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Somatic POLE exonuclease domain mutations in endometrial cancer : Insights into the biology of POLE-mutant tumors

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Chapter 1

Introduction

INTRODUCTION

Personalized medicine concerns the tailoring of prevention, diagnosis, and treatment of disease to the individual's characteristics, needs, and preferences. The concept of personalized medicine is not new. From the time of Hippocrates (~400 B.C.E.), doctors have been aware that patients with similar symptoms may suffer from different diseases; and similarly, that patients with the same disease may respond differently to given treatments.[1] In cancer care, clinicians have long used the location of the tumor and the stage of disease with which a patient presents to direct initial and adjuvant treatment. The stage of disease can be summarized by the extent to which the tumor has spread beyond its original site. For patients with a localized or low-stage tumor, surgical resection can be curative. In contrast, when the cancer has spread throughout the body, surgery alone is not effective, whereas systemic therapy such as chemotherapy may be. Over time, tumor location and disease stage have been combined with multiple other clinicopathological features to provide an increasingly comprehensive profile of a specific tumor. Such features include the histological tumor type and grade (a measure of the appearance and growth patterns of tumor cells), the invasion of cancer cells into lymph and/or blood vessels, and the expression of specific proteins as determined by immunohistochemical analyses. The resulting clinicopathological profile has since been used to predict how a particular cancer will behave and consequently, to assess how best to treat an individual patient.

While the concept of personalized medicine based on clinicopathological characteristics in cancer is not new, the genomic data that can be incorporated into clinical decision making is a rapidly expanding field. Sanger sequencing has been the traditional method of genomic analysis: with this method, 500 to 600 base pairs of DNA can be sequenced per reaction (to put this into perspective, the human genome consists of three billion base pairs). In the past two decades, newer methods of genomic analysis have been introduced that can sequence longer fragments of DNA in a single run. As multiple DNA sequencing platforms were developed around the same time, fierce competition between manufacturers arose to increase the length and output of the sequences, and at the same time, to lower the costs. These new and cheaper sequencing technologies enabled an unprecedented expansion of our knowledge of the human genome.[2,3]

Cancer can, in essence, be considered a disease of the genome in a specific organ or tissue. Much of the contemporary research on cancer has focused on using the genomic data to identify and understand the molecular basis of this disease. This has led to a more advanced form of personalized medicine, now often also termed precision medicine, in which the individual's tumor characteristics not only include clinicopathological

data but also the molecular profile of the specific cancer. Such a combination can even more accurately predict a patient's prognosis and the likelihood of response of the tumor to conventional treatment (e.g. chemo- or radiotherapy). Moreover, it may predict response to specific targeted therapies, directed against molecular alterations identified in the patient's tumor.

The benefits through the application of precision medicine have been clearly demonstrated in breast cancer. In this tumor type, molecular profiling has been implemented to provide more precise estimates of patient outcomes. Together with clinical and morphological features (including age, tumor size, grade, and lymph node status), determination of hormone receptor status and of human epidermal growth factor 2 (HER2) expression (overexpression as surrogate marker for gene amplification) are the standard of care. Both markers are used to predict a patient's prognosis and to stratify for conventional and targeted (adjuvant) treatments.[4] Moreover, this integrated molecular approach has been further refined by the use of gene expression profiles such as the MammaPrint or OncotypeDX. These profiles provide additional prognostic information, and, in certain patients, allow for tailored treatments.[5-9]

In summary, the use of molecular biomarkers has the potential to improve treatment response and prognostic forecasting. Although for some cancers, molecular profiling has already been implemented in clinical practice, for other cancer types, including endometrial cancer, this is not yet the standard of care. This chapter describes the epidemiology, diagnosis, and (adjuvant) treatment options in endometrial cancer. Moreover, it describes our current knowledge of molecular alterations and how these may improve personalized medicine in endometrial cancer.

