Clinical phenotype of Endometrial Carcinoma in Lynch Syndrome: *MSH2* mutation carriers.

By

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Abstract

Objective

The purpose of this study is to compare histological and clinical variables of individuals with Lynch Syndrome associated Endometrial Carcinoma with a cohort with sporadic Endometrial Carcinomas derived from the general population with sporadic Endometrial Carcinomas. The patients in the Lynch Syndrome cohort were genetically confirmed carriers of *MSH2* mismatch repair gene mutations all with previously diagnosed Endometrial Carcinoma.

Methods

Clinical data was abstracted retrospectively from the medical charts of 46 women with endometrial caner who had a known *MSH2* mismatch repair mutation confirmed through genetic sequencing. Clinical variables abstracted from the medical files of these patients included (1) Age at diagnosis (2) International Federation of Gynecology and Obstetrics (FIGO) Stage (3) International Federation of Gynecology and Obstetrics Grade and (4) Cell type of endometrial carcinoma. The characteristics of the *MSH2* carriers were subsequently compared to the clinically relevant variables of sporadic endometrial cancers that were retrieved from the Newfoundland and Labrador Cancer Care Registry (NLCCR) diagnosed between 2000 and 2010. The Newfoundland and Labrador Cancer Care Registry is a provincial cancer care program and database operated by Eastern Health that combines 5 core cancer programs and registries. The NLCCR includes the provinces Colon, Breast and Cervical screening programs and the provincial tumour and systemic therapy surveillance programs.

Results

The mean age at diagnosis of Endometrial Cancer (EC) in the *MSH2* Lynch syndrome mutation carriers was 46.3 years vs. 60.9 years in the sporadic cohort (p=<0.001). The Lynch Syndrome ECs were diagnosed more frequently prior to 55 years of age (p=<0.001). Comparing local and advanced stages of disease, the Lynch Syndrome cohort had more advanced disease at diagnosis (p=0.047). The prevalence of papillary serous cell type carcinomas in the Lynch Syndrome (23.7%) cohort was statistically more frequent than in the sporadic cohort (3.6%) (p=< 0.001). Clear cell carcinomas were observed more frequently in Lynch Syndrome related EC (7.9%) compared to the sporadic cohort (0.8%) (p=<0.001). The prevalence of grade 3 tumours in the Lynch Syndrome related EC cohort was higher compared to the sporadic cohort; 32.4% and 11.9% respectively (p=0.001). Merger of low-grade (1/2) tumours compared to high-grade (3) tumours observed the Lynch Syndrome cohort to present with higher-grade tumours. In the Lynch Syndrome cohort 69.6% had endometrial carcinoma as a sentinel cancer. Survival after diagnosis of EC was similar in each cohort (p=0.068). Logistic regression models indicated that a diagnosis of EC prior to age 55 and a histological diagnosis of papillary serous/clear cell carcinoma to were independently associated with LS (p=<0.001 and p=<0.001 respectively). Multivariate analysis demonstrated that grade, stage of disease, age and cell type were independently associated with a diagnosis of LS.

Conclusion

This is a preliminary study focusing on the clinical features present in Lynch Syndrome related endometrial carcinomas in women carrying *MSH2* mismatch repair mutations. This study serves as a pilot study for a larger, population-based study of the genetics and epidemiology of endometrial carcinomas in Newfoundland and Labrador. We have concluded that Lynch Syndrome associated endometrial cancers are diagnosed at a younger age than the endometrial cancers in the general population, and that prevalence of cell types with unfavorable prognosis was higher in Lynch Syndrome related endometrial carcinomas. The stage of cancer in the Lynch syndrome related endometrial carcinoma cohort at diagnosis was more advanced, and was associated with a higher histological grade. Multivariate analysis found these characteristics to be predictive of LS**.** Lynch Syndrome related EC patients demonstrated no difference in survival ($p=0.068$) when compared to the sporadic cohort via Kaplan-Meier survival analysis.

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Chapter 1: Introduction

In a 1966 paper published by Dr. Henry T. Lynch described two large kindreds with familial cancer syndrome from the Midwestern United States; Families "M" and "N". These two families were remarkably similar to a large pedigree (Family "G") published by Warthin *et al* (1913). These three families in conglomeration with enumerable similar families over the decades led to the description of the classic phenotype and subsequent genotype of Lynch Syndrome still being described today.

The term Lynch Syndrome has often been confused with and misused as being synonymous with Hereditary Non-Polyposis Colorectal Cancer (HNPCC) throughout the literature. This is most likely due to the initial recognition of the familial cancers that were predominantly colorectal adenocarcinomas, in turn leading to genetic discovery of LS. HNPCC is a clinical diagnosis of an individual or family that satisfies the Amsterdam or the revised Amsterdam II criteria, conversely LS is defined by the presence of a germline mutation in a DNA mismatch repair (DNA-MMR) gene; these genes include *MSH2*, *MSH6*, *MLH1*, and *PMS2*. This is incredibly important for maintaining clarity within the research field and in order to make a clear comparison between studies that tend to use these terms interchangeably. Not differentiating LS from HNPCC leads to ambiguity in research findings and difficulty when it comes to applying research to patient management recommendations including risk stratification (Kravochuck, 2014). Moving forward, it is of the upmost importance that researchers make a clear distinction between the two terms in order to further develop the understanding of the genotype-phenotype relationship in LS as well as increasing the comparability of studies and their

cohorts. Moving forward in this study the term LS will be used to describe probands with a confirmed DNA-MMR mutation (Kravochuck, 2014).

Lynch Syndrome is defined in terms of the presence of a germline mutation in DNA mismatch repair (MMR) genes including mutations at the *MSH2*, *MSH6*, *MLH1*, or *PMS2* loci. LS is characterized by vertical progression through pedigrees demonstrating autosomal dominant inheritance pattern as well as early age of diagnosis of ECs and colorectal cancer. Many individuals affected with LS are observed to have an increased prevalence of synchronous and metachronous neoplasms. Additionally, this hereditary cancer syndrome has been associated with a spectrum of neoplasms besides colorectal and endometrial cancers that include cancers of the genitourinary tract, ovaries, biliary tract, pancreas, and the brain (Stuckless, 2007). LS demonstrates incomplete penetrance in that not all individuals carrying a MMR gene mutation will present with the disease phenotype; penetrance is influenced by the mutation possessed by probands. The lifetime risk of developing an EC in individuals with LS is estimated to be 30-70% (Stuckless 2007); certain studies demonstrate the risk of EC is equal to or exceeds the risk of compared to colorectal cancer.

Current literature describing the genotype-phenotype relationship in LS carriers has collectively compared all variations of LS mutations to sporadic control groups without mutation status. While this is understandable given the low incidence and prevalence of mutation carriers this provided an opportunity in research for this particular study. The province of Newfoundland and Labrador presents a unique and valuable

population for studying the propagation of autosomal dominant mutations. The island of Newfoundland was populated in the late 1700 or early 1800's by immigrants from either the Southwest of England or the Southeast or Ireland with very little migration within the island up until recently with modern transportation. The province currently has a population of over 500,000 people of which approximately 90% can trace their roots back to these original 20-30,000 settlers (Parfrey *et al*, 2002). The benefit of conducting a study of the clinical features of a cancer predisposition syndrome such as Lynch Syndrome in a region such as Newfoundland and Labrador (NL) originates from the events that led to the colonization of the province. Newfoundland is the most easterly Canadian province, and is an island situated in the North Atlantic. The island is characterized by the founder effect, large family size and little in or out migration since the original founding populations settled in the late 18th and early 19th century (Parfrey *et al*, 2002).

The groundwork for this current study originated from previous investigations of large Newfoundland families with autosomal dominant disorders in the province (Parfrey *et al*, 2002). In this particular population of the homogeneity of the environmental exposures experienced by the probands, diminishes their potential confounding effects on measured clinical outcomes. These previous studies have also been facilitated by individuals eager to participate in research, and true population-based health care with one tertiary care centre, one provincial medical genetics service and a single tumour registry. Almost 50% of all colorectal probands in this province meet the Revised

Bethesda and/or Amsterdam II criteria representing the highest rate of familial colorectal cancer in the world (Parfrey *et al*, 2002).

In 1993 a large multi-generational NL family with colorectal and other cancer participated in the original study that located a locus on chromosome 2p demonstrating the hereditary link this locus and a predisposition to colorectal cancer (Peltomaki, 1993). This discovery, subsequently lead to the identification of the germline mutation in the *MSH2* gene in the 5' splice site of intron 5 resulting in an in-frame deletion of exon 5 in messenger RNA. This *MSH2* mutation was subsequently found in 12 other Newfoundland families originally from Bonavista North. Initial investigations into the genotype-phenotype relationship of this germline mutation identified males as being at higher risk of colorectal cancer and death than to female mutation carriers (Green, 2002). Continued analysis of other high-risk colorectal and other cancer kindreds lead to the discovery of two additional germline mutations in the *MSH2* gene in families from Newfoundland and Labrador. A genomic deletion in exon 8 was present in these families and an exon 4-16 deletion was found in another family. The tumours associated with these mutations lack expression of the *MSH2* mismatch repair protein that helps regulate and correct errors in DNA during replication.

Currently, research has not well described a specific genotype-phenotype relationship between all identified germline mismatch repair (MMR) protein mutations has not been well described, and whether carriers of different MMR genes have different associated cancer risks is unclear. Lynch Syndrome carriers have been shown to be at risk

for a spectrum of difference carcinomas however gynecological cancers have been identified as being at the top in terms of risk for female probands, above colorectal adenocarcinomas. Gynecological cancers have been termed as the *sentinel* or first-diagnosed cancer, in a population of carriers that have been diagnosed with both colorectal and gynecological (uterine/ovarian) carcinomas (Lu, 2005).

Most recent studies demonstrating the association between Lynch Syndrome mutations and EC have combined cohorts of germline mutation-carriers consisting of different proportions of carriers of *MSH2*, *MSH6*, *MLH1*, and *PMS2* mutations. The current study was undertaken in order to determine the genotype-phenotype relationship of the three variants of the *MSH2* MMR-gene mutations in this province. We sought to evaluate the possible effects the specific mutation has on the clinical and pathological characteristics of the cancer, as well as the overall survival of mutation carriers.

Chapter 2: Literature Review

2.1 Background

The uterus is a pear-shaped organ located in the pelvis between the bladder and the rectum. The uterus is composed of three layers; the serous layer of the perimetrium that covers the outer surface of the organ; the central or middle layer, the myometrium which is composed primarily of smooth muscle as well as stromal vascular tissue, and the lining of the uterine cavity called the endometrium that is composed of the basalis and functionalis layers. The endometrium is the most dynamic layer as it is greatly influenced by the fluctuation of hormones that occur in the normal female reproductive or menstrual cycle. During periods of abnormal cellular function, secondary to hormonal influence, hereditary errors in cellular proteins, or in other pathways of carcinogenesis not yet described, can result in abnormal growth of endometrium along with the ability of these cells to proliferate uncontrollably (Blausteins, 2011). Uncontrolled division when normal cell regulatory inhibition or apoptosis has been evaded can lead to endometrial adenocarcinoma. The neoplasm can spread locally within the cavity of the uterus, invade through the myometrium, or in some cases metastasize throughout the body of the affected individual.

Endometrial adenocarcinoma (EC) is the most common malignancy of the female genital tract of the Western World, and is the fourth most common cancer in women behind breast, lung and colorectal cancer (Canadian Cancer society, 2017). Based on 2017 data, an estimated 1 in 36 women will be diagnosed with EC in their lifetime, and 1 in 156 women will die from the disease. The vast majority of women will be diagnosed

between 50 to 80 years of age, with an average age of 65. The incidence of EC will lead to approximately 7,300 Canadian women being diagnosed, and 1,150 of these women will succumb to the disease annually, based on data for 2017. The incidence of EC is gradually increasing in Westernized countries, and in Canada rates have increased 2.6% since 2004 which is a similar to the trend highlighted from the United States (Canadian Cancer Society, 2017).

