

Please specify

43a. How old were you when you first took tamoxifen, raloxifene or other anti-estrogen medication?

age when first taken ___ ___ or
year when first taken ___ ___ ___ ___
O don't know

43b. Were you still using tamoxifen, raloxifene or other anti- estrogen medication about one year before your recent cancer diagnosis?

O yes
O no
O don't know

43c. In total, how long did you take tamoxifen, raloxifene or other anti-estrogen medication? If you started and stopped and then started again, please count only the time you were taking this medication.

___ ___ number of months or
___ ___ number of years
O don't know

Diet

44. About one year before your recent cancer diagnosis, on average, how often did you eat a piece serving of fruit?
(A serving of fruit is: 1 medium-sized fresh fruit; ½ cup of chopped, cooked or canned fruit; ¼ cup of dried fruit; 6 ounces of fruit juice (50%-100% pure juice).) Please choose one of the following.

___ ___ servings per day or
___ ___ servings per week or
___ ___ servings per month
O don't know

45. About one year before your recent cancer diagnosis, on average, how often did you eat a piece serving of vegetables?
(A serving of vegetables is: 1 medium-sized fresh vegetables; ½ cup of chopped, cooked or chopped vegetables; 6 ounces of vegetable juice (50%-100% pure juice).) Please choose one of the following.

___ ___ servings per day or
___ ___ servings per week or
___ ___ servings per month
O don't know

46. About one year before your recent cancer diagnosis, on average, how often did you eat a serving of red meat (not chicken or fish)?

(A serving of red meat is: 2-3 ounces of red meat (a piece of meat about the size of a deck

of cards). Red meats include: beef, steak, hamburger, prime rib, ribs, beef hot dogs, beef-based processed meat, veal, pork, bacon, pork sausage, ham, lamb, venison.)

___ ___ servings per day or
___ ___ servings per week or
___ ___ servings per month
O don't eat red meat → Please go to #47
O don't know

46a. About one year before your recent cancer diagnosis, on average, how often did you eat a serving of red meat that was cooked by broiling, grilling, barbecueing or pan-frying (not stir-fried or deep-fried)? Please choose one of the following.

___ ___ servings per day or
___ ___ servings per week or
___ ___ servings per month
O don't eat red meat that was cooked by these methods → Please go to #47
O don't know

46b. On average, when you ate red meat cooked by these methods, which of the following best describes its appearance?

What was its outside appearance?	What was its inside appearance? (how well done it was)?
O lightly browned	O red (rare)
O medium browned	O pink (medium)
O heavily browned or blackened	O brown (well-done)
O don't know	O don't know

47. About one year before your recent cancer diagnosis, on average, how often did you eat a serving of chicken? Please do not include turkey or any other bird.
(A serving of chicken is: 2-3 ounces of chicken meat; 1 drumstick; 1 thigh; half a breast; 2 wings; 3 nuggets.) Please choose one of the following.

___ ___ servings per day or
___ ___ servings per week or
___ ___ servings per month
O don't eat red meat that was cooked by these methods → Please go to #48
O don't know

47a. About one year before your recent cancer diagnosis, on average, how often did you eat a serving of chicken that was cooked by broiling, grilling, barbecueing or pan-frying (not stir-fried or deep-fried)? Please choose one of the following.

___ ___ servings per day or
___ ___ servings per week or
___ ___ servings per month
O don't eat chicken that was cooked by these methods → Please go to #48
O don't know

47b. On average, when you ate chicken cooked by these methods, which of the following best describes its appearance?

What was its outside appearance?

O lightly browned
O medium browned
O heavily browned or blackened
O don't know

We would like you to think back to when you were in your 20s and remember the physical activities you participated in then.

48. In your 20s, did you participate regularly in physical activity for a total of at least 30 minutes a week? Please describe your activities below.

		For how many years?	During those years, for many months per year?	During those months, on average, for how many minutes or hours per week?
Walking	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Jogging (running slower than a mile in 10 minutes)	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Running (running faster than a mile in 10 minutes)	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Bicycling (including using an exercise bicycle	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Swimming laps	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Tennis, squash racquetball	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Calisthenics, aerobics, vigorous dance (including ballet), using a rowing machine, lifting weights	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Football, soccer rugby, basketball	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Heavy household work (examples: using a non-power mower, shoveling, moving heavy loads, scrubbing floors)	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week

In your 20s, did you do any other strenuous activities? Strenuous activity means something that really increased your heart rate, make you hot, and caused you to sweat. Some examples are: skiing, skating, hockey, hunting, shedding or tobogganing, water-skiing.

Activity Please specify		For how many years?	During those years, for many months per year?	During those months, on average, for how many minutes or hours per week?
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
		___ ___ years	___ ___ months	__ minutes per week / __hours per week

49. When you were in your 20s, what was your usual occupation? (When mean what you did for the longest time, including any paid or unpaid employment, such as being a student or housewife of being unemployed.)