Endometrial cancer

Epidemiology

Endometrial cancer arises from the epithelial lining of the uterus, called the endometrium. It is the most common gynecological malignancy in the Western world, accounting for approximately 1900 new cases in the Netherlands each year.[4] Due to the increasing obesity and ageing of the population, two important risk factors for the development of endometrial cancer, the incidence of endometrial cancer has been rising rapidly (36% increase since 2000 in the Netherlands, Figure 1).[10-13] This disease typically afflicts older women - incidence peaks between 65 and 75 years of age - who present with postmenopausal bleeding.[4,11] This symptom is recognized by both patients and physicians as an indication for prompt referral and investigation, and leads to diagnosis of endometrial cancer in an early stage of disease in the majority (~75%) of patients.[11,12] Prognosis for these patients is favorable, with a five-year overall survival of 85-90%. For women with

recurrent or metastatic disease, however, outcomes are poor, with a five-year overall survival of ~20% for patients with distant metastases.[12] Because of the favorable prognosis of early-stage disease, the overall mortality rate is relatively low: approximately 460 women die of endometrial cancer in the Netherlands each year. This contrasts with other gynecological malignancies such as ovarian cancer, where ~75% of patients die of the disease.[11]

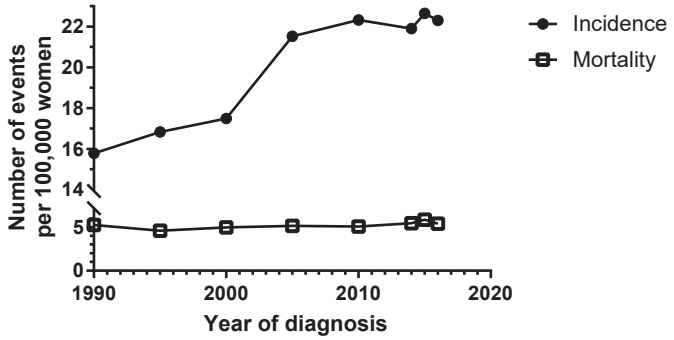

Figure 1. Incidence and mortality of endometrial cancer in the Netherlands per 100,000 women in the period 1990 – 2016.[11]

Diagnosis and primary treatment

Endometrial cancer is diagnosed by histopathological assessment of endometrial tissue obtained through sampling of the endometrium by biopsy or curettage. After diagnosis, standard treatment consists of surgery, during which the uterus, both ovaries and fallopian tubes are removed (abdominal or laparoscopic total hysterectomy and bilateral salpingo-oophorectomy).[4] The indication for adjuvant treatment is currently based on a patient's risk of disease recurrence as estimated by clinicopathological risk factors. Risk factors include tumor type, tumor grade, stage of disease, age of the patient, and lymphovascular space invasion. These factors are described in more detail below.

Clinicopathological risk factors

Histological type

Historically, endometrial carcinomas can be divided morphologically into two main tumor types: endometrioid and non-endometrioid endometrial cancers (Figure 2).[14] Endometrioid carcinomas, the most common subtype (~80% of cases), are cancers that morphologically resemble the normal endometrium. This subtype is associated with unopposed estrogen stimulation and usually arises in the background of endometrial hyperplasia. In contrast, non-endometrioid endometrial cancers are estrogen-independent tumors that often arise in atrophic endometrium and have various histological subtypes, including serous and clear cell carcinomas.[14,15] Hamilton et al., among others,

reported that while 13% of endometrial cancers showed non-endometrioid histology in their study, these cases accounted for 47% of endometrial cancer deaths.[16] Thus, compared to endometrioid-type tumors, non-endometrioid endometrial cancers have an aggressive clinical course and account for a disproportionate number of endometrial cancer deaths.