Endometrial carcinoma (EC) refers to a number of different cancers that develop in the endometrium or epithelial lining of the uterine cavity. From a pathogenic viewpoint, there are two clinicopathologic subtypes of EC that are currently recognized as alternate pathways of carcinogenesis of disease. Type I or endometrioid EC, and Type II or non-endometrioid EC. Type I endometrioid ECs are estrogen-related tumours that typically develop in the pre and peri-menopausal period. These malignancies often coexist with or are preceded by atypical endometrial hyperplasia. Atypical endometrial hyperplasia is a condition of excessive proliferation or multiplication of the cells in the lining of the endometrium and is often a result of exposure to higher than normal levels of endogenous estrogen; a precursor for Type I cancers. Factors that result in an increase of endogenous estrogen (e.g. obesity, estrogen-replacement therapy, or estrogen-secreting tumours) will increase the risk for developing type I tumours, whereas factors that decrease the exposure to endogenous sources of estrogen or increase the exposure to progesterone (e.g. multiparity and oral contraceptives) can decrease an individuals risk (Okuda, 2010). In the Western World approximately 80% of all diagnosed ECs are of endometrioid cell type. Endometrioid tumours are on average diagnosed in women 65

years of age, of which, approximately 70% are confined to the corpus of the endometrium at the time of diagnosis (Creasman, 2006). Endometrioid tumours are reported to have a relatively good prognosis, as their progression rate is slow, and their detection rate is high; the cardinal symptom being abnormal vaginal bleeding. Type I tumours are associated with a 5-year overall-survival rate of 83% (Creasman, 2006).

Non-endometrioid or Type II tumours are more commonly diagnosed in postmenopausal women with an average age of diagnosis of 67 years. The development of these tumours, unlike Type I, is not associated with an increased exposure to estrogen. Type II tumours will account for approximately 10-20 percent of all ECs (Creasman, 2006). Type II tumours include all tumours with non-endometrioid cell histology; clear cell carcinoma, papillary serous, mucinous, squamous, transitional cell, and undifferentiated tumours are considered within the non-endometrioid classification. Type II tumours are found to be more aggressive than their Type I counterparts, and as a result approximately 50% of non-endometrioid tumours have spread beyond the corpus of the uterus at the time of diagnosis. A malignancy that has breached the serosa of the body of the uterus is in turn classified as a more advanced stage of disease and with that carries a less favorable prognosis. The 5-year survival for cases varies according to cell type; clear cell carcinoma and papillary serious having a 5-year survival rate of 62% and 53% respectively (Creasman, 2006).

Following the Federation of Gynecology and Obstetrics (FIGO) meeting in 1988, it was concluded that all ECs should be surgically staged in order to assess prognosis, and

future treatment for patients. Stage of an EC is the variable most closely correlated with predicting prognosis, and patient survival. The stage of an EC is classified based on the invasiveness of the cancer into the uterus, as well as migration to local and distal sites in the body. An early stage of disease is when the malignancy is localized to the corpus or cavity of the uterus. This early classification of disease is limited to stages I/II with varying degrees advancement including the depth of myometrial invasion as well as the presence of cervical involvement (Blausteins, 2011). An advanced stage of endometrial cancer is defined by a tumour expanding through the full thickness of the myometrium, and exiting the body of the uterus through the exterior epithelial serosal layer into surrounding organs, and structures within the pelvic cavity. As the tumour exits the uterus into the pelvic cavity the tumour may invade surrounding structures such as the fallopian tubes, ovaries (i.e. adnexa), the vagina/parametrium, bladder or bowel and potentially metastasize to distal sites of the body through the lymphatic system. The progression of the tumour to distal sites results in a diagnosis of an advanced stage of disease, and in turn drastically affecting patient prognosis. Although stage is the variable most tightly correlated in predicting patient prognosis, there are a number of other prognostic variables that exhibit an influence on prognosis among patients with the same stage of disease (Blausteins, 2011).

Tumour grade is a variable evaluated by a clinical pathologist in order to assess a tumour or neoplasm appearance at the cellular level under light microscopy. Grade is a reflection of the level of differentiation or anaplasia of tumour cells. Anaplasia is the degree to which cells have dedifferentiated, or the degree in which the morphological and

functional characteristics of a mature cell have been lost compared to a native cell in that tissue or organ. In other words, grade is an assessment of how specialized or differentiated a cell has become in comparison to a cell that has gone through normal cellular development in the same tissue or origin. Grade is evaluated by a number of criteria that point to the possibility of malignant transformation and is scaled from Grade 1 to 4 (or G1-G4). Cells have become more undifferentiated while ascending the scale; meaning that as you move up the grade scale, cells are becoming visually and functionally increasingly different from a normal cell in a given tissue (Blaustein, 2011). Tumour cells that appear well organized, have similar structure and function to the normal/native cells of the uterus are classified as grade 1 or 2 (well and moderately differentiated tumours respectively). Grade 1 and 2 low-grade tumours are characteristically much less invasive than tumours with cell types that present on the other end of the grade scale. Grades 3 and 4 (poorly differentiated and undifferentiated tumours) are considered high-grade tumours. They are comprised of abnormal cells and disorganized tissue structure. These tumours are typically more aggressive, and are associated with a higher incidence of advanced stage of disease and metastases (Lippincott, 2010).

Identification of an individual with a potential EC is usually suspected upon the presentation of abnormal vaginal bleeding, most commonly found in postmenopausal women. Abnormal vaginal bleeding is the cardinal symptom associated with EC that occurs in seventy-five to ninety percent of all cases. Therefore, women who present with abnormal vaginal bleeding should proceed to have an outpatient endometrial pipelle

biopsy. If the initial biopsy is not successful or sample is inadequate or returns with a negative result, a more sensitive test is recommended completed. The most common procedure to definitively diagnose an EC carcinoma is a dilation and curettage under general anesthesia, and is considered the gold standard. A dilation and curettage is performed by initially dilating the cervical os by mechanical means in order to gain access to the body of the uterus. If a mechanical dilation fails, Misoprostol may be used as an adjunct to help dilate the cervical os. (Blaustein, 2011). The second step of the procedure involves the surgical removal of the lining or contents of the uterus in order for pathological review. The histological findings that result from the endometrial pipelle biopsy or dilation and curettage will decide whether a referral to a gynecological oncologist is necessary and will determine the course of treatment for the individual.

Despite the incidence of EC affecting Westernized countries including the Canadian population, there is currently no evidence for screening asymptomatic women for this malignancy. The Papanicolaou smear (pap smear) that is currently used to screen for atypical pre-cancerous or cancerous cells of the cervix and endocervix, will detect an endometrial cancer approximately 50-percent of the time. Women detected with pre-cancerous or malignant endometrial cells using this method are shown to have deeper myometrial invasion, higher tumour grade and more advanced stage of disease; all of these characteristics are predictive of poor patient outcomes (Lippincott, 2010). Screening for endometrial cancer or its precursor cells is warranted in certain high-risk individuals; including postmenopausal women who are taking exogenous estrogens without progestins, premenopausal women with anovulatory cycles, and women with a

positive family history are classified as higher risk individuals. HNPCC criteria state that women who have been diagnosed with a mismatch repair gene mutation or Lynch Syndrome should receive regular screening for endometrial cancers and potentially other types of cancers.

2.2 Lynch Syndrome

Colorectal cancer or large bowel cancer were the malignancies originally associated with and studied as the primary malignancy associated with families that fell in the criteria of HNPCC. As research proliferated in the field, and the body of knowledge surrounding HNPCC expanded, there was a realization that these hereditary DNA-MMR mutations were linked to not just colorectal cancer. Malignancies within the Lynch Syndrome spectrum include cancers of the colon, endometrium, ovary, stomach, upper urinary tract, small-bowel, skin and brain. The identification of this array of malignancies and subsequent identification of the genes involved in what is known to be LS characterize this cancer predisposition syndrome named after Dr. Henry T. Lynch, who was one of the first researchers to describe the hereditary cancer syndrome (Lynch, 2009).

Lynch Syndrome and its associated cancer susceptibilities are diagnosed through confirmatory germline analysis of one or more of four DNA-mismatch repair (DNA-MMR) gene mutations, *MLH1* (MutL homolog 1; chromosome 3p21), *MSH2* (MutS homolog 2; chromosome 2p16), *MSH6* (MutS homolog 6; chromosome 2p16), and *PMS2* (postmeiotic segregation 2; chromosome 7p22). Mutations in these genes result in a

deficient mismatch repair (MMR) protein system in the cells of the body. The MMR protein system functions to preserve the structural, and functional integrity of the DNA alpha-helix through the correction of insertion/deletion nucleotide mutations that occur during DNA-replication. There are two heterodimeric proteins that recognize nucleotide mismatches in a functioning MMR system. The MutS-α heterodimeric protein complex is formed by the *MSH2* and *MSH6* proteins, and is involved in the correction of single nucleotide mismatches. The MutS-β protein complex is comprised of the *MSH2* and *MSH3* proteins, and is more commonly involved with the recognition of larger insertion/deletion loops (Masuda, 2011). The deactivation of a proper functioning MMR system results in an increased rate and accumulation of uncorrected mutations in the DNA structure. Insertion/deletion mutations typically occur in microsatellites regions that are composed of tandem dinucleotide repeats within the DNA that comprise approximately 3% of the gross human genome (Baudhuin, 2005). The increased frequency of mutations in microsatellite regions results in a structural imbalance in the DNA alpha helix resulting in slippage or bowing of DNA structure manifesting as microsatellite instability (MSI); a signature characteristic of a proportion of LS-related cancers. The increased frequency of mutation as a result of the impeded MMR-system is theorized to lead to the alteration of many other nucleotides involved in numerous cellular pathways that limit cell growth, regulate cell death, and together accumulate to form the driving force behind the carcinogenic process in LS related malignancies. Given the different carcinogenic pathway associated with LS related malignancies as compared to Type I and Type II sporadic endometrial cancers, it has been of interest to researchers to evaluate the clinical phenotype of LS based endometrial cancers to observe differences

in that area as well.

Recent population estimated Lynch Syndrome mutation prevalence in the region of 1 in 300 to 1 in 500 in the general population. Lynch Syndrome is characterized by an autosomal dominant pattern of inheritance, in that offspring of a proband have a fifty percent chance of an inheriting an inactivating mutation of mismatch repair (MMR) genes. The recognition of Lynch Syndrome families through pedigree and detection guidelines is vitally important for the proper care and future screening of family members found to have a germline mutation. The identification of these families allows for implementation of proven cancer prevention strategies and provides the opportunity for further research into LS families**.**

2.2.1 Age

Lynch Syndrome was originally categorized by early onset of cancerous tumours developing in the proximal end of the colon. Further research in the field of LS led to the discovery that extra-colonic regions were also at an associated increased risk of developing neoplasms. In a paper published in 2005 by Lu *et al,* ECs were reported to be most often the first or *sentinel cancer* for women with this cancer susceptibility syndrome. Lu observed that of 101 patients that were diagnosed with two primary synchronous cancers of gynecological origin (endometrial carcinoma/ovarian cancer) and the other a colorectal cancer, that 51% (n=52) of the time these women had been diagnosed with EC or ovarian cancer prior to the diagnosis of colorectal cancer. The mean age of the probands diagnosed with endometrial/ovarian cancer first was 44 years

of age. Conversely, 49% (n=49) of the women diagnosed with colorectal cancer as their first cancer with an average age of 40 years of age at diagnosis. This finding by Lu lead to the term of *sentinel cancer*, suggesting that women with suspected LS would present with a gynecological cancer prior to the development of colorectal cancer more than half of the time. This would indicate that for families with LS or who fall into the classification of HNPCC by the Amsterdam II or Revised Bethesda guidelines, a gynecologist plays a pivotal role in the care of these patients. Additionally, professionals in the field of Obstetrics and Gynecology should be aware of the early onset of gynecological cancers among women with this cancer susceptibility syndrome. The diagnosis of EC in a woman provides the opportunity to find LS and prevent further cancers.

The observation that women presenting at younger ages with EC increase the likelihood of carrying a LS mutation has long been recognized. A cohort of women under the age of 40 that were diagnosed with EC were analyzed via immunohistochemistry in attempts to evaluate their tumour expression for DNA-mismatch repair (DNA-MMR) proteins. Each of the patient tumours were evaluated for four protein markers *MLH1, MSH2, MSH6,* and *PSM2.* In the total 54 tissue blocks that were examined there was loss of expression of at least one MMR protein in 9 of 54 cases (16%). The lack of protein expression slightly favored the *MSH2*/*MSH6* abnormalities (five of nine) compared with loss of *MLH1*/*PMS2* (four of nine). Though this study demonstrates a correlation between younger age of diagnosis of EC and lack of MMR protein expression this does not qualify as a diagnosis of LS. A continuation of this study evaluating germline mutations

in DNA-MMR genes associated with LS would have greatly enhanced this study.

