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

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# Chapter 1

## General introduction

# 1. General introduction

## 1.1. Case presentation and introduction outline

A 50 year old woman without a personal cancer history visits the clinical geneticist because her sister recently got diagnosed with a hereditary mutation in the BReast CAncer gene 1 (*BRCA1)*. After genetic testing, it turns out she carries the same germline mutation in *BRCA1 (gBRCA1)*. *gBRCA1* mutations are associated with the hereditary breast and ovarian cancer (HBOC)-syndrome, a syndrome characterized by severely increased life-time risks of developing breast cancer (BC) and tubo-ovarian cancer (OC).[1] To reduce the BC and OC risk, she decides to undergo risk-reducing surgery, including both a bilateral mastectomy and a bilateral risk-reducing salpingo-oophorectomy (RRSO). Three years later, postmenopausal vaginal bleeding occurs. She gets diagnosed with (postsurgical) stage IV uterine serous carcinoma (USC) based on a supraclavicular lymph node metastasis. USC is a rare histologic subtype of endometrial cancer (EC) associated with poor clinical outcome.[2] She undergoes a total abdominal hysterectomy and dissection of the iliac and para-aortal lymph nodes, followed by six cycles of adjuvant carboplatin and paclitaxel chemotherapy. She is still disease free three and a half years later. The occurrence of an EC after RRSO raised the question whether EC is a *BRCA*-associated disease and whether this could have been prevented.

In this introduction, first, a general overview of the main DNA damage response pathways and tumor development is given, with emphasis on homologous recombination repair, the DNA repair pathway for which BRCA1 and BRCA2 are crucial. Second, the molecular alterations in EC will be discussed, with emphasis on similarities between EC and the histologic subtypes of BC and OC that frequently occur in *gBRCA1/2*-mutation carriers. Also, hereditary cancer syndromes associated with EC will be described. Third, the clinical implications for patients of having the *gBRCA1/2*-associatied HBOC-syndrome will be discussed. Finally, the aims and thesis outline will be described.

## 1.2. DNA repair pathways and tumor development

During life, the DNA of every living organism is continuously being exposed to both endogenous (e.g. reactive oxygen species, deamination) and exogenous (e.g. UV-radiation, chemicals, ionizing radiation, cigarette smoke) genotoxic agents, causing different forms of DNA damage. Adequate recognition and repair of this DNA damage is essential for the maintenance of genomic integrity. If DNA damage persists through replication, this can lead to mutations. Mutations in key regulatory genes might lead to the accumulation of additional mutations, with subsequent uncontrolled cell growth and loss of protective apoptotic and cell cycle control checks, facilitating cancer development.[3, 4] Not surprisingly, genomic instability is an important hallmark of tumor cells.

To prevent the induction of DNA mutations due to the presence of DNA damage, cells have many (interwoven) DNA damage response (DDR) pathways. These DDR-pathways are responsible for the maintenance of genomic integrity, involving the detection of DNA damage, recruitment of DNA repair factors to the site of damage and the actual repair of the lesion.[4, 5] Cells have different DNA damage repair pathways to repair different kinds of DNA damage, Table 1.[4, 5]

Mutations that are acquired during life, are called "somatic mutations". These mutations are only present in cells derived from the mutated cell. Mutations can also be present in gametes. These are the only mutations that can be passed on to the offspring. These mutations will be present in every cell of the offspring and are called "germline mutations".

A subset of familial cancer syndromes have been associated with germline mutations in genes involved in the different DDR-pathways, making family members more prone to cancer development. Table 1 shows the main DNA-damage repair pathways and associated hereditary syndromes.[4-6]

**Table 1. DNA-damage repair pathways essential for maintenance of genomic stability**

| DNA lesion | Repair mechanism | (subset of) genes involved | Associated hereditary syndromes | Associated tumor types |
|---|---|---|---|---|
| Single strand breaks | Base excision repair | PARP1, XRCC1, Ligase 3, MUTYH | | Colorectal |
| Double strand breaks | Homologous recombination | BRCA1, BRCA2, PALB2, ATM, CHEK1, CHEK2, RAD51 | Hereditary breast and ovarian cancer (HBOC)-syndrome, Fanconi anemia | Breast, ovarian, pancreatic leukemia |
| | Non-homologous end joining | KU70/80, DNA-PK, ligase IV, XRCC4 | Severe Combined ImmunoDeficiency (SCID) | |
| | microhomology-mediated end joining | polymerase theta | | |
| Bulky adducts | Nucleotide excision repair | ERCC4, ERCC1, ERCC2 | Xeroderma pigmentosum | Skin |
| Base mismatches, insertions, deletions | Mismatch repair | MLH1, PMS2, MSH2, MSH6 | Lynch syndrome | Colorectal, endometrial |
| Base alkylation | Direct reversal repair | MGMT | | Glioma |

