Exploring the role of homologous recombination deficiency and BRCA1/2 mutations in endometrial cancer

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Chapter 7

Discussion and future perspectives

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BRCA1 and *BRCA2* are tumor suppressor genes that are essential for the maintenance of genomic integrity.[1, 2] They play, together with other genes (e.g. *PALB2, ATM, CHEK2, RAD51C, RAD51D, BRIP1),*[3-5] a crucial role in homologous recombination repair (HR). HR is important for the high-fidelity repair of DNA double strand breaks (DSBs) and restoration of lesions that stall the DNA replication fork.[1, 2] *BRCA1/2*-deficient tumors are not capable of performing HR and are homologous recombination deficient (HRD), resulting in genomic alterations, called "genomic scars" or "mutational signatures (**chapter 1)**.[1, 6-14]

As discussed in **chapter 1**, women with the *gBRCA1/2*-associated hereditary breast and ovarian cancer (HBOC)-syndrome are especially at increased life-time risk to develop basal-like breast cancer (BC) and high-grade serous tubo-ovarian cancer (HGSOC). These tumor types frequently harbour *BRCA1/2* mutations (both somatic and germline) and/or are HRD, and are further characterized by frequent *TP53* mutations and a high number of somatic copy number alterations (SCNA).[15-20] Interestingly, the p53-abnormal/SCNA-high molecular subgroup of endometrial cancer (EC) resembles HGSOC and basal-like BC both molecularly and clinically, suggesting a similar origin and having potential clinical consequences with regard to adjuvant treatment choices and genetic testing.[15-20]

In the first part of this thesis, we aimed to assess whether HRD occurs in EC. Evidence that this DNA repair pathway is abrogated in a subset of EC would support a potential role for *BRCA1/2* (and/or other HR) gene defects in the carcinogenesis of these tumors. In the second part of this thesis, by performing an in depth molecular and morphological characterization of EC that occurred in *gBRCA1/2* mutation carriers, we sought for recurring characteristics further supporting a causal relationship. In addition, we performed a systematic literature search and meta-analysis, and assessed the EC risk stratified by histologic a molecular subgroup in a large nationwide cohort of *gBRCA1/2* mutation carriers with most EC events reported to date. The ultimate goal was to elucidate whether EC is part of the *gBRCA1/2* mutation associated HBOC-syndrome, and to provide further risk estimates that can be used for genetic counselling. In the third and final part of this thesis, we sought for a more efficient way to screen for somatic and germline *BRCA1/2* mutations in tumor specimens, now that this analysis is being routinely requested for women with epithelial ovarian cancer.

7.1. Homologous recombination deficiency in endometrial cancer

In **chapter 2**, we performed a pilot study in which functional assessment of HR was performed in a prospectively collected series of EC. By assessing the ability of proliferating tumor cells to accumulate RAD51 protein at DNA double-strand breaks after *ex vivo* irradiation, we provided evidence that HRD is a frequent event in the p53-abnormal/SCNA-high molecular subgroup of ECs, with 50% of these tumors being HRD. In our series, all HRD-ECs were of non-endometrioid

histology (either uterine serous carcinomas (USC) or uterine carcinosarcomas (UCS)). These results provide evidence that HRD is an important mechanism in tumor development of *TP53*-mutated EC, and provides a rational for treating these patients with therapies exploiting this defect (e.g. platinum compounds, Poly (ADP Ribose) Polymerase (PARP) inhibitors).[12, 21-28]

In our cohort, HRD was only observed in USC and UCS, which was likely the consequence of the small cohort size. Analysis of ECs from the TCGA-cohort presented in the same study showed that *BRCA*-associated genomic scars were present in endometrioid EC as well, though with lower frequencies (50% versus 4%-12% respectively). Also, in **chapter 4,** we showed that a large proportion of *gBRCA1/2*-associated EC were of endometrioid histology (all being p53-abnormal/SCNA-high).[29] That HRD occurs in EC is further supported by Ashley and colleagues,[30] who found mutational signature 3 (associated with HRD) to be the dominant signature in 15%, and second dominant signature in an additional 20% of p53-abnormal/SCNA-high EC, including both USC, endometrioid EC and mixed carcinomas. Furthermore, Jönsson and colleagues[31] found 53% of USC to be HRD (HRD score >42).