FIGO grade

Endometrioid endometrial cancers are graded according to the International Federation of Gynecology and Obstetrics (FIGO) classification based on the percentage of non-squamous solid growth and on the degree of nuclear atypia in comparison to normal endometrium (Figure 2A-B).[12,15,17] An endometrioid endometrial cancer consisting of a predominantly glandular architecture with no or minimal solid growth (≤5%) is considered grade 1, whereas endometrial cancers with 6-50% or >50% solid growth are designated grade 2 and 3, respectively. When nuclear atypia is notable (e.g. nuclei differ greatly in size and shape) and when this atypia does not correspond to the architectural

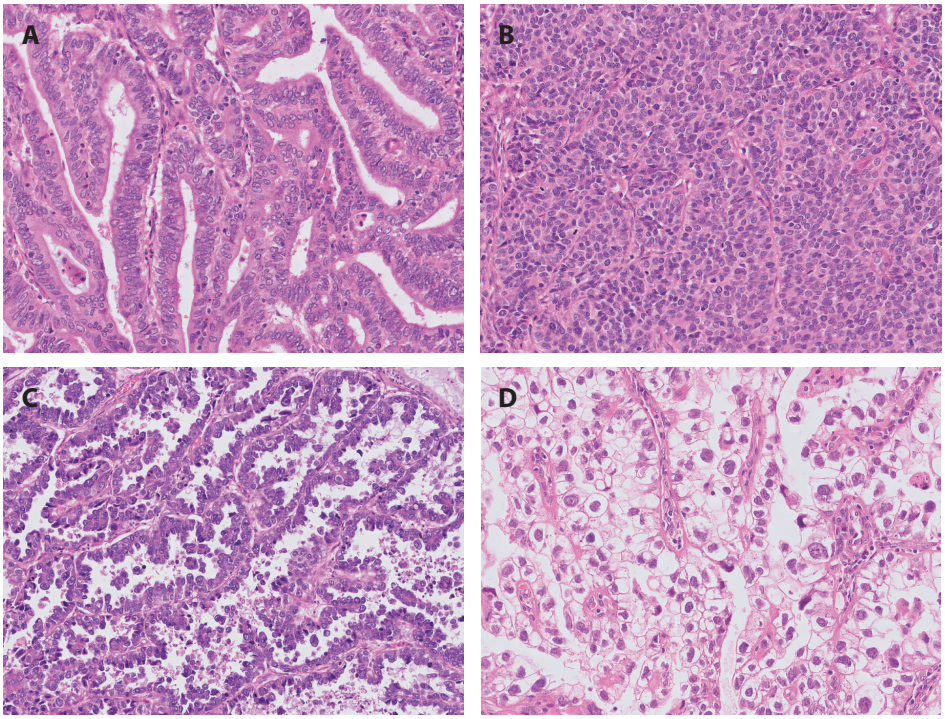

Figure 2. Histological classification of endometrial cancers.
Common histological subtypes of endometrial cancer are endometrioid-type (A and B), serous (C), and clear cell (D) carcinomas. Furthermore, endometrioid endometrial cancers are graded based on the presence of solid growth and nuclear atypia: examples of a grade 1 (A) and grade 3 (B) endometrioid endometrial cancer are shown. Figures A, B, and D are at x20 magnification, C at x10.

grade, the grade of the endometrial cancer can be raised by one. For every stage of disease, high-grade endometrial cancers (grade 3) have a poorer clinical outcome.[12,18]

FIGO stage

The FIGO staging system uses clinical, surgical and pathological findings to classify endometrial cancers into four stages based on the extent of tumor growth (Table 1). This classification takes invasion of the tumor in the myometrium into account, invasion of tumor in cervical stroma and/or serosa, involvement of the adnexae, of pelvic and/or para-aortic lymph nodes, and of surrounding or distant organs and tissues. Evidently, survival decreases with increasing stage.[12]

Table 1. FIGO 2009 staging of endometrial cancer.[12,17]