_____ occupation

O don't know

If you are younger than 31, please go to the next section (Alcohol Consumption) on page 25. Otherwise, please continue with #50.

Now, please think back to your 30s and 40s.

50. In your 30 and 40s, did you participate regularly in physical activity for a total of at least 30 minutes a week? Please describe your activities below.

		For how many years?	During those years, for many months per year?	During those months, on average, for how many minutes or hours per week?
Walking	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Jogging (running slower than a mile in 10 minutes)	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Running (running faster than a mile in 10 minutes)	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Bicycling (including using an exercise bicycle	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Swimming laps	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Tennis, squash racquetball	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Calisthenics, aerobics, vigorous dance (including ballet), using a rowing machine, lifting weights	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Football, soccer rugby, basketball	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Heavy household work (examples: using a non-power mower, shoveling, moving heavy loads, scrubbing floors)	O yes → O no	___ ___ years	___ ___ months	__ minutes per week / __hours per week

In your 30s and 40s, did you do any other strenuous activities? Strenuous activity means something that really increased your heart rate, make you hot, and caused you to sweat. Some examples are: skiing, skating, hockey, hunting, shedding or tobogganing, water-skiing.

Activity Please specify		For how many years?	During those years, for many months per year?	During those months, on average, for how many minutes or hours per week?
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
		___ ___ years	___ ___ months	__ minutes per week / __hours per week

51. When you were in your 30s and 40s, what was your usual occupation? (When mean what you did for the longest time, including any paid or unpaid employment, such as being a student or housewife of being unemployed.)

_____ occupation

O don't know

If you are younger than 31, please go to the next section (Alcohol Consumption) on page 25. Otherwise, please continue with #50.

Now, please think back to since you turned 50s.

52. In your 50s, did you participate regularly in physical activity for a total of at least 30 minutes a week? Please describe your activities below.

		For how many years?	During those years, for many months per year?	During those months, on average, for how many minutes or hours per week?
Walking	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week

Jogging (running slower than a mile in 10 minutes)	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Running (running faster than a mile in 10 minutes)	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Bicycling (including using an exercise bicycle	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Swimming laps	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Tennis, squash racquetball	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Calisthenics, aerobics, vigorous dance (including ballet), using a rowing machine, lifting weights	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Football, soccer rugby, basketball	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week
Heavy household work (examples: using a non-power mower, shoveling, moving heavy loads, scrubbing floors)	O yes → O no →	___ ___ years	___ ___ months	__ minutes per week / __hours per week

In your 50s, did you do any other strenuous activities? Strenuous activity means something that really increased your heart rate, make you hot, and caused you to sweat. Some examples are: skiing, skating, hockey, hunting, shedding or tobogganing, water-skiing.

Activity Please specify		For how many years?	During those years, for many months per year?	During those months, on average, for how many minutes or hours per week?
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week

_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
_____	→	___ ___ years	___ ___ months	__ minutes per week / __hours per week
		___ ___ years	___ ___ months	__ minutes per week / __hours per week

53. When you were in your 50s, what was your usual occupation? (When mean what you did for the longest time, including any paid or unpaid employment, such as being a student or housewife of being unemployed.)

_____ occupation

O don't know

We would like you to think back to when you were in your 20s.

54. In your 20s, did you ever consume any alcoholic beverages at least once a week for 6 months or longer? Please describe your consumption below.

		For how many years?	During those years, how much did you typically consume?
Beer, hard cider (at least 3% alcohol)	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 12 ounce cans or bottles O per day O per week O don't know
Wine	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 4 ounce glasses of wine O per day O per week O don't know
Sake, sherry, port	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 1 ounce servings O per day O per week O don't know

Spirits, liquor mixed drinks, brandy, liqueurs	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 1 ounce shots liquor or spirits O per day O per week O don't know

55. When you were in your 20s, how many years in total did you consume at least one alcoholic beverage (of any type) a week?

___ ___ years consumed
O never consumed alcohol

56. On average, how many alcoholic beverages a week did you consume during those years? That is, how many 4 ounce glasses of wine or 12 ounce cans or bottles of beer or hard cider, or 1 ounce servings of sake, sherry, port, or spirits, mixed drinks and cocktails.

___ ___ years consumed
O never consumed alcohol

If you are younger than age 31, please go to the next section (Smoking) on page 28.
Otherwise, please continue with #57.

Now, please think back to your 30s and 40s.

57. In your 30s and 40s, did you ever consume any alcoholic beverages at least once a week for 6 months or longer? Please describe your consumption below.