Adapted from Lord 2012[4] and Cold Spring Harb Perspect Biol 2012;4:a012773.[6]

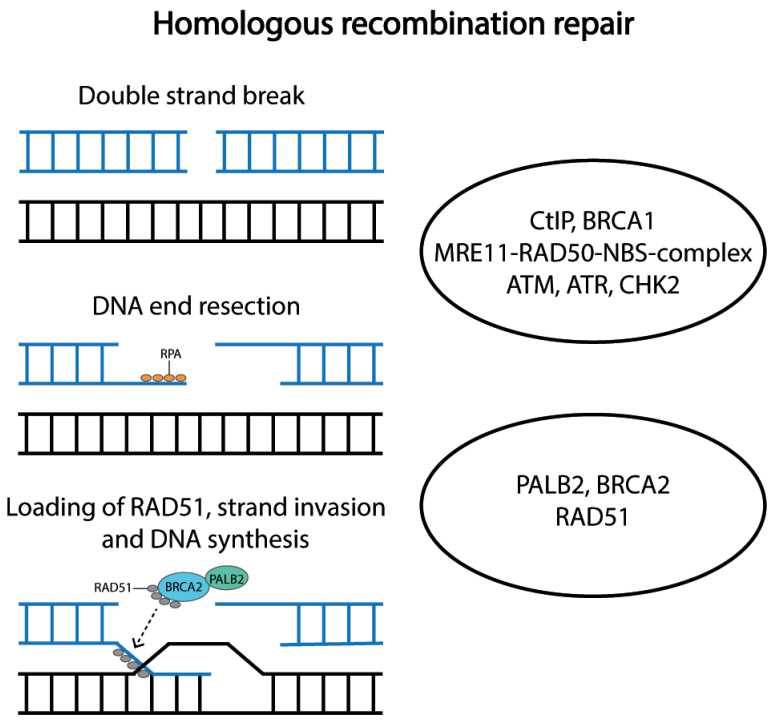



**Figure 1: Simplified overview of homologous recombination repair (HR).** HR is important for the high fidelity repair of DNA double strand breaks and consists of a series of sequential steps. It is active in the S/G2-phases of the cell cycle, in which a sister chromatid can be used as a template for the DNA repair. In the different steps multiple proteins are involved, some of which are shown in Figure 1. BRCA1, ATM, ATR, CHK2 and the MRE11-RAD50-NBS1 complex are important for the detection of the DNA double strand breaks and the resection the 5' DNA sides to create single strand DNA (ssDNA) ends at the break sites. This ssDNA is subsequently coated with RPA. PALB2/BRCA2 is responsible for the replacement of RPA and subsequent loading of RAD51 to the now exposed ssDNA region. RAD51 forms a nucleoprotein filament with the ssDNA region allowing the DNA to invade the homologous DNA helix, so that it can be used as a template for DNA synthesis to restore the double strand break. Adapted from Roy et al, 2012, Lord and Ashworth, 2016 and Vanderstichele, 2017.[7, 8, 13]

### 1.2.1. *BRCA1, BRCA2* and homologous recombination repair

*BRCA1* and *BRCA2* are tumor suppressor genes that are essential for the maintenance of genomic integrity.[7, 8] They both play a crucial role in homologous recombination repair (HR), a DNA repair pathway that is active during the S and G2 phases of cell cycle, and which is important for the high-fidelity repair of DNA double strand breaks (DSBs) and restoration of lesions that stall the DNA replication fork.[7, 8] *BRCA1/2*-deficient tumors are not capable of performing HR, and are therefore considered to be homologous recombination deficient (HRD).

