Radiotherapy for endometrial cancer: improved patient selection, techniques and outcomes

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CHAPTER 7

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

7. GENERAL DISCUSSION

The primary aim of this thesis was to evaluate the role of radiotherapy and to improve quality of treatment for women with endometrial cancer. The second aim of this thesis was to improve treatment selection and reduce over- and undertreatment by the integration of clinicopathological and molecular risk factors into the adjuvant treatment guidelines for women with endometrial cancer.

The guidelines for adjuvant treatment of women with endometrial cancer were largely based on results of the PORTEC, GOG and ASTEC/EN5 trials.[1-3] Conclusions from these trials were that external beam radiotherapy (EBRT) provides excellent locoregional control for women with early stage, intermediate and high-intermediate risk endometrial cancer, however, without a survival benefit and with added treatment-related toxicity, mostly gastro-intestinal symptoms. In the PORTEC-2 trial, vaginal brachytherapy was shown to be equally effective for local control and survival compared to EBRT, with reduced toxicity and better quality of life.[4-6] Long-term analysis of the PORTEC-2 trial showed persistent efficacy with a 10-year vaginal recurrence rate of 3.4% and overall survival of 69.5%, compared to 2.4% and 67.6% after EBRT, respectively, and the importance of new prognostic risk factors **(chapter 2)**. The pelvic recurrence rate was slightly higher after vaginal brachytherapy (6.3% versus 0.9% after EBRT), however these recurrences were mostly combined with distant metastasis, which highlights the need of improved understanding of tumour behaviour based on both clinicopathologic and molecular risk factors.

7.1 Risk factors in endometrial cancer

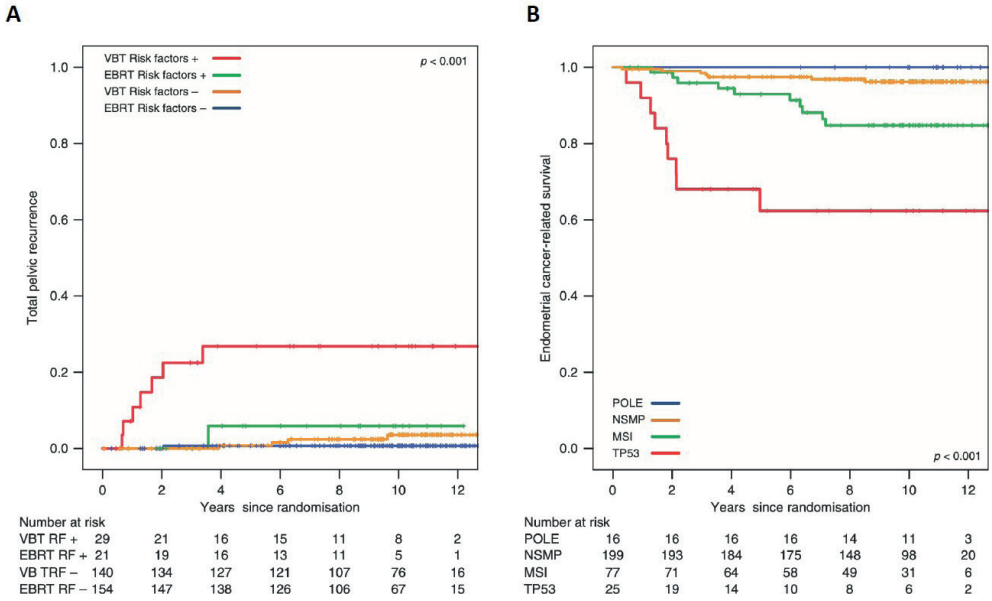
Well-known clinicopathological risk factors in endometrial cancer, associated with increased risk of disease recurrence, are age, histologic type and grade and FIGO stage. More recently discovered major risk factors such as (substantial) lymph-vascular space invasion (LVSI) and L1-cell adhesion molecule (L1CAM) overexpression, and molecular risk factors as defined by the Cancer Genome Atlas group (TCGA) and subsequently detected by surrogate markers in standard pathology specimens (*POLE*mut, NSMP, MMRd and p53abn), and ß-catenin (*CTNNB1*) exon 3 mutation are currently being implemented in the risk classification and treatment guidelines.[7-12] LVSI is associated with the risk of (microscopic) nodal and distant metastases and higher rates of recurrence, and reduced cancer-specific survival, both in the presence and absence of lymph node metastases.[13, 14] LVSI quantified as substantial, compared to no or focal LVSI, showed to be the strongest risk factor for pelvic and distant recurrences.[9, 15, 16] In *CTNNB1*-mutated endometrial cancer, the Wnt signalling pathway is activated by nuclear accumulation of ß-catenin that may result in endometrial cancer progression, and by abnormal expression of cell proliferation and progression genes.

