Identification of RAD51 foci in cancer-associated circulating cells of patients with high-grade serous ovarian cancer: association with treatment outcomes

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ABSTRACT

Objective Fifty percent of patients with high-grade serous ovarian cancer harbor defects in the homologous recombination repair pathway. RAD51 foci form where DNA is damaged, indicating its involvement in repairing double-stranded breaks. High levels of RAD51 in ovarian cancer tissue have been associated with a poorer prognosis.
Objective To demonstrate RAD51 foci in circulating cancer-associated cells of patients with ovarian cancer and their association with clinical outcomes.
Methods One hundred and twenty-four patients with high-grade serous ovarian cancer had blood samples taken at strategic points during treatment and follow-up. Cells were stained using WT1 and RAD51 antibodies with immunofluorescence and reviewed under Leica camera microscopy; RAD51 foci were counted. Correlations were made between numbers of RAD51 foci and treatment response, *BRCA* status, and progression-free survival.
Results RAD51 foci were identified in all patients (n=42) with wild-type *BRCA*. *BRCA* mutant/homologous recombination deficiency-positive patients (n=8) had significantly lower numbers of RAD51 foci (p=0.009). Responders to treatment (n=32) had a reduction in circulating cells (p=0.02) and RAD51 foci (p=0.0007). Numbers of RAD51 foci were significantly higher in the platinum-resistant population throughout treatment: at the start of treatment, in 56 platinum-sensitive patients there was a mean of 3.6 RAD51 foci versus 6.2 in 15 platinum-resistant patients (p=0.02). Patients with a high number of RAD51 foci had worse median progression-free survival: in 39 patients with a mean of <3 RAD51 foci at treatment start, median progression-free survival had not been reached, compared with 32 patients with >3 RAD51 foci whose progression-free survival was 13 months (p=0.04).
Conclusions Levels of RAD51 foci in circulating cancer-associated cells of patients with high-grade serous ovarian cancer are associated with clinical outcomes and may be a more pragmatic method of determining a homologous repair-deficient population.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Establishing which patients with ovarian cancer have homologous repair deficiencies facilitates decisions about optimal treatments and discussions about prognosis and inheritability. Excluding those with recognizable *BRCA* mutations, current methods for the identification of such patients within the *BRCA* wild-type population (Myriad MyChoice homologous recombination deficiency testing) requires sequencing of DNA from suitable cancer tissue and does not accurately predict expected clinical outcomes. The presence of RAD51 foci, a conglomeration of proteins, is considered a direct functional assay of homologous repair, but has only been described in *ex vivo* settings.

WHAT THIS STUDY ADDS

⇒ The first demonstration of RAD51 foci, identified in circulating cancer-associated cells of patients with high-grade serous ovarian cancer. Numbers of RAD51 foci change according to clinical characteristics and treatment outcomes for a 'normal' population with high-grade serous ovarian cancer.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Following validation, this functional assay may prove to more accurately identify an homologous recombination- deficient population. The ability to retest during treatment will reveal changes in DNA repair abilities over the course of an individual journey of a patient with ovarian cancer.

INTRODUCTION

Approximately 50% of high-grade serous ovarian cancers are associated with anomalies in the homologous recombination pathway, resulting in defective DNA repair; this is known as homologous recombination deficiency.[1] Many proteins are involved in this pathway, but the best recognized are mutations associated with the BReast CAncer (*BRCA*) genes, affecting ~15% patients with ovarian cancer.[2 3] The *BRCA* genes encode for proteins, which are key to repairing DNA. Irreparable cells undergo senescence or apoptosis, to remove damaged DNA and minimize the risk of cancer.[3] The reduced ability of cells in patients with *BRCA*1/2 mutations to repair DNA, increases their risk of cancer.[4]

Cytotoxic treatments such as chemotherapy/radiotherapy cause damage to DNA—for example, double-stranded breaks. Double-strand breaks activate the homologous recombination DNA repair pathway which initiates repair.[5] Ataxia telangiectasia mutated and ataxia telangiectasia RAD3 proteins phosphorylate BRCA1. BRCA2 has been shown to directly interact with RAD51, another protein key of this pathway. It is thought that RAD51 binds to the C-terminus of the BRCA2 protein, allowing RAD51 to localize in the nucleus at sites of DNA damage, where it binds to the sites of strand breaks to form nucleoprotein filaments.[6] The BRCA1–BRAD1 complexes stabilize RAD51 nucleoprotein filaments which contain specific enzymes to help synthesize DNA for strand formation/repair and can be visually identified as foci using immunofluorescent antibodies to RAD51.[7 8] These can be viewed microscopically and increase in number following exposure to DNA-damaging agents. Thus RAD51 foci can be used as an indicator of double-strand breaks and a marker of certain repair functions.[9]