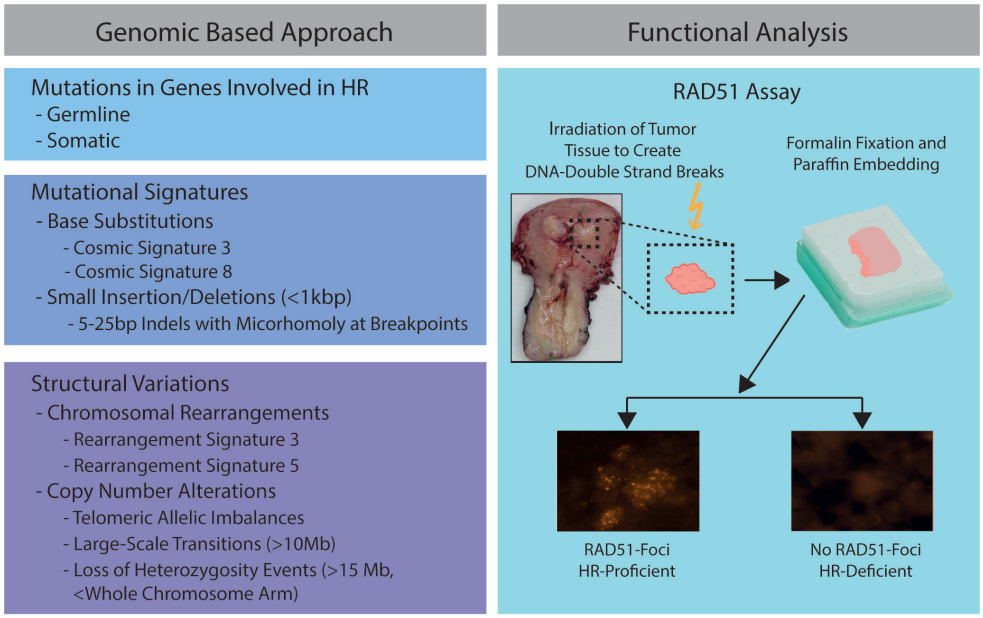


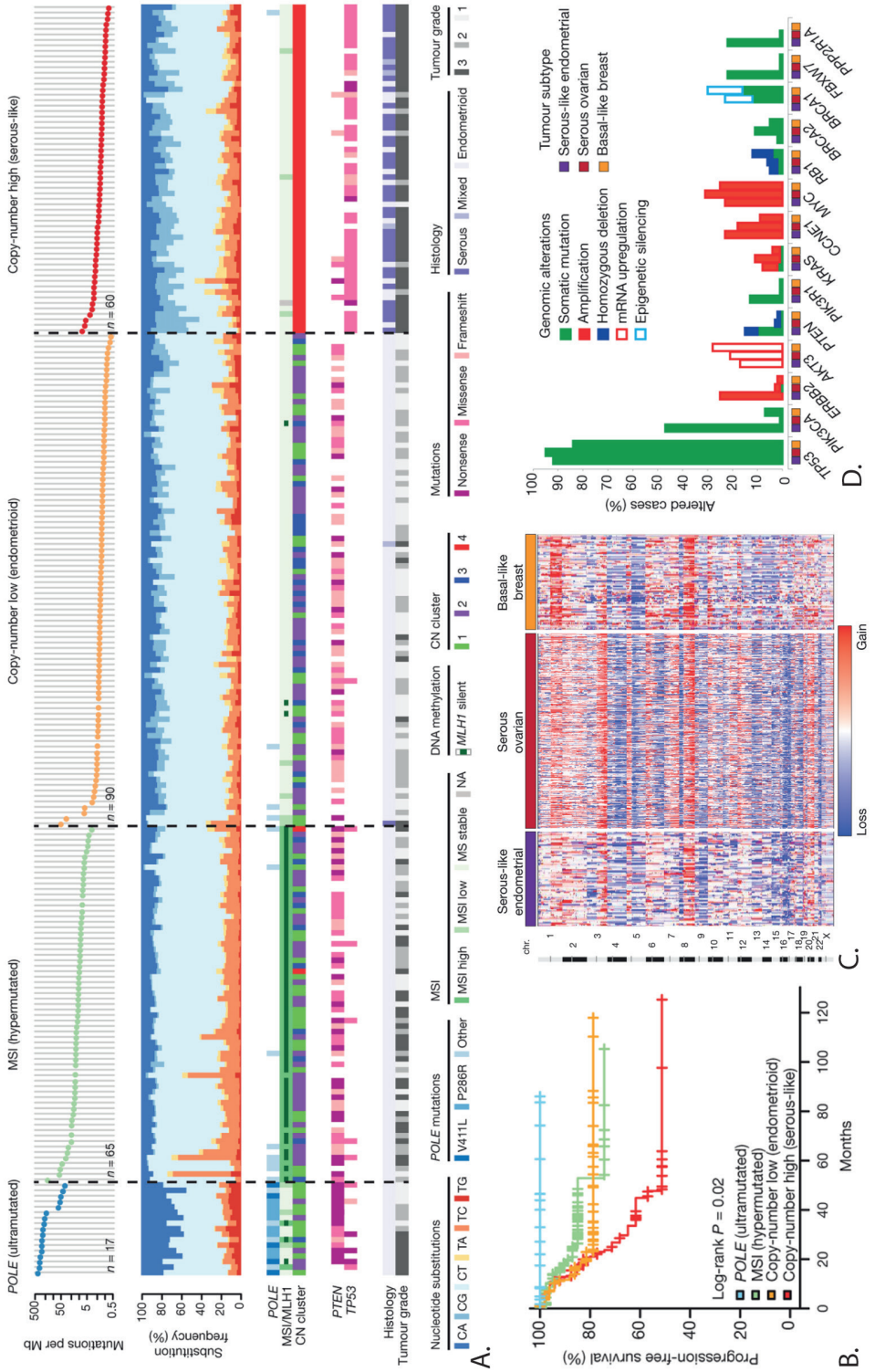

**Figure 2:** Examples of biomarkers used to indirectly (**A**) and directly (**B**) assess homologous recombination capacity tumor samples.

Besides BRCA1 and BRCA2, multiple other proteins are involved in HR.[7-9] Germline mutations in some of these HR genes also give rise to increased breast- and/or ovarian cancer risk (*PALB2, ATM, CHEK2, RAD51C, RAD51D, BRIP1)*, with risks varying dePending on the gene involved.[10-12] Figure 1 gives an overview of HR and a subset of proteins involved.

### 1.2.2. *BRCA1/2*-deficient tumors and genomic scars

Mutations in *BRCA1* and *BRCA2* can both be of somatic and germline origin. BCs and OCs with *BRCA1/2* mutations (both germline and somatic) generally show loss of heterozygosity (LOH) of the wild-type allele, resulting in complete loss of *BRCA1* or *BRCA2* function. LOH is an important step in *BRCA1/2*-associated carcinogenesis, as studies showed that BCs and OCs that occurred in *gBRCA1/2* mutation carriers but in which no LOH was present did not show the typical genomic alterations observed in *gBRCA1/2*-associated carcinomas. These tumors instead showed genomic alterations more similar to sporadically occurring, non-*BRCA1/2*-associated carcinomas.[14]

The combination of genomic alterations, called “genomic scars” or “mutational signatures”, that are observed in tumors that are *BRCA*-deficient (“*BRCA*-null tumors”) can be attributed to the accumulation of DNA-DSBs and the use of alternative, error-prone DSB repair pathways like non-homologous end-joining and alternative non-homologous end-joining,


