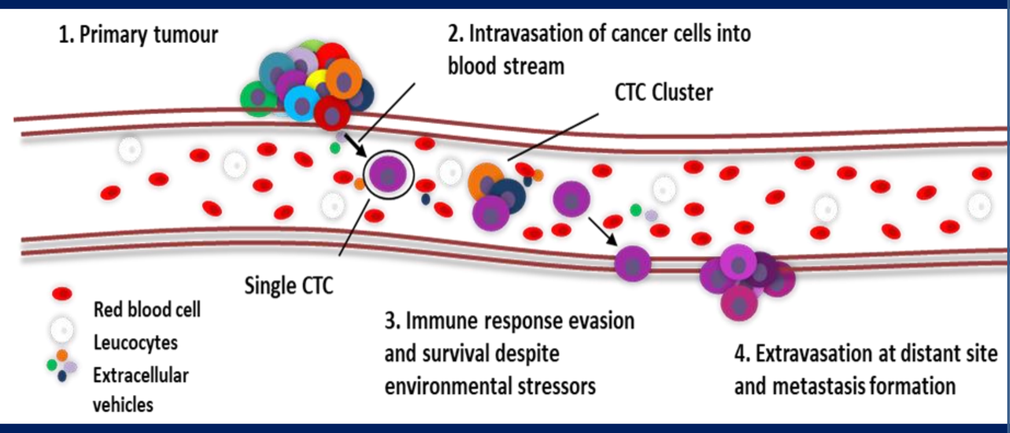
Circulating Tumour Cell Analysis to Evaluate Docetaxel Treatment Response and Resistance Markers in Prostate Cancer

Caitlin Davies[1], Elzbieta Stankiewicz[1], Lei Xu[1], Xeuying Mao[1], Edwina Burke[1], Tianyu Guo[1], Prabhakar Rajan[1,2], Greg Shaw[2], Glenda Scandura[1], Daniel Berney[1], Johnathan Shamash[1,2], Yong-Jie Lu[1]

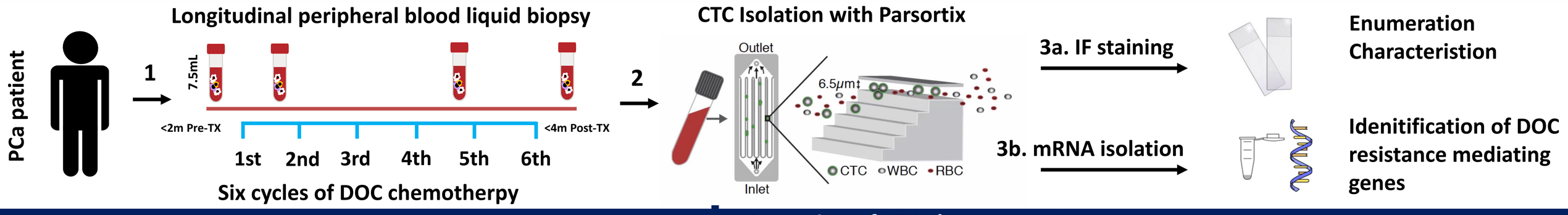
1. Barts Cancer Institute, Queen Mary University of London, 2. St Bartholomew's Hospital, Barts Health NHS Trust

Introduction

- Docetaxel (DOC) treatment has been shown to significantly improve overall survival (OS) in metastatic castration-resistant prostate cancer (mCRPC), and recently is used as chemo-hormonal therapy in metastatic hormone-sensitive prostate cancer (mHSPC) (Here we focus on data from mHSPC patients only).
- However, a proportion of patients treated with DOC have inherent/acquired resistance.
- This project investigates circulating tumour cells (CTCs) in blood liquid biopsies as a novel tool for predicting and/or monitoring DOC response.
- CTCs were captured using the Partsortix, an epitope independent, size based CTC isolation system and downstream CTC enumeration, characterisation and mRNA analysis was performed

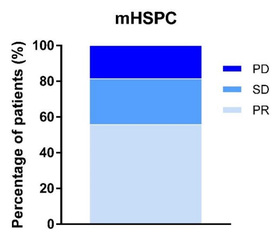


Methods

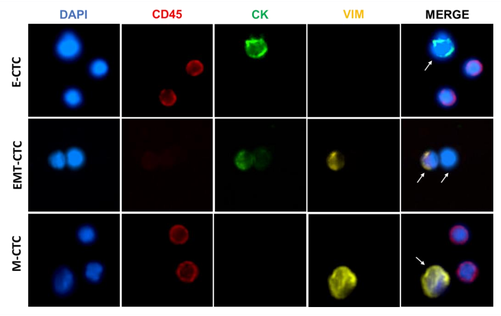


1. mHSPC patient sample collection

mHSPC	Patient #	Sample #
Partial response (PR)	24	81
Stable disease (SD)	11	41
Progressive disease (PD)	8	23



2. Detection of CTCs by immunoflouresence



Three distinct subsets of CTCs were routinley identified in 7.5mL mHSPC PCa patient pheripheral blood samples: CK+/VIM-/CD45- (Epithelial), CK+/VIM+/CD45- (EMTing) and CK-/VIM+/CD45- (Mesenchymal). Patient samples in which ≥1 CK+/VIM-/CD45- cell and/or ≥1 CK+/VIM+/CD45- cell and/or ≥4 CK-/VIM+/CD45- cells were detected were given a score as CTC 'positive', all other samples were scored CTC 'negative'.