7.1.1. Clinical implications

Although most EC have good prognosis, p53-abnormal/SCNA-high EC are still associated with poor clinical outcome.[19, 32-34] The observation that HRD frequently occurs in p53-abnormal/SCNA-high EC provides mechanistic rational for treating these patients with both existing and new treatment strategies.

The best biomarker beyond *BRCA1/2* mutations for predicting efficacy of HRD-directed precision medicine is currently not known.[22, 23, 35-37] Since the presence of HRD can be assessed in multiple ways (e.g. presence of mutations in key HR genes, functional RAD51 assay, presence of "genomic scars" associated with HRD, **chapter 1**; Fig. 2), ideally, a study should be performed in which predictive value of the different available HRD biomarkers is examined side by side. This could for example be performed retrospectively in large already available (combined) cohorts of (recurrent) HGSOC patients treated with PARP inhibitors (e.g. Study 19/NCT00753545, ENGOT-OV16/NOVA trial/NCT01847274, ARIEL2/NCT01891344), and for which formalin-fixed paraffin embedded (FFPE)-tumor blocks are available. The most clinically applicable and predictive biomarker could then be used in future studies.

An advantage of RAD51-based tests above "genomic scar" assays is that it displays the current HR status of the tumor, and that it is rapid and cheap. A disadvantage of RAD51-based tests is the need for fresh tumor specimens/effusions for *ex vivo* irradiation to induce DNA double strand breaks, limiting clinical applicability.[38-42] Interestingly, recent studies suggested that the RAD51 assay could reliably be performed on diagnostic FFPE-tumor tissue without the need for prior induction of DNA damage via *ex vivo* irradiation. This test showed to be predictive for PARP inhibitor sensitivity and discriminative for defects in HR-genes,[43, 44] indicating that

endogenous DNA damage might be sufficient for reliable analyses of HR status. Furthermore, a pilot study presented at the ESGO 2019 (EP1230; http://dx.doi.org/10.1136/ijgc-2019-ESGO.64) in which the results of the RAD51 assay performed on fresh tumor tissue after *ex vivo* irradiation were compared with the RAD51 assay directly performed on diagnostic FFPE-tumor specimens using endogenously present DNA damage (presence of DNA double-strand breaks confirmed with gamma-H2AX staining) showed 100% concordance between both tests. If these findings are confirmed in larger series, the FFPE-RAD51 assay would be an ideal marker to retrospectively investigate the prevalence of HRD in archival diagnostic FFPE tumor specimens, and could be used on larger study cohorts to simultaneously investigate the prevalence of HRD, prognostic and predictive value.