POLE (ultramutated)
MSI (hypermutated)
Copy-number low (endometrioid)
Copy-number high (serous-like)
n = 17
n = 65
n = 90
n = 60
Mutations per Mb
Substitution frequency (%)
POLE
MSI/MLH1
CN cluster
PTEN
TP53
Histology
Tumour grade
A.
Nucleotide substitutions
CA CG CT TA TC TG
POLE mutations
V411L P286R Other
MSI
MSI high MSI low MS stable NA
DNA methylation
MLH1 silent
CN cluster
1 2 3 4
Mutations
Nonsense Missense Frameshift
Histology
Serous Mixed Endometrioid
Tumour grade
3 2 1
B.
Progression-free survival (%)
Months
Log-rank P = 0.02
POLE (ultramutated)
MSI (hypermutated)
Copy-number low (endometrioid)
Copy-number high (serous-like)
C.
Serous-like endometrial
Serous ovarian
Basal-like breast
chr.
Loss
Gain
D.
Altered cases (%)
TP53
PIK3CA
ERBB2
AKT3
PTEN
PIK3R1
KRAS
CCNE1
MYC
RB1
BRCA2
BRCA1
FBXW7
PPP2R1A
Genomic alterations
Somatic mutation
Amplification
Homozygous deletion
mRNA upregulation
Epigenetic silencing
Tumour subtype
Serous-like endometrial
Serous ovarian
Basal-like breast

**< Figure 3: A.** Molecular characterization of endometrial cancer in four molecular subgroups as proposed by The Cancer Genome Atlas Group (TCGA). **B.** Prognostic significance of the different molecular subgroup proposed by the TCGA. **C.** Somatic copy number alterations in the copy-number high/serous-like endometrial cancer subgroup, serous ovarian cancer and basal-like breast cancer. **D.** Genomic alterations frequently present in serous-like EC, serous ovarian cancer and basal-like breast cancer. Figures A, B, C, D are adapted from the TCGA, Nature 2013 (reprinted under the Creative Commons License).[28]



