Review

Not peer-reviewed version

BRAC1 & *BRCA2* Methylation as a Prognostic and Predictive Biomarker in Cancer: Implementation in Liquid Biopsy in the Era of Precision Medicine

Maria Panagopoulou *, Theodoros Panou, Anastasios Gkountakos, Gesthimani Tarapatzi, Makrina Karaglani, Ekaterini Chatzaki

Posted Date: 23 July 2024

doi: 10.20944/preprints202407.1744.v1

Keywords: *BRCA1*; *BRCA2*; promoter methylation; breast cancer; ovarian; cancer; liquid biopsy; PARP inhibitors

Review

BRAC1 & BRCA2 Methylation as a Prognostic and Predictive Biomarker in Cancer: Implementation in Liquid Biopsy in the Era of Precision Medicine

Maria Panagopoulou [1,2,†], Theodoros Panou [1,†], Anastasios Gkountakos [1], Gesthimani Tarapatzi [1], Makrina Karaglani [1,2] and Ekaterini Chatzaki [1,2]

[1] Laboratory of Pharmacology, Department of Medicine, Democritus University of Thrace, 68100 Alexandroupolis, Greece
[2] Institute of Agri-Food and Life Sciences, University Research and Innovation Centre, Hellenic Mediterranean University, 71003 Heraklion, Greece
* Correspondence: mpanagop@med.duth.gr
† These authors contributed equally to this work.

Abstract: BReast CAncer gene 1 (BRCA1) and BReast CAncer gene 2 (BRCA2) encode for tumor suppressor proteins which are critical regulators of the homologous recombination (HR) pathway, the most precise and important DNA damage response mechanism. Dysfunctional HR proteins cannot repair double-stranded DNA breaks in mammalian cells, a situation called HR deficiency. Since their identification, pathogenic variants and other alterations of *BRCA1* and *BRCA2* genes have been associated with an increased risk of developing mainly breast and ovarian cancer. Interestingly, HR deficiency is also detected in tumors not carrying *BRCA1/2* mutations, a condition termed "BRCAness". One of the main mechanisms causing the BRCAness phenotype is the methylation of the *BRCA1/2* promoters and this epigenetic modification is associated with carcinogenesis and poor prognosis mainly among patients with breast and ovarian cancer. *BRCA1* promoter methylation has been suggested as an emerging biomarker of great predictive significance, especially concerning Poly (ADP-ribose) Polymerase inhibitors (PARP inhibitor-PARPi) responsiveness, along with or beyond *BRCA1/2* mutations. However, as its clinical exploitation is still insufficient, the impact of *BRCA1/2* promoter methylation status needs to be further evaluated. The current review aims to gather the latest findings about the mechanisms that underline BRCA1/2 function as well as the molecular characteristics of tumors associated with BRCA1/2 defects, by focusing on DNA methylation. Furthermore, we critically analyze their translational meaning and the validity of *BRCA* methylation biomarkers in predicting treatment response and we suggest a diagnostic pipeline that could be implemented in liquid biopsy to aid precision pharmacotherapy in *BRCA*-associated tumors.

Keywords: BRCA1; BRCA2; promoter methylation; breast cancer; ovarian; cancer; liquid biopsy; PARP inhibitors

Introduction

BReast CAncer gene 1 (BRCA1) and BReast CAncer gene 2 (BRCA2) encode for proteins that are well-known mediators of DNA damage response and particularly of double-strand breaks (DSBs) through homologous recombination (HR) [1,2]. Since the discovery of *BRCA1* and *BRCA2* genes in the early 1990s, it was demonstrated that individuals carrying germline *BRCA1/2* mutations had a much higher lifetime risk of developing a malignancy such as Breast Cancer (BrCa), Ovarian Cancer (OvCa), Prostate Cancer (PrCa), and Pancreatic Cancer (PaCa) compared to the general population [3–7]. Interestingly, the risk probability of carcinogenesis varies and depends on the type and position of the actual mutation within the *BRCA1/2* genes [8,9]. While the correlation between *BRCA1/2* mutations and higher risk for tumorigenesis is well-established, the conclusions regarding impact on

 doi:10.20944/preprints202407.1744.v1

the survival are still under debate and characterized by conflicting results [10–14]. Later, the major importance of *BRCA1* promoter methylation was also highlighted in different types of cancer as it represents an alternative silencing mechanism of the *BRCA1* gene [15–17]. In general, aberrant epigenetic regulation affecting gene expression independently of DNA sequence is very common in cancer [18]. Specifically, hypermethylation of the 5′ promoter region of genes is a frequent epigenetic event in cancer cells leading generally to gene silencing [19,20]. Interestingly, *BRCA1* promoter methylation was identified only in tumor tissue, indicating its potential oncogenic role [15,16]. Different clinical studies have demonstrated that patients with BrCa, OvCa, PrCa, and PaCa harboring *BRCA1/2* mutations or other aberrations leading to malfunction could receive clinical benefit with the use of PARPi), such as olaparib and rucaparib, thus succeeding a major advance of precision medicine for these tumor types [21–27]. Today, additional pieces of clinical research work have shown the *BRCA1/2* impact on a person's lifetime risk of developing specific types of cancer and highlight the potential of the aberrant methylation of these genes as prognostic and predictive biomarkers [28–31]. Therefore, on the eve of precision medicine, the understanding of the tumors with *BRCA1/2* aberrations and their distinct traits remains of utmost significance.

BRCA1/2 molecular mechanism of DNA damage response during Homologous Recombination

BRCA1 and BRCA2 are proteins with a critical role in maintaining genomic stability by responding to Double-Strand Breaks (DSBs) through the HR pathway [1,32]. It is of note, that BRCA1 targets effectively every DSB through HR[1]. Repair through HR takes place in the late S phase and G2 of the cell cycle [33]. The ATR and ATM kinases recognize this DNA damage and initiate the repair process by phosphorylating downstream DNA repair-related targets such as BRCA1 [34]. BRCA1 is a multifunctional nuclear phosphoprotein composed of diverse domains such as BRCA C-terminal (BRCT) domain, which participates in many biological processes [1,35]. HR is considered an error-free DNA damage response mechanism and is mediated by BRCA1/2 and other effectors, as follows: BRCA1 binds to the DSBs through a protein complex composed of Mre11, Rad50, and NBS1 (MRN complex) as well as CtIP [36]. Then, this BRCA1-containing multi-protein complex promotes DNA resection at the 5′ end of DSBs, creating single-strand DNA (ssDNA) [37]. Then, BRCA1 employs Rad51, an important factor with recombinase function, through its interaction with BRCA2 and PALB2 and drives it to the ssDNA, where it takes place the HR process [38]. A schematic representation of the main events of HR is illustrated in Figure 1.

 doi:10.20944/preprints202407.1744.v1

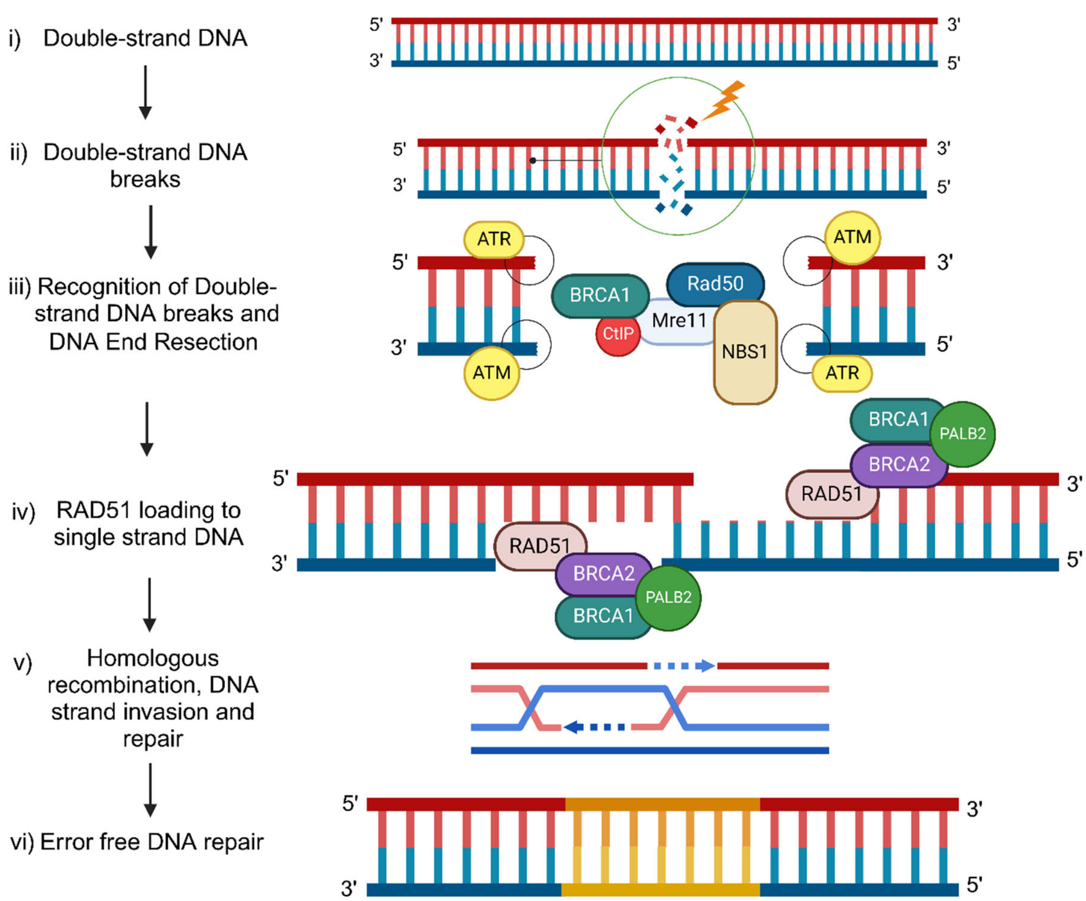

Figure 1. BRCA1 and BRCA2 molecular mechanisms of DNA damage response during in homologous recombination. i) A double-strand DNA molecule without errors. ii) The DSB of DNA is typically caused by external factors such as ionizing radiation or chemotherapeutic drugs but also naturally due to the accumulation of reactive oxygen species. Most of the time, this DNA damage leads to the uneven loss of numerous nucleotides resulting in two DNA strands that are incompatible near the breakage point. iii) ATM and ATR kinases are activated in the presence of DSB initiating the repair process by phosphorylating a set of DNA repair targets. One of the main phosphorylation targets is the BRCA1, which in turn associates with the Mre11, Rad50, and NBS1 (MRN complex) as well as CtIP, eventually forming a multi-protein BRCA1 complex. This complex approaches the DSBs and initiates DNA end resection, creating single-strand DNA (ssDNA) overhangs. iv) After DNA end resection is completed, BRCA1 recruits PALB2 and BRCA2 which in turn promotes the loading of the recombination enzyme RAD51 to the ssDNA. v) The second BRCA1-based protein complex starts the DNA strand invasion and homologous repair mechanism, restoring the genetic information lost at the breakage. vi) After this multi-step but accurate process is successfully completed, two identical or almost identical DNA double strands are formed.