		For how many years?	During those years, how much did you typically consume?
Beer, hard cider (at least 3% alcohol)	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 12 ounce cans or bottles O per day O per week O don't know
Wine	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 4 ounce glasses of wine O per day O per week O don't know
Sake, sherry, port	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 1 ounce servings O per day O per week O don't know
Spirits, liquor mixed drinks, brandy, liqueurs	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 1 ounce shots liquor or spirits O per day O per week O don't know

58. When you were in your 30s and 40s, how many years in total did you consume at least one alcoholic beverage (of any type) a week?

___ ___ years consumed
O never consumed alcohol

56. On average, how many alcoholic beverages a week did you consume during those years? That is, how many 4 ounce glasses of wine or 12 ounce cans or bottles of beer or hard cider, or 1 ounce servings of sake, sherry, port, or spirits, mixed drinks and cocktails.

___ ___ years consumed
O never consumed alcohol

If you are younger than age 51, please go to the next section (Smoking) on page 28.
Otherwise, please continue with #60.

Now, please think back to since you turned 50s.

60. In your 50s, did you ever consume any alcoholic beverages at least once a week for 6 months or longer? Please describe your consumption below.

		For how many years?	During those years, how much did you typically consume?
Beer, hard cider (at least 3% alcohol)	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 12 ounce cans or bottles O per day O per week O don't know
Wine	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 4 ounce glasses of wine O per day O per week O don't know
Sake, sherry, port	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 1 ounce servings O per day O per week O don't know
Spirits, liquor mixed drinks, brandy, liqueurs	O yes → O no O don't know	___ ___ years consumed	___ ___ number of 1 ounce shots liquor or spirits O per day O per week O don't know

61. When you were in your 30s and 40s, how many years in total did you consume at least one alcoholic beverage (of any type) a week?

___ ___ years consumed
O never consumed alcohol

62. On average, how many alcoholic beverages a week did you consume during those years? That is, how many 4 ounce glasses of wine or 12 ounce cans or bottles of beer or hard cider, or 1 ounce servings of sake, sherry, port, or spirits, mixed drinks and cocktails.

___ ___ years consumed
O never consumed alcohol

Smoking

63. Have you ever smoked at least one cigarette a day for 3 months or longer?

O yes
O no → Please go to #64
O don't know → Please go to #64

63a. When did you first start smoking at least one cigarette a day?

age at first use ___ ___ or
year of first use ___ ___ ___ ___
O don't know

63b. During periods when you smoked regularly, how many cigarettes did you typically smoke in a day?

___ ___ ___ ___ cigarettes per day
O don't know

63c. About one year before your recent cancer diagnosis, were you still smoking at least one cigarette a day?

O yes
O no
O don't know

63d. Do you still smoke at least one cigarette a day?

O yes
O no → Please go to #63f
O don't know → Please go to #63f

63e. When did you stop smoking at least one cigarette a day (we mean stop smoking permanently)?

age at first use ___ ___ or
year of first use ___ ___ ___ ___
O don't know

63f. How many years, in total, did you smoke at least one cigarette a day for 3 months or longer? (If you have stopped and restarted at least once, count only the time when you were smoking.)

64. Have you ever smoked at least one cigar a month for at least 3 months?

O yes
O no → Please go to #65
O don't know → Please go to #65

64a. When did you first start smoking at least one cigar a month?

age at first use ___ ___ or
year of first use ___ ___ ___ ___
O don't know

64b. During periods when you smoked regularly, how many cigar did you typically smoke in a month?

___ ___ ___ ___ cigarettes per month
O don't know

64c. About one year before your recent cancer diagnosis, were you still smoking at least one cigar a month?

O yes
O no
O don't know

64d. Do you still smoke at least one cigar a month?

O yes
O no → Please go to #64f
O don't know → Please go to #64f

64e. When did you stop smoking at least one cigar a month (we mean stop smoking permanently)?

age at first use ___ ___ or
year of first use ___ ___ ___ ___
O don't know

64f. How many years, in total, did you smoke at least one cigar a month for 3 months or longer? (If you have stopped and restarted at least once, count only the time when you were smoking.)

___ ___ ___ ___ total number of years
O don't know

___ ___ ___ ___ total number of years
O don't know

65. Have you ever smoked at least one pipe a month for at least 3 months?

O yes
O no → Please go to #66
O don't know → Please go to #66

65a. When did you first start smoking at least one pipe a month?

age at first use ___ ___ or
year of first use ___ ___ ___ ___
O don't know

65b. During periods when you smoked regularly, how many pipe did you typically smoke in a month?

___ ___ ___ ___ pipe per month
O don't know

65c. About one year before your recent cancer diagnosis, were you still smoking at least one pipe a month?

O yes
O no
O don't know

65d. Do you still smoke at least one pipe a month?

O yes
O no → Please go to #65f
O don't know → Please go to #65f

65e. When did you stop smoking at least one pipe a month (we mean stop smoking smoking permanently)?

age at first use ___ ___ or
year of first use ___ ___ ___ ___
O don't know

65f. How many years, in total, did you smoke at least one pipe a month for 3 months or longer? (If you have stopped and restarted at least once, count only the time when you were smoking.)