also called microhomology-mediated end joining. By using techniques like next-generation sequencing (NGS), array-based comparative genomic hybridization (aCGH) and single nucleotide polymorphisms (SNP)-based assays, these genomic scars can be assessed.[7, 13, 15-22] Mutational signatures shown to be overrepresented in *BRCA1/2*-null tumors are for example base-substitutions signature 3 and 8, deletions of >3 base pairs with microhomology at breakpoints, certain genomic rearrangement like rearrangement signatures 3 (small tandem duplications <10 kb) and 5 (deletions <100 kb), and an increased number of somatic copy-number alterations (sCNA) including widespread loss of heterozygosity of areas larger than 15Mb but shorter than the whole chromosome (HRD-LOH), increased number of telomeric allelic imbalances (NtAI) and large-scale state transitions (LST), see Figure 2.[7, 13, 15-22]

There are multiple ways to determine whether a tumor is HRD, see Figure 2. An indirect way is to determine the presence of mutations (either germline or somatic) in key genes involved in HR, like *BRCA1* and *BRCA2,* which will likely result in an HRD-phenotype in the presence of LOH of the wild-type allele. Another indirect way is to assess the presence of beforementioned "genomic scars"[13, 23] that have occurred as a result of the HRD-phenotype. However, assessing the presence of these genomic scars is still costly and not easily implementable in routine diagnostics.

A more direct way to determine HR capacity of tumor cells is by directly measuring the ability of these cells to perform HR. This can be assessed by functional analysis, in which the capacity of tumors cells to recruit RAD51 to ionizing radiation induced DNA DSBs can be measured.[24, 25] As shown in Figure 1, RAD51 is being recruited to the site of the DSB during HR.[8] HRD tumor cells will not be able to recruit RAD51 to the DNA DSBs, and this can thereby be used as a readout for HR capacity, see Figure 2B. The RAD51-assay has already shown to be able to reliably identify cell lines, xenografts and fresh human tumor tissue with defective HR without the necessity of performing expensive genomic analyses.[24-27]

## 1.3. Molecular alterations in endometrial cancer and similarities with HBOC-associated breast- and ovarian cancer

In 2013, the Cancer Genome Atlas Research Network (TCGA) divided EC in four distinct molecular subgroups based on the comprehensive genomic analyses of 373 endometrioid endometrial carcinomas (EECs), uterine serous carcinomas (USCs), and mixed carcinomas. By integrating tumor mutation burden (TMB), somatic copy number alterations (SCNAs) and

microsatellite instability (MSI) status, the following molecular subgroups with prognostic relevance were identified; (1) the *POLE*/ultramutated group, (2) the microsatellite instability-high (MSI-high)/hypermutated group, (3) the SCNA low/no specific molecular profile (NSMP) group and (4) the SCNA-high/serous-like group, see Figure 3, A-B.[28] Subsequent studies could reproduce these molecular subgroups with similar prognostic relevance using more clinically applicable surrogate markers,[29, 30] and showed that these molecular subgroups were also applicable to other histologic subtypes; uterine carcinosarcomas (UCS), clear cell carcinomas (CCC), undifferentiated carcinomas and dedifferentiated carcinomas.[31-34]

When looking more closely to the "SCNA-hi/serous-like" molecular subgroup, these tumors show striking similarities with high-grade serous tubo-ovarian cancer (HGSOC) and basal-like BC, both being tumors frequently associated with *BRCA1/2* mutations. Molecularly, these tumors for example all harbor a high number of SCNAs and frequent *TP53* mutations, see Figure 3, C-D.[28, 35-39] Furthermore, USC, the most common histologic subtype in the SCNA-hi/serous-like group, and HGSOC are morphologically indistinguishable, see Figure 4. Clinically, both USC and HGSOC generally are at advanced stage of disease at presentation, show frequent intraperitoneal spread and are associated with poor clinical outcome.[2, 40-42] These similarities suggest that a subset of ECs of the SCNA-high/serous-like group could be *BRCA1/2*-associated, and/or harbor other genomic defects causing HRD.