7.1.2. Platinum-based chemotherapy

Our data suggest that p53-abnormal/SCNA-high EC will benefit from platinum-based chemotherapy. The presence of mutations in HR genes, genomic scars associated with HRD, and functional HRD already have shown to be predictive for platinum-based chemotherapy response and to correlate with improved progression free survival and overall survival (OS) in women with ovarian cancer (OC) or breast cancer (BC).[12, 25, 26, 41, 45] Up to recently, prognostic risk group allocation and adjuvant treatment recommendations for EC patients (radiotherapy, chemotherapy) were solely based on clinicopathological risk factors (FIGO stage, grade, histologic subtype, age, lymphovascular space invasion),[46, 47] thereby selecting a histologically and molecularly heterogenous group of tumors. This likely contributed to the heterogenous results of previous clinical trials with regard to the presence of an OS and recurrence free survival (RFS) benefit when adding (platinum-based) chemotherapy to pelvic radiotherapy (CTRT) compared with pelvic radiotherapy (RT) alone, with the absolute benefit being limited for studies that found a positive effect.[48-50] Recently, Leon-Castillo and colleagues[34] investigated the predictive value of the four previously defined molecular subgroups for CTRT benefit in patients with high-risk EC from the randomized PORTEC-3 trial. They found a highly significant absolute benefit (5-year RFS: 22.4%; 5-year OS: 23.1%) when women with p53-abnormal/SCNA-high EC were treated with CTRT compared to RT alone, whereas no (clear) benefit was observed for the remainder molecular subgroups (*POLE*-mutated, mismatch repair deficient (MMRd) and no specific molecular profile (NSMP) group). These findings are in line with our expectations, and it would be interesting to further subdivide the p53-abnormal/SCNA-high EC group of the PORTEC-3 trial by HRD status. By doing this, both the prevalence of HRD in this molecular subgroup could be determined, as well as whether HRD status might be of additional predictive value in selecting patients that benefit most from CTRT. Also, HRD prevalence could be assessed in the other molecular subgroups to determine whether HRD is indeed restricted to the p53-abnormal/SCNA-high molecular subgroup. The FFPE-RAD51 assay would be a promising candidate biomarker for HRD as it is fast, cheap and it can easily be performed on the available FFPE-tissue blocks.

7.1.3. PARP inhibitors

The high prevalence of HRD in p53-abnormal/SCNA-high EC provides rational for treating these women with PARP inhibitors.[21-24, 28] Trials assessing the efficacy PARP inhibitor monotherapy in recurrent or metastatic EC are on their way[51] (Table 1) and results have to be awaited.

Based on our studies, PARP inhibitor effect is to be expected in the p53-abnormal/SCNA-high molecular subgroup, and more specifically, the HRD-group within this subgroup. Since only an estimated 18-26% of unselected EC is expected to be p53-abnormal/SCNA-high,[19, 33] the majority of beforementioned studies might not be able to show an effect, and therefore, might not be able to answer the question whether (a subset of) EC patients benefit from PARP inhibitors. Furthermore, three of four studies exclude carcinosarcomas (NCT03016338, NCT03745950, NCT03745950, NCT04080284), a histotype likely benefitting from PARP inhibitors as studies showed carcinosarcomas to be associated with the p53-abnormal/SCNA-high molecular subgroup, an HRD phenotype, and to be enriched in *gBRCA1/2* mutation carriers.[29, 42]

Ideally, studies assessing the effect of PARP inhibitors in EC should include EC of the p53-abnormal/SCNA-high molecular subgroup, and should randomize this group for either platinum-based CTRT with parp inhibitors (intervention arm) or platinum-based CTRT alone (control arm). Primary outcomes should include OS and RFS. Furthermore, differences in toxicity between the treatment-arms should be registered and evaluated. Finally, diagnostic FFPE-tumor tissue of all included EC should be centrally collected to assess the predictive value of HRD in predicting PARP inhibitor response. A promising trial that is currently in the

Table 1. Trials investigating monotherapy with PARP inhibitors in endometrial cancer

Trial	Patient population	Intervention-arm	control-arm
NCT03016338-Phase 2 (*n=44)*	recurrent/advanced endometrial cancer after at least one line of prior platinum based chemotherapy.	Cohort 1; Niraparib (n=22) Cohort 2; Niraparib and TSR-042[a] (n=22)	n.a.
NCT03617679–Phase 2 (*n=138)*	recurrent/metastastic endometrial cancer after 1-2 prior lines of (chemo) therapy	Rucaparib	Placebo
NCT03745950–Phase 2[b] (*n=147)*	Advanced/metastatic endometrial cancer after 1 line of platinum based chemotherapy	Olaparib	Placebo
NCT04080284 Phase II	Advanced, platinum-sensitive recurrent USC	Niraparib	

[a]anti-PD1 inihibitor, [b]Secondary outcome includes to determine time from response rate according to IHC P53, MMR, NGS BRCA/HRD, MSI

developmental phase and which is planning to assess PARP inhibitor efficacy in the p53-abnormal/SCNA-high molecular subgroup is the RED-trial (p53-abnormal EC) of the Refining Adjuvant treatment IN endometrial cancer Based On molecular features (RAINBO)-program.