CTNNB1 exon 3 mutation is associated with decreased overall survival.[17, 18] L1CAM is a membrane glycoprotein with an important role in tumour cell adhesion and migration. L1CAM overexpression (>10%) on immunohistochemistry is reported in approximately 16-28% of endometrial cancers and is associated with presence of *TP53* mutations, non-endometrioid histology, histological grade 3, and with LVSI. L1CAM overexpression was shown to be independently related with an increased risk of locoregional and distant spread, and decreased overall and relapse-free survival.[10-12, 19-21]

7.2 Combining clinicopathologic and molecular risk factors to guide adjuvant treatment

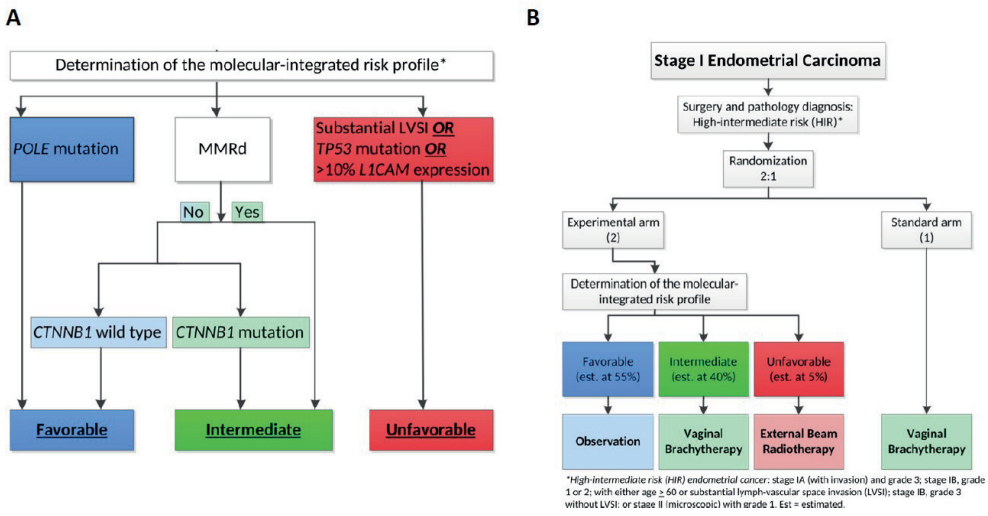
Analysis of the combination of clinicopathologic and molecular risk factors within the PORTEC-2 population showed that substantial LVSI, p53abn and L1CAM overexpression were all strongly associated with the risk of pelvic and distant recurrence and impaired endometrial cancer-related survival **(chapter 2)**. Patients with any of these risk factors who were treated with pelvic EBRT were found to have significantly improved pelvic control over those treated with vaginal brachytherapy (*Figure 1A*). These findings illustrate that within the (high-)intermediate risk group some women with risk factors as LVSI, L1CAM overexpression or p53abn may benefit from pelvic EBRT over VBT.

Figure 1. A) Total pelvic recurrence by unfavourable risk factors (substantial LVSI, p53abn or L1CAM overexpression). **B)** Endometrial cancer-related survival by 4 molecular subgroups.



In a combined analysis of the pooled PORTEC-1 and 2 trial cohorts of (high-)intermediate risk endometrial cancer treated with either EBRT, brachytherapy or observation, the TCGA subclasses and other molecular risk factors as *CTNNB1* mutation were combined with presence of substantial LVSI and L1CAM overexpression into an integrated molecular profile.[8, 9, 22] With the combination of all these risk factors, three subgroups of (high-)intermediate risk endometrial cancer with favourable, intermediate and relatively unfavourable outcomes could be defined. The favourable profile, comprising endometrial cancers with either *POLE* mutation or with absence of all the other risk factors had an excellent prognosis, with low risk of recurrence even without adjuvant treatment, and for this subgroup it was suggested that adjuvant treatment could safely be omitted (*Figure 1B*). For the small group with unfavourable profile with either p53abn, L1CAM overexpression or substantial LVSI, which was strongly associated with higher risk of locoregional and distant spread, adjuvant treatment by EBRT reduced pelvic recurrence as compared to vaginal brachytherapy. For those with an intermediate profile with either MMRd or *CTNNB1* exon 3 mutation, the most benefit of adjuvant vaginal brachytherapy is expected.[23] This molecular-integrated risk profile with corresponding consequences for adjuvant treatment is being investigated in the international, multicentre randomised PORTEC-4a trial for women with high-intermediate risk endometrial cancer (*Figure 2AB*).[24] In the PORTEC-4a trial women in the experimental arm receive adjuvant treatment based on their integrated molecular profile: those with a favourable profile receive no adjuvant treatment; those with an intermediate profile receive vaginal brachytherapy; and those with an unfavourable receive pelvic EBRT, while those in the control group all receive the standard adjuvant brachytherapy (*Figure 2B*).