## 1.4. Endometrial cancer and hereditary cancer syndromes

As previously mentioned, inheritance of genetic alterations can predispose individuals to hereditary cancer syndromes. Hereditary cancer syndromes are characterized by;

- multiple family members at the same side of the family being affected by cancer
- affected family members having increased cancer risks
- affected family members having early age of cancer onset
- affected family members having multiple and/or bilateral primary cancers.[43]

### 1.4.1. Lynch syndrome

The most well-known hereditary cancer syndrome associated with EC is Lynch syndrome, or hereditary nonpolyposis colorectal cancer (HNPCC) syndrome. Lynch syndrome is an autosomal dominant syndrome, caused by a germline mutation in one of the DNA mismatch repair genes; *MLH1, PMS2, MSH2, MSH6* or the epithelial cell adhesion molecule gene*, EPCAM.*[44] Patients with Lynch syndrome are at highest increased risk to develop colorectal cancer (life-time risk to 70 years; 25-75%) and, for women, EC (life-time risk to 70 years; 12-71%), with risks varying dependent on the mutated gene.[44, 45]

Tumors arising in women with Lynch syndrome are mismatch repair deficient and are characterized by a high tumor mutation burden caused by the inability to recognize and

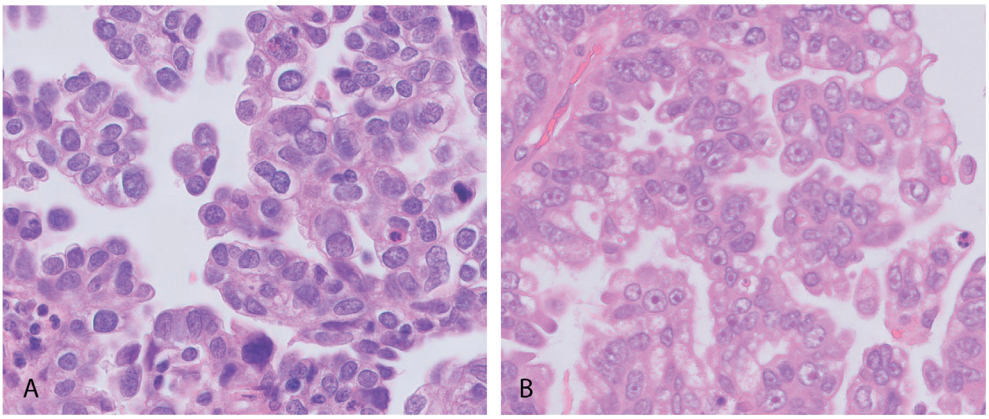



**Figure 4: A.** Example of an H&E slide of uterine serous carcinoma. **B**. Example of an H&E slide of a high-grade serous tubo-ovarian carcinoma.

repair DNA mismatches, giving rise to hypermutated tumors defined by >10 mutations per megabase.[28, 46] ECs that arise in the context of Lynch syndrome are of the MSI-high/ hypermutated molecular subgroup.

### 1.4.2 Cowden syndrome

Another hereditary cancer syndrome associated with EC is the Cowden syndrome. This syndrome is caused by a germline mutation in the phosphatase and tensin homolog (*PTEN)* tumor suppressor gene. It is a rare autosomal dominant syndrome in which patients develop hamartomatous tumors in multiple organ systems. The estimated life-time risk for EC is between 10-28%.[47]

### 1.4.3 *BRCA1/2*-associated HBOC-syndrome

As previously mentioned, women with the g*BRCA1/2*-associated HBOC-syndrome have severely increased life-time risks to develop BC and OC, with reported cumulative risks at age of 70 years for g*BRCA1*-mutation carriers of 50-59% and 34-45%, and for g*BRCA2*-mutation carriers of 42-51% and 13-21% respectively.[1] Other cancer types reported to be increased in *BRCA2*-mutation carriers are pancreatic cancer (both men and women), and prostate cancer (men). Furthermore, there might be an increased risk for stomach and oesophageal cancer (*BRCA1/BRCA2),* uveal melanoma (*BRCA2),* bone (*BRCA2)* and pharyngeal cancer (*BRCA2).*[48-50]

EC is currently not being considered to be part of the HBOC-syndrome. However, beforementioned similarities between a subset of ECs, HGSOCs and basal-like BCs suggest that there might be a role for *BRCA1/2* mutations and HRD in the development of ECs.

Although some studies have reported that women with a *gBRCA* mutation are at increased risk to develop EC, especially USCs[51-55], others were not able to find this association.[56, 57] If a subset of EC is part of the *gBRCA1/2*-associated HBOC, this might have important clinical consequences with regard to the availability of screening for *gBRCA1/2* mutations in EC patients, the extent of risk-reducing surgery (RRSO with/without hysterectomy) and, it might impact treatment strategy choices.