The reduced ability for homologous DNA repair, in patients with *BRCA*1/2 mutations, potentiates the activity of DNA-damaging treatments, such as platinum, radiation, and the novel poly-(ADP-ribose) polymerase inhibitors (PARPi). Such agents lead to better survival in *BRCA* mutant patients than in those with wild-type *BRCA (BRCAwt)*.[4] However, some *BRCAwt* patients also benefit from PARPi, because of malfunction/absence of other genes/proteins involved in homologous DNA repair. Some of these less common genes can be tested for—for example, *PALB2, RAD51C/D, BRIP1A*, although their rarity makes this less routine, but many of these components are not yet known.[9] There is an urgent need to identify a more sensitive biomarker for aberrant repair pathways to optimally use PARPi.

RAD51 is overexpressed in many cancers including ovarian cancer. Higher levels of RAD51 protein indicate greater efficiency of the DNA repair process. Cancer cells replete with RAD51 protein are seemingly resistant to therapy and patients have poorer survival outcomes as they are able to repair DNA adeptly.[10] In a cohort of 126 patients with ovarian cancer, tissue immunohistochemistry for RAD51, demonstrated significantly higher expression in platinum-resistant patients (<6 months to progression from last platinum treatment) than those still sensitive to platinum (>6 months to progression).[11] Hoppe et al demonstrated similar findings: high levels of RAD51 (assessed by quantitative immunohistochemistry on formalin-fixed paraffin embedded material) were associated with poorer survival outcomes and platinum resistance.[12] However, acquisition of multiple paired tissue samples in practice is challenging and uncomfortable for patients, therefore, we investigated a blood-based approach. We sought to identify the presence/absence of RAD51 foci and associations of these with mutant BRCA (*BRCAm*)/homologous recombination deficiency status, treatment response, and survival, in circulating cancer-associated cells in patients with serous ovarian cancer.

MATERIALS AND METHODS

Patient Selection

All patients in this study gave informed consent and enrolled into the CICATRIx study: a biomarker study in patients with various cancers (RD2016-08, approved by West Midlands–South Birmingham ethics committee: reference 16/WM/0196), at Mount Vernon Cancer Center. One hundred and twenty-four patients with serous ovarian cancer were recruited between 2017 and 2020 and all had blood samples taken at strategic points during treatment. These samples were analyzed within 6 days of venesection, as described previously.[13] Twenty-five were excluded from analyses for a variety of reasons—for example, insufficient cells, no active treatment, wrong time point (Online Supplemental Figure 1).

Immunofluorescence

Whole blood (1 mL) was mixed with 9 mL of red blood cell lysis buffer, inverted 10 times and incubated for 10 min with gentle agitation to lyse and remove red blood cells. The solution was centrifuged for 10 min at 2500 rpm. The supernatant was removed, and the process repeated having resuspended the pellet in 3 mL of lysis buffer. The supernatant was again aspirated, and the resultant pellet washed and resuspended in 1 mL of phosphate-buffered saline. Two hundred microliters of this solution was then placed on a slide in the Cytospin for 5 min at 800 rpm. Cells adherent to the slide were fixed in 4% phosphate-buffered saline for 10 min at room temperature. Cell membranes were permeabilized with 0.2% Triton X-100 solution for 10 min and incubated for an hour at room temperature, with 100 µL 5% bovine serum albumin (blocking buffer) covered with parafilm. RAD51 and WT1 antibodies conjugated to Alexa Fluor 488 (Santa Cruz, California, USA), and Alexa Fluor 594 (Novus Biological), respectively, were used in 1:100 dilution with 5% bovine serum albumin. Slides were incubated for 1 hour in a dark chamber at 4°C.