___ ___ ___ ___ total number of years
O don't know

Height and Weight

66. About how tall are you, without your shoes on?

___ feet ___ ___ inches
or
___ ___ ___ centimeters
O don't know

67. How much did you weigh about one year before your recent cancer diagnosis?

___ pounds
Or
___ ___ ___ kilograms
O don't know

Additional Information

69. Previous to this study, have you and your relatives ever taken part in any family health studies?

O yes
O no
O don't know

Background Information

70. What is the highest level of education that you completed?

O less than 8 years
O 8 to 11 years
O high school graduate
O vocational or technical school
O some college or university
O bachelor's degree
O graduate degree
O don't know

71. Country of birth sometimes affects disease risk. Please fill in country of birth for yourself, you parents and your grandparents.

In addition, scientists have found that some genetic traits are more common or less common among Jewish people of different ethnic backgrounds. Please answer the questions about Jewish descent for each person.

	Country of birth	Is this person of Jewish descent?	Ashkenazi (East European)	Sephardic	Other	Don't know
You	_____	O yes O no O don't know	O	O	O	O
Your mother	_____	O yes O no O don't know	O	O	O	O
Your father	_____	O yes O no O don't know	O	O	O	O
Your mother's mother	_____	O yes O no O don't know	O	O	O	O
Your mother's father	_____	O yes O no O don't know	O	O	O	O
Your father's mother	_____	O yes O no O don't know	O	O	O	O
Your father's father	_____	O yes O no O don't know	O	O	O	O

72. How many years have you lived in Canada?

O all my life
___ ___ number of years
O don't know

73. Ethnicity and race sometimes affect disease risk. Scientists have found that some genetic traits are more common or less common among people of different backgrounds. We would like to know if this is true for genes associated with colorectal cancer.

Please fill in the background for yourself, your parents and your grandparents.
Please tick all that apply.

	You	Your mother	Your father	Your Mother's mother	Your Mother's father	Your Father's mother	Your Father's Father
Black, From Africa	O	O	O	O	O	O	O
Black, from Caribbean (Trinidad, Jamaica, Haiti)	O	O	O	O	O	O	O
Black from North America	O	O	O	O	O	O	O
Black, other	O	O	O	O	O	O	O
White	O	O	O	O	O	O	O
First Nations (Indian, Inuit)	O	O	O	O	O	O	O
North African (Egyptian)	O	O	O	O	O	O	O
Middle East (Iranian)	O	O	O	O	O	O	O
Filipino	O	O	O	O	O	O	O
Japanese	O	O	O	O	O	O	O
Korean	O	O	O	O	O	O	O
Chinese	O	O	O	O	O	O	O
Other South East Asian (Vietnamese)	O	O	O	O	O	O	O
South Asian (East Indian, Pakistani)	O	O	O	O	O	O	O
Other: Please specify	_____	_____	_____	_____	_____	_____	_____
Don't know	O	O	O	O	O	O	O

74. Which of the following categories best describes your total annual household income about one year before your recent diagnosis?

O no income
O less than $6,000
O $6,000 - $11,999
O $12,000 - $19,999
O $40,000 - $49,999
O $50,000 - $59,999
O $60,000 - $69,999
O $70,000 - $79,999

O $20,000 - $29,999
O $30,000 - $39,999
O $80,000 +
O don't know

75. In case we need to contact you in the future and you have moved, could we have the name of someone who is not living with you to whom we might write or call for your new address?

Name of relative or friend: _____
His or her address: _____
_____
His or her telephone number: (___ ___ ___) ___ ___ ___ - ___ ___ ___ ___

Thank you very much for taking the time to fill out this questionnaire.
We appreciate your participation.

Please mail this completed questionnaire in the return envelope provided.

Appendix 2. Food Frequency Questionnaire

Canadian Study of Diet and Health

Who this questionnaire is for and what it asks about:

This questionnaire is to be completed by the person taking part in this study:

Part I asks about the foods you ate about **one year before your diagnosis**.

Part II asks about vitamins and other dietary supplements that you may have used.

If possible, please return this questionnaire within two weeks.

The completed questionnaire should be sealed in the pre-paid envelope and mailed back to:

CRC-IHRT,
Room 1758E, Level 1, Health Science Centre,
300 Prince Phillip Drive,
St. John's, NL, Canada, A1B 9Z9.

If you have any questions about this form or the study, call our toll-free number, **1-888-908-4988.**

The information given to us in this questionnaire will be kept confidential.

Thank you for your time and assistance.

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HOW TO COMPLETE THIS QUESTIONNAIRE

We would like to know how often you ate certain foods **about one year before diagnosis,** and their amounts.

Section A ***(lists foods and portion sizes)***
Amounts are described in various ways, including the number of:

cups, teaspoons (tsp), ounces (oz), inches ("), pieces (e.g., 1 apple)
grams (gm), tablespoons (tbsp), millilitres (ml), centimetres (cm).