## 1.5. Clinical consequences of *gBRCA1/2* mutations, HRD and genetic testing

### 1.5.1 Early cancer detection and prevention

Because of the severely increased life-time risks of developing BC and OC, women with a *gBRCA1/2* mutation can opt for intensified screening programs to identify BC at an early stage.[58, 59] Additionally, women can opt for risk-reducing bilateral mastectomy, which has shown to be highly effective in reducing BC risk, with reported rates by some studies even up to 100%.[60-62]

Screening for early detection of OC has failed to result in survival advantage.[58, 63] The only available effective strategy to prevent OC is by performing a RRSO, which is recommended for *gBRCA1*-mutation carriers at an age of 35-40 years, and for g*BRCA2*-mutation carriers at an age of 40-45 years.[58, 63] Studies showed RRSO to be highly effective in preventing OC/fallopian tube cancer, with cancer reduction rates varying between 71% and 96%.[64] Additionally, studies showed a BC reduction rate after RRSO of approximately 50%, though this protective effect might have been an overestimation because of bias in studies analyzing this effect.[65]

Since patients with *gBRCA1/2* mutations are not considered to be at increased risk to develop EC, it is currently not recommended to routinely perform a risk-reducing hysterectomy concurrently with the RRSO. However, since this is based on small studies, this might change if larger future studies show that *gBRCA1/*2-mutation carriers are at increased risk to develop (a subset of) EC.

### 1.5.2 Treatment in *gBRCA1/2*-associated carcinomas

Studies showed that *gBRCA1/2*-associated BC and OC are particularly sensitive to drugs that cause DNA damage that is normally repaired via HR, leading to massive genomic instability that is inconsistent with cell viability.[4, 8] Platinum salts (cisplatin and carboplatin) for example are chemotherapeutic agents that cause inter- and intrastrand crosslinks that stall the progression of the replication fork. DNA damage caused by these agents is normally repaired via HR and nucleotide excision repair.[4, 8] Studies showed that patients with carcinomas that harbor mutations in HR genes (including *BRCA1/2*), or which harbored genomic patterns associated with HRD, showed increased platinum-sensitivity and improved progression-free survival, and overall survival.[20, 66-68]

A more recently approved class of drugs are the poly(ADP-ribose) polymerase (PARP)-inhibitors. The PARP enzyme is involved in the repair of singe-strand DNA breaks (SSBs) through the base excision repair. PARP-inhibitors cause SSBs to persist and PARP to be "trapped" to the damaged chromatin site, generating secondary DSBs during the S-phase, which require HR for repair.[4, 8, 69] PARP-inhibitors already showed promising results as maintenance treatment for relapsed platinum-sensitive HGSOC, with most benefit being observed for *BRCA1/2* mutated tumors (both somatic and germline) or tumors with genomic alterations associated with HRD, and for *gBRCA*-mutated BC.[70-73] More recently, PARP-inhibitors have also shown to be highly effective as first-line maintenance treatment for platinum-sensitive *BRCA1/2*-mutated (both germline and somatic) HSGOC.[74]

Currently, PARP-inhibitors are not indicated for treatment of EC. If a subset of EC, especially the SCNA-high/serous-like EC, which have poorest clinical outcome, are indeed frequently *gBRCA1/2*-associated or HRD, PARP-inhibitors might be a new treatment strategy for these women.

### 1.5.3. Referral and genetic testing for *gBRCA1/2* mutations

Given the major clinical consequences for both patients and family members, it is important to identify patients with the HBOC-syndrome. Table 2 describes the indications for referral to the clinical geneticist for, amongst other, g*BRCA1/2* mutation screening. As can be seen, the main indications are early onset of BC, epithelial OC or a family history of, amongst others, early BC.[59, 63] EC is currently not included as an indication for *gBRCA1/2* mutation testing.

The gold-standard for germline mutation testing is analysis performed on high-quality blood-derived leukocyte-DNA. Depending on the gene analyzed and the mutation sought for, a combination of different techniques is used (next generation sequencing (NGS), sanger sequencing, copy number multiplex ligation probe amplification (CN-MLPA).[75-78] Since 2015, all women with epithelial OC are eligible for *gBRCA1/2* testing,[63, 79] which has significantly increased the referral rates of patients to clinical geneticist.

A more efficient way to select women for referral to the clinical geneticist, would be by preselecting women via mutation analysis performed on tumor-derived DNA. Since *BRCA1/2* mutations are only present in approximately 20% of HGSOC (+/- 14% germline, +/- 6% somatic mutations),[42] such a "tumor-first approach" could possibly prevent referral of around 80% of OC patients to the clinical geneticist and prevent unnecessary patient distress.