Following incubation, slides were washed three times with Tris-buffered saline with Tween 20 (pH 7.5 Sigma-Aldrich) at 3 min intervals at room temperature, then dehydrated in an ethanol series (70%, 90%, and 100% v/v). After air-drying, 15 µL of 4′,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, UK) was added, a cover slip placed and sealed with clear nail varnish. Cells were viewed under fluorescence microscopy—Leica camera DM4000. Cells were identified from their antibody staining in different confocal laser channels: RAD51–fluoroscein isothiocyanate (495–519 nm) and WT1-Cy3 (535–610 nm). The number of positive cells was counted per 100 cells and the number of RAD51 foci assessed using a fluorescein isothiocyanate channel. Patients were categorized according to the number of RAD51 foci seen: 0, 0–3, >3–10, and >10. Data were correlated with clinical outcomes.

Clinical Analysis

Histological diagnosis, stage, grade, *BRCA* and homologous recombination deficiency status (where known), cancer antigen 125 (CA125), and types of treatments were collected for all patients. Times of blood sampling in relation to treatment were collected. 'Start' of treatment was defined as some point within the first two cycles (6–8 weeks) of treatment and the 'end' of treatment was defined as some point within the last two cycles (6–8 weeks) of treatment. Blood samples from patients who had more than just 'start' and 'end' time points were correlated with CA125 changes: patients with a falling CA125 during active treatment were responders and those with rising CA125 were progressors in accordance with Gynecological Cancer InterGroup CA125 criteria.[14] The numbers of RAD51/WT1 positive cells and RAD51 foci were correlated with clinical and CA125 outcomes in all possible patients. Patients were additionally classified as platinum-sensitive or resistant. At the time of

Table 1 Baseline patient characteristics

Characteristics	No. of patients (n=99)	%
FIGO stage		
I	2	2%
II	7	7%
III	65	66%
IV	25	25%
Grade		
1 (low-grade serous)	2	2%
3 (high-grade serous)	97	98%
Treatment setting		
First line*	60	61%
Relapse	39	39%
BRCA/HRD status		
*gBRCA*1 mutant	9	9%
*sBRCA*1 mutant	1	1%
gBRCA 2 mutant	3	3%
gBRCAwt/HRD positive	4	4%
gBRCAwt/HRD negative	8	8%
gBRCA wt/HRD unknown	74	75%
Number of blood samples		
Patient with 1 sample	46	46%
Patient with >1 sample	53	54%

*First-line includes patients receiving neoadjuvant or adjuvant chemotherapy.
FIGO, International Federation of Gynecology and Obstetrics; *gBRCA* 1/2 mutant, germline *BRCA* 1/2 mutant; HRD, homologous recombination deficiency; *sBRCA 1* mutant, somatic *BRCA 1* mutant.

recruitment, routine homologous recombination deficiency and somatic *BRCA* tissue testing was not being undertaken, so was only available for 12 patients. Progression-free survival was calculated in two ways. For the samples taken at the start of treatment, it was calculated in the standard way—that is, from the date of the first treatment (eg, primary surgery or first cycle of chemotherapy) to relapse. However, for the samples taken at the end of treatment, progression-free survival was calculated from the date of the last cycle of treatment to disease relapse.

Statistical Analysis

All statistical analyses were calculated using GraphPad software with statistical significance $p<0.05$, using Student's t-test. Kaplan-Meier and log-rank tests were used to calculate survival curves.

RESULTS

Baseline Characteristics

Baseline characteristics for the 99 patients analyzed are shown in Table 1. All bar two patients had high-grade serous ovarian carcinomas (98%), were at stage III/IV (91%), the average age was 66 years (range 32–84). Two patients had low-grade serous ovarian cancer (2%). Forty-six percent of patients had only one sample available for analysis. Seventeen percent of patients had a *BRCA* mutation and/or homologous recombination deficiency, 83% were germline BRCA wild-type (*gBRCAwt)* and/or able to undertake homologous recombination or had unknown homologous recombination status. Further information pertaining to treatments received by patients appears in Online Supplemental Table 1.