DSB: Double-Strand Breaks (DSB)

***BRCA1*/2-mutated cancers**

BRCA1-mutated tumors include all tumors which exhibit a pathogenic mutation and not a Variant of Unknown Significance (VUS) in the *BRCA1* gene [39]. Mutations in *BRCA1* gene are detected in different cancers, such as in BrCa (about 5% to 10% of all cases), OvCa (about 20% of cases), PaCa (about 5% to 10% of all cases), and to a lesser extent in PrCa (about 1% to 5% of all cases) [40–45]. In clinicopathological settings, these tumors display some distinct features: *BRCA1*-mutated BrCa is more often associated with the basal-like triple-negative phenotype (ER-/PR-/HER2-),

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 July 2024 doi:10.20944/preprints202407.1744.v1

mutated *p53*, immune cell infiltration (mainly T-cell lymphocytes), whereas *BRCA2*-mutated BrCa presents the following features: luminal type, ER+/PR+/HER2- profile, intense immunogenicity and better survival rates [39,46–48]. Apart from mutations, other genetic aberrations of *BRCA1/2* take an active role in carcinogenesis. For example, researchers analyzed 36 Formalin-Fixed Paraffin-Embedded (FFPE) OvCa samples by next-generation sequencing (NGS) and found 15 *BRCA1* and 12 *BRCA2* variants as well as important loss-of-function due to CNV of *BRCA1/2* genes [49].

Loss of heterozygosity (LOH) is also a key concept in tumorigenesis. It refers to the loss of an allele, usually through a mutation, and then the loss of the second allele due to genetic imbalance/rearrangements, epigenetic regulation, or other mechanisms [50]. LOH is strongly correlated to BRCA1/2 status as it was found to be relatively frequent in BrCa and OvCa bearing *BRCA1/2* mutations [51]. Moreover, *BRCA1* mutation carriers presented *BRCA1* promoter methylation and to a great extent exhibited also LOH. In *BRCA1/2* mutation carriers, LOH is associated with better survival rates and therapeutic implications, as the absence of BRCA1/2 function due to LOH renders tumors sensitive to PARPi and mainly to platins [51], as LOH is necessary for tumor sensitivity to platins and LOH absence is connected with a worse prognosis under this type of treatment [51].

BRCA1-like cancers

Following the establishment of the pivotal role of *BRCA1/2* mutations in carcinogenesis, another emerging group of tumors was associated with BRCA1 dysfunction, the so-called *BRCA1*-like (or BRCAness) tumors, which do not exhibit a distinct mutation in the *BRCA1* gene but share altogether common traits [52,53]. *BRCA1*-like tumors are HR deficient and present chromosomal breaks, DNA methylation, copy number variations (CNV), and genomic instability [52,54]. Recent studies focus on describing every aspect of the *BRCA1*-like tumors, in order to identify cancer subgroups with distinct characteristics rendering them candidates for efficient therapeutic strategies [48,54].

A broad spectrum of DNA damage response genes directly or indirectly linked to HR status has been identified including *ATM*, *STK11*, *TP53*, *PTEN*, *CDH1*, *CHEK2*, *BARD1*, *BRIP1*, *MRE11*, *RAD50*, *NBS1*, *RAD51C/D*, *ATR*, *BAP1*, *BLM*, *CDK12*, *FANCA*, *FANCC*, *FANCD2*, *KRAS*, and *PALB2* [3,53,55]. However, only a few of the aforementioned genes are found often mutated, such as *TP53*, which is mutated in 84% of all *BRCA1*-like tumors and could serve as a valuable biomarker for stratifying *BRCA1*-like tumors [48]. Takamatsu et al, showed that BRCA1/2 wild-type cancers which present alteration in other HR genes associated with elevated genomic scar scores (model predicting homologous recombination deficiency). This score differed significantly by sex and the presence of somatic TP53 mutations and was associated with HR deficiency and

treatment response to DNA-damaging agents [56]. Alternatively, the evaluation of foci formation (a biomarker of HR repair) of 4 key HR proteins (BRCA1, Rad51, γH2AX and 53BP1) on DNA is recommended in order to detect possible HR deficiency and *BRCA1*-like tumors [57]. As mentioned above, BRCA1 and Rad51 are key mediators of HR and thus their foci formation is present in HR-proficient cells, whereas γH2AX and 53BP1 as conventional DNA damage markers build foci in HR-deficient cells [58]. Interestingly, researchers proved that the positive BRCA1 and RAD51 foci formation is associated with non-response to olaparib therapy in a study featuring Patient Derived Xenograft (PDX)-derived Triple Negative Breast Cancer (TNBC) samples with BRCA1/2 defect and could be used as a predictive marker in the TNBC [59]. The above studies, pointing out the importance of analyzing a panel of HR genes to identify HR deficiency. Also, identifying a *BRCA1*-like tumor and distinguishing it from a *BRCA1*-mutated tumor is not as simple as anticipated on the genetic level, making it clear that a multidimensional approach would be more suitable in studying *BRCA1*-like tumors.

BRCA1/2 promoter methylation in cancers

It is widely accepted that aberrant gene promoter methylation represents an epigenetic event exhibiting an oncogenic role by repressing gene expression in numerous cancers [60–63]. Specifically, locus-specific hypermethylation takes place on sites rich in CGs of the promoter region of tumor suppressor genes such as *BRCA1*, leading to *BRCA1* transcripts and BRCA1 protein levels downregulation [64]. In BrCa tissues, *BRCA1* promoter hypermethylation has been identified in 9 to 24% of all cases [65,66]. In particular, the prevalence of *BRCA1* promoter methylation is increased in TNBC [67–69]. In general, an individual with *BRCA1* promoter methylation, an event being particularly encountered in East Asia than Caucasians, has a 4.6 higher risk of developing BrCa than baseline, according to a meta-analysis [28] including 19,084 individuals, which associated *BRCA1* promoter methylation with BrCa occurrence, recurrence, prognosis, and therapy response [28,70]. *BRCA1* promoter methylation was present in 44.4% of malignant and 9.7% of normal tissues [28]. Despite the strong evidence that hypermethylation of *BRCA1* promoter is detected mainly in cancer tissue, several studies presented contradictory results, suggesting that *BRCA1* promoter methylation levels in normal tissue might be equal to or exceed the methylation levels of cancer tissues [71–73].

On the other hand, *BRCA2* promoter hypermethylation is rarely encountered in BrCa and OvCa and no statistically significant correlation has been observed to clinical end-points [28], whereas in ovarian tumor samples, *BRCA2* promoter methylation was confirmed in only 4.6% of the cases [31].

From a technical point of view, the overall approach for the quantification of *BRCA1* promoter methylation differs between studies likely due to the determination of different cut-offs, different handling and pre-analytical procedures, lack of a common validation assay and quality of the biomaterial, eventually leading to discrepancies in calling a sample hypermethylated or not [74]. For example, in one study, 5% of the TNBC tissue samples showed methylation levels over 80% and were classified as high-methylated while 25% of them demonstrated methylation levels between 30% and 80% respectively, classified as low-methylated [75]. In another study focusing on OvCa tissues, researchers considered as a cut-off value the 15% methylation for calling a sample methylated [31]. There are also different methodologies to determine methylation (pyrosequencing, Methylation Specific PCR, restriction enzyme-based methods, droplet digital PCR, Genome-Wide Methylation Assays) and therefore the results have to be interpreted according to the used assay to avoid discrepant results between studies [31,64]. It is clear that *BRCA1* promoter methylation should be examined quantitatively and in relation to methylation zygosity, as samples, many times are misidentified as hypermethylated without adequate methylation levels [64]. Methylation zygosity describes the methylation status of all epialleles (alleles that are variably expressed due to epigenetic modifications). 'Homozygous methylation' refers to the situation when all epialleles in a cell have highly methylated promoters resulting in gene silence. 'Heterozygous methylation' describes a mix of highly methylated and unmethylated epialleles coexisting within each cell. In these cells, gene expression is active due to the presence of unmethylated epialleles, despite the presence of highly methylated epialleles [76]. An important factor that affects the methylation rate determination is neoplastic cellularity. Tumor cells exhibit drastically different methylation levels thus, sufficient tumor cellularity will lead to higher mean methylation in cancerous in relation to healthy tissue [29,31,64]. Collectively, for what is concerned with measuring *BRCA1* methylation, for valid conclusions to be drawn, the establishment of a widely accepted unified analytical procedure is of utmost importance.

BRCA1/2 promoter methylation in different cancer types

The following section will provide insight into current studies linking the *BRCA1/2* promoter methylation status and other *BRCA1/2*-related genetic modifications to certain cancer types. Data from major studies assessing *BRCA1* promoter methylation levels among patients with different types of cancer have been included, to frame the whole spectrum of *BRCA1* promoter methylation applications in clinical settings. Table 1 presents the percentages of *BRCA1* methylation reported in BrCa, OvCa, PrCa, and PaCa and correlations that have been made with the disease state.

Table 1. BRCA1 methylation percentages in tissue and blood cells among cancer types and their correlations with the disease's clinically significant end-points.