Stage	Description
Stage I	Tumor confined to the corpus uteri
IA	No or less than one half myometrial invasion
IB	Invasion equal to or greater than the outer one-half of the myometrium
Stage II	Tumor invades the cervical stroma but does not extend beyond the uterus
Stage III	Local and/or regional spread of the tumor
IIIA	Tumor invades the serosa and/or adnexae
IIIB	Vaginal and/or parametrial involvement
IIIC	Metastases to pelvic and/or para-aortic lymph nodes
IIIC1	Metastases to pelvic lymph nodes
IIIC2	Metastases to para-aortic lymph nodes with or without positive pelvic lymph nodes
Stage IV	Tumor invades bladder and/or bowel mucosa, and/or distant metastases
IVA	Tumor invades the bladder or bowel mucosa
IVB	Distant metastases, including intra-abdominal metastases and/or inguinal lymph nodes

Age at diagnosis

The age of the patient is another determinant of prognosis in endometrial cancer. Age is associated with other prognostic factors such as tumor type: endometrial cancers of elderly women are more often of non-endometrioid histology. Despite this association, although it remains to be clarified how this is reflected in the underlying tumor biology, age is an independent prognostic factor in endometrial cancer. Patients with an age at diagnosis of 60 years or more have a higher risk of both locoregional (vaginal or pelvic) and distant recurrences and more often succumb to endometrial cancer than those younger than 60 years at diagnosis.[19-22]

Lymphovascular space invasion

Finally, over the past three decades, many independent studies have shown the prognostic significance of lymphovascular space invasion in endometrial cancer.[23-30] Lymphovascular space invasion can be defined as the presence of cancerous cells in a space lined by endothelial cells outside the immediate invasive border.[24] The presence of substantial lymphovascular space invasion is an important risk factor for lymph node involvement, local recurrence (even in the absence of lymph node metastases) and distant metastases.[23-30] Although a standard definition and an optimal scoring method of lymphovascular space invasion remain to be developed, a comparison of different scoring methods determined a three-tiered scoring system to be the most straightforward and to have the strongest prognostic power.[24]

Adjuvant therapy

Together, these clinicopathological risk factors are used to group patients according to their risk of endometrial cancer recurrence with consequent implications for adjuvant treatment (Table 2).[31] In this risk assessment, patients with early-stage, endometrioid-type endometrial cancers are stratified into low-, (high-)intermediate-, and high-risk groups. Patients with more advanced-stage disease and women with non-endometrioid-type cancers are all classified as being at high risk. For patients with low-risk endometrial cancer, no additional treatment is recommended as their risk of recurrence after hysterectomy is low.[19,32-34] Therapeutic options considered in the adjuvant setting for (high-) intermediate and high-risk disease are radiotherapy (either vaginal brachytherapy or external beam radiotherapy), chemotherapy or a combination.

In patients with intermediate-risk endometrial cancer, large randomized controlled trials showed that external beam radiotherapy significantly decreases the risk of locoregional recurrence (vaginal and/or pelvic recurrences): in the PORTEC-1 trial, the five-year risk was 14% without additional treatment and 4% with radiotherapy. However, radiotherapy does not improve survival in early-stage disease: overall survival is approximately 80-85% after five years, both with and without adjuvant treatment. In addition, most recurrences are local (predominantly found at the vaginal vault), and the majority can be effectively salvaged.[19,33,34] In the PORTEC-2 trial, vaginal brachytherapy has been shown to reduce the risk of vaginal recurrence as effectively as external beam radiotherapy: five-year recurrence rates were less than 2% for both vaginal brachytherapy and external beam radiotherapy.[35] However, vaginal brachytherapy causes significantly less bowel toxicity than external beam radiotherapy, and quality of life remains high, with rates similar to a norm population matched for age and gender.[36,37] Therefore, vaginal brachytherapy is currently the standard adjuvant treatment for patients with (high-)intermediate-risk endometrial cancer.[35,38]

Table 2. Risk groups in endometrial cancer to guide the use of adjuvant treatment according to the ESMO-ESGO-ESTRO consensus guidelines 2016.[31]