Changes in RAD51-Positive and WT1-Positive Circulating Cancer-associated Cells with Treatment

Our first objective was to identify RAD51 foci in circulating cancer-associated cells. Images of cells were identified using immunofluorescence, with different antibody staining used to identify them (ie, RAD51/WT1/DAPI positive).[13]

RAD51 foci were counted in sequential samples taken during a patient's treatment. Figure 1 demonstrates an example of a patient with multiple RAD51 foci. Sixty percent of the population was chemotherapy naïve, most receiving neoadjuvant chemotherapy prior to interval debulking surgery. A few had had primary surgery. There was no difference in the numbers of RAD51 foci seen at the start of chemotherapy between these two groups. Confirmation will be sought from a larger primary surgery cohort given the small numbers.

Changes in numbers of RAD51/WT1 positive cells and the quantity of RAD51 foci within these cells were compared with changes in CA125 at the start and end of treatment in 40 evaluable patients, five of whom were *BRCA* mutant or homologous recombination

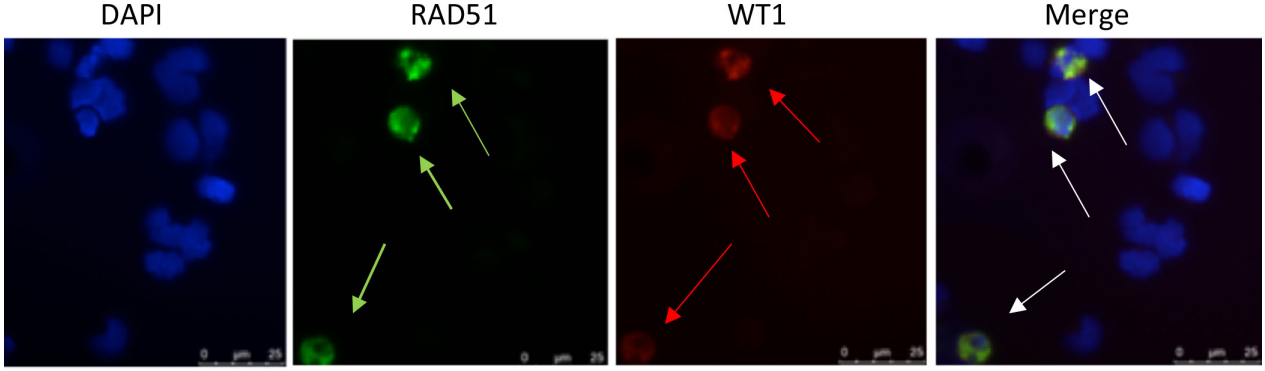

Figure 1 Leica images x100 magnification. DAPI (blue) nuclear stain, RAD51 (green) ~3 RAD51 foci depicted in cells with green arrows, WT1 (red). Merged image: RAD51 foci (green) in WT1-positive cells (red) with DAPI nuclear staining (blue). DAPI, 4,6-diamidino-2-phenylindole; WT1, Wilms tumor gene 1.

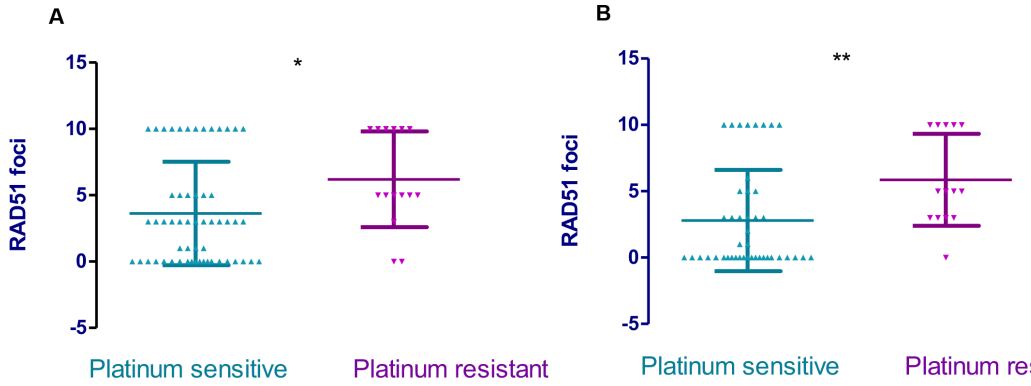

Figure 2 Mean number of RAD51 foci at start/end of treatment for platinum-sensitive (blue)/-resistant (purple) patients. (A) Mean number of RAD51 foci at treatment start in 71 patients: 3.6 foci for those >6 months from last platinum treatment (n=56); 6.2 foci for those <6 months from last platinum treatment (n=15) (*p=0.02, 95% CI −4.8 to −0.35). (B) Mean number of RAD51 foci at the end of treatment in 58 patients: 2.8 foci for those >6 months from last platinum treatment (n=44); 5.9 foci in those <6 months from last platinum treatment (n=14) (**p=0.01, 95% CI −5.4 to −0.8).