The younger age of diagnosis of ECs in LS probands has been utilized to aid in determining the prevalence of loss of MMR protein expression in younger populations leading to potential LS families. In a study conducted by Lu *et al* (2007) that compiled a cohort of women diagnosed with EC prior to 50 years of age, germline mutation analysis was performed in order to evaluate deletions in *MLH1*, *MSH2*, and *MSH6* genes. Tumour studies conducted included immunohistochemistry analysis for expression of MMR proteins (*MSH2, MSH6,* and *MLH1*), microsatellite instability, and hypermethylation of the *MHL1* promoter region. The analysis found that of the 100 women involved in the study nine (9%) were found to have deleterious mutations in DNA-MMR genes. The majority of the nine deleterious mutations were in *MSH2* with a total of seven *MSH2* mutations followed by a single mutation each in the *MLH1* and the *MSH6* gene variants. The study also concluded that in addition to younger age of onset of ECs, that Body Mass Index (BMI) could also help identify LS probands as they present with lower BMIs. In a retrospective research study performed by Shih *et al* (2011) a pathological review was carried out where pathologic data of patients (n=56) diagnosed with EC before or at 40 years of age in order to perform DNA-MMR immunohistochemistry analysis on the endometrial tumours to try to detect presumptive-LS probands. This study also examined clinical risk factors as well as outcomes for these patients with EC under the age of 40 with EC. The researchers identified nine patients (16%) of the 56 with loss of expression of MMR proteins via immunohistochemistry. The germline mutation status of the individuals involved in this analysis were unknown. Though this does not constitute a

diagnosis of LS, this study further emphasizes the link between a dysfunctional MMR system and early age of diagnosis of ECs. Similar to other study findings, lower BMI was significantly associated with loss of expression of tumour MMR proteins (Lu 2007).

A study conducted by Walsh *et al* (2010) on a cohort of patients with early-onset EC with diagnosis prior to 50 years of age, had tumour sections undergo histopathological review via light microscopy. The tumours were, immunostained and evaluated for *MHL1*, *MSH2*, *MSH6*, and *PSM2* MMR proteins. The tumours were also evaluated for hypermethylation of the *MLH1* promoter region as well as microsatellite instability. Individuals in the cohort were given a diagnosis of presumptive-LS when they presented with loss of expression of at least one MMR protein, and tested negative for *MLH1* hypermethylation. Presumptive-LS was identified in 26 (18%) of the 146 analyzed tumours. Family history was assessed in cases where available, and presumptive-LS cases were more likely to be associated with a positive family history when compared with families meeting the Amsterdam Criteria II ($p < 0.05$). The authors conclude strongly recommending screening for LS MMR mutations in women who present with ECs at or prior to 45 years of age.

Although the younger age of diagnosis has been utilized as a possible LS identifier in aiding the search for individuals with MMR gene mutations, there currently exists no clear-cut diagnostic strategy for identifying LS carriers. A diagnostic strategy was the purpose of a study performed by Leenen *et al* (2012). The prospective study evaluated 179 consecutive ECs diagnosed in patients less than 70 years of age. The study

examined tumour tissue samples from the patients in the cohort in order to assess MMR protein expression, microsatellite instability status, and BRAF-mutations status. The tumours that were identified as microsatellite instability high (MSI-H) cancers, and showed loss of expression of *MLH1* proteins, underwent an additional testing for hypermethylation of the *MLH1*-promoter region. This additional test was conducted in order to distinguish if the loss of protein expression was due to a true germline mutations at the *MLH1* promoter region or if the region was hypermethylated and in turn silenced or under-expressed. After the molecular analysis of the 179 consecutive ECs eleven (6.1%) patients were diagnosed with presumptive-LS. The average age of the eleven women with the presumptive-LS status was 59 years at diagnosis, which did not differ from the median age of sporadic MSI-H tumours ($p = 0.19$) or microsatellite stable (MSS) tumours ($p = 0.46$). All of the eleven women were then referred to the department of clinical genetics in order to undergo germline analysis. Ten of the eleven women accepted the referral for confirmatory genetic testing. Seven of the ten women sent to clinical genetics were found to have germline mutations in a DNA-MMR gene. Mutations in *MSH6* DNA-MMR gene were found in six individuals with an average age of diagnosis of 55 years. The single *PSM2* mutation carrier was diagnosed at the age of 69 years. This study found that seven (4%) of 179 women diagnosed ≤ 70 years of age were found to have germline mutations in MMR genes.

Lynch Syndrome demonstrates variable penetrance and expressivity in those with a specific DNA-MMR gene mutation; variation in phenotype is also seen among different MMR mutations. In a research study conducted by Pérez-Cabornero *et al* (2013) the

researchers set out to identify clinical variations among the different LS mutations. Statistical analysis was conducted on a cohort of 46 individuals from 22 unrelated families with deleterious mutations in *MSH2*, *MSH6*, or *MLH1* DNA-MMR genes. The patients with EC in this study had varying ages of diagnosis depending on which mutation individuals carried. Women with *MSH2* gene mutations had an average age of diagnosis of 48.2 years of age. In one individual carrying a *MSH6* DNA-MMR mutation the age of onset was delayed to more than a decade later at 60 years of age demonstrating variation among mutations. Similarly, in a study conducted by Ramsoekh *et al* (2009), the researchers set out to evaluate the lifetime risk of cancer in carriers of *MLH1*, *MSH2*, and *MSH6*. The results uncovered by the researches were that the *MSH6* mismatch repair gene carriers were diagnosed with EC much later in life, close to 5-10 years in most cases when compared to the *MLH1* and *MSH2* carriers. The later age of diagnosis of EC reached significance in carriers with a *MSH6* mutation (56 years of age) when compared with *MSH2* (46 years of age; p=0.001) and *MLH1* (51 years of age; p=0.02) mutation carriers. The identification of a genotype-phenotype relationship among LS probands is an essential consideration when developing a surveillance program as well as when to consider prophylactic surgery for patients.

2.2.2 Histology

In contrast to the pathologic characteristic of Lynch Syndrome associated colorectal cancer, the pathological and clinical features of EC in Lynch Syndrome have not been as well described. The ability to identify Lynch Syndrome mutation carriers through LS-associated ECs could increase our ability for recognition of probands with a LS mutation. This in turn could aid in prevention of subsequent morbidity and mortality

in the identified patient as well as their potential offspring.

In a study performed by Broaddus *et al* (2006), fifty hematoxylin and eosin slides from four different hereditary cancer registries of patients formerly diagnosed with EC were reviewed. Reports and as well as the pathological blocks were reviewed from each of the cases in order to highlight any pathological or clinically relevant molecular features present in these ECs. A single gynecological pathologist examined tumour histology for International Federation of Gynecology and Obstetrics (FIGO) stage and grade, depth of myometrial invasion, lymphovascular invasion, and cervical involvement when available. Each of the patient slides that were reviewed had previously confirmed mutations in either *MLH1* (n=3) or *MSH2* (n=47) DNA-mismatch repair (DNA-MMR) genes. As a comparison the LS (HNPCC used in paper but met LS diagnosis standards) cohort was compared to two different groups with sporadic ECs. The first control group consisted of patients younger than 50 years of age, diagnosed with EC and with no known mutation or family history of LS. The second group consisted of women of all ages with tumours demonstrating microsatellite instability (MSI) secondary to hypermethylation and silencing of the *MLH1* promoter region resulting in loss of expression in the *MLH1* mismatch repair (MMR) protein. The cohort compiled of women diagnosed prior to the age of 50 was used because research has demonstration that LS associated ECs occur at younger average age than their sporadic counterparts, and the *MLH1* hypermethylated group was chosen in order to have a group with similar protein mechanism defect resulting in microsatellite instability secondary to a defective MMR protein system. The researchers found that the tumours in the LS cohort included a more

variable histological array of non-endometrioid cell types compared to the other two sporadic groups. There were a total of seven (14%) non-endometrioid tumours found in the LS cohort, and these histologies were found exclusively in patients carrying *MSH2* mutations. The cell type breakdown for the non-endometrioid tumours were clear cell carcinoma (n=3), papillary serous with a clear cell component (n=3) and malignant mixed müllerian (n=1). The data from this study is suggesting a potential genotype-phenotype relationship in which a deleterious mutation in the *MSH2* DNA-MMR gene is associated with a variable histological spectrum of ECs that could with other distinguishing factors aid in the identification of LS-related neoplasms. Non-endometrioid tumours in sporadic cases are associated with worse clinical outcomes in relation to survival (Broaddus, 2006).

The pathological characteristics of LS-related ECs were evaluated in a study Carcangui *et al* (2010) that compared a cohort of mutation proven LS carriers to a cohort of age-matched individuals. For every mutation proven LS proband two sporadic ECs with no family history of LS were matched for comparison. The researchers found a correlation between non-endometrioid histology and LS-associated ECs. Despite the younger age of the LS-associated ECs the cohort was found to have a higher frequency of non-endometrioid histologies. The LS cohort was composed of 43.5% of non-endometrioid endometrial carcinomas (*neec*) compared to the sporadic cohort with 4.3% (p=0.001). Of the 10 *neec* identified, eight individuals were found in individuals that carried a *MSH2* DNA-MMR gene mutation. The remaining two individuals were found in probands with a proven *MLH1* mutation. Five clear cell carcinomas, one of the

papillary serous carcinomas, and the small cell neuroendocrine carcinoma had a small component of endometrioid architecture. The mixed pattern cell types were included in the non-endometrioid category as long as they composed of greater than 25% non-endometrioid component, as their prognosis and behavior have been shown to behave similar to that of pure *neec*. Additionally, all the endometrioid endometrial carcinomas in the LS cohort were noted to have a higher frequency of higher-grade tumours compared to their sporadic counterparts (P=0.0368).

In an attempt to compare the pathological features of EC in patients with loss of expression of mismatch repair (MMR) proteins to those with maintained expression Grzankowski *et al* (2012) compiled a cohort of patients diagnosed with EC less than 60 years of age. Patients with pure sarcomas and without appropriate available tissue were not included in the study. 158 patients were identified for inclusion in the study population. Immunohistochemistry analysis was performed for *MLH1*, *MSH2*, *MSH6*, and *PMS2* proteins identifying 31 patients with loss of expression of MMR proteins. The remaining 127 patients maintained MMR protein expression. 25.8% of patients identified with loss of MMR protein expression were found to have non-endometrioid tumours versus 13.4% of patients that maintained expression; this did not reach statistical significance. Loss of MMR protein expression was also noted to present without the typical characteristics associated with Type I ECs. These cancers presented with higher frequencies of Grade III tumours, more advanced stage of disease, and positive lymph node metastases.

Walsh *et al* (2008) published a study where they examined a cohort of patients under the age of 50, diagnosed with EC who had their tumours analyzed for expression of MMR proteins. Loss of expression of these proteins results in a diagnosis of presumptive-LS; Amsterdam criteria II was applied when family history was available. This study was conducted in order to evaluate the prevalence of presumptive-LS in women diagnosed at younger age. Similar to other studies the presumptive-LS cohort had a higher frequency of Grade III tumours and a more advanced FIGO staging (Grzankowski, 2012).

Research published by Boks *et al* (2002) noted that the HNPCC cohort in their study demonstrated no statistical difference in the frequency of tumour histological cell types when compared to a sporadic cohort. The frequency of non-endometrioid tumours in the HNPCC cohort (8%) was lower than the sporadic cohort (12%). The inclusion criteria for the HNPCC cohort in this study were from families that harbor a germline LS mutation or that have met the Amsterdam Criteria II; the study reported none of the MMR mutations of individuals in the HNPCC cohort that were germline tested for LS. This study did not release information regarding which DNA-MMR genes were included in the HNPCC cohort.

2.2.3 Stage

The stage of EC reflects the progression of disease at the time of diagnosis and is the variable most tightly correlated with patient prognosis. Along with other pathological characteristics stage is the most important component used in formulating medical and surgical treatment for patients diagnosed with this neoplasm. Patients diagnosed with ECs

are typically diagnosed early in the progression of the disease due to the identifiable cardinal symptom of abnormal vaginal bleeding. Seventy-five to 90% of women diagnosed with EC present with this symptom, and 68% of these carcinomas are confined within the uterus at the time of diagnosis. Patients identified at this stage of disease would be classified as Stage I disease, which is associated with a 96% five-year survival rate. Twenty-percent of women are diagnosed with a more advanced stage of disease where the cancer has spread to the lymph nodes (stage II), and 8% are diagnosed with distant metastases (Stage III/IV) (Blaustein, 2012). Lynch Syndrome clinical characteristics are currently being researched in order to assess for possible differences in disease at the time of diagnosis compared to sporadic ECs in turn furthering our understanding of the aggressiveness of disease and prognosis.

