Detection of DNA damage in circulating tumour cells harvested from blood samples of ovarian and prostate cancer patients

Amina Mezni [1], Jessica Kimber [1], Rachel Downes [1], Laila Vlietinck [1], Mariacristina Ciccioli [1], Anne-Sophie Pailhes-Jimenez [1]

[1] ANGLE Europe Limited, 10 Nugent Road, Surrey Research Park, Guildford, Surrey GU2 7AF United Kingdom

ANGLE

EACR 2024 10-13 June 2024 Rotterdam Netherlands

EACR2024-0926

Introduction

Gamma H2AX (γ-H2AX) and Phospho KAP1 (pKAP1) are predictive biomarkers that can be used to identify the induction of the DNA damage response (DDR) pathway (Figure 1). Monitoring the activation of the DDR pathway can be valuable when evaluating effectiveness of DNA damage-inducing therapies, with the standard method of assessment involving testing DDR marker expression on tumour biopsies. However, obtaining tissue biopsies is invasive, challenging and often non-repeatable. Circulating tumour cells (CTCs), enriched from a liquid biopsy, offer an alternative method allowing for minimally invasive, repeatable and real-time assessments of treatment response. ANGLE has developed a Research Use Only (RUO) workflow for the identification of DNA Damage on CTCs. In this study we aimed to assess the performance of ANGLE's immunofluorescence (IF) assay for the identification of epithelial, mesenchymal and transitioning CTCs and for the determination of the DNA Damage status (targeting either pKAP1 or γ-H2AX) on the identified CTCs, by combining it with the Parsortix® technology, an epitope-independent microfluidic device that enriches and harvests CTCs from blood based on their size and deformability.

For Research Use Only. Not For Use In Diagnostic Procedures.

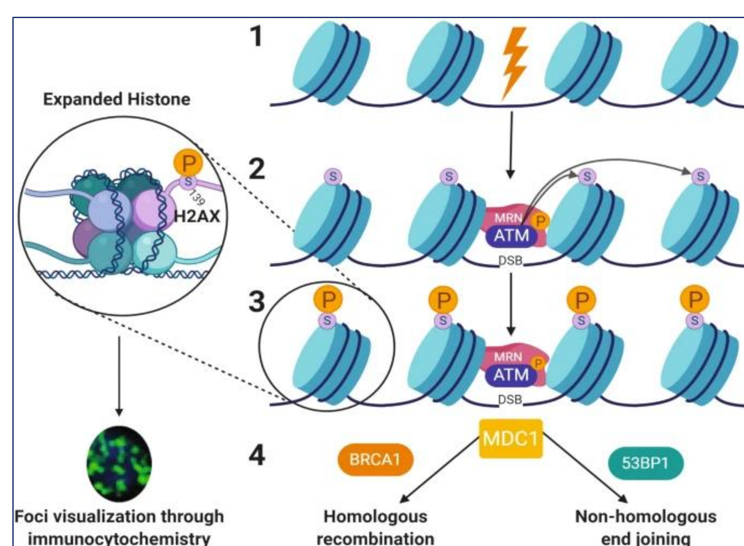


Figure 1. DNA damage and repair pathway. Figure adapted from Noubissi. FK et al (2021). Detection and quantification of γ-H2AX using a dissociation enhanced lanthanide fluorescence immunoassay. Scientific Reports

Workflow

- For analytical verification, blood samples from healthy volunteers were collected into Streck Cell-Free DNA Blood Collection tubes (BCTs) and spiked with DNA Damage-induced H226 or MCF7 cancer cell lines. Samples were processed between 96 and 144 hours post draw, as per workflow in **Figure 2,** and used to assess analytical sensitivity, specificity and linearity of both assays.
- Patient samples from 14 Ovarian and 15 Prostate cancer patients, on a variety of treatment programmes, including platinum treatment, were collected into Streck Cell-Free DNA BCTs and processed as shown in **Figure 2**. Blood was collected for up to six draws per patient, with two tubes collected per draw, each processed between 72-144 hours post draw and stained using the ANGLE's IF-based CTC identification assay combined with DDR marker detection.