deficiency positive (Online Supplemental Figure 2). Responding patients, with decreasing CA125, had a statistically significant reduction in both mean numbers of RAD51/WT1 positive cells (p=0.006, 95% CI 1.21 to 6.79) and mean numbers of RAD51 foci (p=0.0028, 95% CI 1.1 to 4.7) (Online Supplemental Figure 2A). By contrast, patients with progressive disease, identified by a rising CA125, had no significant change in either mean numbers of positive RAD51/WT1 cells (p=0.9, 95% CI −4.3 to 4.8) or mean RAD51 foci (p=0.8, 95% CI −3.7 to 4.4) (Online Supplemental Figure 2B).

Platinum Sensitivity and Resistance

Mean numbers of RAD51 foci were counted at the beginning and end of treatment for patients classified as platinum-sensitive (n=56 (start) and n=44 (end)) and resistant (n=15 (start) and n=14 (end)) (Figure 2). Figure 2A demonstrates the difference in the mean number of RAD51 foci in the sensitive group (3.6) versus the resistant group (6.2), p=0.02, (95% CI −4.8 to −0.35) for patients with samples taken at the start of treatment. Figure 2B demonstrates the difference in mean number of RAD51 foci for the sensitive group (2.8) versus the resistant group (5.9) for patients with samples taken at the end of treatment, p=0.01, 95% CI −5.4 to −0.8). There were significantly fewer RAD51 foci in platinum-sensitive patients than in platinum-resistant patients.

BRCA Mutation/Homologous Recombination-deficient Patients

The numbers of RAD51 foci in 50 of 60 treatment-naïve patients were matched with *gBRCA* and homologous recombination status, where known. Figure 3 demonstrates the mean number of RAD51 foci according to *BRCAm*/homologous recombination deficient (mean=1.25) versus *BRCAwt*/homologous recombination unknown (mean=5.55, p=0.009). Only one patient in the eight *BRCA*m and *BRCAwt*/homologous recombination deficient group had any RAD51 foci; this patient was *BRCAwt* but homologous recombination deficient.

Progression-free Survival

Numbers of RAD51 foci were counted in 71 patients, where samples were taken at the start, and 58 patients where samples were taken at the end of treatment. Results were correlated with progression-free survival, which was calculated from the first treatment, for patients where numbers of RAD51 foci were counted at the start of treatment (Figure 4A). For 39 patients with <3 RAD51 foci, median progression-free survival has not yet been reached, but in 32 patients with >3 RAD51 foci median progession-free survival was 13 months (p=0.04, 95% CI 0.19 to 0.95). For the 58 patients evaluated from samples taken at the end of treatment, progression-free survival was calculated from the last treatment to subsequent relapse. Median progression-free survival in 32 patients with <3 RAD51 foci group was 12 months in contrast to 26 patients with >3 RAD51 foci where it was 3 months (p=0.001, 95% CI 3.5 to 4.5).