Figure 2. A) Decision tree of the molecular-integrated risk profile. **B)** Study design of the PORTEC-4a trial.



The design of the PORTEC-4a trial showed a satisfactory patient acceptance rate of 35% even in the initiation phase of the trial, which ranged from 0 to 57% per institute **(chapter 3)**. Another challenge of the PORTEC-4a trial was the determination of the molecular profile within a narrow timeframe of 2 weeks, as patients had to start treatment within 6-8 weeks from surgery. The overall time between randomisation and determination of the molecular risk profile was 10.2 calendar days, and without the local LUMC cases for which no time was needed to send the tumour samples over, 12.2 days. In 15.6% of all cases pathology review lasted more than 2 weeks, mostly because of delayed receipt of the requested materials and the turnaround time of the NGS. Overall, this analysis showed that determining the molecular profile within time was a logistical challenge. Ideas to further optimise the logistical process may be a regional platform with digital image sharing, a joint laboratory information system and further implementation of molecular testing at regional hospitals.

The PORTEC-4a trial has recently completed accrual. In case the PORTEC-4a trial results will be positive, direct assessment of the molecular profile at the local cancer centre could greatly facilitate the logistical process. This can be achieved by using more widely available, faster and cheaper methods. MMR protein expression and p53 status assessed by immunohistochemistry showed high concordance and low interobserver variation, and first results of a faster and low-cost *POLE* test by PCR assays also showed high sensitivity and specificity.[25-27] Other pathology items that could be assessed at the local pathology lab are LVSI, quantified according to the three-tiered scoring system (no, focal or substantial LVSI), and L1CAM overexpression by immunohistochemistry. Determining the integrated molecular profile locally ensures timely decisions on adjuvant treatment for each individual patient.

7.3 Radiotherapy quality assurance

Within the PORTEC-4a trial an extensive quality assurance (QA) programme was performed to ensure uniform high-quality treatment, as institutes had limited experience with delineating on CT- or MRI-scans for single channel vaginal brachytherapy. Of 21 institutes participating in the dummy run of the PORTEC-4a trial, 15 (71.4%) needed minor or major adjustments of the vaginal brachytherapy plan **(chapter 4)**. Most common reasons for revisions were delineation of the CTV or OAR, dose planning and applicator reconstruction. With the revised brachytherapy plans, the range and standard deviation in dose parameters narrowed down to a more acceptable variation. Thereafter, during annual QA, still 5 out of 27 brachytherapy plans had major protocol deviations and in addition, several institutional changes were observed, such as change in treatment planning system, applicator set, type of afterloader and staff, all essential aspects of brachytherapy treatment. With these findings, QA in radiotherapy trials shows to be essential to

prevent trial protocol deviations and therewith possible impaired trial outcomes.[28, 29] Extensive QA increases treatment uniformity and ensures optimal treatment in the trial which leads to more reliable trial outcomes.[30-37] In a previous review on radiotherapy QA in randomised trials major protocol deviations were observed in 11.0–48.0% of cases, and were reported to be associated with impaired overall survival and local control and potentially increased treatment related toxicity.[28, 38] Besides the positive effect on treatment quality and uniformity within a trial, QA for vaginal brachytherapy will additionally result in increased reliability of data on dose parameters that can be used for evaluation of treatment effectiveness and brachytherapy related toxicity. For external beam radiotherapy, with the introduction of more conformal radiotherapy techniques as IMRT or VMAT, QA is of major importance, as delineation and treatment planning variations can result in significant alteration of dose distribution to the target volumes or OARs. In future radiotherapy trials, a comprehensive QA program, including a pre-trial dummy run and annual QA, should be strongly considered to be included in the trial design. Even though continuous QA within radiotherapy trials comes with additional costs and is labour-intensive to perform, the benefit of continuous QA seems to outweigh these disadvantages. Review of one case per institute per year could already increase protocol adherence, and therewith treatment quality and uniformity. In the near future, digital platforms for central review of radiotherapy data and the use of artificial intelligence for case-specific QA can facilitate fast QA procedures in future radiotherapy trials, for example by using statistical models to detect outliers of target volume delineation or dosimetry.[39-41]