Cancer type	Biomaterial	*BRCA1* methylation (%)	Correlation	Reference
BrCa	Tissue	9.1	Diagnosis at a young age	Birgisdottir et al [65]
		3.0	Improved Survival after chemotherapy	Stefansson et al [68]
		12.4	Incidence of TNBC	Lonning et al [77]
		26.0	Worse Survival	Chen et al [78]
		24.1	Improved Survival after chemotherapy	Glodzik et al [66]
TNBC	Tissue	20.6	Improved Survival	Brianese et al [67]
OvCa	Tissue	16.3	Young age, Advanced stage, Improved Survival	Kalachand et al [43]
		19 (high methylation) 14 (low methylation)	High methylation with GIS and PARPi treatment option	Durand et al [79]
		89.9	None	Pradjatmo et al [80]
		5.2	Partially BRCAness prediction	Aref-Eshghi et al [49]
		19.3	None	Sahnane et al
HGSOC	Tissue	14.8	Young age	Ruscito et al [81]
PrCa	Tissue	100.0	None	Rabiau et al [17]
		0.0	None	Bednarz et al [82]
PaCa	blood lymphocytes	0.3	None	Zhou et al [83]
	Tissue	60.3	Poorer tumor differentiation, protein expression levels	Peng et al. [84]
PaCa	Tissue	0.0	None	Abdalah et al. [85]
PaCa	Tissue & Blood lymphocytes	3.6	None	Zhen-Lin et al. [86]

Abbreviations: BrCa: Breast Cancer; TNBC: Triple-Negative Breast Cancer; OvCa: Ovarian Cancer; HGSOC: High-Grade Serous Ovarian Cancer, PaCa: Pancreatic Cancer, GIS: Genomic Instability; PARPi: PARP inhibitor.

BrCa

BrCa is the most frequently diagnosed malignancy in women globally. In addition to the study mentioned above [65], a meta-analysis by Wu et al. featuring data from 3,205 women suffering from BrCa, reported that *BRCA1* methylation in tumor tissues was statistically significantly correlated to poor prognosis in terms of overall survival [87]. Interestingly, the researchers also concluded that the handling and storage of cancerous tissue could affect the tissue quality influencing the methylation results [87]. In another study by Chen et al., 139/536 (26.0%) tumor samples deriving from patients with sporadic BrCa exhibited *BRCA1* promoter methylation. Interestingly, the scientists observed a worse 5-year Disease Free Survival (DFS) for patients bearing tumors with *BRCA1* methylation in a statistically significant manner [78]. A meta-analysis in patients with BrCa showed that *BRCA1* promoter methylation status was similar between tumor tissue and peripheral blood cells, thus encouraging its potential use as a blood-based biomarker [28]. However, a study that analyzed *BRCA1* methylation in the blood of early BrCa in younger patients found that only 2 out of 154 blood cell samples presented hypermethylation of *BRCA1* promoter [88]. According to these findings, someone can speculate that *BRCA1* promoter methylation is a rare event in the early onset of BrCa, but more studies are needed for definite conclusions to be drawn. On the other hand, BRCA2 methylation has a very low incidence, about 4% and no correlation was observed with BrCa, according to a meta-analysis [28]. TNBC is a subtype of BrCa lacking the ER, PR, and HER-2 receptors, and thus not responding to hormonal therapy (like tamoxifen or aromatase inhibitors) or therapies that target HER2 receptors (like Herceptin) [89]. TNBC accounts for about 10% to 20% of all BrCa cases and may be either hereditary or sporadic [90]. TNBC is stimulated by mechanisms, such as point mutations, large rearrangements, and gene promoter methylation, and interestingly shares the same clinicopathological characteristics with the *BRCA1*-mutated tumors [28,67]. Multiple studies confirmed that *BRCA1* promoter methylation and *BRCA1* mutation status are almost mutually exclusive, thus tumors featuring *BRCA1* promoter methylation are not linked to *BRCA1* gene mutations, although there are some rare exceptions observed [28,29,31,43,67–69,91–93]. Interestingly, according to a study, 62% of *BRCA1*-mutated and 50% of *BRCA1* promoter methylated cancers appear to be TNBC, whereas 40% to 70% of TNBC is estimated to be HR deficient [68]. Another study analyzed 237 TNBC tissues identifying hypermethylation of *BRCA1* promoter in the 57/237 (24.1%) of samples [66]. Interestingly, 89.5% of the hypermethylated cases harbored concurrent LOH of *BRCA1* and patients with TNBC harboring *BRCA1* promoter methylation presented a significantly longer DFS than non-altered patients [66]. An immense potential of *BRCA1* methylation as an early biomarker for TNBC (also HGSOC), was highlighted in a study showing that *BRCA1* promoter methylation aberrations can be detected in white blood cells almost 5 years earlier than usually expected, paving the way for timely interventions and a better therapeutic outcome [69].

It is clear that *BRCA1* promoter methylation is a strong candidate both as a prognostic and a predictive biomarker; nevertheless, intratumor heterogeneity and differences in epialleles render *BRCA1* promoter methylation as a marker only partially effective. It is well-known that the dynamic evolution of a tumor leads to different tumor cell subpopulations with distinct genetic, epigenetic, and phenotypic traits. The different epialleles in these subpopulations could determine the response to treatment as in the case of *BRCA1* mutations. Scientists now focus their attempts on deep sequencing to catch all sample epialleles. In a relevant study, researchers using bisulfite sequencing found lower methylation in epialleles of core breast tumors than in tumor periphery, [94]. These methylation differences were rendered to the hypoxic microenvironment of the tumor's, core leading to this heterogeneous phenotype; such tumor biology aspects need to be considered for developing effective treatment schemes [94]. On the other hand, the combination of the *BRCA1* promoter methylation status with other markers has been used to assess prognosis and therapy response with more accuracy. In TNBC, researchers revealed that the combination of low pRb expression levels, high p16 expression levels, PTEN absence, and *BRCA1* promoter methylation exhibited a similar phenotype to *BRCA1*-mutated tumors [70].

Collectively, *BRCA1* promoter methylation is detected frequently in BrCa, especially in TNBC, and has been associated with survival and other prognostic and therapy response end-points. Further

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 July 2024 doi:10.20944/preprints202407.1744.v1

studies analyzing all epialleles at a cellular level and/or combined with additional markers are awaited towards the establishment of *BRCA1* promoter methylation as a useful tool in the clinical management of BrCa.

OvCa

Although first identified in breast, *BRCA1* mutations and other gene aberrations were soon shown to have a significant role also in OvCa [95]. The presence of germline *BRCA1* and *BRCA2* mutations in patients with OvCa ranges from 5% to 20%, also somatic mutations are rare (2% and 8%, respectively) [95]. OvCa is the second cancer type that has been extensively studied as regards to *BRCA1* promoter methylation status. A recent meta-analysis of 15 studies concluded that *BRCA1* promoter methylation was present in 430/2636 tumors (16.3%). However, methylation percentages were not consistent between studies, ranging from 6.2% to 73.7% [43], and this is probably attributed similarly to breast cancer to variations in analytical methods and different methylation cut-offs used in each study. Nevertheless, *BRCA1* promoter-methylated tumors share similar clinicopathological characteristics with the *BRCA1*-mutated as they are associated with younger age and advanced disease but no correlation with survival or platinum sensitivity has been reported [43]. In general, studies are not in agreement regarding a possible correlation between *BRCA1* methylation and survival [79–81]. OvCa patients with homozygous *BRCA1* promoter methylation showed higher PFS than patients bearing *BRCA1*-mutated tumours [43,49]. Another study showing *BRCA1* promoter hypermethylation in 17/88 (19.3%) OvCa and *BRCA2* methylation in 4/86 (4.6%) reported no correlation with clinicopathological characteristics (age, stage, histology type) [31]. Interestingly, *BRCA1/2* promoter methylation is never observed in non-neoplastic ovarian tissue at any histological type, confirming its cancer-specific role [31].

HGSOC, a most lethal OvCa subtype accounting for 70% to 80% of OvCa cases is linked to rapid intraperitoneal spread [96]. The majority of *BRCA1* promoter methylation cases concern younger patients with HGSOC of advanced stage [43]. A study including 172 HGSOC tissues, concluded that the combined examination of *BRCA1/2* sequencing, CNVs, and methylation could lead to a more accurate diagnosis of "BRCAness" phenotype, with an estimated Area Under the Curve (AUC) of 0.77 and accuracy of 0.75, thus worthy to be validated in bigger cohorts of patients [49]. Interestingly, another study using HGSOC-derived PDX models harboring *BRCA1* mutations showed a response to rucaparib and so did two chemo-naive HGSOC-PDX models with homozygous *BRCA1* methylation [97]. Moreover, the donor-patients responded to rucaparib as well [97]. On the other hand, two PDX models with heterozygous *BRCA1* methylation presented some *BRCA1* mRNA and protein expressions and failed to respond to the rucaparib, suggesting that it is homozygous *BRCA1* methylation that predicts PARPi sensitivity [97]. The above results again highlight the significance of assessing *BRCA1* methylation zygosity very carefully to predict clinical outcomes. The zygosity status is thus considered an emerging factor of clinical significance to support decisions for different therapeutic strategies [29,49].

Collectively, these results point out a potential predictive and to a lesser extent prognostic role for *BRCA* gene methylation in OvCa. Survival rates in relation to *BRCA1* methylation should be further studied for conclusions to be drawn. For sure, a determining factor is the quantitative analysis in terms of methylation zygocity as it is of utmost importance for guiding treatment options.

Prostate Cancer (PrCa)

PrCa is the most frequent cancer in men. Although the majority of PrCa cases present an indolent clinical course, PrCa remains a leading cause of cancer-related deaths [98]. Germline mutations in *BRCA1/2* genes increase significantly the risk of developing PrCa. Although *BRCA2* mutations have been found only in 1-3% of cases, *BRCA2* mutation carriers are two-fold to four-fold more likely to develop an aggressive tumor at a younger age compared to the general population [99]. Genetic alterations affecting *BRCA1* gene and representing part of BRCAness phenotype also seem to play a role in PrCa development and metastasis [100]. In PrCa, *BRCA1* promoter methylation status has not been considered of the same clinical importance as in BrCa and OvCa, as there are controversial

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 July 2024 doi:10.20944/preprints202407.1744.v1

results between studies. *BRCA1* promoter methylation was absent in all of the 31 prostate cancer samples examined, although other *BRCA1* aberrations, such as *BRCA1* imbalance, could bear some value in evaluating PrCa prognosis [82]. Another study examined BRCA1 promoter methylation both in non-malignant and malignant tissues, reporting contrasting results; Specifically, *BRCA1* promoter methylation was observed in all malignant tissues (prostate intraepithelial neoplasia, peri-tumor tissue, and adenocarcinoma) but also in 15/17 normal samples [17]. Clearly, further studies are required to enlighten the topic and reveal any significance.