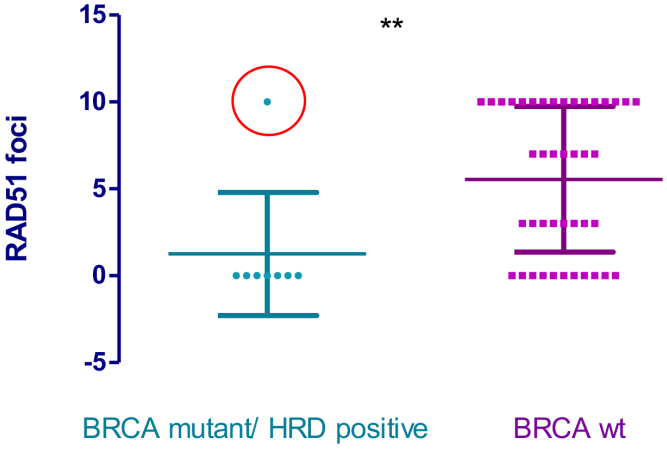

Figure 3 Number of RAD51 foci according to *gBRCA* status: On the left (green): five patients with *gBRCA1m* and three patients with *gBRCAwt* but HRD positive (Myriad MyChoice) mean number of RAD51 foci 1.25. The one patient who had any RAD51 foci in the *gBRCAm*/HRD-positive group was *gBRCAwt*/HRD positive (>10 foci)—circled in red. On the right (purple): 42 patients with *gBRCAwt*/unknown HRD; mean number of RAD51 foci 5.55 (p=0.009, 95% CI −7.5 to −1.1). *gBRCA*, germline *BRCA*; *gBRCA1m*, germline *BRCA1* mutant; *gBRCAwt*, germline *BRCA* wild-type; HRD, homologous recombination deficiency.