7.4 Treatment-related toxicity and modern radiotherapy techniques

Vaginal brachytherapy is a treatment with very limited toxicity. Within the PORTEC-2 trial, of all women who received vaginal brachytherapy 5.6% reported quite a bit to very much diarrhoea after treatment, compared to 22.7% of those who received EBRT.[5] After vaginal brachytherapy lower scores of faecal leakage were reported six months after treatment, compared to EBRT. Both of these symptoms resulted in limitations of daily activities due to bowel problems for 6% of women after vaginal brachytherapy, versus 22% after EBRT.[42] Long-term analysis showed that, besides more bowel symptoms after EBRT, urinary urgency was significantly more frequent after EBRT compared to vaginal brachytherapy.[43] Symptoms of vaginal dryness, vaginal shortening/tightening or dyspareunia were not significantly different between vaginal brachytherapy or EBRT, even though a higher rate of grade 1 and 2 mucosal atrophy was observed from 6 months onwards after vaginal brachytherapy; 35% versus 17% at 3-years after vaginal brachytherapy versus EBRT, respectively. Toxicity of EBRT, however, has decreased over the past decade due to the implementation of new radiotherapy techniques. EBRT techniques have developed

from 3D-conformal radiotherapy towards more modern techniques as intensity-modulated radiotherapy techniques as IMRT or VMAT. Analysis of the EBRT techniques in the PORTEC-3 trial for high risk endometrial cancer showed that IMRT resulted in lower rates of grade $\geq$ 2 adverse events (67.7% versus 74.0%), which were mostly gastro-intestinal or haematological, and fewer patient-reported bowel symptoms, compared to 3D conformal radiotherapy **(chapter 5)**. Besides reducing the doses to the lower gastro-intestinal and genito-urinary tract, IMRT and VMAT can also better spare the bone marrow.[44-47] Previous studies showed that reduced radiation dose to the pelvic bone marrow resulted in significant fewer haematological adverse events, which in turn may result in improved clinical outcomes by increased tolerance for chemotherapy.[48-51] For future perspectives of EBRT, development and implementation of new modern radiotherapy techniques can result in fewer treatment-related toxicities and studies are ongoing. Exciting opportunities for the improvement of radiotherapy treatment can be expected from daily MR-guided adaptive radiotherapy, CT-based adaptation based on 4D cone-beam CT, and fast, automated treatment planning software. For vaginal brachytherapy, increased availability and use of CT or MRI can result in better visualisation of the target volume and organs-at-risk for each fraction. By using in-room CT, brachytherapy procedures could be performed more efficiently and patient-friendly.

All aforementioned developments can lead to decreased treatment margins, increased precision and decreased radiated volume of the organs-at-risk and therewith reduced treatment-related toxicity and patient-reported symptoms. Other radiotherapy modalities as proton beam radiotherapy are being introduced for gynaecological malignancies and may reduce dose to organs-at-risk even further, including bowel and bone marrow.[52-55] With these developments the future of radiation therapy beholds fewer toxicity and increased quality of life by more precise and image-guided adaptive therapy with improvement of clinical outcomes.

7.5 Pathology review

Pathology review by expert gynaecological pathologist in diagnosis and treatment of endometrial cancer is frequently performed, as previous studies have shown that pathology assessment of the female reproductive tract has the highest rates of discrepancies between the original and review pathology assessment.[56] Results of the pathology review of the PORTEC-1 and -2 trials showed that 14-24% of patients would not have been eligible for the trial based on the pathology review, mostly due to a shift in histological grade. This was also confirmed in a study of a large high grade endometrial cancer cohort.[57] **Chapter 6** describes the results of the upfront pathology review of the PORTEC-3 trial, before inclusion and randomisation. After reviewing 1226 pathology specimens, 102 patients (8.3%) were found not eligible and therefore not included in the trial. These findings show that without pathology review, 8% of patients could have been under or overtreated, and

could have received unnecessary toxic treatment. An additional benefit of upfront pathology review within clinical trials is that the trial population will consist of a truly eligible patients, which increases the reliability of the trial results.[56, 58, 59] Remaining challenges of routine pathology review by dedicated pathologists are the time-consuming aspect, the costs, and the logistical difficulties. These challenges can partly be solved by a reduction of inter-observer variation by subspecialisation of pathologists and increased use of fast digital pathology consults.