Pancreatic Cancer (PaCa)

Generally, PaCa is characterized by poor prognosis [101]. Pancreatic Ductal Adenocarcinoma (PDAC), the predominant form of PaCa is a highly aggressive tumor with rising incidence and the lowest survival rate amongst all the major cancers. Germline *BRCA1/2* mutations are detected in approximately 5–10% of cases of hereditary PDAC and approximately 3% of cases of sporadic PDAC [45]. Similarly, with PrCa, *BRCA2* mutations seem to be associated with an increased risk of PDAC development [45]. Regarding *BRCA* mutations and survival, the few studies exploring possible associations have presented controversial findings [45,102–104]. Moreover, the findings supporting the role of *BRCA1* promoter methylation in PaCa are not conclusive yet. Indeed, Peng et al. examined surgical samples of PDAC, reporting *BRCA1* promoter methylation in more than half of the cancerous samples (60.3%) [84]. However, Zhou et al. evaluated the promoter methylation status of *BRCA1* and *BRCA2* in the peripheral blood lymphocytes of 655 patients suffering from PaCa and reported *BRCA1* promoter methylation levels ranging from 0.0% to 3.3%, and the *BRCA2* from 0.0% to 7.6%. As the mean values were extremely low (0.3% and 0.1% respectively), the researchers considered the occurrence of *BRCA1* and *BRCA2* promoter methylation in PaCa as highly unlikely [83]. Abdallah et al. assessed the promoter methylation levels of *BRCA1* in 121 FFPE PDAC samples by using different analytical methods to exclude possible low sensitivity and observed no methylation in any of the PDAC samples [85]. In 2022, Zhen-Lin et al. examined tissue samples from patients with PDAC and reached similar conclusions. The mean *BRCA1* promoter methylation levels were found to be low (3.62%). To ensure the results, an additional detection method was used by which the unmethylated status of *BRCA1* promoter was confirmed. Thus, they concluded in concordance with previous studies that *BRCA1* promoter methylation was rather unusual [86].

BRCA1/2 methylation in liquid biopsy as a predictive biomarker

The emergence of liquid biopsy has revolutionized clinical oncology, introducing an alternative to traditional tissue sampling for exploring genetic aberrations and dynamic changes in the tumor [105–108]. Some of its most significant advantages are its non-invasive character and the powerful potential for effective disease monitoring by repeated sampling for controlling therapy efficacy and resistance onset [109,110]. In cancer, circulating tumor DNA (ctDNA) is an important blood component released in the bloodstream by dying tumor cells, reflecting molecular patterns of the cancer cells. It is mainly comprised of around 150 bp nucleic acid fragments and because of its relatively short length, an increased tumor volume is required for accurate assessment [88,110]. The application of liquid biopsy in assessing the *BRCA1* promoter methylation status is on the rise, especially in OvCa. A study evaluating *BRCA1* promoter methylation status in plasma cfDNA from patients with OvCa before and during treatment observed occurrence at 60% before treatment, and a 24% epigenetic shift to the unmethylated state during treatment, which was correlated to OvCa recurrence. Researchers concluded that *BRCA1* promoter methylation in cfDNA can be used as a marker for treatment monitoring (Elazezy et al. 2021). In a relevant study, researchers found cfDNA hypermethylated *BRCA1* in about 57% of OvCa patients of all cancer stages, suggesting its use as a diagnostic and prognostic marker [111]. Similarly, the hypermethylation of *BRCA1* and *RASSF1A* was detected in 68% of the tumor tissues but also in the corresponding cfDNA in all stages of OvCa, being present in the majority of early-stage OvCa cfDNAs, suggesting an early event in OvCa [112] and making *BRCA1* an ideal marker for OvCa monitoring in liquid biopsy. Melnikov et al, used the methylation of a five-gene panel (*BRCA1, HIC1, PAX5, PGR* & *THBS1*) for OvCa detection in cfDNA,

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 July 2024 doi:10.20944/preprints202407.1744.v1

reaching a sensitivity of 85% and a specificity of 61% [113]. These results indicate the importance of using multiple methylation biomarkers in cfDNA to achieve maximum effectiveness in cancer detection.

As far as BrCa concerns, studies of *BRCA1/2* methylation in liquid biopsy are less. Cristall et al, introduced the mDETECT method for detecting ctDNA to manage TNBC. This assay examined many common hypermethylated genome regions including *BRCA1* promoter, reaching an AUC of 0.97 for detecting a tumor with a sensitivity of 93% and a specificity of 100%. Interestingly, *BRCA1* promoter methylation was present in cfDNA of about 25% of TNBC cases and 5% of healthy samples [114]. Liu et al found that cfDNA methylation frequency was higher (but still low) in patients with breast ductal cancers than in healthy individuals [115]. Low cfDNA *BRCA1* methylation frequency (below 5%) was also reported in BrCa by Sturgeon et al. However, *BRCA1* methylation was more often present in lymph-node-positive patients [116]. According to a meta-analysis, the hypermethylation of *BRCA1* in cfDNA, among other markers, was associated with poor prognosis in ER+/PR+ BrCa [117]. In a recent work by Yen et al, researchers introduced the Guardant INFINITY, a cfDNA-based test that simultaneously examined *BRCA1* methylation and genomic alterations for the management of advanced BrCa. In specific, 3% of patients had germline mutations in *BRCA1*, *BRCA2*, or *ATM* and almost 9% of patients had methylated the *BRCA1* gene. Only one patient presented concomitant methylation and mutation at the *BRCA1* gene [118]. Interestingly, methylation of *BRCA1* was not detected in the 3210 cancer-free samples, implying the great specificity of *BRCA1* methylation as a biomarker for cancer detection and monitoring.

In PaCa, only one recent study in cfDNA is available. Unlike PaCa tissue where methylation is low, Koukaki et al identified high methylation levels of *BRCA1* and *BRCA2* in plasma cfDNA, ranging between 46% and 63% in a group of 105 PaCa patients, associated further with poorer survival [119]. The evaluation of CTCs, although challenging as CTCs are extremely few (1 cancer cell:10 billion healthy cells) [120] presents another liquid biopsy alternative *BRCA1* loss is linked to vimentin and cytokeratin-positive CTCs, showing an EMT stimulation through *BRCA1* loss [59,65]. Unfortunately, there is no available study examining the BRCA1 methylation status in CTCs. This could be due to technical reasons as CTCs counts are low. Perhaps, analysis of methylation in CTCs could be applicable in metastatic cancer where CTCs are more abundant.

Based on these limited observations presented above, the highest *BRCA1/2* methylation percentages in liquid biopsy were reported in PaCa. Then, was more often detectable in OvCa than BrCa, but more studies are needed to confirm results. In OvCa, *BRCA1* methylation correlated with diagnosis and treatment monitoring but in BrCa the detectable methylation was correlated to specific cancer subtypes and poor prognosis. We believe that it is of utmost importance the design of new larger liquid biopsy-based studies in those and other cancer types, such as in PrCa, to explore BRCA1/2 methylation as predictive liquid biopsy biomarkers to aid treatment decisions in a minimally invasive manner, which also allows dynamic monitoring.

BRCA1/2 methylation and treatment strategies

Through the evaluation of *BRCA1* promoter methylation in tumor tissue or liquid biopsy and as this assessment becomes more concrete in terms of methylation zygosity and methylation levels, specific groups of patients are identified, who are likely to experience clinical benefit from a specific treatment strategy [31]. PARPis (including olaparib, rucaparib, veliparib, talazoparib, niraparib) are considered a primary treatment option for patients with *BRCA1* mutations and especially for TNBC and HGSOC [29,30,48,49,59,69,75,121–123]. However, not all tumors in these cancer subtypes are sensitive to PARPi due to tumor heterogeneity [124,125]. Consequently, not all TNBC patients carrying *BRCA1* Wild Type (WT) will benefit from PARPi, as much as non-TNBC patients, carrying a *BRCA1* mutation [46,124]. It has been shown that PARPi is also effective in those patients presenting homozygous *BRCA1* methylated tumors [39,49,75,123,126,127]. Thus, all alleles of *BRCA1* must be evaluated. Homozygous *BRCA1* methylation carriers (and not heterozygous) show similar treatment outcomes as *BRCA1* mutation carriers [128]. In general, *BRCA1*-methylated tumors present similarities to *BRCA1*-mutated tumors as regards to the HR pathway activity but are substantially

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 July 2024 doi:10.20944/preprints202407.1744.v1

less differentiated according to their pathological traits [68,69]. A study recommends that methylation levels for multiple genes engaged in the HR pathway need to be evaluated, to recognize eligible patients for PARPi treatment [49]. Interestingly, secondary *BRCA1* mutations occurring within the *BRCA1* ring domain can lead to platinum and PARPi resistance [129,130]. Partially predictive for PARPi effectiveness are also the LOH status of*BRCA1/2* mutations implying a defective HR [39,75]. To identify a possible correlation between *BRCA1* promoter methylation status and LOH, studies in PDX models were conducted using a suitable scoring system for measuring LOH. They confirmed that LOH is linked to homozygous *BRCA1* promoter methylation that could induce sufficient HR deficiency to permit PARPi activity [29]. The truth is that heterozygous *BRCA1* promoter methylation carriers cannot have a significantly improved clinical status under PARPi treatment due to remaining BRCA1 activity. It is of note, that low methylation levels may be attributed either to a monoclonal cancer with heterozygous *BRCA1* promoter methylation status or a heterogenous cancer with some cells exhibiting homozygous *BRCA1* promoter methylation status [51,75]. The complete or almost complete loss of BRCA1/2 system activity is a requirement for HR deficiency and thus PARPi sensitivity [51]. According to a study, BRCA1/2 deficient status and consequently HR deficiency can be determined through the absence of BRCA1 and Rad51 [59,88]. Other studies suggest the simultaneous evaluation of *BRCA1* methylation and BRCA1 protein expression or *PALB2* promoter methylation alone as predictive for therapy response [59,124,131–133].

In OvCa, patients that have BRCA1 hypermethylation are very likely to have high genomic instability, being good candidates for PARPi therapy. On the other hand, low levels of methylation were associated with poor outcomes post-platinum [79]. In a relevant study, TNB patients with *BRCA1*-methylated tumors were sensitive to adjuvant chemotherapy and had better survival as compared with TNB patients with BRCA1-unmethylated tumors [30]. A patient with TNBC presenting high *BRCA1* promoter methylation levels and a *BRCA2* VUS experienced a complete response after Olaparib/Eribulin combination treatment [75]. Rucaparib was evaluated in 9 cell lines of BrCa, OvCa and PaCa of various *BRCA1/2* statuses such as methylation, LOH, and mutation [134]. Particularly, cytotoxic effect was caused in UACC3199, a BrCa cell line methylated at *BRCA1* promoter, being equal to or even exceeding carboplatin efficiency. The importance of *BRCA1* promoter methylation for PARPi efficiency is thereby confirmed. Furthermore, a study noted that *BRCA1* and *BRCA2* methylation frequencies varied between CpG sites across their promoters. Some CpG sites were methylated more frequently in *BRCA1/2* mutated cancers, while others were more often methylated in sporadic carcinomas, suggesting the use of BRCA methylation as a screening test to identify patients with BRCA germline mutation or BRCAness who may benefit from therapies such as PARPi [135].