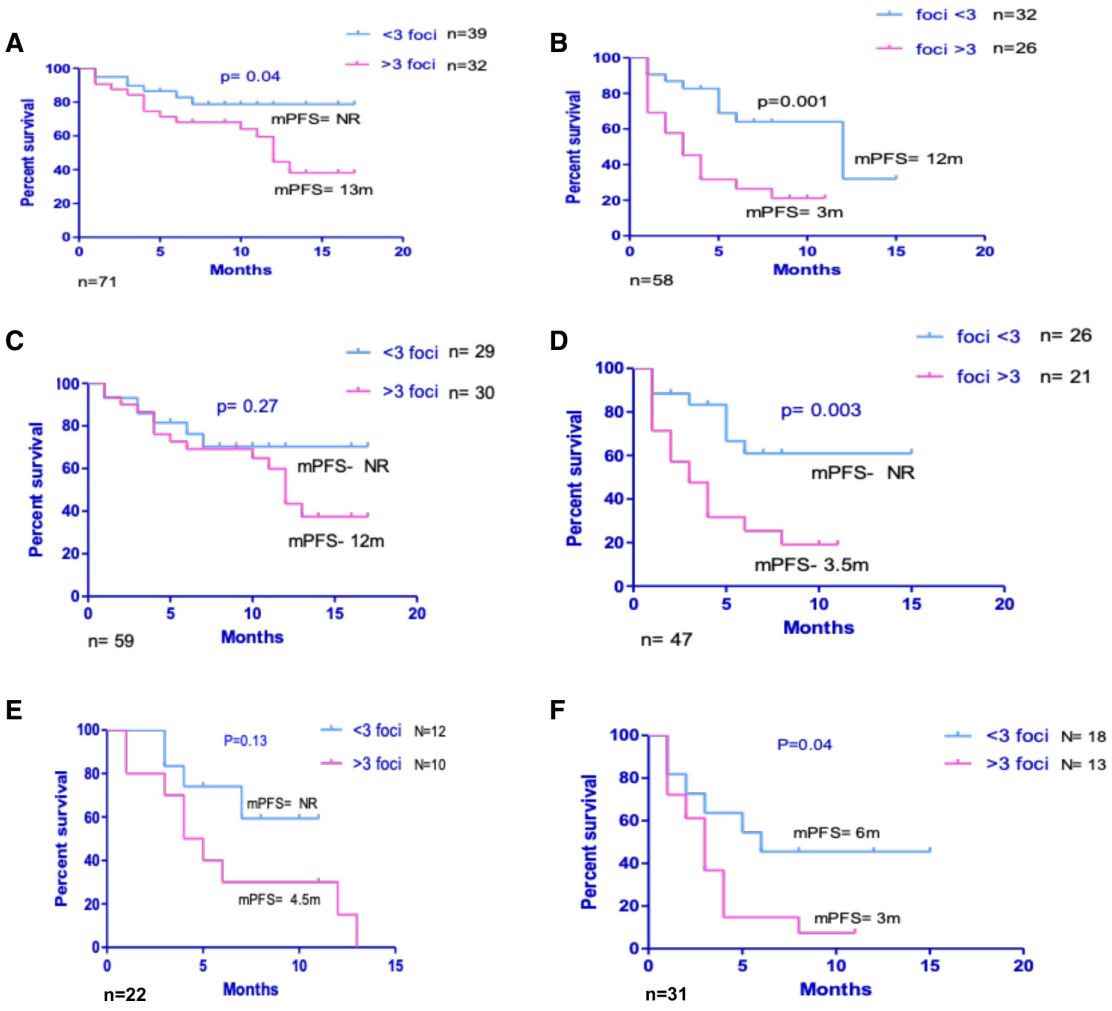

Figure 4 Progression-free survival correlated with numbers of RAD51 foci observed per 100 cc at the start/end of treatment. (A) Whole group progression-free survival. Start: survival in relation to numbers of RAD51 foci in cc from 71 patients taken at the start of treatment. <3 RAD51 foci were seen in 39 patients—median progression-free survival not reached (blue); >3 RAD51 foci were seen in 32 patients—median progression-free survival 13 months (pink), (*p=0.04, 95% CI 0.19 to 0.95). (B) Whole group progression-free survival. End: survival in relation to numbers of RAD51 foci in cc from 58 patients taken at the end of treatment. >3 RAD51 foci in 32 patients—median progression-free survival 12 months; <3 RAD51 foci in 26 patients—median progression-free survival 3 months (**p=0.001, 95% CI 3.5 to 4.5). (C) Excluding *BRCAm* and known homologous recombination deficiency-positive patients progression-free survival. Start: <3 RAD51 foci seen in 29 patients—mPFS not reached (blue); >3 RAD51 foci seen in 30 patients—median progression-free survival 12 months (p=0.27). (D) Excluding *BRCAm* and known homologous recombination deficiency-positive patients progression-free survival. End: <3 RAD51 foci seen in 26 patients—median progression-free survival not reached (blue); >3 RAD51 foci seen in 21 patients—median progression-free survival 3.5 months. (E) Patients with relapse high-grade serous ovarian cancer, progression-free survival Start: <3 RAD51 foci seen in 12 patients, median progression-free survival not reached (blue); >3 RAD51 foci in 10 patients—median progression-free survival 4.5 months (pink), (p=0.13, 95% CI 0.1 to 1.3). (F) Patients with relapse high-grade serous ovarian cancer, progression-free survival. End: <3 RAD51 foci seen in 18 patients—median progression-free survival 6 months (blue); >3 RAD51 foci in 13 patients—median progression-free survival 3 months (pink), (p=0.04, 95% CI 1.6 to 2.4). *BRCAm*, mutant *BRCA*; mPFS, —median progression-free survival.

Figure 4C,D are survival data for the group without *gBRCAm*/homologous recombination-deficient patients. Twenty-nine and 26 patients with <3 foci at the start and end of treatment, respectively, had not reached median progression-free survival at median follow-up of 18 months. By contrast, 30 and 21 patients with >3 RAD51 foci at the start and end of treatment, respectively, had median progression-free survival of 12 months and 3.5 months.

Patients with relapsed ovarian cancer have shorter progression-free intervals. Figure 4E,F show significantly different median survival data for 31 relapse patients. Although not significant, there was a progression free survival difference for the 22 patients where samples were taken at the start of treatment (Figure 4E). From samples taken at the end of treatment, 18 patients with <3 RAD51 foci had a median progression-free survival of 12 months compared

with 13 patients with >3 RAD51 foci, where it was 3 months (p=0.04) (Figure 4F).

DISCUSSION

Summary of Main Results

This is the first demonstration of a functional RAD51 assay in circulating cancer-associated cells. RAD51 foci are present in these cells of patients with high-grade serous ovarian cancer, although five with *BRCA1* mutations had no foci. Reduced numbers of circulating cells and RAD51 foci correlated with clinical response. Numbers of RAD51 foci were significantly higher in a platinum-resistant population, and patients with >3 RAD51 foci had worse median progression-free survival.

Results in the Context of Published Literature

Enumeration of circulating cancer cells has generally involved an enrichment process, based on various biological (epithelial markers) or physical properties (eg, size, deformability, invasive capacity). The US Food and Drug Administration (FDA)-approved cell search system which isolates small[1–5] numbers of circulating cells bearing an epithelial cell adhesion molecule; changes in numbers of cells bearing an epithelial cell adhesion molecule are associated with outcomes in breast, colorectal, and prostate cancers. Despite many efforts, there is no such validated assay for ovarian cancer[15 16] where, following various enrichment strategies, 1–5 cells are found in only 14–70%. Adding immunohistochemistry,±gene expression analysis using RT-PCR improves identification to 50–90%,[15] but numbers are too small (1–5 circulating tumor cells/5–7.5 mL whole blood) for clinical use.[17]