We want to know the ***Portion Size*** of your **USUAL SERVING.** We have given an example of an average portion size. If your portion size was different than the average, you can indicate this by putting an **X** or ✓in the circles for ***Smaller*** or ***Larger*** portion sizes. ***Smaller*** than average is about 25% or less than the average portion size, while ***Larger*** than average is about 25% or more than the average size. Leave the circle blank if your typical portion size was average.

Included with this questionnaire is a ***FOOD PHOTOGRAPH PAGE*** that shows small, medium and large portion sizes for vegetables, meat and chicken. Some questions ask you to refer to the photo page to help you choose your usual portion size.

Section B ***(asks about how often you ate certain foods one year before diagnosis)***
For each food item listed, choose **one** column (Per Day, Per Week, Per Month, or Never / Rarely) that best describes ***HOW OFTEN*** you ate or drank that item. For example, if you ate CREAM CHEESE 3 times a month during the year of interest, you would write (3) in the *PER MONTH* column. If you ate SWEET POTATOES only 2 times during the year of interest, you can place a checkmark (✓) in *the NEVER OR RARELY* column.

Section C ***(To be completed only for seasonal foods)***
Some foods (for example fresh fruit and vegetables) are not available throughout the year. **For foods that you do not eat all year round** (i.e. in season only), indicate the number of months of the year that you ate them.

Please complete each question as best you can. We know that it is difficult to recall exactly how often you ate something. If you are not certain, try to give your best estimate.

EXAMPLE		Section A			Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food in Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
1	CREAM CHEESE	2 tbs/ 30 ml/ 1 oz	O	O			3		
2	CANTELOUPE	1/8 or 1 slice	O	O		1			4
3	SWEET POTATOES	1 medium/ ½ cup	O	O				√	

Section A					Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
	Dairy Products								
24	EGG (boiled, poached)	1 egg	O	O					
25	EGG (fried, scrambled, omelette)	1 egg	O	O					
26	CREAM CHEESE, Regular fat	2 tbs/ 30 ml/ 1 oz	O	O					
27	CHEESE, Regular fat (such as cheddar, Swiss, processed)	1 slice/ 30 g/ 1oz	O	O					
28	CHEESE, Light (6-15% fat, such as cream cheese, cheddar)	1 slice/ 30 g/ 1oz	O	O					
29	CHEESE, Ultra Light (5% fat or less, such as cheddar)	1 slice/ 30 g/ 1oz	O	O					
30	COTTAGE or RICOTTA CHEESE	125 ml/ ½ cup	O	O					
31	CREAM (coffee, whipping, sour or regular)	1 tbs/ 15 ml	O	O					
32	CREAM (half and half, light sour cream)	1 tbs/ 15 ml	O	O					
33	COFFEE WHITENER (non-dairy)	1 tbs/ 15 ml	O	O					
34	YOGURT, Regular (plain, 2.4% fat or more)	¾ cup/ 175 ml	O	O					
35	YOGURT, Light (plain, less than 2.4% fat)	¾ cup/ 175 ml	O	O					
36	YOGURT, Regular (fruit flavoured or frozen, 2.4% fat or more)	¾ cup/ 175 ml	O	O					
37	YOGURT, Light (fruit flavoured or frozen, less than 2.4% fat)	¾ cup/ 175 ml	O	O					
	Mixed Dishes								
38	SOUPS (creamed)	1 cup/ 250 ml	O	O					
39	SOUPS (non-creamed)	1 cup/ 250 ml	O	O					
40	PEA SOUP	1 cup/ 250 ml	O	O					
41	PASTA with meat sauce (spaghetti, lasagna)	1 cup/ 250 ml	O	O					
42	PASTA with tomato sauce (spaghetti)	1 cup/ 250 ml	O	O					
43	MIXED DISHES with cheese or cheese sauce (macaroni and cheese)	1 cup/ 250 ml	O	O					
44	PIZZA with meat	1 Medium slice	O	O					
45	PIZZA with vegetable only	1 Medium slice	O	O					