In contrast to mutations, methylation status can change due to tumor microenvironment over the lifespan of a tumor or during treatment [136]. This might lead to the emergence of PARPi treatment resistance either during treatment or at recurrence [39,43,93,137]. Retention of homozygous *BRCA1* methylation, a shift to heterozygous *BRCA1* methylation, or complete loss of *BRCA1* methylation may be observed following chemotherapy, e.g. under cisplatin/rucaparib treatment [29]. Loss of *BRCA1* promoter methylation restores BRCA1 function and thus HR activity [29,93,137], driving PARPi treatment resistance. Methylation reversion in recurrent tumors is associated with resistance and shorter PFS, as illustrated in studies of paired primary-recurrent ovarian tumors [137]. To the best of our knowledge, studies analyzing *BRCA1* methylation in cfDNA in relation to treatment response are missing. Only in one recent report, researchers used methylation and mutation analysis to assess how clinical resistance to PARPis developed in a cohort of 35 metastatic BrCa bearing *BRCA1/2* mutations. Guardant INFINITY (explained above) was employed to analyze tumors' DNA and corresponding cfDNA. Results showed that the most common resistance mechanism was *BRCA1/2* reversion mutation and less frequent alterations in the 53BP1-Shieldin pathway [138]. Liquid biopsy seems to be also promising in PrCa, but available data concern only gene sequencing results and no methylation. In a phase II study of abiraterone acetate in chemotherapy-naive metastatic castration-resistant prostate cancer patients, the targeted sequencing

 doi:10.20944/preprints202407.1744.v1

of *BRCA1*, *BRCA2*, and other 11 genes in cfDNA after one cycle of treatment could be indicative of cancer prognosis and treatment response [139].

Conclusions

BRCA1 promoter methylation status is a promising predictive and prognostic biomarker in BrCa and OvCa but also in PrCa and PaCa is worthy of further attention. Apart from germline/somatic *BRCA1/2* mutations, other aberrations can lead to tumors bearing similar features, a phenotype called *BRCA1*-like or "BRCAness". Specifically, *BRCA1* promoter methylation, a cancer-specific mechanism, accounts for most cases of BRCA1-like tumors. It has become clear from several studies that to predict treatment response in PARPi, *BRCA1* promoter methylation needs to be assessed quantitatively, both concerning methylation levels and in terms of methylation zygosity. This is why some researchers point out the term hypermethylation, thus showing that methylation levels must exceed a certain cut-off, to be of clinical, prognostic, or therapeutic significance. A combination of a comprehensive evaluation of *BRCA1* methylation, Rad51 foci formation, and BRCA1 protein expression analysis in tumor samples is considered predictive for "BRCAness", although other genes may be of significance as well, e.g. *PTEN*. Currently, liquid biopsy as a cancer monitoring tool has attracted particular interest in clinical oncology. Evaluating BRCA1/2 in tumor-derived material in the blood can demonstrate an early diagnosis and predict therapy response thus, leading to personalized solutions for effective treatment. The analysis of BRCA1/2 methylation in liquid biopsy could reveal how methylation patterns are influenced by cancer evolution and treatment and moreover, define patient subgroups at different time-points which may benefit from PARPi. In Figure 2, we suggest a diagnostic pipeline that could be implemented in liquid biopsy to aid precision pharmacotherapy in BRCA-associated tumors. PARPi is a relatively new therapy with a particular effect in tumors with identified BRCA1/2 or HR deficiency. PARPi therapy is often combined with other chemotherapy agents and stands in the epicenter targeting the underlying molecular mechanisms. As genetic testing becomes less expensive and more comprehensive, validation, optimization, and unifying of assays analyzing *BRCA1/2* methylation alone or combined with other biomarkers in a clinical setting are expected to change the scenery in prognosis and predicting treatment response in multiple cancer types.

 doi:10.20944/preprints202407.1744.v1

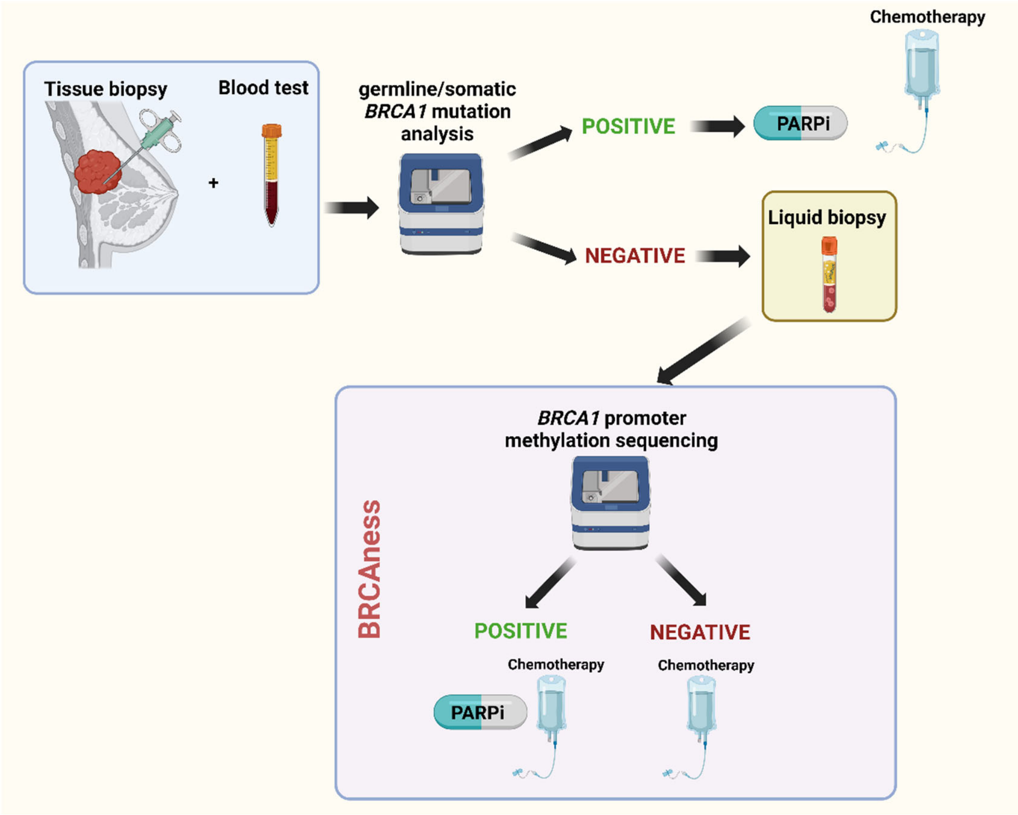

Figure 2. A suggested diagnostic pipeline to identify cancer patients who will benefit from PARPi treatment. Abbreviation: PARPi: PARP inhibitors

References

1 Roy, R., Chun, J. & Powell, S. N. BRCA1 and BRCA2: different roles in a common pathway of genome protection. *Nat Rev Cancer* **12**, 68-78, doi:10.1038/nrc3181 (2011).

2 Prakash, R., Zhang, Y., Feng, W. & Jasin, M. Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins. *Cold Spring Harb Perspect Biol* **7**, a016600, doi:10.1101/cshperspect.a016600 (2015).

3 Guo, M. & Wang, S. M. The BRCAness Landscape of Cancer. *Cells* **11**, doi:10.3390/cells11233877 (2022).

4 Miki, Y. *et al.* A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* **266**, 66-71, doi:10.1126/science.7545954 (1994).

5 Black, D. M. & Solomon, E. The search for the familial breast/ovarian cancer gene. *Trends Genet* **9**, 22-26, doi:10.1016/0168-9525(93)90068-S (1993).

6 Wooster, R. & Weber, B. L. Breast and ovarian cancer. *N Engl J Med* **348**, 2339-2347, doi:10.1056/NEJMra012284 (2003).

7 Pilarski, R. The Role of BRCA Testing in Hereditary Pancreatic and Prostate Cancer Families. *Am Soc Clin Oncol Educ Book* **39**, 79-86, doi:10.1200/EDBK_238977 (2019).

8 Rebbeck, T. R. *et al.* Association of type and location of BRCA1 and BRCA2 mutations with risk of breast and ovarian cancer. *JAMA* **313**, 1347-1361, doi:10.1001/jama.2014.5985 (2015).

9 Kuchenbaecker, K. B. *et al.* Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. *JAMA* **317**, 2402-2416, doi:10.1001/jama.2017.7112 (2017).

10 Copson, E. R. *et al.* Germline BRCA mutation and outcome in young-onset breast cancer (POSH): a prospective cohort study. *Lancet Oncol* **19**, 169-180, doi:10.1016/S1470-2045(17)30891-4 (2018).

11 Goodwin, P. J. *et al.* Breast cancer prognosis in BRCA1 and BRCA2 mutation carriers: an International Prospective Breast Cancer Family Registry population-based cohort study. *J Clin Oncol* **30**, 19-26, doi:10.1200/JCO.2010.33.0068 (2012).

12 Schmidt, M. K. *et al.* Breast Cancer Survival of BRCA1/BRCA2 Mutation Carriers in a Hospital-Based Cohort of Young Women. *J Natl Cancer Inst* **109**, doi:10.1093/jnci/djw329 (2017).

13 Kurian, A. W. *et al.* Association of Genetic Testing Results With Mortality Among Women With Breast Cancer or Ovarian Cancer. *J Natl Cancer Inst* **114**, 245-253, doi:10.1093/jnci/djab151 (2022).

14 Bolton, K. L. *et al*. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. *JAMA* **307**, 382-390, doi:10.1001/jama.2012.20 (2012).

15 Dobrovic, A. & Simpfendorfer, D. Methylation of the BRCA1 gene in sporadic breast cancer. *Cancer Res* **57**, 3347-3350 (1997).