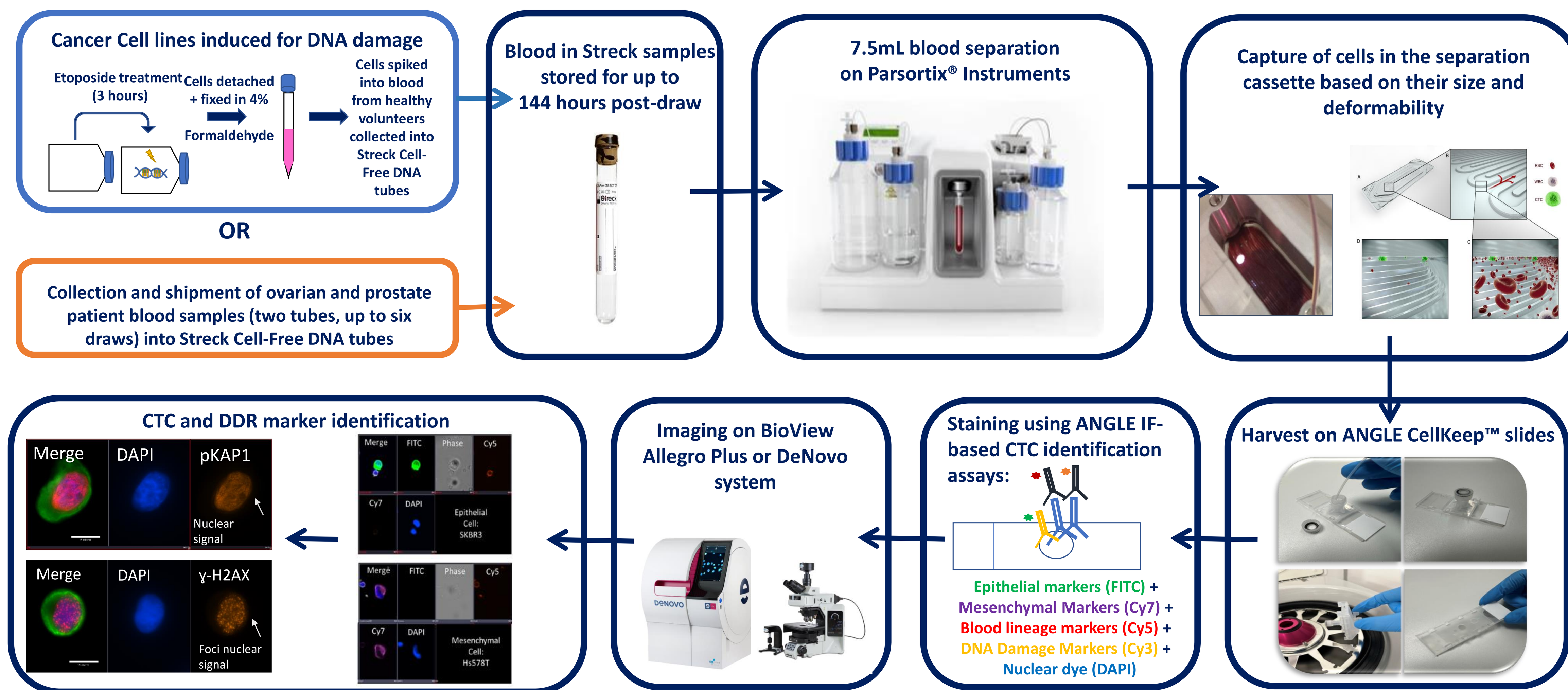
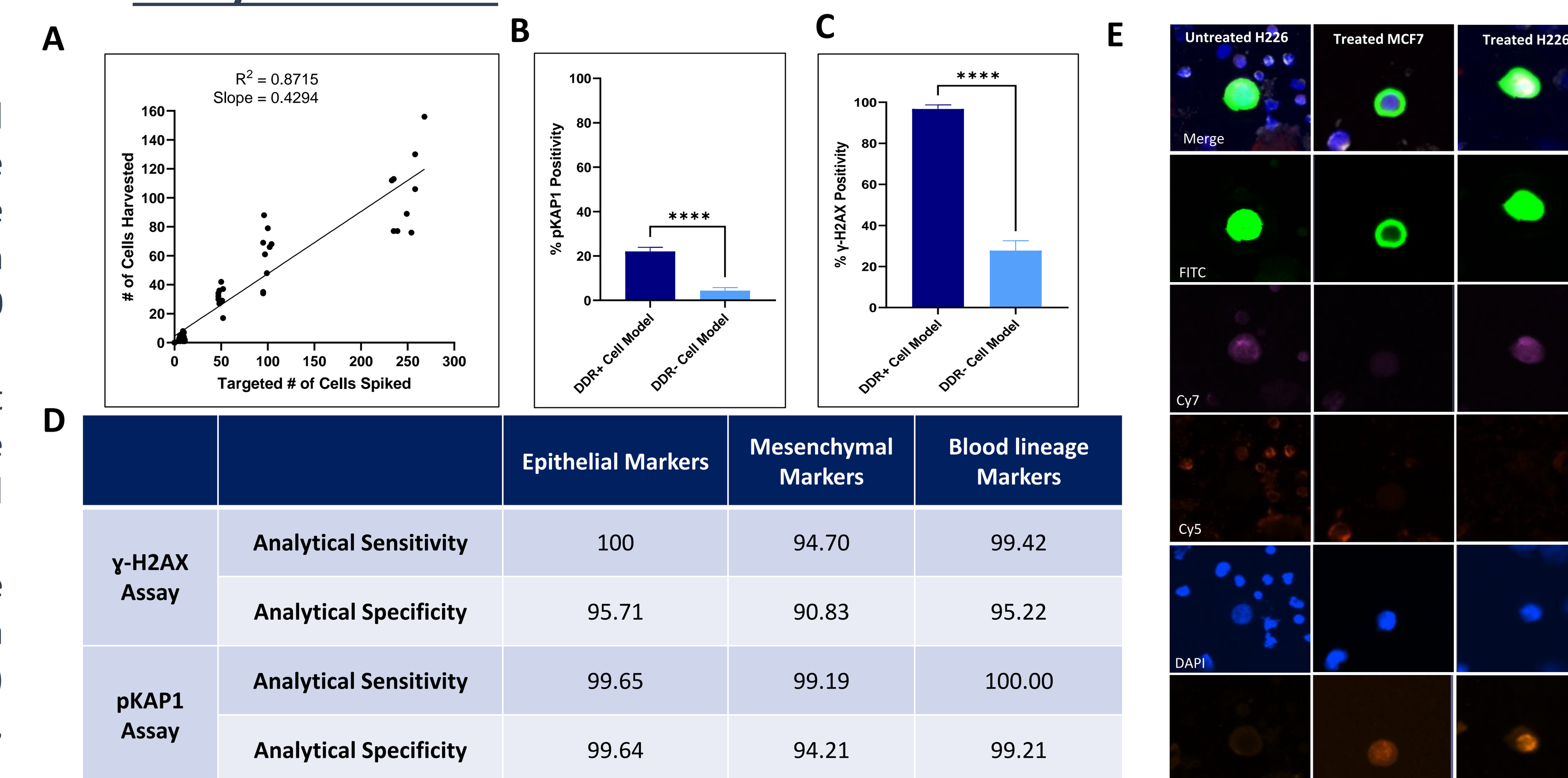