Risk group	Description	Adjuvant treatment recommendations
Low	Stage I endometrioid, grade 1-2, <50% myometrial invasion, LVSI negative	No adjuvant treatment recommended
Intermediate	Stage I endometrioid, grade 1-2, ≥50% myometrial invasion, LVSI negative	Vaginal brachytherapy No adjuvant treatment is an option, especially for patients aged <60 years
High-intermediate	Stage I endometrioid, grade 3, <50% myometrial invasion, regardless of LVSI status	External beam radiotherapy when LVSI positive
	Stage I endometrioid, 1-2, LVSI unequivocally positive, regardless of depth of myometrial invasion	Vaginal brachytherapy when grade 3 and LVSI negative
High	Stage I endometrioid, grade 3, ≥50% myometrial invasion, regardless of LVSI status	External beam radiotherapy
	Stage II endometrioid	Consider adjuvant and concurrent chemotherapy (standard: carboplatin and paclitaxel)
	Stage III endometrioid, no residual disease	Consider vaginal brachytherapy boost when stage II
	Non-endometrioid (serous, clear cell, undifferentiated, carcinosarcoma)	

In a pooled analysis of long-term outcomes of the PORTEC-1 and -2 trials, patients with early-stage grade 3 disease and/or with substantial lymphovascular space invasion had significantly increased pelvic regional and distant recurrence rates. For the 5% of patients who had substantial lymphovascular space invasion, the risk of pelvic regional recurrence was 15% after five years, compared to 2% for those without lymphovascular space invasion.[24] These patients are therefore considered at high-intermediate risk in the current risk classification according to the recent ESMO-ESGO-ESTRO consensus guidelines. For patients with high-intermediate-risk endometrial cancer with substantial lymphovascular space invasion, external beam radiotherapy is recommended to maximize pelvic control.[31]

Controversy remains concerning the optimal adjuvant therapy for patients with high-risk endometrial cancer, which includes those with early-stage grade 3 disease with deep invasion, those with more advanced stages, and those with non-endometrioid-type cancers. Two trials compared pelvic external beam radiotherapy to chemotherapy (consisting of three to five cycles of cyclophosphamide, doxorubicin and cisplatin) and found no difference between the arms regarding overall and progression-free survival.[20,21] In a multicenter randomized trial, sequential external beam radiotherapy and different re-

gimes of chemotherapy (predominantly platinum-based) was compared to radiotherapy alone; the combination resulted in a significantly higher five-year progression-free survival (78% vs. 69%).[39] Two randomized controlled trials in which combined radiotherapy and chemotherapy is compared to either external beam radiotherapy (PORTEC-3) or chemotherapy (GOG-0258) alone, have recently presented preliminary results. The GOG-0258 trial for stage III-IVA endometrial cancer found that, although chemotherapy combined with radiotherapy reduced the risk of vaginal, pelvic, and para-aortic recurrences, it did not increase the recurrence-free survival compared to chemotherapy alone.[40] In the PORTEC-3 trial, which included stage I-III high-risk disease, a trend towards an improved five-year failure-free survival (i.e. relapse or endometrial cancer-related death) was found with concurrent chemotherapy and radiotherapy. In women with stage III disease, the addition of chemotherapy significantly improved the failure-free survival by 11% after five years.[41] While awaiting the long-term outcomes of these studies, external beam radiotherapy is recommended to maximize locoregional control. To decrease the rate of distant metastases, adjuvant platinum-based chemotherapy can be considered, especially for stage III disease and for those with non-endometrioid-type cancers. Current literature supports the use of combined radiotherapy and chemotherapy rather than either modality alone.[31]

Despite the use of this clinicopathological risk assessment, over- and undertreatment remain an important clinical problem. On one hand, approximately seven endometrial cancer patients need to be treated with vaginal brachytherapy to prevent one vaginal recurrence. This results in unnecessary treatment-associated costs and toxicities: six of out of the seven patients treated would not have had a recurrence regardless of the vaginal brachytherapy.[32,35,42] On the other hand, approximately 7% of patients with intermediate-risk endometrial cancer develop distant metastases within five years that might have been prevented with tailored adjuvant treatment.[32] Similarly to the model used in breast cancer described at the beginning of this chapter, recent studies proposed the use of molecular biomarkers in an integrated risk model to more accurately predict the clinical outcome of endometrial cancer patients.