Another advantage of this "tumor-first approach" would be the simultaneous detection of both somatic and germline mutations. Though the presence of a germline/somatic *BRCA1/2* mutations is not a prerequisite for PARP-inhibitor maintenance treatment of recurrent platinum-sensitive high-grade OC anymore, the presence of such a mutation is a prerequisite

**Table 2. Indications for referral of women to clinical geneticist for (amongst others) *BRCA1/2* mutation testing**

| |
|---|
| **Women with history of breast cancer** |
| BC and a family member with a pathogenic g*BRCA1/2* mutation |
| BC diagnosis <40 years |
| Bilateral BC, of which the first BC was diagnosed <50 years |
| Multiple primary BC on one side, of which the first BC was diagnosed <50 years |
| Triple negative BC <60 years |
| BC < 50 years and one or more first[a] degree relatives with BC <50 years |
| BC <50 years and first degree family member with prostate cancer <60 years |
| BC and two or more first or second[b] degree family members with BC, of which one was diagnosed <50 years (same side of family) |
| **Women with a history of epithelial ovarian/tubal cancer** |
| All patients, irrespective of age at diagnosis (not including borderline tumors) |
| **Women without a cancer history** |
| First or second degree family member with *BRCA1/2* mutation (man or women) |
| First degree family member with; |
| BC<40 years |
| bilateral BC, of which the first BC was diagnosed <50 years |
| multiple primary BC on one side, of which the first BC was diagnosed <50 years |
| triple negative BC <60 years |
| First degree male relative with BC |
| First degree family member with BC <50 years and first degree family member with prostate cancer <60 years (same side of family) |
| Two or more first degree family members with BC <50 years |
| Three or more first or second degree family members with BC, of which one was diagnosed <50 years (same side of family) |
| First degree family member with OC, irrespective of age |

[a]First degree family member: Parents, Children, Siblings. [b]Second degree family member: grandparents, grandchildren, uncles, aunts, half-siblings, children of siblings from same side of the family Abbreviations: BC; breast cancer

for first-line maintenance treatment of platinum-sensitive high-grade OC. Thereby, a "tumor-first approach" would prevent additional mutation analysis for women that tested negative for a *gBRCA1/2* mutation, but who are eligible for first-line maintenance treatment with a PARP-inhibitor treatment in case a somatic *BRCA1/2* mutation would be present.

However, before such a tumor-first approach can implemented as pre-selection tool for referral to the clinical geneticist, the reliability of this method needs to be proven. Tumor tissue used

for pathology diagnoses and DNA-isolation is formalin-fixed and paraffin embedded (FFPE). FFPE-derived tumor DNA is of lower quality than blood-derived DNA, as formalin causes the DNA to be high-fragmented, making mutation analyses more technically challenging.



## 1.6. Subjects, aims and thesis outline

Whether women with g*BRCA1* and *gBRCA2* mutations are at increased risk to develop EC remains topic of debate, mainly because literature shows contradictory results.[51-57] These contradictory findings are likely attributable to small cohort sizes, limited follow-up and subsequent limited events, and the lack of pathology review. Accurate risk predictions are not only essential for genetic counselling and risk-reducing strategies, but may also provide evidence that a subgroup of EC is HRD, which subsequently provides new treatment options, like PARP-inhibitors.[70-72, 74] Furthermore, now all patients with epithelial OC are being offered genetic testing and having a germline and somatic *BRCA1/2* mutations is a prerequisite for first-line PARP-inhibitor maintenance therapy, more efficient *BRCA1/2* (pre-) screening pathways are desirable.

Therefore, the aims of this thesis were

- to determine whether (a subset of) ECs harbor deficits in the homologous recombination repair pathway.
- to determine whether (a subset of) EC should be considered part of the *gBRCA1/2*-associated HBOC-syndrome.
- to determine whether *BRCA1/2* analyses can reliably be performed on FFPE-derived tumor DNA.

**Chapter 2** reports on the occurrence of HRD in EC using a functional *ex vivo* assay. In **chapter 3,** a systematic review and meta-analyses is performed to determine the frequency of *gBRCA1/2* mutations in USCs compared with what would be expected based on population frequencies. **Chapter 4** describes an in depth molecular and morphological characterization of ECs that occurred in women with *gBRCA1/2* mutations. **Chapter 5** reports on the uterine cancer risk in a large cohort of women with a proven *gBRCA1/2* mutation compared with both non-*gBRCA1/2*-mutation carriers and population incidence rates. In **chapter 6,** we validated *BRCA1/2* tumor testing performed on DNA isolated from FFPE-tissue and compared the performance with *BRCA1/2* analyses on leukocyte DNA, which is the gold standard. **Chapter 7** provides a general discussion on the results obtained in this thesis, focusing on potential clinical implications and future perspectives.

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