In a study conducted by Broaddus *et al* (2005) in order to assess the pathologic features of Lynch Syndrome related EC, a cohort of women with mutation-proven Lynch Syndrome (probands testing positive for a *MSH2* or *MLH1* mutation) related ECs was compared to two other cohorts. The two cohorts consisted of patients with sporadic ECs diagnosed before the age of 50 and another of patients of all ages with tumours demonstrating microsatellite instability-high (MSI-high) tumours secondary to methylation of the *MLH1* gene. In LS cohort in the study 78% of the patients were diagnosed with Stage I, 10% were diagnosed with Stage II, and 12% were diagnosed with either Stage III or IV disease. When compared to the other two cohorts there was no statistical difference in the stage of disease.

Clinical, molecular, and pathologic variables were evaluated in a study performed by Walsh *et al* (2008) in order to help improve clinicians alertness to the phenotype of LS mutation carriers, and to ameliorate their identification. A total of 146 patients met the inclusion criteria for the study. The surgical histological sections from these patients were immunologically stained, and assessed for expression of mismatch repair (MMR) proteins *MLH1*, *MSH2*, *MSH6*, and *PMS2*. Tumour DNA was also assessed for microsatellite instability, and methylation of *MHL1* promoter region. Patients were classified as Presumptive Lynch Syndrome patients if tumour analysis showed lack of expression in one of the MMR proteins, and negative for *MLH1* methylation. In the cohort of 146 women, 26 (18%) had tumours that did not express for at least one MMR protein. The remaining 120 women in the cohort were classified as non-Lynch and compared to the Presumptive-LS cases. The study found that the presumptive-LS cases showed a statistical trend towards more advanced FIGO stage in comparison to the non-Lynch cohort (P=0.029).

This was a similar result to a study by Grzankowski *et al* (2012) that examined the pathologic features of patients under the age of 60 with EC that demonstrated loss of expression of MMR (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) proteins as verified by a single pathologist from archived tissue blocks using immunohistochemical analysis. One hundred and fifty-eight patients were identified from a single tumour registry at Queens Medical Center, and 31 of these patients were found to have at least the loss of expression of one or more MMR proteins. The breakdown of the 31 patients included 10

women with the loss of expression of *MSH2*/*MSH6*, and 21 women with the loss of both *MLH1*/*PSM2* MMR proteins. Methylation of the *MLH1* gene was not conducted to identify possible sporadic methylation and silencing of the respective gene. The patients found to have loss of expression of MMR proteins were then compared to the women with tumours that maintained MMR protein expression. Statistical analysis identified that the patients that showed loss of MMR expression were more probable to have a more advanced stage of EC than those that maintained protein expression (P= 0.0079). The tumours with loss of expression were also found to have more unfavorable disease characteristics including greater myometrial invasion (P= 0.0019), increased incidence of lymph node metastases (P=0.0157), and increased likelihood of lymphovascular invasion (P=0.0020).

Additionally, Garg *et al* (2009) conducted a study in attempts to define a pathological phenotype for patients with presumptive LS. Out of a total of 2000 possible hysterectomies performed at the Memorial Sloan Kettering Cancer Center between 1993 to 2008 a total of 70 patients met the inclusion criteria of this study; a diagnosis of EC aged 40 years or younger. Of the 70 women that met the inclusion criteria, 54 had the appropriate tumour blocks available for immunohistochemical analysis for MMR protein expression. Out of the 54 cases, nine (16%) were identified to have loss of expression of at least one MMR protein, slightly favoring the loss of *MSH2*/*MSH6* (fine of nine), compared to loss of *MLH1*/*PMS2* (four of nine). The patients with abnormal or loss of expression of MMR proteins were compared to the patients with normally expressed MMR proteins on numerous clinical variables. The tumours with loss of MMR protein

expression were frequently more associated with lymphovascular invasion (P=0.012), often presented with more advanced stages of disease (P=0.005), and were noted to be more often located in the lower uterine segment.

The clinical phenotype and pathological presentation of EC in Lynch Syndrome carriers can vary tremendously. Carcangui *et al* (2010) an analysis of 23 Lynch syndrome carriers (*MSH2* n=16*, MLH1* n=6, and *MLH1/MSH2* n=1) as confirmed by the detection of causative germline mismatch repair (MMR) gene mutations, and diagnosed with EC. The Lynch Syndrome cohort was analyzed on clinical variables of grade, and International Federation of Gynecology and Obstetrics (FIGO) stage, as well as histological type, and survival. This study found that the frequency of FIGO stage did not differ between the Lynch Syndrome associated EC cohort, and the control cohort. Despite not finding a statistical difference in stage between the two cohorts it was worth noting that intravascular invasion was identified in 13 (56.5%) of the LS cohort, compared to 9 (19.5%) of the controls, as in study by Garg et al 2009.

2.2.4 Survival

Prognosis and survival can be difficult to predict with many variety of neoplasms including EC. In order to assess prognosis, the oncologist must take into consideration numerous characteristics of the neoplasm and base their predictions on previous neoplasms with the same clinical and pathological characteristics. The primary characteristics involved in the prediction of patient survival are focused around the International Obstetrics and Gynecologist Federation (FIGO) Stage and histological characteristics of the neoplasms at the time of diagnosis (Blaustein's, 2011). The

prognosis for EC tends to be favourable as most of these carcinomas are endometrioid cell type and diagnosed at a relatively early stage. This early diagnosis is usually due to the cardinal symptom of irregular vaginal bleeding in postmenopausal patients. Lynch Syndrome related ECs have been identified to deviate from the standard clinical phenotype of sporadic varieties, and it has long been recognized that these mutation-related cancers may be diagnosed decades prior to their sporadic counterparts. Researchers are currently evaluating the potential survival differences between Lynch Syndrome-related ECs and those found in non-mutation carrying sporadic patients with no family history of Lynch Syndrome-related cancers.

The study conducted by Terada *et al* (2013) evaluated the prognosis, and survival of LS-related ECs. This retrospective study compiled a cohort of patients who had been diagnosed with LS-related EC between 1998 and 2009 and who had their primary tumours evaluated through immunohistochemical analysis for mismatch repair (MMR) proteins (*MLH1*, *MSH2*, *MSH6*, and *PMS2*). Patients in this study that tested negative for expression of *MLH1* MMR protein were not tested for hypermethylation of this promoter region. The inclusion criteria for the study included ECs that tested positive for lymphovascular invasion between stages I-IIIA. International Obstetrics and Gynecologist Federation (FIGO) stage IIIC-IV were included if lymph node metastases were present on biopsy. An upper age boundary of 70 years of age was used so as to exclude older patients with increased medical co-morbidities, and to remove these variables, and their possible influence on overall survival. Sixty-six patients were identified, and met the inclusion criteria. Forty of the inclusion patients were found to

have normal expression of MMR proteins in tumour tissue. Negative or under expression MMR protein status was defined as tumours stained with respective proteins with less than 5% of tumours cells staining positive. Twenty-six of the patients were found to have deficient expression of MMR proteins in their tumours; 4 were found to have loss of expression of *MSH6* and *MSH2* proteins, the other 22 were found to have deficient expression in either *MLH1* or *PSM2* protein. Kaplan Meier, and log-rank analysis was performed in order to statistically evaluate survival. The overall survival analysis between tumours with maintained MMR protein expression, and the cohort that did not maintain MMR protein expression were statistically significant (P=0.03). Patients that showed deficient expression in MMR proteins were found to have better survival than the patients that maintained normal MMR protein expression. A subgroup Kaplan Meier survival-analysis was performed including the patients in each group with an FIGO stage IIIC cancer. This analysis showed the same trend in that the patient with deficient expression had better overall survival (P=0.01). Due to the poor prognostic association with FIGO stage IV cancers, an additional subgroup analysis was performed with these cancers removed from each of the cohorts. The same statistical significance was found with the overall survival of the patients with deficient MMR protein expression (P=0.05), although better disease specific survival was not found (P=0.08).

A survival analysis of fifty patients accumulated from 46 HNPCC families that were identified as harboring a germline mutation or fulfilling the Amsterdam Criteria II were compared to a cohort of individuals with EC with no outstanding history of HNPCC related cancers. In this study initiated by Boks *et al* (2002) the fifty patients that were in

the HNPCC cohort were age, and stage-matched with two sporadic ECs that acted as controls. Germline analysis of the HNPCC cohort was confirmed in 38 of the 50 cases that comprised the HNPCC group. Sixteen patients were identified to carry a *MSH2* mismatch repair (MMR) gene mutation, three were found to carry a *MSH6* MMR gene mutation, and 19 were confirmed in carrying a *MLH1* MMR gene mutation. The germline mutation status in the remaining 12 patients was unknown. Survival analysis was complete, and observation time of the analysis was from the date of diagnosis until death or until the end of the study date. Survival analysis was complete using log-rank analysis. Student t-test was conducted in order to compare the case to the control cohort. The only significant difference found between the two groups was the age of the patients with Stage IIIA and IIIC ECs. The HNPCC cohort had an average age of diagnosis of 48.0 years, and the sporadic cohort had an average age of diagnosis of 59.0 years of age (P=0.009). The cohorts did not vary significantly in any other baseline characteristics. The log-rank analysis demonstrated that the survival between the HNPCC related ECs, and the age and stage matched sporadic controls were not significantly different. The overall cumulative survival rates for the HNPCC and sporadic cohorts were 88% and 82% respectively (P=0.59). Subgroup analysis of stage I (A, B, and C) ECs between LS, and sporadic cohorts resulted in survival rates of 92%, and 91% respectively (P=0.90). Another subgroup analysis of Stages IIIA and IIIC showed no significance difference between the two cohorts (P=0.35).

Lynch Syndrome related EC survival and behavior is not well described in the literature. In a study by Carcangui *et al* (2010) a cohort of 23 patients with Lynch

Syndrome related ECs were compared to sporadic EC patients with no personal history of Lynch Syndrome associated cancers. The 23 probands in the Lynch Syndrome cohort had previously received a germline mutation analysis for at least one mutation of DNA-mismatch repair (DNA-MMR) gene. The case cohort of 23 consisted of 16 *MSH2*, 6 *MHL1*, and one carrier with both *MLH1*/*MSH2* mutations. The study compared the LS cohort to a cohort of sporadic EC patients. Two aged matched controls with no family history of LS-related cancers were compared to each Lynch Syndrome proband. The study found a significant difference in histology between the two groups, noting that the Lynch Syndrome cohort had an increased frequency of non-endometrioid cell types. The study then compared the patients in the LS cohort with endometrioid ECs to those with non-endometrioid ECs using log-rank analysis that showed that histology did not affect overall survival. Despite not affecting the overall survival, the hazard ratio, though not statistically significant, was higher (HR=4.86 [0.054-437]; P=0.1583) for the non-endometrioid patients.

The overall effect of a non-functional MMR protein system on survival of probands is an area of interest for the study conducted by Shikama *et al*. Consecutive ECs (n=221) were analyzed via immunohistochemistry for MMR proteins. Based on the findings of the immunohistochemical analysis the patients were categorized as either sporadic or Probable Lynch Syndrome (PLS). Probable LS was classified as tumour that maintained all MMR proteins (*MLH1, MSH2, MSH6, and PMS2*) or was found to have *MLH1* loss of MMR proteins with hypermethylation at the *MHL1* promoter region. Patients with tumours that demonstrated loss of expression of *MSH2*, *MSH6*, or *PMS2* MMR proteins were considered PLS. Patients were also considered PLS if tumours

showed loss of expression of *MLH1* proteins without methylation at the *MLH1* promoter region. There were 28 (13%) cases that were classified as PLS and the remaining 193 (87%) were classified as sporadic ECs. Survival analyses revealed that PLS group had significantly better overall survival than the sporadic group ($p = 0.038$). In an additional analysis to compared the prognosis of PLS and sporadic cohorts, the study found that the favorable overall survival of the PLS cohort continued in demonstrating a stronger association in more advanced stages of disease compared to early stages (hazard ratio, 0.044 [95% CI 0–25.6] vs. 0.49 [0.063–3.8] respectively).

Chapter 3: Materials and Methods

3.1 Design

The aim of this pilot study is to compare the clinical features and outcomes of EC associated with a Lynch Syndrome mutation with sporadic EC that are presumed mutation negative cases and representative of the general population. The ultimate goal of the study is to construct a phenotype or clinical picture of how these cancers differ from non-LS associated cancers, and as well in the pathological setting in order to improve the identification of mutation-based cancers. In turn, we hope to improve the probability of identifying LS-related cancers at earlier stages. With increased sensitivity of identifying these mutation-based cancers comes improved surveillance programs for probands as well as immediate family members that are at an inherited increased risk for a spectrum of neoplasms given the autosomal dominant inheritance pattern of LS mutations.