In the near future, significantly less inter-observer variation is expected when the molecular risk factors are implemented in the treatment guidelines which may lead to reduced need for pathology review. Only for rare (non-)endometrioid histologic subtypes with unusual molecular-histology combinations, pathology review should still be performed, especially in clinical trials.

7.6 Prognostic significance of molecular risk factors in endometrial cancer trial cohorts

The analysis of molecular risk factors within the PORTEC-2 trial population, as described in **chapter 2**, showed that out of 344 (high-)intermediate risk endometrial cancer samples 7.3% were p53abn, 4.7% *POLE*mut, 22.4% MMRd, 57.8% had no specific molecular profile and 2.9% were multiple classifiers. Similar analysis has been performed in the high risk population of the PORTEC-3 trial and showed quite a different distribution of the molecular subgroups. Of 423 high risk endometrial cancer samples 22.7% were p53abn, 12.4% *POLE*mut, 33.4% MMRd, 31.5% had no specific molecular profile and 7.1% were multiple classifiers. With this molecular subdivision, remarkable survival differences were observed. Patients with *POLE*mut endometrial cancer had excellent prognosis (98.0% recurrence-free and overall survival), while those with p53abn endometrial cancer had significantly worse prognosis (48.0% and 54.0% recurrence-free and overall survival).[60] Women with p53abn endometrial cancer had the largest benefit of the addition of chemotherapy with an absolute difference of 22.4% and 23.1% for recurrence-free and overall survival at 5 years, while for the MMRd subgroup no benefit of added chemotherapy was observed over EBRT alone. Within the NSMP subgroup the addition of chemotherapy showed a trend for improved recurrence-free survival, similar to the overall trial results; however, due to the limited number of NSMP endometrial cancer in this subanalysis, no definitive conclusions can be drawn for this group. The relatively small subgroup within the NSMP group with negative ER and PR receptors was recently shown to have worse prognosis and more often non-endometrioid histology.[61] Future challenges remain the further specification of risk characteristics within the NSMP group, and define optimal adjuvant treatment for each individual endometrial cancer patient based on the patient's specific risk factors.

7.7 Improving treatment selection by implementing molecular risk factors in the treatment guidelines

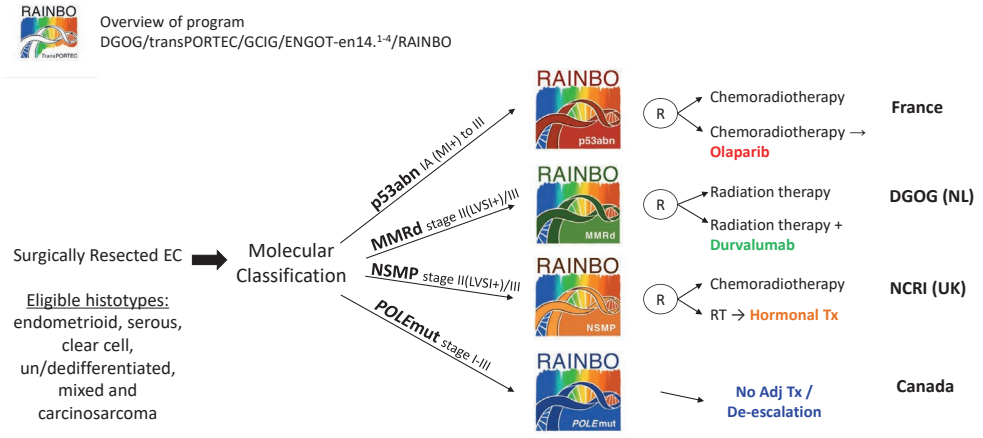
With the knowledge that has been gained on molecular risk factors in endometrial cancer, treatment selection can be improved by implementing these risk factors into the treatment guidelines. p53abn endometrial cancer, even when surgically staged as stage I, should be considered as high risk endometrial cancer and treated accordingly with EBRT and chemotherapy, as investigated by molecular analyses in the PORTEC-3 and in a Danish cohort.[60, 62] The PORTEC-3 trial showed 10% survival benefit for serous cancers of all stages with the addition of chemotherapy, serous cancers comprising about half of all p53abn endometrial cancers. What the exact benefit of added chemotherapy to EBRT will be for stage I (especially stage IA) p53abn endometrioid-type endometrial cancer remains to be investigated further.