16 Catteau, A., Harris, W. H., Xu, C. F. & Solomon, E. Methylation of the BRCA1 promoter region in sporadic breast and ovarian cancer: correlation with disease characteristics. *Oncogene* **18**, 1957-1965, doi:10.1038/sj.onc.1202509 (1999).

17 Rabiau, N. *et al*. Methylation analysis of BRCA1, RASSF1, GSTP1 and EPHB2 promoters in prostate biopsies according to different degrees of malignancy. *In vivo (Athens, Greece)* **23**, 387-391 (2009).

18 Sharma, S., Kelly, T. K. & Jones, P. A. Epigenetics in cancer. *Carcinogenesis* **31**, 27-36, doi:10.1093/carcin/bgp220 (2010).

19 Herman, J. G. & Baylin, S. B. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* **349**, 2042-2054, doi:10.1056/NEJMra023075 (2003).

20 Baylin, S. B. DNA methylation and gene silencing in cancer. *Nat Clin Pract Oncol* **2 Suppl 1**, S4-11, doi:10.1038/ncponc0354 (2005).

21 Tutt, A. N. J. *et al.* Adjuvant Olaparib for Patients with BRCA1- or BRCA2-Mutated Breast Cancer. *N Engl J Med* **384**, 2394-2405, doi:10.1056/NEJMoa2105215 (2021).

22 Arora, S. *et al.* FDA Approval Summary: Olaparib Monotherapy or in Combination with Bevacizumab for the Maintenance Treatment of Patients with Advanced Ovarian Cancer. *Oncologist* **26**, e164-e172, doi:10.1002/onco.13551 (2021).

23 Tung, N. & Garber, J. E. PARP inhibition in breast cancer: progress made and future hopes. *NPJ Breast Cancer* **8**, 47, doi:10.1038/s41523-022-00411-3 (2022).

24 Wu, Y., Xu, S., Cheng, S., Yang, J. & Wang, Y. Clinical application of PARP inhibitors in ovarian cancer: from molecular mechanisms to the current status. *J Ovarian Res* **16**, 6, doi:10.1186/s13048-023-01094-5 (2023).

25 Mateo, J. *et al.* Olaparib for the Treatment of Patients With Metastatic Castration-Resistant Prostate Cancer and Alterations in BRCA1 and/or BRCA2 in the PROfound Trial. *J Clin Oncol* **42**, 571-583, doi:10.1200/JCO.23.00339 (2024).

26 Fizazi, K. *et al.* Rucaparib or Physician's Choice in Metastatic Prostate Cancer. *N Engl J Med* **388**, 719-732, doi:10.1056/NEJMoa2214676 (2023).

27 Reiss, K. A. *et al.* Phase II Study of Maintenance Rucaparib in Patients With Platinum-Sensitive Advanced Pancreatic Cancer and a Pathogenic Germline or Somatic Variant in BRCA1, BRCA2, or PALB2. *J Clin Oncol* **39**, 2497-2505, doi:10.1200/JCO.21.00003 (2021).

28 Li, S. *et al.* The association between the methylation frequency of BRCA1/2 gene promoter and occurrence and prognosis of breast carcinoma: A meta-analysis. *Medicine* **99**, e19345, doi:10.1097/md.0000000000019345 (2020).

29 Kondrashova, O. *et al.* Methylation of all BRCA1 copies predicts response to the PARP inhibitor rucaparib in ovarian carcinoma. *Nat Commun* **9**, 3970, doi:10.1038/s41467-018-05564-z (2018).

30 Xu, Y. *et al.* Promoter methylation of BRCA1 in triple-negative breast cancer predicts sensitivity to adjuvant chemotherapy. *Annals of oncology : official journal of the European Society for Medical Oncology* **24**, 1498-1505, doi:10.1093/annonc/mdt011 (2013).

31 Sahnane, N. *et al.* BRCA Methylation Testing Identifies a Subset of Ovarian Carcinomas without Germline Variants That Can Benefit from PARP Inhibitor. *Int J Mol Sci* **21**, doi:10.3390/ijms21249708 (2020).

32 Gorodetska, I., Kozeretska, I. & Dubrovska, A. BRCA Genes: The Role in Genome Stability, Cancer Stemness and Therapy Resistance. *J Cancer* **10**, 2109-2127, doi:10.7150/jca.30410 (2019).

33 Moynahan, M. E. & Jasin, M. Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis. *Nat Rev Mol Cell Biol* **11**, 196-207, doi:10.1038/nrm2851 (2010).

34 Mandel, P. & Metais, P. [Not Available]. *Comptes rendus des seances de la Societe de biologie et de ses filiales* **142**, 241-243 (1948).

35 Clapperton, J. A. *et al.* Structure and mechanism of BRCA1 BRCT domain recognition of phosphorylated BACH1 with implications for cancer. *Nature structural & molecular biology* **11**, 512-518, doi:10.1038/nsmb775 (2004).

36 Her, J., Soo Lee, N., Kim, Y. & Kim, H. Factors forming the BRCA1-A complex orchestrate BRCA1 recruitment to the sites of DNA damage. *Acta Biochimica et Biophysica Sinica* **48**, 658-664, doi:10.1093/abbs/gmw047 %J Acta Biochimica et Biophysica Sinica (2016).

37 Zhao, F., Kim, W., Kloeber, J. A. & Lou, Z. DNA end resection and its role in DNA replication and DSB repair choice in mammalian cells. *Exp Mol Med* **52**, 1705-1714, doi:10.1038/s12276-020-00519-1 (2020).

38 Zhao, W. *et al.* BRCA1-BARD1 promotes RAD51-mediated homologous DNA pairing. *Nature* **550**, 360-365, doi:10.1038/nature24060 (2017).

39 Shah, J. B. *et al.* Analysis of matched primary and recurrent BRCA1/2 mutation-associated tumors identifies recurrence-specific drivers. *Nat Commun* **13**, 6728, doi:10.1038/s41467-022-34523-y (2022).

 doi:10.20944/preprints202407.1744.v1

40 Fu, X., Tan, W., Song, Q., Pei, H. & Li, J. BRCA1 and Breast Cancer: Molecular Mechanisms and Therapeutic Strategies. *Front Cell Dev Biol* **10**, 813457, doi:10.3389/fcell.2022.813457 (2022).

41 Petrucelli, N., Daly, M. B. & Pal, T. in *GeneReviews((R))* (eds M. P. Adam *et al.*) (1993).

42 Casaubon, J. T., Kashyap, S. & Regan, J. P. in *StatPearls* (2024).

43 Kalachand, R. D. *et al.* BRCA1 Promoter Methylation and Clinical Outcomes in Ovarian Cancer: An Individual Patient Data Meta-Analysis. *Journal of the National Cancer Institute* **112**, 1190-1203, doi:10.1093/jnci/djaa070 (2020).

44 Messina, C. *et al.* BRCA Mutations in Prostate Cancer: Prognostic and Predictive Implications. *J Oncol* **2020**, 4986365, doi:10.1155/2020/4986365 (2020).

45 Lai, E. *et al.* BRCA-mutant pancreatic ductal adenocarcinoma. *Br J Cancer* **125**, 1321-1332, doi:10.1038/s41416-021-01469-9 (2021).

46 Miklikova, S. *et al.* The Role of BRCA1/2-Mutated Tumor Microenvironment in Breast Cancer. *Cancers (Basel)* **13**, doi:10.3390/cancers13030575 (2021).

47 Hill, S. J., Clark, A. P., Silver, D. P. & Livingston, D. M. BRCA1 pathway function in basal-like breast cancer cells. *Mol Cell Biol* **34**, 3828-3842, doi:10.1128/MCB.01646-13 (2014).

48 Severson, T. M. *et al.* BRCA1-like signature in triple negative breast cancer: Molecular and clinical characterization reveals subgroups with therapeutic potential. *Mol Oncol* **9**, 1528-1538, doi:10.1016/j.molonc.2015.04.011 (2015).

49 Aref-Eshghi, E. *et al.* Genetic and epigenetic profiling of BRCA1/2 in ovarian tumors reveals additive diagnostic yield and evidence of a genomic BRCA1/2 DNA methylation signature. *Journal of human genetics* **65**, 865-873, doi:10.1038/s10038-020-0780-4 (2020).

50 Nichols, C. A. *et al.* Loss of heterozygosity of essential genes represents a widespread class of potential cancer vulnerabilities. *Nat Commun* **11**, 2517, doi:10.1038/s41467-020-16399-y (2020).

51 Maxwell, K. N. *et al.* BRCA locus-specific loss of heterozygosity in germline BRCA1 and BRCA2 carriers. *Nat Commun* **8**, 319, doi:10.1038/s41467-017-00388-9 (2017).

52 Byrum, A. K., Vindigni, A. & Mosammaparast, N. Defining and Modulating 'BRCAness'. *Trends Cell Biol* **29**, 740-751, doi:10.1016/j.tcb.2019.06.005 (2019).

53 Murai, J. & Pommier, Y. BRCAness, Homologous Recombination Deficiencies, and Synthetic Lethality. *Cancer Res* **83**, 1173-1174, doi:10.1158/0008-5472.CAN-23-0628 (2023).

54 Schouten, P. C. *et al.* Breast cancers with a BRCA1-like DNA copy number profile recur less often than expected after high-dose alkylating chemotherapy. *Clin Cancer Res* **21**, 763-770, doi:10.1158/1078-0432.CCR-14-1894 (2015).

55 Turner, N., Tutt, A. & Ashworth, A. Hallmarks of 'BRCAness' in sporadic cancers. *Nat Rev Cancer* **4**, 814-819, doi:10.1038/nrc1457 (2004).

56 Takamatsu, S. *et al.* Utility of Homologous Recombination Deficiency Biomarkers Across Cancer Types. *JCO precision oncology* **6**, e2200085, doi:10.1200/po.22.00085 (2022).

57 Cruz, C. *et al.* RAD51 foci as a functional biomarker of homologous recombination repair and PARP inhibitor resistance in germline BRCA-mutated breast cancer. *Ann Oncol* **29**, 1203-1210, doi:10.1093/annonc/mdy099 (2018).

58 Bunting, S. F. *et al.* 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell* **141**, 243-254, doi:10.1016/j.cell.2010.03.012 (2010).

59 Velazquez, C. *et al.* BRCA1-methylated triple negative breast cancers previously exposed to neoadjuvant chemotherapy form RAD51 foci and respond poorly to olaparib. *Frontiers in oncology* **13**, 1125021, doi:10.3389/fonc.2023.1125021 (2023).