In order to fulfill the previously described objectives of this study, it was necessary to conduct this research project in three integrated steps. Each of the steps is described in detail in the following sections, including the necessary ethical approval and considerations in order to conduct research in our health district.

3.2 Ethical Considerations

An application to conduct this research project was initially sent to the Health Research Ethics Authority (HREA) of Newfoundland and Labrador. The Health Research Ethics Authority Act established this non-profit agency on July 1st 2011 governing all

research involving human subjects or their data in the province. The Health Research Ethics Board (HREB) then reviewed the submissions of a *Notification of Research* form, *General application* form, and The *Secondary Use of Data/Chart audit* form. The project was also submitted for Research Proposal Authority Committee (RPAC) approval through Eastern Health. This committee (RPAC) was enacted in order to govern the research taking place within the district of Eastern Health in order to monitor resource allocation, confidentiality of patients/employees, and stewardship of biological samples. Eastern Health is the largest of four regional health authorities in Newfoundland and Labrador, servicing a population of approximately 300,000. The study received full ethical approval that was renewed throughout the duration of the project.

All of the participants in this study have previously consented to participate in a study titled "The Impact of Screening on Individuals from HNPCC Families" and therefore re-contact was not required.

3.3 Data Abstraction

3.3.1 History of Lynch Syndrome Cohort

The cohort of patients involved with this study is a population of forty-six patients with genetic ties to the Northeast coast of Newfoundland and Labrador. These patients were originally identified through the investigation to find a gene associated with HNPCC. In the early 1990's two probands were identified independently and referred to the Provincial Medical Genetics program in the Newfoundland and Labrador due to their significant familial cancer histories. These patients were subsequently investigated and an

extensive six-generation family history was uncovered discovering a common ancestor between these two probands. This large family, named Family C, played an integral part in mapping the first gene in the HNPCC family of mutations, the *hMSH2* gene locus located on chromosome 2p. The mutation identified through Family C was the intron 5 splice *MSH2* mismatch repair mutation; All of the patients in this study were found to be confirmed heterozygotes for this pathogenic variant tested positive for this mutation or for the *MSH2* exon 8 deletion mutation. The patients being studied in this cohort are females that have been identified as mutation carriers either by genetic testing or have been proven to be an obligate carrier through the retrospective analysis of their family pedigree. All of the probands within this study have consented to have all available medical information reviewed in order to extract the details of their respective cancers.

All of the patients included in this cohort have been diagnosed with EC in their lifetimes, and the vast majority, have had other synchronous or metachronous Lynch Syndrome related cancers during their lives.

3.3.2 Abstraction Form

The data necessary in order to fulfill the aims of this study was extracted from patient charts using an abstraction form. The form was constructed in order to organize the clinically relevant data of interest, remove any patient identifying information and to improve confidentiality for the patients involved in the study. The categories included on the abstraction form are as follows:

- Demographic Information
- Onset of symptoms
- Site(s) of Cancer
- Date of Diagnosis
- Method of Diagnosis
- Type of Surgical Procedure
- Pathology Characteristics
- Treatment Received
- Other Cancers
- Date of Death or Last Follow-up

The abstraction form was constructed by Dr. Lesa Dawson, MD, FRCSC in collaboration with the primary investigator Drs. Jane Green and Adam Nichols .

3.3.3 Patient Charts

The patient charts that were used in this study were accumulated by Dr. Jane Green, Professor in the Discipline of Genetics in the Faculty of Medicine at Memorial University of Newfoundland and Labrador. These charts were originally compiled through the study of HNPCC for associated gene discovery. Dr. Jane Green compiled the charts for the previous study by contacting patients directly either by phone or in-person in order to obtain consent to access their medical records. In many cases, the medical records did not exist and written dialogues of proband histories were taken. The charts consisted of copied versions of original medical records and in some cases hand-written notes obtained from probands or family members of the probands regarding medical information. The primary investigator then revisited each individual chart that was stored on behalf of the original study that were kept securely in the Discipline of Genetics in the Faculty of Medicine at Memorial University of Newfoundland and Labrador.

The primary investigator manually searched through each of the 46 proband charts in order to complete an abstraction form for each individual patient. The majority of these charts consisted of medical records relating to each individual neoplasm. This would include visits prior to the diagnosis, confirmation of diagnosis, surgical and potential chemotherapeutic treatment for each neoplasm and pathological validation of the type of cancer for each patient.

3.3.4 Data

The data for this project was abstracted from patient charts, using the abstraction form, for a period lasting from January 2012 to July 2012; solely by the primary investigator. The necessary information came from numerous locations within the patient charts and was based on queries from gynecological oncologists, geneticists, and the literature. The sections included the appointments leading up to and following patient diagnosis of endometrial cancer, the surgical and pathology reports, if surgery was performed, as well as any adjunctive treatments required by patients post-surgery. The patient charts were also reviewed in order to identify any neoplasms that had been diagnosed either before or after the diagnosis of endometrial cancer. This was completed in order to create an individualized cancer history for each patient involved in the study. All the data that was collected for each of the 46 individual patients was manually abstracted by the primary investigator (Dr. Adam Nichols) over the six-month period.

If at any point during the abstraction of patient data there was any concern or ambiguity in the interpretation of the information, the primary investigator would

collaborate with Dr. Lesa Dawson on the research team to ensure the validity of the findings. Secondary validation was also conducted by referring to Dr. Jane Green, the geneticist on the committee, to ensure the accuracy of the information removed from patient charts. As the abstraction forms were completed for each patient, the forms were stored in a locked filling cabinet in an office with access limited to the members of the research team. In order to validate the pathologic data found in the pathology reports a staff pathologist was assigned to the committee in order to confirm the pathological diagnoses when possible. Pathological review was achieved by retrieving patient histology slides from medical storage when they were available. The staff pathologist along with the primary investigator then reviewed the slides manually in order to confirm concordance and compared their findings to the pathological data that was retrieved from the patient charts.

Once completed, the information was subsequently entered into a database and analyzed with IBM Software Package for the Social Sciences (SPSS) version 22. The dataset was constructed from July 2012 to September 2012.

3.3.5 The Sporadic Cohort

The sporadic cohort was comprised of de-identified data acquired from the Newfoundland and Labrador Cancer Registry upon written request and subsequent approval. The sporadic cohort represents the general population or presumed non-mutation based ECs. The data file consisted of consecutive patients diagnosed with EC from June 2000 to September 2010 within Newfoundland and Labrador who were treated at the Dr. H. Bliss Murphy Cancer Center.

The file contained 753 women diagnosed over the ten-year period. The dataset included variables of date of birth, date and age of diagnosis, histological information, stage of cancer as well as information on follow-up and whether or not the patient survived their cancer. The dataset did vary in the amount of information available for each individual patient, as not all the mentioned categories were complete for all individuals. When patient data was missing they were excluded from the respective variables analysis. The majority of the information within the file received from the Cancer Registry was easily deciphered, except the histological information, which was coded as per the International Classification of Disease for Oncology (ICD-O). The information had to be translated back into cell-type information using the ICD-O manual by the primary investigator. This involved searching through the dataset for each of the 753 patients and de-coding the cellular histology for each individual that had the information available. If the information was not present for an individual the information was requested a second time from the Newfoundland and Labrador Cancer Registry; if at that point the information was still not available the patient was excluded from the study.

3.3.6 Validity of the Data

The clinical details regarding the pathological specimens from of the Lynch Syndrome patients were collected and abstracted using patient charts as reviewed by the primary investigator. After the data was extracted from the patient charts an application was submitted to obtain patient surgical/pathological slides from the cancer archive. Not

all surgical specimens were available as surgical specimens are only kept for limited time frames. This was completed in order to have as many of the surgical slides reviewed by a staff pathologist (Dr. Shaikh Mortuza) at the Health Sciences Center in order to validate the diagnosis that was assigned to the patient.

The proposal to retrieve the histological slides was submitted, and all of the slides that were available for the thirty-six LS associated ECs were retrieved, including any other slides that may have been archived from other surgeries on the same patient. Of the total 46 LS probands, 36 had histological information abstracted from the charts in Discipline of Genetics in the Faculty of Medicine at Memorial University of Newfoundland and Labrador. Of the 36 probands with histological information 12 patient specimens were retrieved from the surgical archive. The 12 patient specimens were reviewed by a single staff pathologist (Dr. Shaikh Mortuza) and the primary investigator in order to validate the cell types, when available.

This process involved the primary investigator and a staff pathologist from the Health Sciences Center examining each of the surgical slides under light microscopy. This involved three different sessions that included teaching about histology and pathology related to the endometrium as well as the evaluation of the slides to confirm the cell types that were in the original pathological reports.

3.4 Statistical Analysis

3.4.1 Data Analysis Software

The data analysis software utilized for this project was IBM Software Package for the Social Sciences (SPSS) version 22.

3.4.2 Data Analysis

The statistical analysis for this project was a comparison of the *hMSH2* mutation-carrying Lynch Syndrome cohort with that of a sporadic cohort without a Lynch Syndrome mutation representative of the general population of Newfoundland and Labrador. The primary investigator performed the construction of the datasets by transferring the information from the abstraction form to a database. This involved the manual transcription of abstraction form data into the SPSS Software Package and subsequent coding of the datasets in order to aid analysis. This included classifying the data into different types of data as well as their respective coding into different groups in order to conduct the appropriate analyses. After the completion of both the datasets for the two cohorts, the datasets were merged in order to conduct analytical comparisons between all collected variables. The variables that were compared between the two cohorts included:

1. Age of diagnosis
2. Stage of Endometrial Carcinoma (Local versus Advanced)
3. Cell type
4. Grade
5. Overall Survival.

Comparisons of stage, grade and cell type were all conducted using Chi-Squared analysis. The comparison of age was conducted using the *Student's t-test* and the survival comparison was performed using a *Kaplan-Meier Survival Analysis.*

Chapter 4: Results

The Lynch Syndrome cohort in this study was compiled of 46 women retrieved from the patient charts compiled by Dr. Jane Green, Professor in the Discipline of Genetics in the Faculty of Medicine at Memorial University of Newfoundland and Labrador from January 2012 to July 2012. The LS cohort consisted of probands with a confirmed *hMSH2* mutation that had been diagnosed with EC; there were a total of 46 probands that met these criteria. The information was retrieved from replica photocopies of patient charts that were originally collected for research in the HNPCC screening program in Newfoundland and Labrador. The secondary use of this data was initiated in order to abstract pathological and surgical data that was clinically relevant to the disease and progression in the patients who had been previously diagnosed with EC. This retrospective analysis was conducted in order to evaluate similarities or differences among LS-related ECs and sporadic ECs representative of the general population.

The data that was collected from these charts was additionally cross-referenced when possible to the data that was present in the Newfoundland and Labrador Cancer Registry on the patients that were diagnosed in this province. This was completed in order to ensure that patients that were included in the LS cohort were not concomitantly included in the sporadic cohort. This was completed by physically bringing patient charts to the Newfoundland and Labrador Cancer Registry to ensure that the Medical Care Plan (MCP) numbers did not match. This additional process had to occur as the data sets were created in order to maintain the highest level of security and all probands were de-identified. The comparative data for the sporadic cohort came from the Newfoundland

and Labrador Cancer registry from 2000 to 2012. The cases represented consecutively enrolled patients that were treated at the Dr. H Bliss Murphy Cancer Center. The data from the cancer center were incomplete in that not all patients had data for each of the examined variables. When cases from the cancer center had absent data of a particular variable they were excluded from the respective analysis. At the end of the data collection period the Lynch Syndrome cohort had total of 43 individuals, and the sporadic cohort had a total of 753 individuals.