Figure 2. Schematic representation of the assay workflow. In analytical samples (light blue square), cultured cancer cell lines expressing Epithelial and/or Mesenchymal markers were induced for DNA damage, fixed with 4% Formaldehyde and spiked into healthy volunteer blood samples collected in Streck Cell-Free DNA tubes. For patient-derived samples (orange square), two tubes of 7.5 mL of peripheral blood were collected into Streck Cell-Free DNA tubes from 14 ovarian and 15 prostate cancer patients for up to six draws per donor. Blood was stored up to 144 hours from collection before processing. Blood samples were processed on a Parsortix® instrument, a microfluidic device capable of capturing and harvesting CTCs from bodily fluids based on cell size and lack of deformability. Harvested CTCs were cytospun onto ANGLE's CellKeep™ slides. Slides were stained using ANGLE's IF-based CTC identification assay combined with DDR markers (γ-H2AX and pKAP1) and CTCs were defined as epithelial (FITC+, Cy7-, Cy5-, DAPI+), mesenchymal (FITC-, Cy7+, Cy5-, DAPI+), or EMT transitioning (FITC+, Cy7+, Cy5-, DAPI+). CTCs were then investigated for the presence of DNA damage signal. γ-H2AX positivity was identified mostly by the presence of distinct nuclear foci and, occasionally, diffuse nuclear signal, while pKAP1 positivity was identified by the presence of a distinct diffuse nuclear signal. Stained slides were imaged using a BioView Allegro Plus system or the BioView DeNovo system, a platform equipped with artificial intelligence for automated imaging, CTC candidate identification and reporting.