Molecular (integrated) classification

In 2013, The Cancer Genome Atlas (TCGA) proposed a new classification of endometrial cancer based on genomic, transcriptomic and proteomic analysis of 373 endometrial cancers (Figure 3A-B). This classification included four distinct molecular subgroups with prognostic significance: (1) a group characterized by mutations in the exonuclease domain of the gene encoding the catalytic subunit of DNA polymerase epsilon (*POLE)*, which have a very high mutational burden ('ultramutated'), a specific mutational signature, but also a very good clinical outcome; (2) a microsatellite-unstable (MSI) group,

which shows a hypermutated phenotype, many insertions and deletions due to defects in mismatch repair, and has an intermediate prognosis; (3) a copy-number low group with a lower mutational burden, a microsatellite-stable phenotype, and an intermediate to favorable outcome; and (4) a copy-number high group, which contains the majority of non-endometrioid (mostly serous) cancers, has a low mutation rate, is associated with *TP53* mutations, and has the poorest clinical outcome.[43] The clinical follow-up data in the TCGA study were, however, limited. Furthermore, while the employed methodologies were useful to identify the molecular subgroups, they are costly and cannot easily be implemented in routine clinical practice. Therefore, different studies validated this molecular classification and its prognostic implications in independent cohorts using surrogate markers, which can be analyzed on formalin-fixed paraffin-embedded (FFPE) tissues as described below.[42,44-46]

Stelloo et al. defined the TCGA molecular subgroups on FFPE tumor samples by clinically applicable methods: *POLE*-mutant cases were identified by Sanger sequencing of a part of the *POLE* exonuclease domain, microsatellite-unstable cases by microsatellite instability assay and immunohistochemistry of the mismatch repair proteins, and copy-number high cases by immunohistochemical analysis of p53.[42,45] Identification of these cases leaves a *POLE*-wild-type, microsatellite-stable, p53-wild-type group that was called 'copy-number low' in the TCGA study or 'no specific molecular profile' (NSMP), as no driver mutation has been identified for this group.[45] Similarly to the TCGA study, Stelloo et al. showed highly significant differences in recurrence and survival rates between the molecular subgroups (Figure 3C).[42] The use of surrogate markers in FFPE samples to establish the molecular classification was further supported in subsequent studies, which also replicated its prognostic impact.[44,46]

Both Stelloo et al. and Talhouk et al. compared risk models based on this molecular classification to models based on clinicopathological or combined molecular and clinicopathological characteristics and assessed their ability to predict clinical outcomes.[42,44] Both studies showed that a model integrating both molecular alterations and clinicopathological risk factors most strongly improved the risk assessment of endometrial cancer patients.[42,44] The study from Stelloo et al., based on the pooled PORTEC-1 and -2 trials, proposed the inclusion of lymphovascular space invasion status, expression of L1 cell adhesion molecule, and *CTNNB1* mutational status in addition to the TCGA molecular subgroups in the integrated risk classification. L1 cell adhesion molecule (L1CAM; CD171) is a membrane glycoprotein of the immunoglobulin superfamily, whose expression in endometrial cancer is strongly related to disease progression and recurrence.[47-51] *CTNNB1* exon 3 mutations lead to activation of Wnt/β-catenin signaling, and characterize an aggressive subset of low-grade early-stage endometrial cancers.[52,53] In the resulting