Table 1: Baseline Demographics and Clinical Characteristics

	MSH2	**Sporadic**
	n 46(%)	n 753(%)
Year of Birth		
1910-1950	29 (63.0)	511 (67.9)
After 1950	17 (37.0)	242 (32.1)
Age		
Median (Range in Years)	45 (32-65)	60 (29-92)
Histology		
FIGO Grade 1	14 (37.8)	325 (60.4)
FIGO Grade 2	11 (29.7)	149 (27.7)
FIGO Grade 3	12 (32.5)	64 (11.9)
Cell Type		
Endometrioid	22 (57.9)	532 (70.7)
Papillary Serous	9 (23.7)	27 (3.6)
Clear Cell Carcinoma	3 (7.9)	6 (0.8)
Sarcomas	3 (7.9)	43 (5.7)
FIGO Stage		
Localized (Stages I & II)	30 (76.9)	340 (88.1)
Advanced (Stages III & IV)	9 (23.1)	46 (11.9)
***MSH2* mutation**		
Intron 5 spliced site	33 (73.9)	n/a
Exon deletion 8	11 (26.1)	n/a
Dead (all-cause mortality)		
Yes	23 (54.8)	150 (24.7)
No	19 (45.2)	457 (75.3)

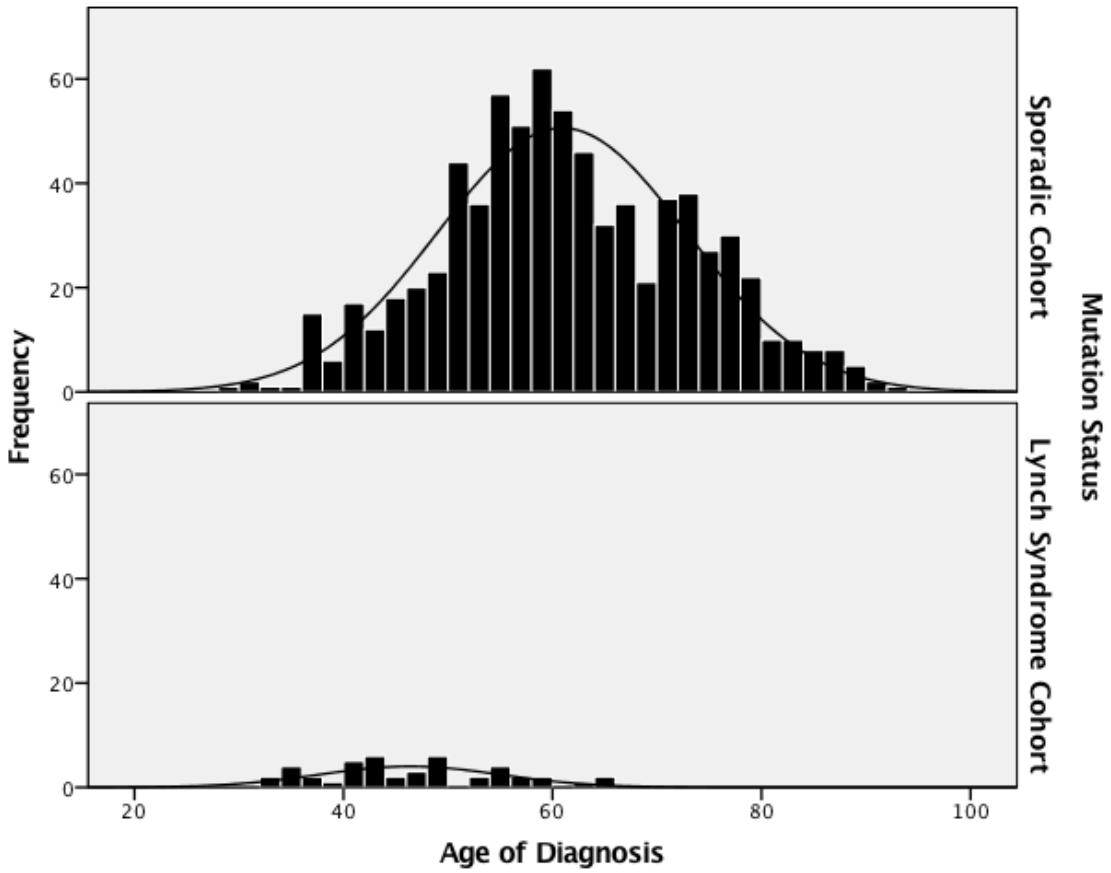
4.1 Age Analysis

The Lynch Syndrome cohort and the sporadic cohort were compared in order to analyzed the average and median age of diagnosis with Endometrial EC. There were 44 and 753 in the Lynch Syndrome and Sporadic cohorts respectively that had information on age of diagnosis. The analysis was executed using the Student's t-distribution test in order to compare the means of the two cohorts. Levene's test for equal variances had a p-value of < 0.05 indicating that the variances for the populations were significantly different however it did not change the outcome data. The Student's t-test distribution between the two cohorts differed significantly ($p=<0.001$). This indicated that the LS-related ECs are diagnosed at a considerably younger average than the sporadic ECs. The mean for the LS cohort was 46.32 years of age (SD- 8.733), with a median age of 45.5. The sporadic cohort had an average age of diagnosis of 60.91 years of age (SD- 11.877), and a median age of diagnosis of 60.0. The median age for the LS and Sporadic cohorts were 45 and 60 respectively.

Table 2: Mean age of Diagnosis of EC in Lynch Syndrome and Sporadic Cohorts

Mutation Status	Mean	N	Std. Deviation
Sporadic Cohort	60.9	753	11.9
Lynch Syndrome	46.3	44	8.7

Figure 1: Distrubution of Age of Diagnosis in Lynch Syndrome and Sporadic Cohorts

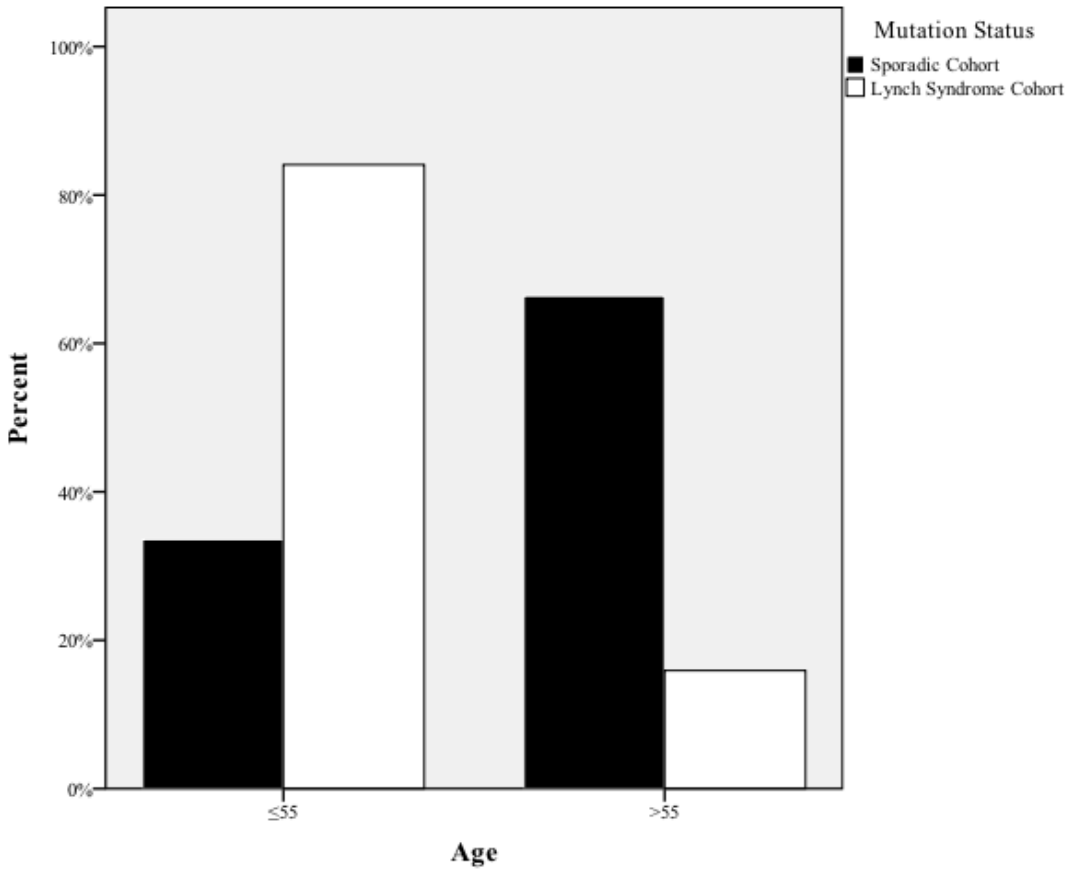


The Lynch Syndrome and sporadic cohorts were evaluated on age of diagnosis before or after fifty-five years of age. The Chi-squared analysis resulted in a Pearson Chi-squared value of 45.787 with an asymptotic significance value of $p < 0.001$. The Phi coefficient is -0.240.

Table 3: LS/Sporadic comparison of EC diagnosis ≤55 years of Age or >

			≤55	>55	
Mutation Status	**No**	Count	253	500	753
		Expected Count	274.0	479.0	753.0
	Yes	Count	37	7	44
		Expected Count	16.0	28.0	44.0
Total		Count	290	507	797
		Expected Count	290.0	507.0	797.0

Figure 2: Distribution of EC Diagnosis ≤55 or >55 Years of Age In Lynch Syndrome and Sporadic Cohorts



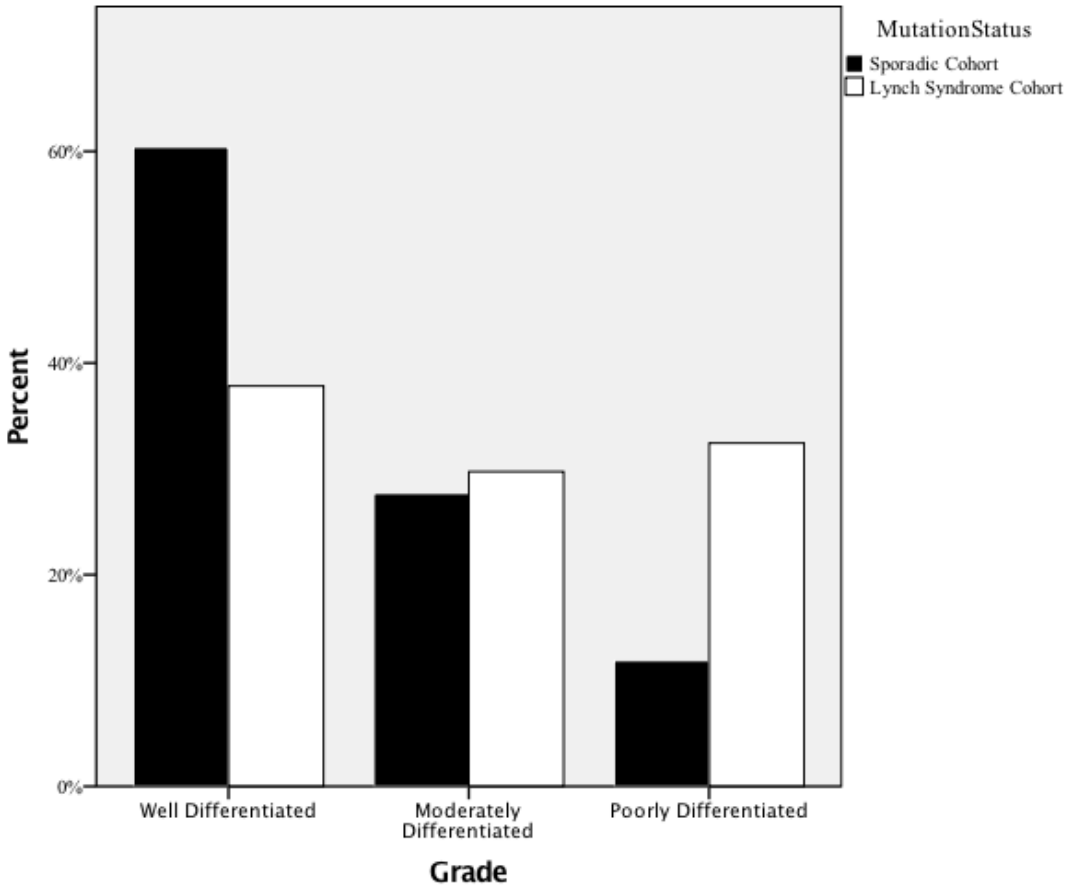
4.2 FIGO Grade Analysis

The analysis of the cohorts was designed in order to identify possible differences between the levels of differentiation or grade in ECs within the Lynch Syndrome cohort, and those patients in the sporadic cohort. A chi-squared analysis was conducted on the LS-related and Sporadic cohorts ECs in order to evaluate for differences in the two groups. The cohorts were analyzed independently based on the level of grade. There were 37 patients in the LS cohort that had information abstracted from their medical history on grade of cancer, and 538 total women from the Sporadic cohort that had information on grade taken from the Newfoundland and Labrador Cancer Registry.