7.1.4. Trastuzumab

Another potential therapeutic target for p53-abnormal/SCNA-high EC is the presence of *ERBB2* amplifications, which encodes for the human epidermal growth receptor 2 (HER2) and which is amplified in 25% of P53-abnormal/SCNA-high EC.[19, 52] In our *gBRCA1/2*-carrier cohort described in **chapter 4,** none of the EC displayed *ERBB2* amplifications. In BC, the *ERBB2*-overexpressing subgroup and the basal-like subgroup are two biologically distinct groups[53], the latter being associated with, amongst others, *BRCA1* defects and HRD.[54] This might indicate that the p53-abnormal/SCNA-high EC could possibly be further divided in an HRD-group and an *ERBB2* amplified group, which would be an interesting topic for future studies.

7.2. Endometrial cancer and the *gBRCA1/2*-associated HBOC-syndrome.

By demonstrating that HRD occurs in EC, we provided mechanistic support that a subset of EC might be a *gBRCA1/2*-associated disease, something that has long been topic of debate. Studies that assessed EC risk in *gBRCA1/2* mutation carriers were either small with limited number of events and follow-up, and/or did not stratify the EC for histologic subtype (**chapter 5,** supplementary Table S1).[55-62] This has resulted in conflicting data with regard to EC risk in *gBRCA1/2* mutation carriers, resulting in divided opinions between clinicians and uncertainty whether these risk should be integrated in counselling and clinical management of these women. In **chapter 3, chapter 4** and **chapter 5** we focused on answering the question whether EC is part of the *gBRCA1/*2-associated HBOC syndrome.

By performing a systematic review and meta-analysis (**chapter 3**), we found that the odds ratio for having a *gBRCA1/2* mutation was increased for women with USC compared to what would be expected based on population frequencies. In addition, we described a case of a *gBRCA1* mutation carrier who developed an USC three years after risk-reducing salpingo-oophorectomy (RRSO). The USC showed loss of heterozygosity of the *BRCA1* wild-type allele and showed an HRD phenotype in the functional RAD51 assay, thereby providing evidence that *BRCA1* was involved in the carcinogenesis of this tumor. In **chapter 4**, we comprehensively histologically and molecularly characterized a unique series of 40 EC that developed in *gBRCA1/2* mutation carriers, and found recurring characteristics, further supporting a causal relationship. Since previous studies demonstrated LOH to be an essential event in carcinogenesis of *BRCA1/2*-associated carcinomas,[63] EC with LOH were considered *gBRCA1/2*-associated, whereas EC without LOH were considered “sporadic” (non-*gBRCA1/2*-associated). Sixty percent of EC in *gBRCA1/2* mutation carriers were *gBRCA1/2*-associated, with the remainder being sporadic tumors that likely developed independently

of the *gBRCA1/2* mutation. *gBRCA1/2*-associated EC were clearly enriched for histotypes associated with unfavourable clinical outcome (79.2% USC, UCS, high-grade endometrioid or ambiguous EC)[64], the p53-abnormal/SCNA-high subgroup molecular subgroup (91.7%), and for Solid, pseudoEndometrioid, and/or Transitional morphology (SET morphology) , a growth pattern already shown to be enriched in HGSOC with *BRCA1*- and HR-gene mutations.[17, 18, 65] Now that we learned that ECs not just occur sporadically in *gBRCA1/2* mutation carriers, **chapter 5** focussed on quantifying the EC risk of *gBRCA1/2* mutation carriers using a large nationwide multicenter cohort. With 58 EC events, this was the largest study to date,[55-62] and analyses were stratified for histologic- (endometrioid, serous-like, clear cell, sarcoma, other) and molecular subgroups (p53-abnormal/SCNA-high versus other) after pathology review. We showed that gBRCA1/2 mutation carriers have a 2 to 3-fold increased risk for developing EC, with highest increased risks being observed for the serous-like histological and p53-abnormal/SCNA-high molecular subgroups (approximately 10-fold). When stratified for mutation type, risks were highest for gBRCA1 carriers. Despite these highly increased risks, absolute risks by 75 years remained low because of the rarity of the disease; overall EC, 3.0%; serous-like EC, 1.1%.