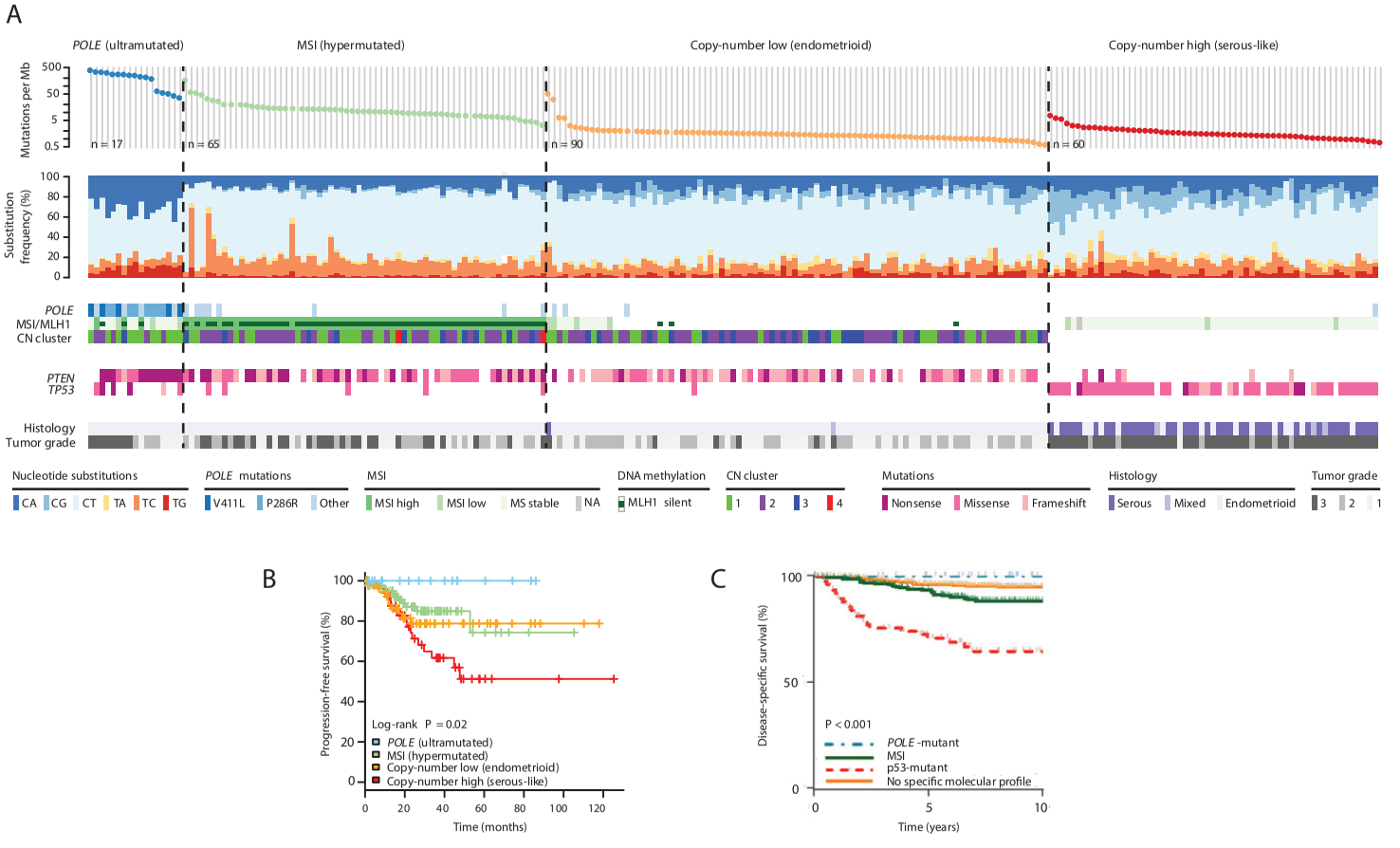

Figure 3. Molecular characterization of endometrial cancer and its prognostic significance in two independent studies.