Results of the PORTEC-4a trial may show the efficacy of EBRT alone on locoregional control rates and disease-free survival for early stage p53abn endometrioid endometrial cancer. In the GOG-249 trial, which included 20% of women with serous and clear cell cancers, pelvic EBRT had similar recurrence-free survival and better pelvic and para-aortic nodal control compared with the combination of vaginal brachytherapy and 3 cycles of chemotherapy.[63] Based on these findings, patients can be counselled in shared decision making about the survival benefit of adding chemotherapy to EBRT versus the extra treatment-related toxicity. Strikingly, for high risk MMRd endometrial cancers no benefit of chemoradiotherapy over EBRT alone was found in the molecular analysis of the PORTEC-3 trial, and adjuvant chemotherapy should not be recommended; ongoing trials are exploring checkpoint inhibition for MMRd cancers. For high risk (grade 3 and/or stage III) NSMP endometrial cancer a trend for improved recurrence-free survival was seen with chemoradiotherapy. For patients with stage I-II EC with substantial LVSI or L1CAM overexpression EBRT should be recommended, as there is an increased risk of locoregional spread of the disease and EBRT showed excellent locoregional control for this subgroup in the PORTEC-2 cohort (**chapter 2**). *POLE*mut endometrial cancer is associated with excellent prognosis, even in the series which are available without adjuvant treatment, and omission or de-escalation of adjuvant treatment should therefore be considered especially for stage I and II *POLE*mut endometrial cancer. For the very rare stage III *POLE*mut cancers more evidence is needed. These findings and treatment principles are the subject of new and ongoing trials, and treatment recommendations based on molecular integrated risk groups have recently been implemented in the updated European guidelines for endometrial cancer of the European Society of Gynaecological Oncology, Radiation Oncology and Pathology (ESGO-ESTRO-ESP) Guidelines Committee (*Table 1*).[64, 65]

7.8 Future targets for treatment related to the molecular classification

The characteristics of the four molecular groups have led to new possibilities for targeted treatments and/or altered treatment strategies. A new research program for women with high risk EC, with a specific trial for each of the four molecular subgroups is the RAINBO study program, which is a collaboration between large international research groups and is based on the molecular classification and translational research within the *Trans*PORTEC research consortium. The RAINBO platform consists of four different studies targeting each different molecular subgroup, with overarching aims of evaluating the impact on survival and quality of life with targeted treatments, and with an overarching biobank for further translational research (*Figure 3*).[66]

Figure 3. RAINBO study program.



RAINBO program supported by GCIG and coordinated by *Trans*PORTEC will allocate EC pts to 4 international academic sub-trials each led by one Gyn-Onc national clinical trial group

Table 1. Risk groups in endometrial cancer according to PORTEC and the ESMO-ESGO-ESTRO guideline, including the molecular-integrated risk groups.

Risk Group	PORTEC (2002-2013)[1]	ESMO-ESGO-ESTRO guideline 2021[65]	ESMO-ESGO-ESTRO guideline 2021 Molecular-integrated risk groups[65]
Low	FIGO stage IA EEC: grade 1-2	FIGO stage IA EEC: grade 1-2, LVSI neg.	FIGO stage I-II EEC: *POLE*mut
			FIGO stage IA EEC: MMRd/NSMP, grade 1-2, no or focal LVSI
Intermediate	FIGO stage IB EEC: grade 1-2, age <60	FIGO stage IB EEC: grade 1-2, LVSI neg.	FIGO stage IA EEC: MMRd/NSMP, grade 3, no or focal LVSI
		FIGO stage IA EEC: grade 3, LVSI neg.	FIGO stage IA NEEC or p53abn EEC: without myometrial invasion
		FIGO stage IA NEEC: no myometrial invasion	FIGO stage IB EEC: MMRd/NSMP, grade 1-2, no or focal LVSI
High-intermediate	FIGO stage IA EEC: grade 3, age ≥60	FIGO stage IA/B EEC: grade 1-3, LVSI pos.	FIGO stage I EEC: MMRd/NSMP, substantial LVSI
	FIGO stage IB EEC: grade 1-2, age ≥60	FIGO stage IB EEC: grade 3, LVSI neg.	FIGO stage IB EEC: MMRd/NSMP, grade 3
		FIGO stage II EEC	FIGO stage II EEC: MMRd/NSMP
High	FIGO stage IB EEC: grade 3	FIGO stage III-IVA EEC without residual disease	FIGO stage III-IVA EEC: MMRd/NSMP
	FIGO stage II-III EEC	FIGO stage I-IVA NEEC: without residual disease	FIGO stage I-IVA EEC: p53abn with myometrial invasion
	FIGO stage I-III NEEC		FIGO stage I-IVA NEEC: MMRd/NSMP with myometrial invasion