Together, by showing that *gBRCA1/*2-associated ECs have a specific phenotype, and providing mechanistic and epidemiologic support for an association between EC and g*BRCA1/2* mutations, we can conclude that ECs, and more specifically ECs of serous-like histology and the p53-abnormal/SCNA-high molecular subgroup, are an integral part of the *gBRCA1/2*-associated HBOC syndrome.

Although our study included most EC events reported to date (**chapter 5,** supplementary table 1), it would be interesting to redo the analysis in 10 years. Despite long follow-up, our cohort was still relatively young, with limited person-years at risk in the age categories above 75-80 years. As can be seen in Figure 1, EC, and especially EC of serous-like histology, is a disease of older age for which incidences remain relatively high, even after the age of 80 years.[66] Therefore, having limited follow-up years and events in these age categories might have influenced the observed increase in risk, especially since we observed a higher EC risk increase for older age categories (table 3, **chapter 5**).[11, 12, 24]

7.2.1 Clinical implications

Now that we provided additional evidence that EC, and more specifically, the rare but aggressive serous-like and p53-abnormal/SCNA-high subgroup of EC, is part of the *gBRCA1/2*-associated HBOC syndrome, the question arises how this should impact current clinical practice.

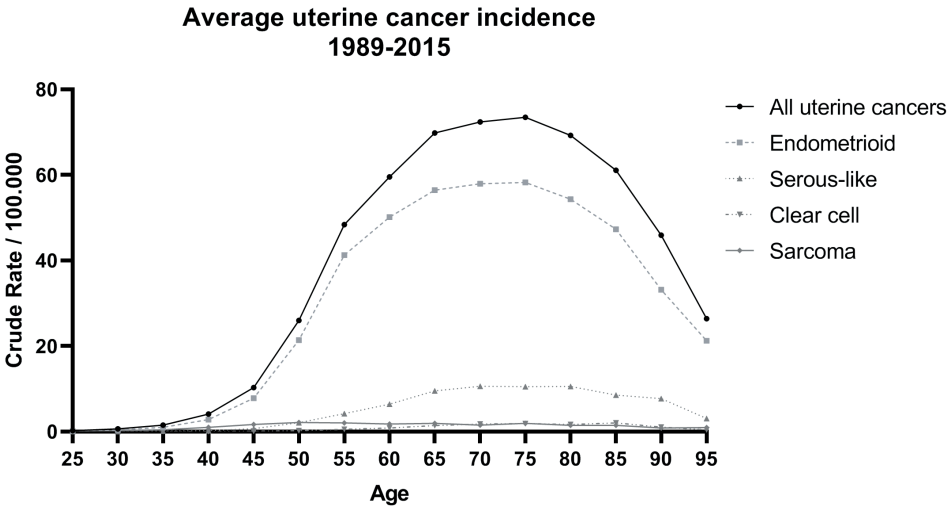

Figure 1. Dutch population uterine cancer incidence, both overall and stratified by histologic subgroup. Data was retrieved from the Dutch Cancer Registry, and was stratified according to histologic subgroups as described in **chapter 5**.