(Figure 3 continued) (A) Genomic, transcriptomic, and proteomic analysis of 373 endometrial cancers by The Cancer Genome Atlas (TCGA) identified four distinct molecular subgroups. Molecular and clinicopathological characteristics are depicted for each molecular subgroup. (B) Progression-free survival for the different molecular subgroups from the TCGA study is shown. (C) Disease-specific survival for the four molecular subgroups, identified with clinically applicable surrogate markers, in a pooled analysis of two large randomized controlled trials (PORTEC-1 and PORTEC-2) of endometrial cancer is depicted. Figures A and B are adapted from TCGA, Nature 2013 (reprinted under the Creative Commons license).[43] Figure C is adapted from Stelloo et al., Clin Can Res 2016 with permission from the American Association for Cancer Research.[42] Abbreviations: CN, copy number.

molecular integrated risk model, stage I endometrial cancers at (high-)intermediate risk could be reclassified to favorable, intermediate, or unfavorable risk groups. This lead to 55% of patients at intermediate risk of recurrence being reassigned to the favorable risk group: these patients may not benefit from adjuvant treatment. Moreover, 15% of patients were reclassified to the unfavorable group: these patients may benefit from more extensive adjuvant treatment.[42] This model is currently prospectively evaluated in the multicenter randomized PORTEC-4a trial and may substantially reduce unnecessary adjuvant treatment in endometrial cancer.[54]

SUBJECT, AIMS AND OUTLINE OF THIS THESIS

Personalized medicine in cancer care can be improved upon by combining molecular biomarkers with clinicopathological characteristics to better predict a patient's prognosis and/or response to treatment. The Cancer Genome Atlas paved the way towards such an even more personalized approach in endometrial cancer: a molecular classification was proposed in which four distinct subgroups were identified with prognostic significance.[43] One of the molecular subgroups in endometrial cancer as proposed by The Cancer Genome Atlas was novel, and was characterized by mutations in the *POLE* proofreading exonuclease domain.[43,55-57] Moreover, The Cancer Genome Atlas found that *POLE* exonuclease domain-mutant cancers are characterized by a very high mutational burden, which is amongst the highest found in human cancers.[43,55,58] Genomic instability and mutability are central to cancer development and progression.[59] Therefore, it was striking that, despite this so-called 'ultramutated' phenotype, multiple independent studies showed that patients with *POLE* exonuclease domain-mutant endometrial cancers have a very good clinical outcome.[42-46,60-62]

The aims of this thesis were to gain insight into somatic *POLE* exonuclease domain mutations in endometrial cancer and especially into the underlying mechanism(s) by which these *POLE* mutations contribute to the observed favorable clinical outcome. These insights may facilitate the implementation of *POLE* exonuclease domain mutations as an

important prognostic biomarker in routine clinical practice. Moreover, it may provide a deeper understanding of molecular mechanisms underlying endometrial cancer, which may lead to the discovery of new therapeutic options. Finally, as *POLE* exonuclease domain mutations are found in a wide variety of human malignancies (for example colorectal cancer), these findings may be generalizable to other cancer types as well.

In **chapter 2**, the current understanding of the mechanisms and the consequences of *POLE* exonuclease domain mutations in human cancers is reviewed. Furthermore, chapter 2 reports on the potential usefulness of these mutations as novel cancer biomarkers and therapeutic targets. **Chapter 3** describes a histopathological and immunohistochemical characterization of *POLE* exonuclease domain-mutant endometrial cancers to aid their detection in routine clinical practice. **Chapter 4** shows how enhanced immunogenicity may contribute to the favorable clinical outcome of *POLE* exonuclease domain-mutant endometrial cancers. **Chapter 5** focuses on validation of the results obtained from chapter 3 and on the possible utility of immunotherapeutic strategies in a series of high-risk endometrial cancer. **Chapter 6** describes the sensitivity to adjuvant treatment strategies of *POLE* exonuclease domain-mutant cancers and its impact on clinical outcome. **Chapter 7** focuses on the timing of *POLE* exonuclease domain mutations in carcinogenesis. Finally, **chapter 8** provides a general discussion of the results of this thesis, focusing on future perspectives.

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