EEC endometrioid endometrial cancer; LVSI lymph-vascular space invasion (neg.: negative, pos.: substantial LVSI); NEEC non-endometrioid endometrial cancer (serous or clear cell carcinoma);

7.8.1 Adjuvant treatment for p53abn endometrial cancer

p53abn endometrial carcinomas are associated with unfavourable prognosis and higher stage of disease; 7.3% of the PORTEC-2 (high-)intermediate risk samples were p53abn versus 22.7% of the high risk endometrial cancer samples of the PORTEC-3. This subgroup has the largest benefit of the addition of chemotherapy (22.4% and 23.1% for RFS and OS).[60] Both amplification of the human epidermal growth factor receptor 2 (Her-2/Neu) and homologous recombination deficiency (HRd) are frequent molecular alterations in p53abn endometrial cancer. Within the PORTEC-3 translational research, 25% of p53abn endometrial cancers were found HER2 positive.[67] The HER-2/Neu receptor is a potential target for therapy, however, studies using HER-2/Neu inhibitors as monotherapy have had disappointing results.[68-71] In a recent study, the combination of trastuzumab with carboplatin and paclitaxel chemotherapy in HER-2/Neu-positive serous carcinomas resulted in a prolonged median progression-free survival in the carboplatin-paclitaxel alone group (13 vs. 8 months, p=0.005), with the largest benefit for stage III-IV disease (17.7 vs. 9.3 months, p=0.005).[72, 73] Homologous recombination is essential for repair of DNA double-strand breaks, which is mediated by (among others) BRCA1 and BRCA2 proteins, and deficiency (HRd) is reported in 46% of p53abn endometrial cancer. Targeting HRd in endometrial cancer by using platinum-based chemotherapy and/or PARP inhibitors seem promising therapeutic options.[74] PARP, or poly (ADP-ribose) polymerase, is involved in DNA damage detection and generation of poly (ADP ribose) chains. These chains facilitate chromatin remodelling and DNA repair. Loss of PARP results in persistent single strand DNA breaks and eventually in double-strand DNA breaks (DSBs). Normally, DSBs are repaired by homologous recombination or other repair mechanisms such as non-homologous end joining. Tumour cells with either loss of PARP or with HRd are still viable, although more faulty DNA repair occurs. However, in case of simultaneous inhibition of both factors, accumulation of DSBs leads to cell death.[75, 76] Response rates of 31-40% to PARP inhibitors (PARPi) have been reported in HRd ovarian carcinoma and BRCA-mutated breast cancer.[77, 78] Within the p53abn-Red trial of the RAINBO program, women with p53abn endometrial cancer will be randomised to chemoradiotherapy versus chemoradiotherapy with the PARP inhibitor olaparib.

7.8.2 Adjuvant treatment of MMR deficient endometrial cancer

The MMRd subgroup comprises 30% of all endometrial cancers and has an intermediate prognosis.[7, 8, 79-82] MMRd endometrial cancers fail to express one or more of the MMR proteins, leading to the accumulation of mismatches, deletions, and microsatellite instability. The majority (~ 75%) of MMRd endometrial cancers are MMR deficient due to MLH-1 promoter hypermethylation with subsequent loss of MLH1 protein expression, and another 10-15% are due to biallelic somatic mutations or other DNA defects.[83] The remaining 10% are caused by germline defects in one of the

MMR genes (Lynch syndrome). Within the entire endometrial cancer population, the frequency of Lynch syndrome varies between 3-6%.[83, 84] MMRd endometrial cancers are hypermutated tumours that harbour higher neoantigen loads, which are associated with increased immune response by cytotoxic CD8+ tumour-infiltrating lymphocytes (TILs).[80-82, 85-93] The presence of CD8+ TILs has been investigated within the PORTEC-1 and 2 cohort and showed that the TIL density was a strong predictor of disease recurrence.[94] MMRd tumours have an increase of PD-1 and PD-L1 expression, which makes these tumours attractive for immune checkpoint inhibitors.