7.2.2. Risk-reducing surgery

Because of the highly increased life-time risks to develop BC and OC (BC: *gBRCA1,* 50- 59% and *gBRCA2,* 42-51%; OC: *gBRCA1,* 34-45% and *gBRCA2,* 13-21%),[67] *gBRCA1/2* mutation carriers can opt for risk-reducing bilateral mastectomy and risk-reducing salpingo-oophorectomy (RRSO).[68] In the Netherlands, it is currently not recommended to perform a concurrent risk-reducing hysterectomy at the time of RRSO since, up to now, EC was not considered to be part of the *gBRCA1/2*-associated tumor spectrum.[68] Although we now showed that EC is part of the *gBRCA1/2*-associated HBOC-syndrome, the low absolute EC risks (overall: *gBRCA1*: 3.4%; *gBRCA2*: 2.0%; serous-like: *gBRCA1*: 1.4%; *gBRCA2*: 0.6%), especially when compared to beforementioned OC and BC risks, support current clinical practice in which routine risk-reducing hysterectomy at the time of RRSO is not routinely recommended. Nevertheless, understanding EC risks is essential for informed decision-making during counselling, and the potential benefits need of performing a hysterectomy should be balanced against the potential hazards.

The main disadvantage of performing an additional risk-reducing hysterectomy at the time of RRSO is the expected increase in surgery-related morbidity. Studies that assessed surgery-related morbidity for total laparoscopic hysterectomy (TLH) that were conducted for benign indications or low-grade malignancy[69], and RRSO[70] reported the following major and minor complication rates (as formulated by the Dutch Society of Obstetrics and Gynecology); major: 4.0% versus 0.6%, and minor: 4.0% versus 3.7% respectively.[69, 70] In addition, de mean length of hospital stay was longer for women that underwent a TLH (4 days, range:2-7)[71, 72] compared

to women that underwent a RRSO (1 day, range: 0-13).[70] To our knowledge, there are no studies that compared complication rates between RRSO with and without risk-reducing hysterectomy in our population of interest, and future studies need to elucidate the true additional morbidity of this procedure.

Reasons to consider a risk-reducing hysterectomy in this population could be the presence of (benign) uterine disease that give symptoms/that will likely give to symptoms in the future, the presence of other risk factors that increase EC risk, anxiety that patients may experience from being at increased EC risk, and, that it is unknown whether there are effective screening modalities to detect early-stage EC in this patient population.

7.2.3. Patient preferences

Although it does not seems rational to routinely perform a risk-reducing hysterectomy at the time of RRSO from a clinical perspective, it would be interesting to conduct a patient preference study to determine patients choice of surgical extent.

This could for example be performed by interviewing patients using a treatment tradeoff method, to assess how patients weigh risk benefits against potential additional complications from extended surgery, and to determine the minimally desired risk benefit from an additional risk-reducing hysterectomy. Figure 2 illustrates an example of a flow-chart that could be used.

7.2.4. PARP inhibitor

The finding that a subset of ECs is *gBRCA1/2*-associated provides additional rationale for treating these women with PARP inhibitors, which was already discussed in paragraph 7.1.3.

7.2.5. Screening for *gBRCA* mutations in uterine cancer patients

DNA testing for hereditary mutations is generally recommended if the expected detection rate is sufficiently high (>5%).[73] Studies that assessed *gBRCA1/2* mutation frequency in an unselected cohort of patients with USC, or EC patients (not selected for histotype) with a history of BC, reported mutation frequencies of 2%74 and 3.8%75 respectively. These data do not support screening for *gBRCA1/2* mutations in EC patients. However, based on our data, highest *gBRCA1/2* mutation frequencies are to be expected in EC with TP53 mutations. Therefore, it would be interesting to perform a future study that determines the *gBRCA1/2* mutation frequency in women that developed TP53-mutated EC, ideally including a subanalyses taking into account mutation incidence when additionally including BC history, family history and/or morphological features enriched in *gBRCA1/2* associated ECs (chapter 4).