Table 4: FIGO Grade Comparison

Phenotype	Lynch Syndrome	Sporadic Cohort	P-value
Grade 1	37.8% (14/37)	60.4% (325/538)	0.018*
Grade 2	29.7% (11/37)	27.7% (149/538)	0.711
Grade 3	32.4% (12/37)	11.9% (64/538)	0.015*

Figure 3: Distribution of Grade of EC in Lynch Syndrome and Sporadic Cohorts



The chi-squared analysis of the Grade 1 or well-differentiated ECs resulted in a Pearson chi-squared statistic of 6.463 (p=0.018) indicating a significant difference between LS-related ECs and the sporadic cohorts. The Chi-Squared analysis of Grade 2 or moderately differentiated ECs between the cohorts had a Pearson Chi-Squared statistic of 0.137 (p=0.711) indicating no significant difference between the two cohorts. The

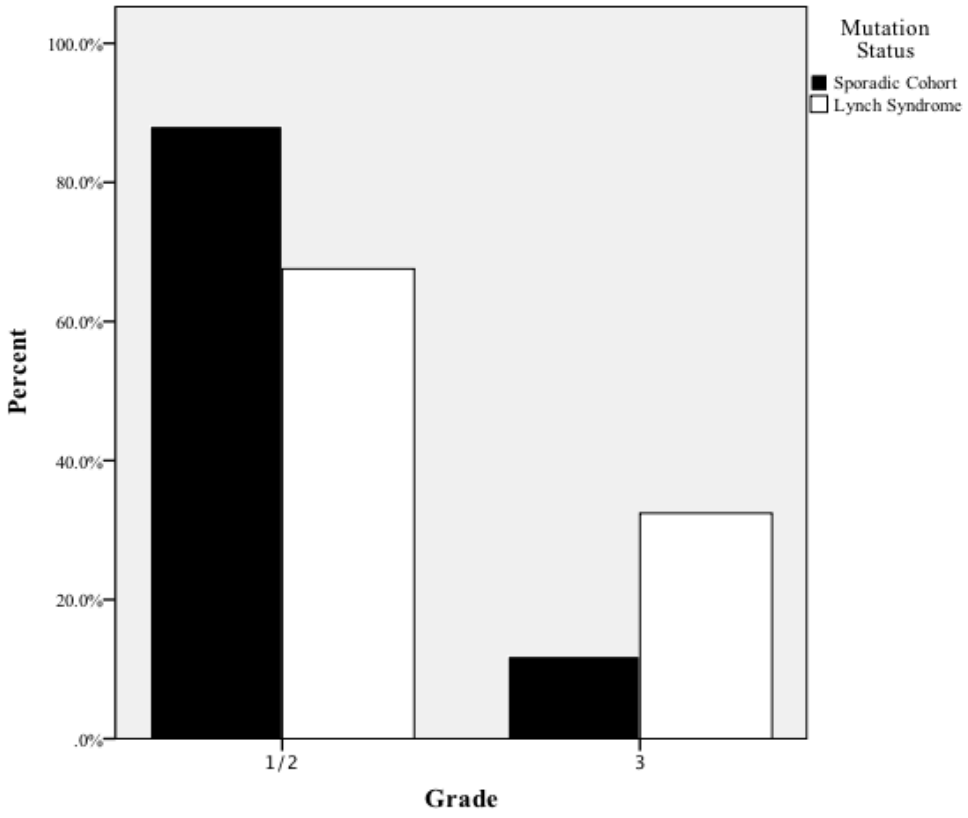
Pearson Chi-Squared analysis on the Grade 3 or poorly differentiated ECs showed a significant association between LS-related ECs and the outcome of developing a poorly differentiated tumour. The analysis resulted in a Pearson Chi-Squared value of 10.343 (p=0.001).

The Fisher's exact test comparing cell grades 1 and 2 combined versus 3 in the Lynch Syndrome and sporadic cohort yielded an exact significance of p=<0.001.

Table 5: Comparison of FIGO Grade 1/2 versus 3 Tumours in LS/Sporadic cohorts

			Grade 1/2	Grade 3	Total
Mutation Status	**No**	Count	474	64	538
		Expected Count	466.9	71.1	538.0
	Yes	Count	25	12	37
		Expected Count	32.1	4.9	37.0
Total		Count	499	76	575
		Expected Count	499.0	76.0	575.0

Figure 4: Distribution of Grade 1&2 versus 3 ECs in Sporadic and Lynch Syndrome Cohorts



4.3 Cell Type Analysis

The cohorts were compared using Pearson Chi-Squared analysis. The Chi-Squared analysis did not reach significance (p=0.094) indicating that there is no difference between mutation-status and developing either an *eec* or *neec* tumour.

The cell types were then broken down into individual sub-classifications of *neec* cell types in order to compare the sub-classes to analyze whether or not LS-related ECs had any predominant cell types compared to sporadic ECs. The *neec* sub-classes included sarcomas, clear cell carcinomas, and papillary serous cell types. The incidences of sarcoma cell types were compared for association to mutation status. The Pearson Chi-Squared value from the analysis yielded a result of non-significance (p= 0.547) indicating that LS-related ECs did not reach a clinically significant difference of frequency of endometrial adenosarcoma compared to the sporadic cohort in this study.

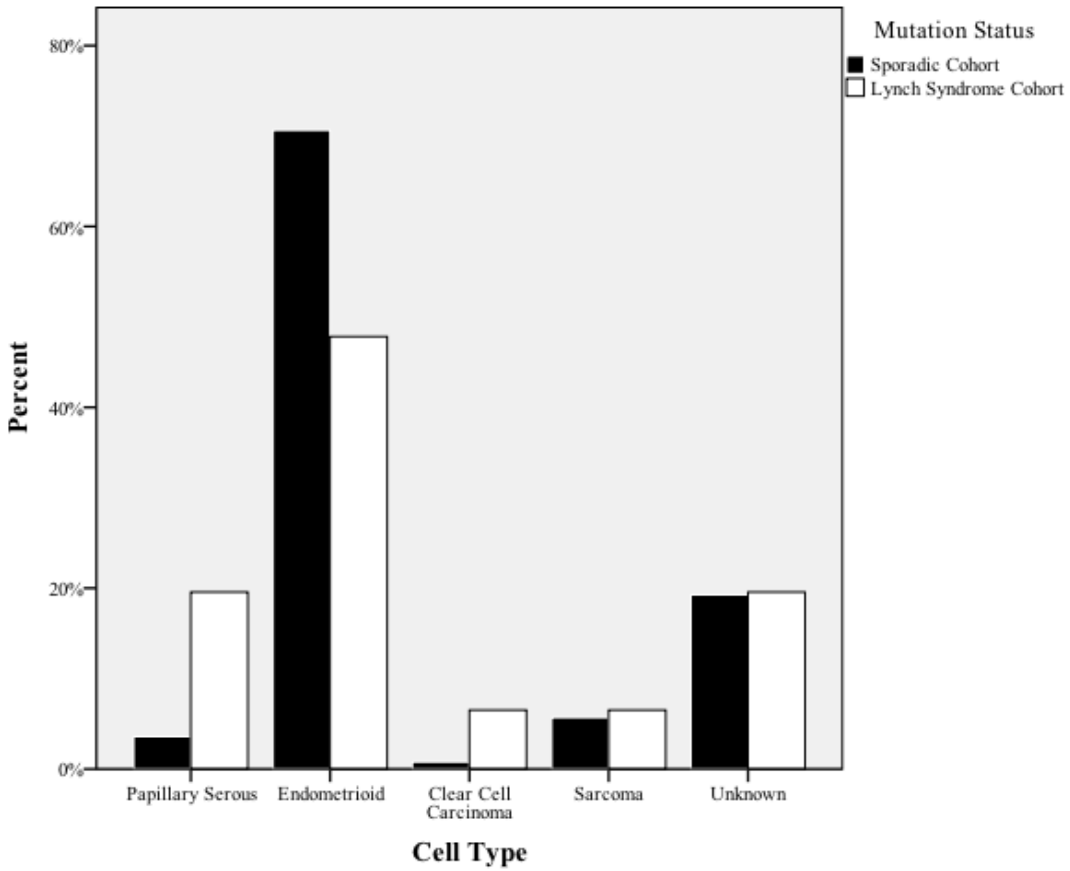
The prevalence of clear cell carcinomas in the LS cohort compared to the sporadic cohort resulting in a statistically significant Pearson Chi-Squared value of 16.202 (p=<0.001). This demonstrated the frequency of clear cell carcinomas in LS-related ECs were more common than in sporadic ECs.

The Pearson Chi-Squared analysis conducted to compare the frequency of papillary serous cell types between the LS and sporadic cohorts resulted in a statistically significant Pearson Chi-Square statistic of 33.638 (p=<0.001).

Table 6: Cell Type Comparison

Phenotype	Lynch Syndrome	Sporadic Cohort	P-Value
Endometrioid	59.4%(22/37)	70.7% (532/753)	0.094
Papillary Serous	24.3%(9/37)	3.6% (27/ 753)	0.045*
Clear Cell Carcinoma	8.1% (3/37)	0.8% (6/753)	<0.011*
Sarcoma	8.1% (3/37)	5.7% (43/753)	0.547

Figure 5: Distribution of Cell Type in Lynch Syndrome and Sporadic Cohorts



Fisher's exact test comparison of grouped cell types (papillary serous and clear cell carcinoma) versus all other cell types (endometrioid and sarcomas) between the LS and sporadic cohorts resulted in an exact significance of p=<0.001.

Table 7: Papillary Serous/ Clear Cell Carcinoma versus other cell types comparison between LS and Sporadic cohorts

			Non-Pap/CCC	Pap/CCC	Total
Mutation Status	No	Count	575	33	608
		Expected Count	565.6	42.4	608.0
	Yes	Count	25	12	37
		Expected Count	34.4	2.6	37.0
Total		Count	600	45	645
		Expected Count	600.0	45.0	645.0

4.4 FIGO Stage Analysis

The Lynch Syndrome cohort had 39 and the sporadic cohort had 387 patients that had appropriate data on stage of EC in order to conduct the analysis. Chi-Squared analysis was completed comparing local stages of disease (stages I/II) to advanced stages of disease (stages III/IV) between the cohorts. The analysis yielded a Pearson Chi-Squared statistic of 3.916 (p=0.048) demonstrating a significant association between mutation status and stage. The resulting Phi coefficient is 0.096.

Table 8: Comparison of Local and Advanced stage disease between LS and Sporadic Cohort

			Local	Advanced	Total
Mutation Status	No	Count	340	46	386
		Expected Count	336.0	50.0	386.0
	Yes	Count	30	9	39
		Expected Count	34.0	5.0	39.0
Total		Count	370	55	425
		Expected Count	370.0	55.0	425.0

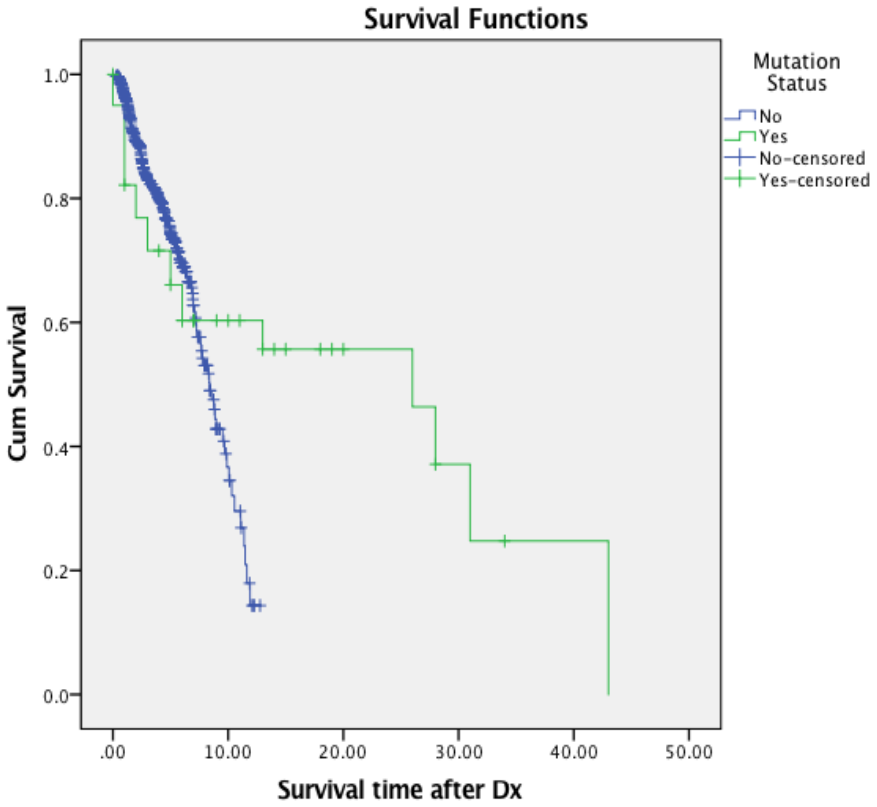
4.5 Survival Analysis

The overall lifetime survival of the Lynch Syndrome, and sporadic cohorts were compared using the Kaplan-Meier Survival analysis. The Lynch Syndrome cohort had 42 individuals with information on date of death or last follow-up, and the sporadic cohort had 616 patients that had information on date of death or last follow up. The overall examination of survival is analyzed using all-cause mortality for these patients after the diagnosis of their ECs. The Kaplan-Meier analysis demonstrated no statistical difference between the cohorts survival (p=0.068). Though the Lynch Syndrome cohort did demonstrate a trend of survival long after their initial diagnosis.