Section A					Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
46	MEAT STEW with carrots, other vegetables	1 cup/ 250 ml/ photo A, medium	O	O					
47	CHILI with meat or Con Carne	1 cup/ 250 ml	O	O					
	Vegetables								
48	POTATOES (mashed, boiled, baked etc)	1 medium/ ½ cup/ 125 ml	O	O					
49	FRENCH FRIES or FRIED POTATOES	1 cup/ 250 ml	O	O					
50	CARROTS (raw or cooked)	1 medium/ ½ cup /125 ml	O	O					
51	BROCCOLI	1 cup/ 250 ml	O	O					
52	CABBAGE, COLESLAW	½ cup/ 125 ml	O	O					
53	CAULIFLOWER	½ cup/125 ml	O	O					
54	CORN	1 ear / ½ cup	O	O					
55	PEAS or LIMA BEANS	½ cup/125 ml	O	O					
56	GREEN or YELLOW BEANS	½ cup/125 ml	O	O					
57	BEANS or LENTILS (baked or boiled beans, kidney beans, chickpeas)	½ cup/125 ml cooked	O	O					
58	SPINACH and other green leafy vegetables (greens, collards, kale, mustard greens etc)	½ cup/125 ml cooked or 1 cup raw	O	O					
59	GREEN SALAD (with lettuce)	1 cup/ 250 ml	O	O					
60	CUCUMBER	½ cup/ 125 ml sliced	O	O					
61	TOMATOES (fresh)	1 medium/ ½ cup/ 125 ml	O	O					
62	TOMATOES (canned, pureed or sauce)	½ cup/125 ml	O	O					
63	ONIONS (raw or cooked)	½ cup/125 ml	O	O					
64	BEETS (boiled or pickled)	½ cup/125 ml	O	O					
65	TURNIPS or RUTABAGAS	1 medium/ ½ cup/125 ml	O	O					
66	OTHER ROOT VEGETABLES (sweet potatoes, yams, radish, etc)	½ cup/125 ml	O	O					
67	YELLOW SQUASH (winter type)	½ cup/125 ml	O	O					

Section A					Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
90	LIVER	85 g/ 3 oz	O	O					
91	FRIED CHICKEN	photo C, medium	O	O					
92	CHICKEN / TURKEY (roasted or stewed)	photo C, medium	O	O					
93	CHICKEN / TURKEY, SKIN REMOVED	photo C, medium	O	O					
94	SALTED/ DRIED MEAT	photo C, small	O	O					
95	PICKLED MEAT (brined)	photo C, small	O	O					
96	SHELLFISH (shrimp, lobster, crab)	85 g/ 3 oz/ photo C, small	O	O					
97	FRIED FISH	175 g/ 6 oz/ photo B, medium	O	O					
98	FISH (baked or broiled)	175 g/ 6 oz/ photo B, medium	O	O					
99	CANNED FISH (tuna, salmon)	½ can/ 48 ml/ 1.7 oz	O	O					
100	SMOKED FISH or LOX	85 g/ 3 oz/ photo C, small	O	O					
101	SALTED/ DRIED FISH	85 g/ 3 oz/ photo C, small	O	O					
102	PICKLED FISH	85 g/ 3 oz/ photo C, small	O	O					
103	SEA-BIRDS, SEAL	85 g/ 3 oz/ photo C, small	O	O					
104	CARIBOU, MOOSE	85 g/ 3 oz/ photo C, small	O	O					
105	PARTRIDGE, OTHER WILD BIRDS	85 g/ 3 oz/ photo C, small	O	O					
	Cereals and Grains								
106	BRAN or GRANOLA CEREALS (including All Bran)	½ cup/ 125 ml	O	O					
107	WHOLE WHEAT CEREALS (such as shredded wheat)	½ cup/ 125 ml/ 1 biscuit	O	O					
108	CEREALS, NOT SUGAR COATED (such as Special K)	½ cup/ 125 ml	O	O					
109	HOT CEREALS (for example: oatmeal)	½ cup/ 125 ml	O	O					
110	SUGAR COATED CEREALS	½ cup/ 125 ml	O	O					
111	OTHER BREAKFAST CEREALS	½ cup/ 125 ml	O	O					
112	SUGAR ON CEREAL	1 tsp	O	O					

Section A					Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
90	LIVER	85 g/ 3 oz	O	O					
91	FRIED CHICKEN	photo C, medium	O	O					
92	CHICKEN / TURKEY (roasted or stewed)	photo C, medium	O	O					
93	CHICKEN / TURKEY, SKIN REMOVED	photo C, medium	O	O					
94	SALTED/ DRIED MEAT	photo C, small	O	O					
95	PICKLED MEAT (brined)	photo C, small	O	O					
96	SHELLFISH (shrimp, lobster, crab)	85 g/ 3 oz/ photo C, small	O	O					
97	FRIED FISH	175 g/ 6 oz/ photo B, medium	O	O					
98	FISH (baked or broiled)	175 g/ 6 oz/ photo B, medium	O	O					
99	CANNED FISH (tuna, salmon)	½ can/ 48 ml/ 1.7 oz	O	O					
100	SMOKED FISH or LOX	85 g/ 3 oz/ photo C, small	O	O					
101	SALTED/ DRIED FISH	85 g/ 3 oz/ photo C, small	O	O					
102	PICKLED FISH	85 g/ 3 oz/ photo C, small	O	O					
103	SEA-BIRDS, SEAL	85 g/ 3 oz/ photo C, small	O	O					
104	CARIBOU, MOOSE	85 g/ 3 oz/ photo C, small	O	O					
105	PARTRIDGE, OTHER WILD BIRDS	85 g/ 3 oz/ photo C, small	O	O					
	Cereals and Grains								
106	BRAN or GRANOLA CEREALS (including All Bran)	½ cup/ 125 ml	O	O					
107	WHOLE WHEAT CEREALS (such as shredded wheat)	½ cup/ 125 ml/ 1 biscuit	O	O					
108	CEREALS, NOT SUGAR COATED (such as Special K)	½ cup/ 125 ml	O	O					
109	HOT CEREALS (for example: oatmeal)	½ cup/ 125 ml	O	O					
110	SUGAR COATED CEREALS	½ cup/ 125 ml	O	O					
111	OTHER BREAKFAST CEREALS	½ cup/ 125 ml	O	O					
112	SUGAR ON CEREAL	1 tsp	O	O					