7.3. Tumor-based screening for *BRCA1/2* (and other HR gene) mutations

Given the high prevalence of *gBRCA1/2* mutations in HGSOC (16%)[16] and triple-negative BC (14%),[15] germline analysis is routinely being offered to all women with OC, and women

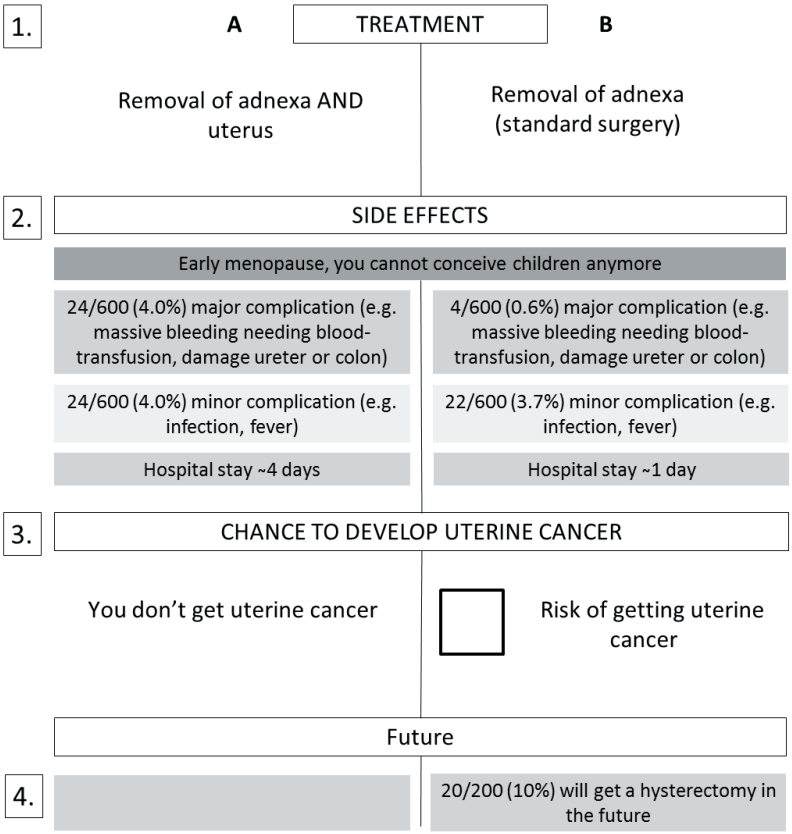

Figure 2: **Example of information on uterine cancer risk and complication rates that could be presented during an interview according to the treatment tradeoff method.**

with triple-negative BC <60 years of age. Furthermore, with the additional registration of PARP inhibitors by the European Medicines Agency as maintenance treatment for first-line platinum-sensitive high-grade epithelial OC in patients with proven *BRCA1/2* mutations (somatic/germline), additional somatic tumor testing will be more regularly required. In **Chapter 6**, we first optimized *BRCA1/2* mutation analysis performed on diagnostic FFPE-tumor tissue in a training cohort of known *gBRCA1/2* mutation carriers, and subsequently validated the tumor test in a prospective cohort of women that developed epithelial OC. We showed that, when using a combination of next-generation sequencing and copy number variant (CNV)-multiplex ligation-dependent probe amplification (MLPA), *BRCA1/2* mutations (both somatic and germline) can reliably be detected. Using this tumor-first approach as pre-screening tool to detect and select patients with *BRCA1/2* mutations for referral to the clinical geneticist could prevent approximately 80% of referrals. Another study in the Netherlands that was simultaneously performed (*BRCA* testing in **O**varian cancer by **Pa**thologist (OPA)-study)

using a different sequencing technique (combination of single-molecule molecular inversion probe-based NGS and CNV-MLPA) also showed the tumor-first approach to be reliable, rapid, feasible in daily practice, and to be appreciated by patients and gynaecologist.[76, 77]

7.3.1. Clinical implications

The tumor-first approach is currently being implemented in different regions of the Netherlands, and is already part of routine diagnostic work-up for all epithelial OC patients (except for women with borderline OC) in other regions (e.g. Leiden, Nijmegen).[68] Because of the consequences of detecting hereditary variants in other HR genes besides *BRCA1/2*, the sequencing panels should also include additional genes (e.g. *ATM, PALB2, CHEK2, RAD51C, RAD51D, BRIP1*).