Table 9: Survival Analysis

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	3.323	1	0.068

Figure 6: Survival after diagnosis of EC in LS and sporadic cohorts



4.6 Predictability Models

Logistic regression including age of diagnosis of EC before or after the age of fifty-five, local versus advanced stage of EC, grade of tumour one and two versus three, and grouped cell types of papillary serous and clear cell carcinoma versus all other cell types resulted in a Hosmer and Lemeshow Chi-square statistic of 3.062 and p= 0.382. Age of diagnosis of EC before or after fifty-five years of age and grouped cell type of papillary serous and clear cell carcinoma were both significant predictors in the regression equation p= <0.001 and <0.001 respectively.

Table 10: Logistic Regression Predicting Lynch Syndrome

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	**Age55**	-2.596	0.541	23.033	1	0.000	0.075
	Stage Advanced Vs Local	0.702	0.538	1.703	1	0.192	2.018
	Grade1/2or3	0.791	0.504	2.463	1	0.117	2.205
	Papillary Serous/CCC Dicho	2.278	0.593	14.745	1	0.000	9.753
	Constant	-2.268	0.668	11.544	1	0.001	0.103

Multivariate analysis including age of diagnosis of EC before or after the age of fifty-five, local versus advanced stage of EC, grade of tumour one and two versus three, and grouped cell type of papillary serous and clear cell carcinoma versus all other cell types resulted in a Pillai's trace value of 0.241 and F-value of 20.668 and significant with p=<0.001. All dependent variables in the in the analysis are significance in predicting Lynch Syndrome as seen in the table below.

Chapter 5: Discussion

Lynch Syndrome associated EC have been identified by research studies to possess unique clinical features compared to sporadic cancers with a functioning MMR system (Backes 2011, Barrow 2009, Baudhuin 2005, Bear 1987, Boks 2002). The findings of the LS-related ECs in our study are consistent with other studies showing a younger median age of diagnosis. Our prediction models further exemplified these findings indicating that patients being diagnosed with EC under the age of fifty-five should be considered for MMR protein analysis. Given the common feature of LS-related ECs being diagnosed at a younger age when compared to sporadic non-mutation carrying patients, it would lead us to speculate that this result is secondary to the lack of expression of mismatch repair (MMR) proteins.

The majority of sporadic ECs diagnosed at a young age are well-differentiated (Grade 1) tumours. The pathogenesis of these cancers is related to excess endogenous estrogen exposure often secondary to body habitus and excess adipose tissue. These neoplasms are often preceded by atypical or complex endometrial hyperplasia and follow a benign clinical course with favourable prognostic outcomes (Bouquier 2011, Broaddus 2006). Earlier studies on LS-related ECs highlighted the increased frequency of endometrioid endometrial carcinomas (*eec*) in these tumours finding the incidence as high as 92% of cases (Carcangiu 2010). Later research began to indicate a different phenotype or clinical picture with studies showing 14% and 43.4 % of non-endometrioid endometrial carcinomas (*neec*) in LS-related ECs (Baudhuin 2005, Bear 1987). This further exemplifies the importance of our data findings. Almost half of the LS-related

ECs in our cohort were found to have non-endometrioid, Type II tumours. This in combination with a young median age at diagnosis deviates from the phenotype of sporadic estrogen driven *eec* that typically comprise eighty percent of sporadic ECs. Most studies demonstrate that *neec* comprise anywhere from 10 to 20% of sporadic ECs, and are typically diagnosed in pre or postmenopausal women with an average age between 65 and 68 years with no identifiable precursor.

The LS-related tumours in our study were associated with a higher frequency of papillary serous, and clear cell carcinoma cell types compared to sporadic cases. In our prediction models when these cell types (papillary serous and clear cell carcinoma) were grouped and compared to all other cell types these cell types were significant predictors of LS-related ECs. The increased in frequency of these cell types in LS-related ECs could serve as a "red-flag" for patients presenting with these pathologies to ensure more in-depth family history analysis and screening with the Amsterdam II and Revised Bethesda Guidelines and in turn genetic testing.

Antecedent studies researching FIGO grade have linked LS-related ECs with an increased frequency of high-grade tumours when compared to sporadic ECs (Baughuin 2005, Bear 1987, Carcangiu 2010, Classics in Oncology 1985). Our study showed that poorly differentiated tumours were also found in higher frequency in LS-related ECs. High-grade ECs are associated with decreased survival and an increased incidence of metastases. Given that the LS cohort was shown to have an increased frequency of high-

grade tumours this would lead us to believe that this would negatively impact proband survival and overall prognosis if they behave similarly to non-mutation related ECs.

The International Federation of Gynecology and Obstetrics (FIGO) stage has been analyzed by previous studies showing higher frequency of stage III tumours in LS-related EC as compared to their control cohorts (Bear 1987, Carcangiu 2010). In our study analysis on local and advanced stages of disease (combination of stages I/II and III/IV respectively) demonstrated that LS-related ECs were more likely to be diagnosed with a more advanced stage of disease. In our prediction models, this was not found to be a significant predictor of LS for screening purposes. Given that our analysis identified that probands in the LS cohort were more likely to have a more advanced stage of disease compared to sporadic variants we hypothesize that this would be attributable to the impairment on the MMR protein system and subsequent effects on cellular regulation.

In Canada the average age of diagnosis of an EC is approximately 60 years of age (Stats Canada, 2017). The average age of diagnosis of EC in our LS cohort was 45 years of age, which is more than a decade earlier when compared to national statistics. Our analysis of ECs diagnosed before of after 55 years demonstrated that the majority of LS-related ECs are diagnosed earlier in life when compared to the general population, mutation negative ECs. Though this is well known in LS literature this is important knowledge for clinicians to consider when diagnosing EC in women with a younger age at diagnosis. In most women, menopause will occur between the ages of fifty and fifty-five years of age with an average age of occurrence at approximately 51.5. The cardinal

symptom for recognition of EC is postmenopausal or abnormal uterine bleeding, which occurs in 90% of patients. Our population of LS-related ECs were on average diagnosed prior to the average age of menopause. This in turn could delay the diagnosis of EC as changes in menstrual bleeding could be attributed to more benign causes by clinicians. Given that the stage of disease at diagnosis in the LS cohort was statistically more advanced than in the sporadic (older on average) cohort; this could result in theoretical delays in diagnosis allowing the progression of the cancers increasing the likelihood of their disease becoming more invasive.

As previously highlighted by Lu *et at* the incidence of LS-related ECs as *Sentinel Cancers* was 51% in their cohort. The LS cohort in our study demonstrated this previously highlighted trend with 69.6% of the women in this cohort diagnosed with EC prior to any other neoplasm. Unlike the study by Lu *et al,* not all the women in our study had a synchronous or metachronous neoplasm. This does further exemplify the importance in specialty areas of gynecology and gynecological oncology of being vigilant towards identifying possible LS-related ECs and referral of the relevant patients to medical genetics for genetic testing Cyr 2012).

The results of research comparing the survival of individuals with LS-related ECs and sporadic cases is limited. Research in this area has had mixed results some studies showing that LS-related ECs have improved survival and others showing that overall survival was unaffected. The inclusion criteria in these studies were vastly different making it difficult to draw conclusions from the analyses. This comparison highlights the

importance of rigid data collection in the clinical and surgical setting. The limitations of previous studies highlight the importance of digital health records. Having a digital catalog of patient information is vitally important for future research in terms of accessibility and storage. Increased quality and accessibility of patient information for research is vital in removing obstacles to the progression of any field of research.

The Kaplan-Meyer survival analysis was conducted using date of death or date of last follow-up for censoring purposes for both the LS and sporadic EC cohorts. The survival analysis demonstrated that the LS-related ECs had the tendency to live longer after diagnosis compared to the sporadic cohort but did not obtain statistical significance result. Interestingly, this is in spite of the LS cohort having worse prognostic indicators at diagnosis. More studies would need to be conducted to assess the reproducibility of these results and subsequent analysis comparing younger sporadic ECs with age matched LS ECs as the younger age at diagnosis could be a factor improving survival.

5.1 Study Limitations

This study was designed as a pilot for a larger study assessing the clinical phenotype of Lynch Syndrome related ECs and subsequent effect on survival compared to disease without a genetic mutation. One of the more significant limitations was the lack of ongoing information available on the Lynch Syndrome cohort. As this was a retrospective analysis and the use of information was granted from a previous study, future studies should involve a new ethics application allowing access to current proband electronic medical files when available. This project has begun thorough review of all

Lynch Syndrome cases in Newfoundland and Labrador. This would help with specific dates of diagnosis and death as well as cause of death in order to enhance the quality of the survival analysis. The data that was gathered from the LS proband charts were limited with regards to the information surrounding death including cause and date of death. This was similar to the limitations that were found in the sporadic cohort data from The Newfoundland and Labrador Cancer Registry. The data from the Newfoundland and Labrador Cancer Registry did not contain information pertaining to follow up and whether or not patients survived their neoplasms. The conclusions that we can draw from our Survival Analysis are limited because of the previous mentioned reasons and further research in this area would greatly improve our understanding of how these neoplasms behave compared to their sporadic counterparts with regards to prognosis and overall survival.

5.2 Conclusions

In conclusion, the Lynch Syndrome-related ECs in this research study presented with a different pathological phenotype and younger age of diagnosis than sporadic neoplasms in individuals without mutations. The Lynch Syndrome ECs presented as higher-grade neoplasms with cell types known to have more aggressive natural histories coupled with increased risk of metastases and poor prognosis. Younger ages of diagnosis in combination with aggressive cell types (papillary serous/clear cell carcinoma) were both predictive of Lynch Syndrome and both these clinical variables should raise the suspicion of clinician when presenting concurrently. The Lynch Syndrome related neoplasms were also associated with more advanced stage of disease at diagnosis and

though this variable was not predictive of LS should warrant a more thorough family history analysis. The overall survival of individuals with Lynch Syndrome related ECs also appeared to be worse as a result of this autosomal dominant mutation.

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Appendix

Amsterdam II criteria

1. Three or more relatives with histologically verified Lynch syndrome-associated cancers (CRC, cancer of the endometrium or small bowel, transitional cell carcinoma of the ureter or renal pelvis), one of whom is a first-degree relative of the other two and in whom familial adenomatous polyposis (FAP) has been excluded.
2. Lynch syndrome-associated cancers involving at least two generations.
3. One or more cancers were diagnosed before the age of 50 years.

Revised Bethesda Criteria

1. Colorectal or uterine cancer diagnosed in a patient how is less than 50 years of age.
2. Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumours, * regardless of age.
3. Colorectal cancer with the MSI-H ** histology *** diagnosed in a patient who is less than 60 years of age.
4. Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumour, with one of the cancers being diagnosed under age 50 years.
5. Colorectal cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumours, regardless of age

Synchronous neoplasias: are defined as 2 or more primary tumours identified in the same patient and at the same time.

Metachronous neoplasias: tumours are defined as primary tumours developing 6 months after the first primary has been resected.

Presumptive-Lynch Syndrome: Patients were conservatively classified as presumptive Lynch syndrome if their tumours showed loss of at least one mismatch repair protein and were negative for methylation of MLH1

Hypermethylation: is an epigenetic control aberration that is important in gene inactivation in the majority of tumour analysis. Hypermethylation of CpG islands have been described in almost all tumour analysis. In the context of this research paper it refers to gene silencing of Mismatch repair genes.