60 Bouras, E. *et al.* Gene promoter methylation and cancer: An umbrella review. *Gene* **710**, 333-340, doi:10.1016/j.gene.2019.06.023 (2019).

61 Panagopoulou, M. *et al.* Circulating cell-free DNA in breast cancer: size profiling, levels, and methylation patterns lead to prognostic and predictive classifiers. *Oncogene* **38**, 3387-3401, doi:10.1038/s41388-018-0660-y (2019).

62 Panagopoulou, M., Fanidis, D., Aidinis, V. & Chatzaki, E. ENPP2 Methylation in Health and Cancer. *International journal of molecular sciences* **22**, doi:10.3390/ijms222111958 (2021).

63 Panagopoulou, M., Esteller, M. & Chatzaki, E. Circulating Cell-Free DNA in Breast Cancer: Searching for Hidden Information towards Precision Medicine. *Cancers* **13**, doi:10.3390/cancers13040728 (2021a).

64 Ibragimova, I. & Cairns, P. Assays for hypermethylation of the BRCA1 gene promoter in tumor cells to predict sensitivity to PARP-inhibitor therapy. *Methods Mol Biol* **780**, 277-291, doi:10.1007/978-1-61779-270-0_17 (2011).

65 Birgisdottir, V. *et al.* Epigenetic silencing and deletion of the BRCA1 gene in sporadic breast cancer. *Breast cancer research : BCR* **8**, R38, doi:10.1186/bcr1522 (2006).

66 Glodzik, D. *et al.* Comprehensive molecular comparison of BRCA1 hypermethylated and BRCA1 mutated triple negative breast cancers. *Nature communications* **11**, 3747, doi:10.1038/s41467-020-17537-2 (2020).

67 Brianese, R. C. *et al.* BRCA1 deficiency is a recurrent event in early-onset triple-negative breast cancer: a comprehensive analysis of germline mutations and somatic promoter methylation. *Breast cancer research and treatment* **167**, 803-814, doi:10.1007/s10549-017-4552-6 (2018).
68 Stefansson, O. A. *et al.* BRCA1 Promoter Methylation Status in 1031 Primary Breast Cancers Predicts Favorable Outcomes Following Chemotherapy. *JNCI cancer spectrum* **4**, pkz100, doi:10.1093/jncics/pkz100 (2020).
69 Lonning, P. E. *et al.* Constitutional BRCA1 Methylation and Risk of Incident Triple-Negative Breast Cancer and High-grade Serous Ovarian Cancer. *JAMA Oncol* **8**, 1579-1587, doi:10.1001/jamaoncol.2022.3846 (2022).
70 Stefansson, O. A. *et al.* CpG island hypermethylation of BRCA1 and loss of pRb as co-occurring events in basal/triple-negative breast cancer. *Epigenetics* **6**, 638-649, doi:10.4161/epi.6.5.15667 (2011).
71 Vu, T. L., Nguyen, T. T., Doan, V. T. H. & Vo, L. T. T. Methylation Profiles of BRCA1, RASSF1A and GSTP1 in Vietnamese Women with Breast Cancer. *Asian Pac J Cancer Prev* **19**, 1887-1893, doi:10.22034/APJCP.2018.19.7.1887 (2018).
72 Parrella, P. *et al.* Nonrandom distribution of aberrant promoter methylation of cancer-related genes in sporadic breast tumors. *Clin Cancer Res* **10**, 5349-5354, doi:10.1158/1078-0432.CCR-04-0555 (2004).
73 Kontorovich, T., Cohen, Y., Nir, U. & Friedman, E. Promoter methylation patterns of ATM, ATR, BRCA1, BRCA2 and p53 as putative cancer risk modifiers in Jewish BRCA1/BRCA2 mutation carriers. *Breast Cancer Res Treat* **116**, 195-200, doi:10.1007/s10549-008-0121-3 (2009).
74 Sahnane, N. *et al.* Pyrosequencing Assay for BRCA1 Methylation Analysis: Results from a Cross-Validation Study. *J Mol Diagn* **25**, 217-226, doi:10.1016/j.jmoldx.2023.01.003 (2023).
75 Kawachi, A. *et al.* BRCA1 promoter methylation in breast cancer patients is associated with response to olaparib/eribulin combination therapy. *Breast Cancer Res Treat* **181**, 323-329, doi:10.1007/s10549-020-05647-w (2020).
76 Geissler, F. *et al.* The role of aberrant DNA methylation in cancer initiation and clinical impacts. **16**, 17588359231220511, doi:10.1177/17588359231220511 (2024).
77 Lønning, P. E. *et al.* Constitutional BRCA1 Methylation and Risk of Incident Triple-Negative Breast Cancer and High-grade Serous Ovarian Cancer. *JAMA oncology* **8**, 1579-1587, doi:10.1001/jamaoncol.2022.3846 (2022).
78 Chen, Y. *et al.* BRCA1 promoter methylation associated with poor survival in Chinese patients with sporadic breast cancer. *Cancer science* **100**, 1663-1667, doi:10.1111/j.1349-7006.2009.01225.x (2009).
79 Blanc-Durand, F. *et al.* Clinical Relevance of BRCA1 Promoter Methylation Testing in Patients with Ovarian Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **29**, 3124-3129, doi:10.1158/1078-0432.Ccr-22-3328 (2023).
80 Pradjatmo, H., Dasuki, D., Anwar, M., Mubarika, S. & Harijadi. Methylation status and immunohistochemistry of BRCA1 in epithelial ovarian cancer. *Asian Pacific journal of cancer prevention : APJCP* **15**, 9479-9485, doi:10.7314/apjcp.2014.15.21.9479 (2014).
81 Ruscito, I. *et al.* BRCA1 gene promoter methylation status in high-grade serous ovarian cancer patients--a study of the tumour Bank ovarian cancer (TOC) and ovarian cancer diagnosis consortium (OVCAD). *European journal of cancer (Oxford, England : 1990)* **50**, 2090-2098, doi:10.1016/j.ejca.2014.05.001 (2014).
82 Bednarz, N. *et al.* BRCA1 loss preexisting in small subpopulations of prostate cancer is associated with advanced disease and metastatic spread to lymph nodes and peripheral blood. *Clinical cancer research : an official journal of the American Association for Cancer Research* **16**, 3340-3348, doi:10.1158/1078-0432.Ccr-10-0150 (2010).
83 Zhou, C. *et al.* Examination of ATM, BRCA1, and BRCA2 promoter methylation in patients with pancreatic cancer. *Pancreatology : official journal of the International Association of Pancreatology (IAP) ... [et al.]* **21**, 938-941, doi:10.1016/j.pan.2021.03.015 (2021).
84 Peng, D. F. *et al.* DNA methylation of multiple tumor-related genes in association with overexpression of DNA methyltransferase 1 (DNMT1) during multistage carcinogenesis of the pancreas. *Carcinogenesis* **27**, 1160-1168, doi:10.1093/carcin/bgi361 (2006).
85 Abdallah, R. *et al.* BRCA1 and RAD51C promotor methylation in human resectable pancreatic adenocarcinoma. *Clin Res Hepatol Gastroenterol* **46**, 101880, doi:10.1016/j.clinre.2022.101880 (2022).
86 Zheng-Lin, B. *et al.* Methylation Analyses Reveal Promoter Hypermethylation as a Rare Cause of "Second Hit" in Germline BRCA1-Associated Pancreatic Ductal Adenocarcinoma. *Molecular diagnosis & therapy* **26**, 645-653, doi:10.1007/s40291-022-00614-1 (2022).
87 Wu, L. *et al.* Promoter methylation of BRCA1 in the prognosis of breast cancer: a meta-analysis. *Breast cancer research and treatment* **142**, 619-627, doi:10.1007/s10549-013-2774-9 (2013).
88 Azzollini, J. *et al.* Constitutive BRCA1 Promoter Hypermethylation Can Be a Predisposing Event in Isolated Early-Onset Breast Cancer. *Cancers* **11**, doi:10.3390/cancers11010058 (2019).
89 de Ruijter, T. C., Veeck, J., de Hoon, J. P., van Engeland, M. & Tjan-Heijnen, V. C. Characteristics of triple-negative breast cancer. *J Cancer Res Clin Oncol* **137**, 183-192, doi:10.1007/s00432-010-0957-x (2011).