Section A					Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
113	100% WHOLE GRAIN or DARK BREAD	1 slice	O	O					
114	60% WHOLE GRAIN, LIGHT RYE	1 slice	O	O					
115	WHITE BREAD	1 slice	O	O					
116	WHITE BREAD ROLLS (including hot dog buns etc)	1 roll	O	O					
117	WHOLE WHEAT ROLLS	1 roll	O	O					
118	CRACKERS (snack or soda type)	2	O	O					
119	BRAN/OAT MUFFIN	1 medium, ½ extra large	O	O					
120	OTHER MUFFIN (plain cake, with berries)	1 medium, ½ extra large	O	O					
121	PANCAKES, WAFFLES	1	O	O					
122	MACARONI, SPAGHETTI, NOODLES (plain)	1 cup cooked/ 250 ml	O	O					
123	RICE	½ cup cooked/ 125 ml	O	O					
124	CRISP SNACKS (potato chips, popcorn, pretzels etc)	small bag or 1 cup	O	O					
	Fruits								
125	APPLE, PEAR	1	O	O					
126	CITRUS FRUITS (orange, grapefruit)	1 orange, ½ grapefruit	O	O					
127	BERRIES (strawberries, blueberries, bakeapples)	½ cup/ 125 ml	O	O					
128	GRAPES	½ cup/ 125 ml	O	O					
129	BANANA	1	O	O					
130	PEACH, PLUM, NECTARINE, APRICOT	1	O	O					
131	CANTALOUPE	1/8 or 1 slice	O	O					
132	WATERMELON	1 wedge, 3" base	O	O					
133	HONEYDEW MELON	1/8 or 1 slice	O	O					
134	MANGO	1	O	O					
135	PAPAYA	1	O	O					
136	APPLESAUCE	½ cup/ 125 ml	O	O					

Section A					Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
137	DRIED FRUITS (raisins, dates, prunes)	2 tbsp/ 2 dates	O	O					
138	CANNED FRUIT (all kinds)	½ cup/ 125 ml	O	O					
139	ALL OTHER FRUIT (fresh kiwi, pomegranate, etc.)	1	O	O					
	Desserts and Sweets								
140	CAKES	1 slice, 2" x 4" x 1"	O	O					
141	PIES and TARTS	1 slice	O	O					
142	DONUTS and SWEET ROLLS	1	O	O					
143	COOKIES	1	O	O					
144	ICE CREAM	½ cup/ 125 ml	O	O					
145	LIGHT or DIET ICE CREAM	½ cup/ 125 ml	O	O					
146	PUDDING	½ cup/ 125 ml	O	O					
147	DIET or LIGHT PUDDING	½ cup/ 125 ml	O	O					
148	JELLO	½ cup/ 125 ml	O	O					
149	POPSICLES, FREEZIES	1	O	O					
150	CHOCOLATE BAR and CHOCOLATE CANDY	1 bar / 50g or 5 candy size	O	O					
151	CANDY (without chocolate)	1 caramel	O	O					
	Miscellaneous								
152	TOFU, TEMPEH	½ cup, 2" x 2" x 1" piece	O	O					
153	KETCHUP	1 tbs	O	O					
154	MAYONNAISE/ MIRACLE WHIP, Regular fat (on bread, salad, meat, etc)	1 tbs	O	O					
155	MAYONNAISE/ MIRACLE WHIP, Light (on bread, salad, meat, etc)	1 tbs	O	O					
156	SALAD DRESSING, Regular fat (French, Italian etc)	1 tbs	O	O					
157	OIL (in cooking)	1 tsp	O	O					