Analytical Results

- **Linearity** was established by plotting the number of stained and harvested Etoposide-treated H226 cells against the number of spiked cells. A linear relationship between the number of harvested and stained cells was confirmed, with R^2= 0.87, slope = 0.43 (**Figure 3A**), over the range of 0-250 cells.
- For both γ-H2AX and pKAP1 markers, a statistically significant increase (*p <0.0001*) in DNA damage was observed in the positive/treated cell model vs the negative/untreated cell model (**Figure 3B,C**).
- **Analytical Sensitivity** and **Specificity**, referring to the percentage of harvested cells known to express/not express a marker that had a mean fluorescence intensity (MFI) above/below the established thresholds for that marker, respectively, were all ≥ 90% in both DDR assays **(Figure 3D, E).**



		Epithelial Markers	Mesenchymal Markers	Blood lineage Markers
γ-H2AX Assay	Analytical Sensitivity	100	94.70	99.42
	Analytical Specificity	95.71	90.83	95.22
pKAP1 Assay	Analytical Sensitivity	99.65	99.19	100.00
	Analytical Specificity	99.64	94.21	99.21

Figure 3. Analytical performance of the IF CTC identification assay combined γ-H2AX and pKAP1. (A) Dot plots shows the number of cancer cells spiked (x-axis) vs the number of cancer cells harvested (y-axis) across spiking levels for Etoposide treated fixed H226 cells harvested onto CellKeep Slides. The trendline equation and R^2 value are included for the first order polynomials. Histograms show the mean and SEM of the mean percentage of **(B)** pKAP1 positive cancer cells and **(C)** γ-H2AX positive cancer cells in respective positive and negative cell models (Unpaired t test, *p**** <0.0001). **(D)** Summary table shows the analytical sensitivity (proportion of cells known to express the marker(s) of interest which were positive) and specificity (proportion of cells known to not express the markers of interest which were marker negative in the assay) of both DDR assays. **(E)** Representative images of untreated H226 cell (**left**) and treated (**right**) H226 cell positive for pKAP1 (FITC+, Cy7+, Cy5-, DAPI+, Cy3+/-) cells and treated (**middle**) MCF7 cell positive for γ-H2AX (FITC+, Cy7-, Cy5-, DAPI+, Cy3+) cells and PBMCs. Epithelial markers (FITC) in green, Mesenchymal markers (Cy7) in magenta, Blood lineage markers (Cy5) in red (white in merge), Nuclear dye (DAPI) in blue and DDR markers (Cy3) in orange.