Previous trials have confirmed this hypothesis and have shown response rates of 13-48% to checkpoint inhibitors such as nivolumab, pembrolizumab and dostarlimab in women with recurrent or metastatic hypermutated tumours, including endometrial cancer.[95-98] The addition of the PD-L1 inhibitor durvalumab to the radiotherapy will be investigated in the MMRd-Green trial of the RAINBO program.

7.8.3 Adjuvant treatment of endometrial cancer with no specific molecular profile (NSMP)

The subgroup with no specific molecular profile is a heterogeneous group of tumours with a low mutational burden, and mostly comprises endometrioid-type cancers of low to intermediate grade. Within this subclass about 85-90% of cancers are hormone receptor positive. Hormone receptor status was found to be an important prognostic factor, and loss of ER or PR expression is related to higher grade tumours, non-endometrioid histology, L1CAM overexpression, substantial LVSI and impaired disease-free survival.[61, 99-101] A recent analysis showed that only among the NSMP group, histological grade is still a significant prognostic factor.[102] Analysis of the PORTEC-3 NSMP subgroup showed that the large majority of tumours were ER and PR positive. Targeting the endocrine receptors by hormonal therapy is currently only used for women that wish the preserve fertility with low grade early-stage disease, and in those with advanced or metastatic low grade disease. As hormonal therapy for women with low grade ER+/PR+ NSMP tumours might effectively reduce relapse with a better toxicity profile than chemotherapy, the addition of hormonal therapy to radiotherapy will be compared with chemoradiotherapy in the NSMP-Orange trial of the RAINBO program.

7.8.4 De-escalation of adjuvant therapy for POLE-mutant endometrial cancer

POLE mutations are more frequently found in relatively younger women with lower BMI and higher grade endometrioid endometrial tumours compared to *POLE* wildtype EC.[7, 8, 79, 103-108] In *POLE*mut endometrial cancers, which are ultramutated cancers, increased antitumour response by peritumoral and tumour-infiltrating CD8+ lymphocytes has been reported, most

probably because the mutated DNA fragments act as neo-antigens that elicit a strong immune response.[88-90, 109, 110] In contrast to the poorly differentiated microscopic appearance of *POLE*mut endometrial cancers, they have consistently been shown to have an excellent prognosis with only an occasional relapse, both with and without adjuvant treatment.[62] It has been suggested that their very favourable outcome is mainly based on their ultramutated phenotype with many mutated DNA fragments, which elicit a strong host immune response.[88] In addition, these ultramutated cells might not be able to function properly and DNA replications and consequently cell division and potential for spread may be impaired. De-escalation or omission of adjuvant treatment could be considered in *POLE*mut endometrial cancers, and this will be prospectively investigated in the POLE-Blue trial of the RAINBO program.[8, 79, 91, 103, 105-107, 111]

8. CONCLUSION

Vaginal brachytherapy has been shown to be the current best adjuvant treatment for women with early stage, (high-)intermediate risk endometrial cancer, balancing maximal local control with lowest toxicity. The risk of recurrence is strongly associated with risk factors as substantial LVSI, L1CAM overexpression and p53abn. Women with these risk factors should be treated with adjuvant external beam radiotherapy instead of vaginal brachytherapy to maximise pelvic control and recurrence-free survival. Using a molecular-integrated risk profile to determine adjuvant treatment in early stage, high-intermediate risk disease might optimize outcomes and spare many women adjuvant treatment. This rationale is currently being investigated in both the PORTEC-4a and TAPER trials, and in the coming years the results will show if molecular risk factors should be used to determine adjuvant treatment.[24, 112] Molecular alterations are frequently found in endometrial cancer and increasing knowledge on their prognostic significance and possible therapeutic options has been gained. Trials investigating (adjuvant) treatment based on molecular alterations for women with (high-)intermediate risk, high risk and recurrent or metastatic endometrial cancer are ongoing. New treatment targets have emerged and are being investigated in trials for localised, advanced and metastatic disease. Moreover, radiotherapy techniques for endometrial cancer have been improving over time. Modern radiotherapy techniques have the ability to increasingly spare the surrounding healthy tissues with similar or improved oncological outcomes and fewer treatment related toxicities. Future developments can be expected in daily image-guided adaptive radiotherapy and improved use of innovative modalities which reduce the dose to the organs-at-risk. Future radiotherapy trials should incorporate adequate quality assurance programs, including dummy run and annual quality assurance, to achieve uniform high-quality treatment. All of these developments will lead to better outcomes and highest quality of life for women with endometrial cancer.

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