Section A					Section B YEAR BEFORE DIAGNOSIS				Section C
	FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
			Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year
158	BUTTER (on vegetables or bread; exclude use in baked and mixed dishes)	1 pat/ 1 tsp	O	O					
159	MARGARINE (on vegetables or bread; exclude use in baked or mixed dishes)	1 pat/ 1 tsp	O	O					
160	PEANUT BUTTER	1 tbs	O	O					
161	PEANUTS	30g/ 1 oz	O	O					
162	OTHER NUTS	30g /1 oz	O	O					
163	JAM, JELLY, HONEY, SYRUP	1 tbs	O	O					
164	GRAVY	4 tbs	O	O					
165	CHOCOLATE or STRAWBERRY SYRUP	1 tbs	O	O					
166	CHOCOLATE SPREADS	1 tbs	O	O					
167	SAUCES (white, cream, Mornay)	30 ml/ 1oz/ 2 tbs	O	O					
168	WHEAT BRAN	1 tbs	O	O					
169	WHEAT GERM	1 tbs	O	O					

Continue on next page ⟶

Now we would like to ask you a few questions about how you prepared certain foods ABOUT ONE YEAR BEFORE DIAGNOSIS and whether you followed any special diets. For the following questions, please check the circle or fill in the appropriate answer:

1. About 1 year before diagnosis, how much of the visible fat on your meat did you eat?

- O Most of it.
- O Some of it.
- O As little as possible.
- O Do not eat meat

2. About 1 year before diagnosis, how often did you eat the skin on chicken?

- O Most of it.
- O Some of it.
- O As little as possible.
- O Do not eat chicken

3. About 1 year before diagnosis, what kind of fat did you usually use for stir/pan frying?

- O Vegetable oil
- O Vegetable shortening
- O Lard/ pork fat
- O Butter
- O Margarine
- O Do not add fat or oil
- O Other, please specify _____

4. About 1 year before diagnosis, what kind of fat did you usually use for deep frying?

- O Vegetable oil
- O Vegetable shortening
- O Lard/ pork fat
- O Butter
- O Margarine
- O Do not fry
- O Other, please specify _____

5. About 1 year before diagnosis, what kind of fat did you usually use for baking?

- O Butter
- O Margarine
- O Vegetable Oil
- O Vegetable shortening
- O Lard/ pork fat
- O Do not bake

6. About 1 year before diagnosis, what type of oil did you use in other preparations (for example, in salad dressings)?

_____

7. About 1 year before diagnosis, what type of the following items did you usually use? *Please check one box per line.*

Mayonnaise/Miracle Whip

O regular O light O both O none

Cream cheese

O regular O light O both O none

8. About 1 year before diagnosis, were you a (*please check one box only*):

- O Non-vegetarian (eats all meat, chicken, fowl)
- O Partly non-vegetarian (eats chicken, fish, no meat)
- O Vegan (eats no dairy, no eggs, no meat)
- O Lacto-vegetarian (eats dairy, no eggs, no meat)
- O Lacto-ovo vegetarian (eats dairy & eggs, no meat)

9. About 1 year before diagnosis, were you on a special diet?

O No O Yes

If yes, what type of diet?

- O To lose Weight
- O To lower cholesterol
- O Diabetes
- O Heart disease
- O Hypertension
- O Gastric ulcer
- O Bowel disease
- O Low fat
- O High fibre
- O Other type: _____

If yes, how long were you on the special diet?

_____

PART 2 - USE OF VITAMINS AND DIETARY SUPPLEMENTS

Now we would like to know about your use of vitamins and dietary supplements.
ABOUT ONE YEAR BEFORE DIAGNOSIS, did you take any of the following? If *Yes*, then specify usual brand and amount and how long you took them.

Vitamin and Amount – if used, ⇨	How many pills did you take per week?	How long had you taken them?
Vitamin C EXAMPLE O None O Below 500 Ø 500-1000 O above 1000 mg	0 5 per week	2 4 months
Multivitamins that include minerals ⇨ O No O Yes If yes, usual brand _____	per week	months
Multivitamins, no minerals O No O Yes If yes, usual brand _____	per week	months
B Complex vitamins O No O Yes If yes, usual brand _____	per week	months
In the following items, DO NOT INCLUDE use of the above MULTIVITAMINS		
Vitamin A O None O Below 10000 O 10000-15000 O above 15000 IU	per week	months
Vitamin C O None O Below 500 O 500-1000 O above 1000 mg	per week	months
Vitamin E O None O Below 400 O 400-800 O above 800 IU	per week	months
Beta-carotene O None O Below 10000 O 10000-15000 O above 15000 IU	per week	months
Folic acid O None O Below 1.0 O 1.0 mg O above 1.0 mg*	per week	months
Calcium O None O Below 250 O 250-500 O above 500 mg	per week	months
Iron O None O Below 100 O 100-200 O above 200 mg	per week	months
Other dietary supplements (e.g., yeast, cod liver oil, etc) O No O Yes, specify type: _____	per week	months
_____	per week	months

* 1 mg = 1000 micrograms

We welcome any other information or comments that you would like to give us:

_____

_____

_____

THANK YOU VERY MUCH for your assistance in this research!

For Office Use Only

Study #: _____

Interviewer: _____

Date completed (D/M/Y): _____