Patient Results

A	Ovarian	Prostate
N (Donors)	14	15
N (Samples)	102	116
N Donors with ≥1 CTC	13 (93%)	14 (93%)
N Samples with ≥1 CTC	67 (66%)	52 (45%)
CTC range	1-254	1-227
CTC mean	17	24
CTC median	3	6
Donors with ≥1 Epithelial only CTCs	0 (0%)	0 (0%)
Donors with ≥1 Epithelial and Mesenchymal CTCs	4 (31%)	4 (28.5%)
Donors with ≥1 EMT CTCs	2 (15%)	4 (28.5%)
Donors with ≥1 Mesenchymal only CTCs	7 (54%)	6 (43%)
Donors with ≥ 1 CTC with DNA damage (N and %)	8 (62%)	10 (77%)
Samples with ≥ 1 CTC with DNA damage (N and %)	21 (31%)	17 (33%)
Samples % DNA damage concordance in matched samples	80% (12/15)	77% (10/13)

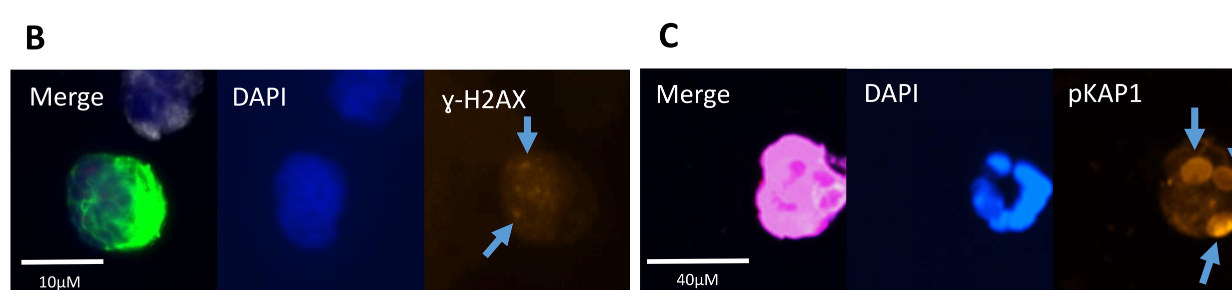
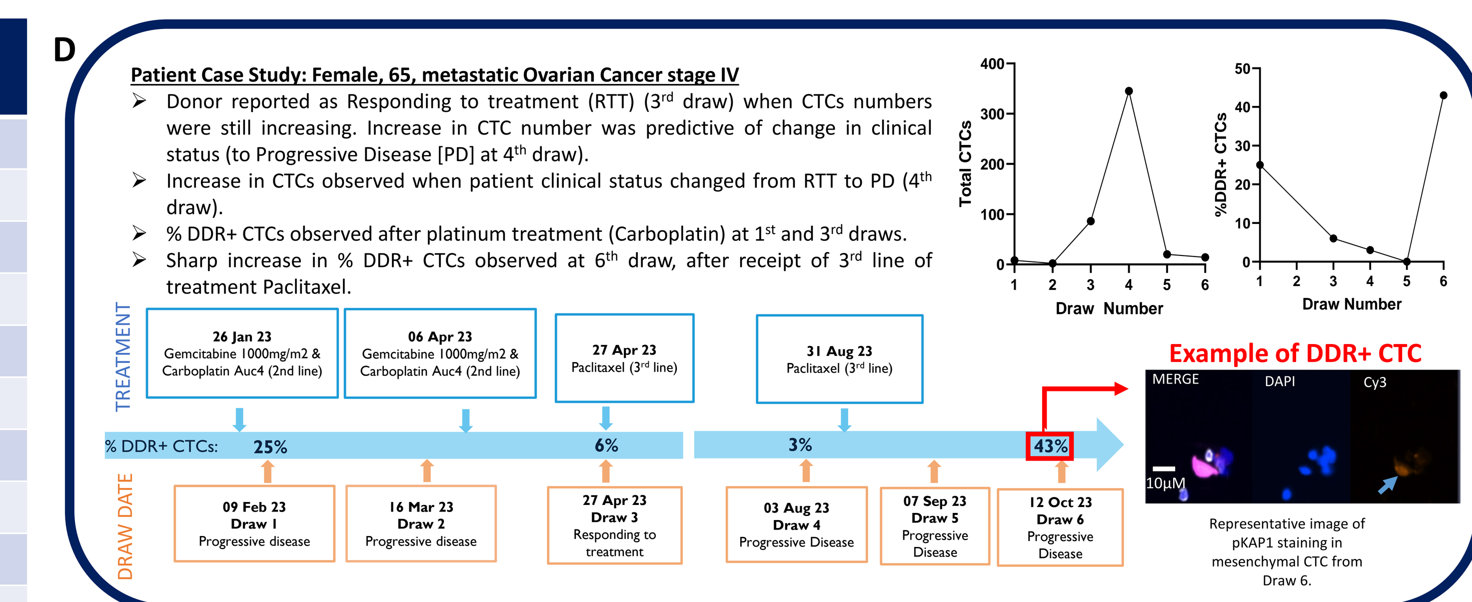