90 Almansour, N. M. Triple-Negative Breast Cancer: A Brief Review About Epidemiology, Risk Factors, Signaling Pathways, Treatment and Role of Artificial Intelligence. *Front Mol Biosci* **9**, 836417, doi:10.3389/fmolb.2022.836417 (2022).
91 Bednarz-Knoll, N., Eltze, E., Semjonow, A. & Brandt, B. BRCAness in prostate cancer. *Oncotarget* **10**, 2421-2422, doi:10.18632/oncotarget.26818 (2019).
92 Swisher, E. M. *et al.* Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. *Lancet Oncol* **18**, 75-87, doi:10.1016/S1470-2045(16)30559-9 (2017).
93 Elazezy, M. *et al.* BRCA1 promoter hypermethylation on circulating tumor DNA correlates with improved survival of patients with ovarian cancer. *Mol Oncol* **15**, 3615-3625, doi:10.1002/1878-0261.13108 (2021).
94 Wang, Y. *et al.* Intratumor heterogeneity of breast cancer detected by epialleles shows association with hypoxic microenvironment. *Theranostics* **11**, 4403-4420, doi:10.7150/thno.53737 (2021).
95 Ashour, M. & Ezzat Shafik, H. Frequency of germline mutations in BRCA1 and BRCA2 in ovarian cancer patients and their effect on treatment outcome. *Cancer management and research* **11**, 6275-6284, doi:10.2147/cmar.S206817 (2019).
96 Bowtell, D. D. The genesis and evolution of high-grade serous ovarian cancer. *Nat Rev Cancer* **10**, 803-808, doi:10.1038/nrc2946 (2010).
97 Nesic, K. *et al.* BRCA1 secondary splice-site mutations drive exon-skipping and PARP inhibitor resistance. *medRxiv : the preprint server for health sciences*, doi:10.1101/2023.03.20.23287465 (2023).
98 Litwin, M. S. & Tan, H. J. The Diagnosis and Treatment of Prostate Cancer: A Review. *JAMA* **317**, 2532-2542, doi:10.1001/jama.2017.7248 (2017).
99 Rajwa, P. *et al.* Prostate cancer risk, screening and management in patients with germline BRCA1/2 mutations. *Nat Rev Urol* **20**, 205-216, doi:10.1038/s41585-022-00680-4 (2023).
100 Omari, A. *et al.* Somatic aberrations of BRCA1 gene are associated with ALDH1, EGFR, and tumor progression in prostate cancer. *Int J Cancer* **144**, 607-614, doi:10.1002/ijc.31905 (2019).
101 Bilici, A. Prognostic factors related with survival in patients with pancreatic adenocarcinoma. *World journal of gastroenterology* **20**, 10802-10812, doi:10.3748/wjg.v20.i31.10802 (2014).
102 Lal, G. *et al.* Inherited predisposition to pancreatic adenocarcinoma: role of family history and germ-line p16, BRCA1, and BRCA2 mutations. *Cancer Res* **60**, 409-416 (2000).
103 Iqbal, J. *et al.* The incidence of pancreatic cancer in BRCA1 and BRCA2 mutation carriers. *Br J Cancer* **107**, 2005-2009, doi:10.1038/bjc.2012.483 (2012).
104 Golan, T. *et al.* Overall survival and clinical characteristics of BRCA mutation carriers with stage I/II pancreatic cancer. *Br J Cancer* **116**, 697-702, doi:10.1038/bjc.2017.19 (2017).
105 Tivey, A., Church, M., Rothwell, D., Dive, C. & Cook, N. Circulating tumour DNA - looking beyond the blood. *Nat Rev Clin Oncol* **19**, 600-612, doi:10.1038/s41571-022-00660-y (2022).
106 Panagopoulou, M. *et al.* Circulating cell-free DNA release in vitro: kinetics, size profiling, and cancer-related gene methylation. *J Cell Physiol* **234**, 14079-14089, doi:10.1002/jcp.28097 (2019).
107 Panagopoulou, M. *et al.* ENPP2 Promoter Methylation Correlates with Decreased Gene Expression in Breast Cancer: Implementation as a Liquid Biopsy Biomarker. *International journal of molecular sciences* **23**, doi:10.3390/ijms23073717 (2022).
108 Papadakis, V. M. *et al.* Label-Free Human Disease Characterization through Circulating Cell-Free DNA Analysis Using Raman Spectroscopy. *International journal of molecular sciences* **24**, doi:10.3390/ijms241512384 (2023).
109 Nikanjam, M., Kato, S. & Kurzrock, R. Liquid biopsy: current technology and clinical applications. *J Hematol Oncol* **15**, 131, doi:10.1186/s13045-022-01351-y (2022).
110 Heidrich, I., Ackar, L., Mossahebi Mohammadi, P. & Pantel, K. Liquid biopsies: Potential and challenges. *Int J Cancer* **148**, 528-545, doi:10.1002/ijc.33217 (2021).
111 S, S. K., Swamy, S. N., Premalatha, C. S., Pallavi, V. R. & Gawari, R. Aberrant Promoter Hypermethylation of RASSF1a and BRCA1 in Circulating Cell-Free Tumor DNA Serves as a Biomarker of Ovarian Carcinoma. *Asian Pacific journal of cancer prevention : APJCP* **20**, 3001-3005, doi:10.31557/apjcp.2019.20.10.3001 (2019).
112 Ibanez de Caceres, I. *et al.* Tumor cell-specific BRCA1 and RASSF1A hypermethylation in serum, plasma, and peritoneal fluid from ovarian cancer patients. *Cancer research* **64**, 6476-6481, doi:10.1158/0008-5472.Can-04-1529 (2004).
113 Melnikov, A., Scholtens, D., Godwin, A. & Levenson, V. Differential methylation profile of ovarian cancer in tissues and plasma. *The Journal of molecular diagnostics : JMD* **11**, 60-65, doi:10.2353/jmoldx.2009.080072 (2009).
114 Cristall, K. *et al.* A DNA methylation-based liquid biopsy for triple-negative breast cancer. *NPJ Precis Oncol* **5**, 53, doi:10.1038/s41698-021-00198-9 (2021).
115 Liu, L. *et al.* Quantitative detection of methylation of FHIT and BRCA1 promoters in the serum of ductal breast cancer patients. *Bio-medical materials and engineering* **26 Suppl 1**, S2217-2222, doi:10.3233/bme-151527 (2015).

116 Sturgeon, S. R. *et al.* Detection of promoter methylation of tumor suppressor genes in serum DNA of breast cancer cases and benign breast disease controls. *Epigenetics* **7**, 1258-1267, doi:10.4161/epi.22220 (2012).
117 de Ruijter, T. C. *et al.* Prognostic DNA methylation markers for hormone receptor breast cancer: a systematic review. *Breast Cancer Research* **22**, 13, doi:10.1186/s13058-020-1250-9 (2020).
118 Yen, J. *et al.* Abstract 6603: BRCA1 promoter methylation in sporadic breast cancer patients detected by liquid biopsy. *Cancer research* **83**, 6603-6603, doi:10.1158/1538-7445.AM2023-6603 %J Cancer Research (2023).
119 Koukaki, T. *et al.* Prognostic significance of BRCA1 and BRCA2 methylation status in circulating cell-free DNA of Pancreatic Cancer patients. *Journal of Cancer* **15**, 2573-2579, doi:10.7150/jca.93184 (2024).
120 Vasseur, A., Kiavue, N., Bidard, F. C., Pierga, J. Y. & Cabel, L. Clinical utility of circulating tumor cells: an update. *Molecular oncology* **15**, 1647-1666, doi:10.1002/1878-0261.12869 (2021).
121 Stordal, B. *et al.* BRCA1/2 mutation analysis in 41 ovarian cell lines reveals only one functionally deleterious BRCA1 mutation. *Mol Oncol* **7**, 567-579, doi:10.1016/j.molonc.2012.12.007 (2013).
122 Cortesi, L., Rugo, H. S. & Jackisch, C. An Overview of PARP Inhibitors for the Treatment of Breast Cancer. *Target Oncol* **16**, 255-282, doi:10.1007/s11523-021-00796-4 (2021).
123 Moschetta, M., George, A., Kaye, S. B. & Banerjee, S. BRCA somatic mutations and epigenetic BRCA modifications in serous ovarian cancer. *Ann Oncol* **27**, 1449-1455, doi:10.1093/annonc/mdw142 (2016).
124 Jacot, W. *et al.* BRCA1 promoter hypermethylation, 53BP1 protein expression and PARP-1 activity as biomarkers of DNA repair deficit in breast cancer. *BMC Cancer* **13**, 523, doi:10.1186/1471-2407-13-523 (2013).
125 Zhu, X. *et al.* Efficacy and mechanism of the combination of PARP and CDK4/6 inhibitors in the treatment of triple-negative breast cancer. *J Exp Clin Cancer Res* **40**, 122, doi:10.1186/s13046-021-01930-w (2021).
126 Gelmon, K. A. *et al.* Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase 2, multicentre, open-label, non-randomised study. *Lancet Oncol* **12**, 852-861, doi:10.1016/S1470-2045(11)70214-5 (2011).
127 Veeck, J. *et al.* BRCA1 CpG island hypermethylation predicts sensitivity to poly(adenosine diphosphate)-ribose polymerase inhibitors. *J Clin Oncol* **28**, e563-564; author reply e565-566, doi:10.1200/JCO.2010.30.1010 (2010).
128 Kondrashova, O. *et al.* Methylation of all BRCA1 copies predicts response to the PARP inhibitor rucaparib in ovarian carcinoma. *Nature communications* **9**, 3970, doi:10.1038/s41467-018-05564-z (2018).
129 Dimitrova, D. *et al.* Germline mutations of BRCA1 gene exon 11 are not associated with platinum response neither with survival advantage in patients with primary ovarian cancer: understanding the clinical importance of one of the biggest human exons. A study of the Tumor Bank Ovarian Cancer (TOC) Consortium. *Tumour Biol* **37**, 12329-12337, doi:10.1007/s13277-016-5109-8 (2016).
130 Drost, R. *et al.* BRCA1185delAG tumors may acquire therapy resistance through expression of RING-less BRCA1. *J Clin Invest* **126**, 2903-2918, doi:10.1172/JCI70196 (2016).
131 Jacot, W. *et al.* BRCA1 Promoter Hypermethylation is Associated with Good Prognosis and Chemosensitivity in Triple-Negative Breast Cancer. *Cancers (Basel)* **12**, doi:10.3390/cancers12040828 (2020).
132 Buisson, R. *et al.* Cooperation of breast cancer proteins PALB2 and piccolo BRCA2 in stimulating homologous recombination. *Nat Struct Mol Biol* **17**, 1247-1254, doi:10.1038/nsmb.1915 (2010).
133 Annunziata, C. M. & O'Shaughnessy, J. Poly (ADP-ribose) polymerase as a novel therapeutic target in cancer. *Clin Cancer Res* **16**, 4517-4526, doi:10.1158/1078-0432.CCR-10-0526 (2010).
134 Drew, Y. *et al.* Therapeutic potential of poly(ADP-ribose) polymerase inhibitor AG014699 in human cancers with mutated or methylated BRCA1 or BRCA2. *J Natl Cancer Inst* **103**, 334-346, doi:10.1093/jnci/djq509 (2011).
135 Vos, S., Moelans, C. B. & van Diest, P. J. BRCA promoter methylation in sporadic versus BRCA germline mutation-related breast cancers. *Breast Cancer Research* **19**, 64, doi:10.1186/s13058-017-0856-z (2017).
136 Guo, M., Peng, Y., Gao, A., Du, C. & Herman, J. G. Epigenetic heterogeneity in cancer. *Biomark Res* **7**, 23, doi:10.1186/s40364-019-0174-y (2019).
137 Prieske, K. *et al.* Loss of BRCA1 promotor hypermethylation in recurrent high-grade ovarian cancer. *Oncotarget* **8**, 83063-83074, doi:10.18632/oncotarget.20945 (2017).
138 Harvey-Jones, E. *et al.* Abstract 6094: Longitudinal analysis of PARP inhibitor and platinum resistance in BRCA1/2m breast cancer using liquid biopsy. *Cancer research* **83**, 6094-6094, doi:10.1158/1538-7445.AM2023-6094 %J Cancer Research (2023).
139 Jayaram, A. *et al.* Plasma tumor gene conversions after one cycle abiraterone acetate for metastatic castration-resistant prostate cancer: a biomarker analysis of a multicenter international trial. *Annals of Oncology* **32**, 726-735, doi:https://doi.org/10.1016/j.annonc.2021.03.196 (2021).