Sequencing of *BRCA1/2* is challenging. *BRCA1/2* are large genes with a wide mutation spectrum. Because FFPE-derived tumor DNA is highly fragmented, created amplicons are shorter compared to when leucocyte-derived DNA is used, thereby increasing the chance of mutations being located at amplicon ends or primer binding sites, increasing the chance for detection errors. Furthermore, large genomic *BRCA1* deletions (e.g. exon 22 deletion) are common founder variants in the Netherlands.[78] These large deletions are easily overlooked when only using next generation sequencing, making it necessary to perform additional copy number analysis.[79] Furthermore, once variants are detected, interpretation can be difficult, especially when it considers variants of uncertain significance.[80] Because of these challenges, we think *BRCA1/2* analysis should be restricted to academic hospitals with sufficient sequencing experience and in which there is a close collaboration between the pathology department and the clinical genetics department. Also, despite this tumor-first approach having many advantages, it is important that clinicians are aware that because of technical limitations (depending on the technique(s) that is/are used), some variants will not be detected unless additional analyses are being performed (**Chapter 6).** Therefore, if there is a high suspicion for a hereditary variant, patients should always be referred to the clinical geneticist, even if there is no variant detected in the tumor test.

Whether it is necessary to screen all women with epithelial OC (currently recommended by the Dutch guideline)[68] remains topic of debate. Table 2 summarizes the *gBRCA1/2* mutation frequency among different histologic OC subtypes found by two studies. Both studies included pathology review by gynaecologic pathologists and reported highest *gBRCA1/2* mutation incidences in women with HGSOC.[81, 82] Although Alsop and colleagues almost exclusively found *gBRCA1/2* mutations to be associated with high-grade serous histologic subtype after pathology review, Norquist and colleagues also found high incidences in other histologic subtypes, especially carcinosarcomas and high-grade endometrioid carcinomas. Based on these findings, it seems reasonable to exclude women with mucinous OC and grade 1 endometrioid OC from screening for *gBRCA1/2* mutations.

Table 2. *gBRCA1/2* mutation frequency stratified by ovarian cancer histotype

Norquist and colleagues[81]	***gBRCA1/2* mutation frequency (%)**
High-grade serous	16.1
Low-grade serous	5.7
High-grade endometrioid (gr 2/3)	10.9
low-grade endometrioid (gr 1)	0
Carcinosarcoma	13.9
Clear cell	6.9
Alsop and colleagues[82]	
High-grade serous	22.6
Endometrioid (grade not specificied)	1.7[a]
Carcinosarcoma	0
Clear cell	1.6[b]

[a]Originally 8.4%. Eight out of ten (80%) cases were reclassified as serous or unspecified adenocarcinoma after pathology review.
[b]Originally 6.3%. Three out of four (75%) reclassified as high-grade serous carcinoma with focal clear cell alteration after pathology review.

7.3.2. Tumor-based screening in other cancer types

Another major advantage of the tumor-first approach is that it could easily be implemented for other tumor types for which germline mutations have been described in a subgroup of cases (e.g. prostate cancer, pancreatic cancer, (*TP53*-mutated) EC, BC), because referral to the clinical geneticist will only be necessary if a mutation is detected.

7.4. Conclusion

In this thesis, we provided mechanistic, morphologic and epidemiologic evidence that serous-like or p53-abnormal/SCNA-high ECs belong to the *gBRCA1/2*-associated HBOC syndrome. In addition, we demonstrated that HR is frequently abrogated in this molecular subgroup, also in the absence of *BRCA1/2* mutations, thereby providing a strong rationale for future clinical trials assessing the efficacy of treatment strategies exploiting this repair defect in these tumors. Finally, by showing that *BRCA1/2* mutations can reliably be detected in diagnostic FFPE-material, we provided a basis for a more efficient genetic work-up pathway for OC patients, which can also be extended to other tumor types.

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