Figure 4. CTC identification and phenotyping in metastatic ovarian and prostate cancer patient samples. (A) Table showing number (N) of donors and samples, percentage of CTC+ donors (≥1 CTC), mean, median, range of total CTCs and different CTC phenotypes by donor, percentage of donors with ≥1 DDR+ CTC over the CTC+ donors and draws, percentage concordance between γ-H2AX and pKAP1 assays. Representative images from patient samples of **(B)** γ-H2AX foci signal in an epithelial CTC (image taken using 60x objective lens) and **(C)** pKAP1 pan-nuclear signal in a mesenchymal cluster of CTCs (image taken using 10x objective lens). DDR signal indicated by blue arrows. Epithelial markers (FITC) in green, Mesenchymal markers (Cy7) in magenta, Blood lineage markers (Cy5) in white, Nuclear dye (DAPI) in blue and DDR markers (Cy3) in orange. **(D)** Case study of one ovarian cancer patient with changes in CTC numbers and DDR+ expression in CTCs identified across draws.



- 93% of both metastatic Ovarian and Prostate cancer donors showed at least one CTC, in at least one draw, with a CTC range of 1-254 and 1-227 CTCs, respectively, in positive samples (**Figure 4A**).
- 54% of Ovarian and 43% Prostate cancer donors had only mesenchymal CTCs, 31% and 28.5% showed both mesenchymal and epithelial CTCs and 15% and 28.5% displayed transitioning (EMT) CTCs respectively (**Figure 4A**).
- 62% and 77% of Ovarian and Prostate cancer donors had at least one DDR+ CTC, with a comparable rate of 31% and 33% observed across all samples respectively (**Figure 4A**). Example images of localised nuclear γ-H2AX and pKAP1 signal is shown in **Figures B** and **C,** respectively.
- Concordance between γ-H2AX and pKAP1 was high and was observed in 77-80% of matched samples (**Figure 4A**).
- A case study from the Ovarian cancer cohort (**Figure 4D**) demonstrates the potential clinical utility of CTCs and DDR+ CTCs. CTC numbers in the patient increased at the 3rd draw, despite a reported clinical status of responding to treatment (RTT). Increase in CTC numbers may be predictive of a clinical status change to progressive disease (PD) at subsequent draws. DDR+ CTCs were observed after receiving platinum treatment, with a sharp increase recorded in 6th draw a few weeks after 3rd line of treatment Paclitaxel.

Conclusions

- Analytical verification demonstrated that both DDR assays produce linear data, with high analytical sensitivity and analytical specificity for epithelial, mesenchymal, blood lineage and DDR markers.
- ANGLE's DDR assay workflow identified CTCs in ovarian and prostate cancer patient cohorts, with ≥1 CTC observed in 93% of donors. γ-H2AX and pKAP1 markers were also successfully detected in CTCs in both cancer cohorts, on a variety of treatment programmes (donors with ≥1 DDR+ CTC were 62% and 77%, respectively).
- This study demonstrated the possibility of using the DDR workflow to monitor the number of CTCs and DDR+ CTCs over time. Applied to a clinical setting, this workflow can potentially allow for minimally invasive monitoring of DNA damage targeting therapies.