We identified large numbers of cells without any enrichment except red cell lysis. Positive staining with appropriate antibodies (pan-cytokeratin, WT1) reveals abundant circulating cancer-associated cells per milliliter whole blood from patients with stage III/IV or relapsed high-grade serous ovarian cancer.[13] WT1, used to confirm microscopy appearances of serous ovarian cancer in tissue samples, has also been employed in many 'liquid biopsy' studies: Zhang et al reported finding circulating cells in 93% of 36 patients with early stage I/II ovarian cancer, despite ~40% having normal CA125 levels.[17]

Expression of RAD51 correlates with efficient homologous recombination DNA repair, a process aberrant in ~50% patients with high-grade serous ovarian cancer. Patients with low levels of RAD51 expression, as demonstrated by quantitative immunohistochemistry in tissue, are unable to repair DNA this way and have improved survival.[11 18] High levels of RAD51 denote cells with intact homologous recombination DNA repair pathways and are linked to poorer treatment response and reduced progression-free survival.[9] Thus the presence/absence and numbers of RAD51 foci are considered a functional assay for homologous recombination DNA repair.[19 20] However, RAD51 functional assays have only been undertaken on fresh tissue or ascitic fluid cells, which have then been treated *ex vivo* with irradiation or PARPi.[21 22] Here, we demonstrate a functional assay *in vivo*—that is, in blood from patients with high-grade serous ovarian cancer undergoing treatment.

Patients whose disease progresses despite treatment maintain numbers of RAD51 foci and WT1+ cells (Online supplemental figure 2), suggesting efficient repair of DNA damage via the homologous recombination pathway and consequent treatment resistance.[11] However, we cannot conclude that patients who have <3 RAD51 foci at any time point represent a better prognostic group. Hoppe et al, described and validated a nuclear expression score using tissue from patients with ovarian cancer, recruited onto a clinical trial assessing two doses of carboplatin. In line with our results where platinum-resistant patients have higher numbers of RAD51 foci in their circulating cells compared with platinum-sensitive patients, high RAD51 nuclear expression scores predicted early relapse confirming RAD51 as a marker of platinum resistance.[12]

Strengths and Weaknesses

An obvious limitation of our work is the possibility that RAD51 foci are simply a reflection of increased proliferation through common regulatory pathways in patients with a poorer prognosis.[23] We plan to incorporate geminin or Ki67 in an appropriate number of prospectively recruited patients to exclude this possibility and to begin validation. The test failure rate needs to be determined. Concomitant homologous recombination deficiency (Myriad, MyChoice) tests will be sought in patients to correlate with numbers of cells/RAD51 foci and outcomes. Tighter sampling requirements in individual patients, over multiple treatment lines, are planned to determine if increasing numbers of RAD51 foci denote developing treatment resistance, as is reported *in vitro* with established cell lines.[24 25] Finally we need to incorporate recognized factors of prognosis, such as residual disease after surgery, with the RAD51 foci findings.

Implications for Practice and Future Research

The identification of patients with high-grade serous ovarian cancer with homologous recombination deficiency is currently undertaken by scoring genetic instability seen in tumor DNA. The Myriad MyChoice test is the current 'gold standard' but was only available for UK patients from mid-2020, hence in this patient population, only 12 were tested. Conflicting results from large phase III studies of maintenance PARPi for patients with ovarian cancer demonstrate inadequacies in this scoring system. Using numbers of RAD51 foci in circulating cells may provide a more accurate, real-time assessment of homologous recombination efficiency.

RAD51 foci were also found in circulating cells of two patients with low-grade serous ovarian cancer; patients with other malignancies, especially where repair of double-strand DNA breaks is thought important (eg, breast, prostate, pancreas, colorectal, and lymphoid cancers), may benefit from functional RAD51 assays.[26]

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con MH, EK: conceived the work; MH, TMD, SK, ZA: identified and recruited patients, and collected clinical data; ZA, SS processed samples; ZA, SS, EK: analysed data. All authors contributed to the writing of the manuscript. MH accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by West Midlands–South Birmingham ethics committee (reference 16/WM/0196). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Datasets will be made available on reasonable request from the corresponding author.

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