3. Pre-TX CTC enumeration as predictive biomarkers of treatment response in mHSPC patients

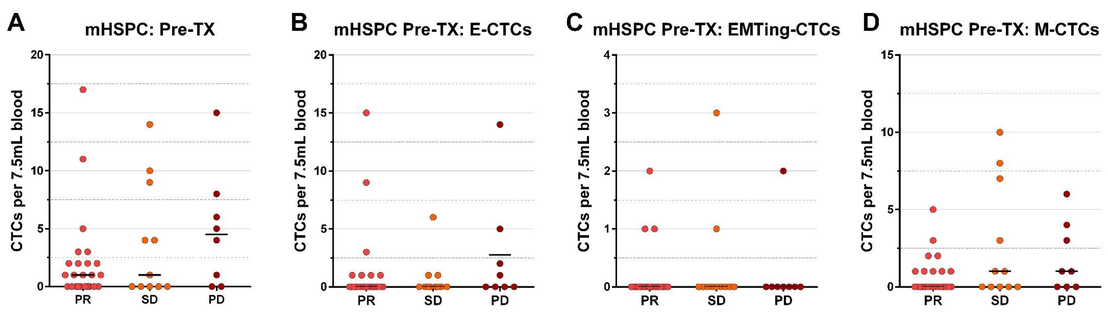


Figure 1: The detection of CTCs pre-TX. A-B) Median total CTC number and E-CTC number was highest in patients with PD. **C)** Very few EMTing-CTCs were detected pre-TX. **D)** Median M-CTC number was lowest in patients with PR

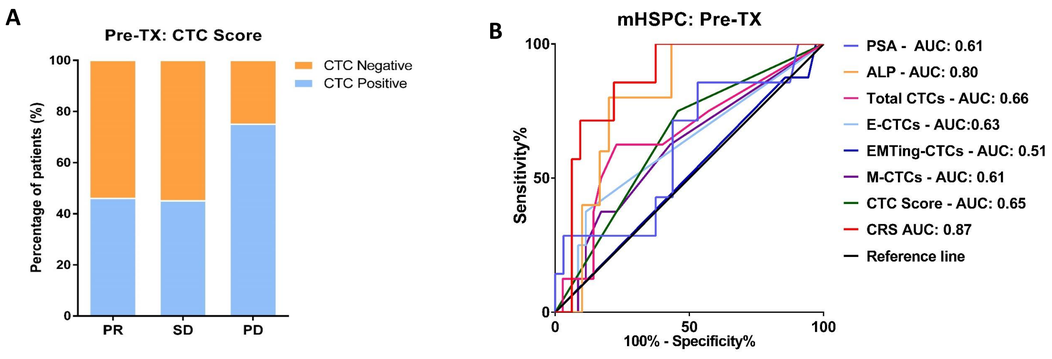


Figure 2: CTC Score is indicative of PD. A) Pre-TX 75% of patients with PD had a CTC Positive Score (≥1 E-CTC and/or ≥1 EMTing-CTC and/or ≥4 M-CTCs). **B)** Individual CTC parameters alone were not significantly predictive of inital clinical response to DOC, however a combined risk-score (CRS) of PSA+ALP+CTC Score resulted in an AUC of 0.87, p= 0.0027.

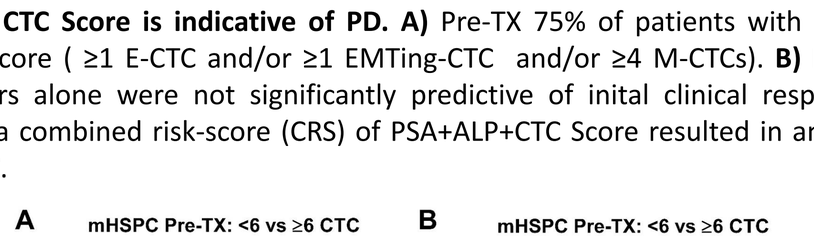
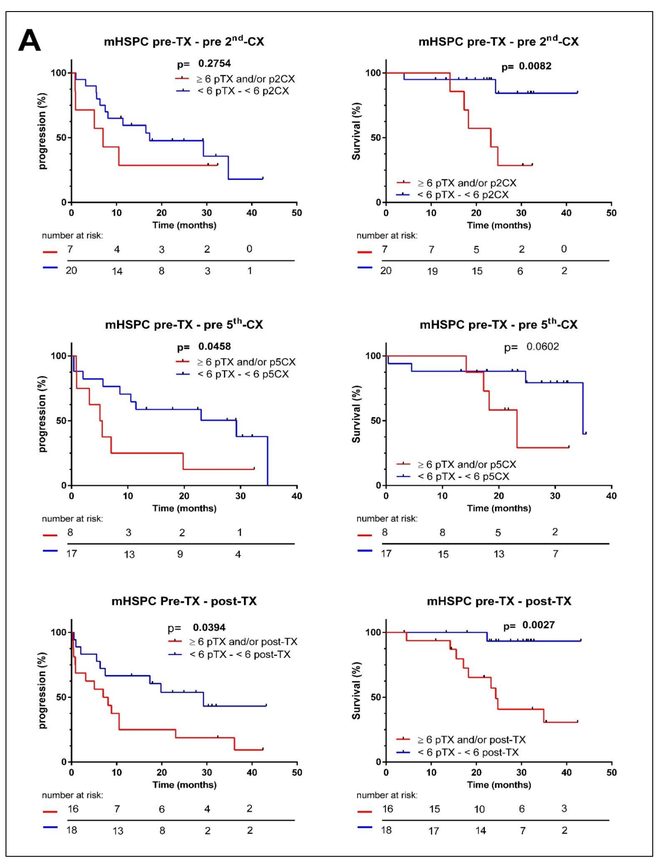
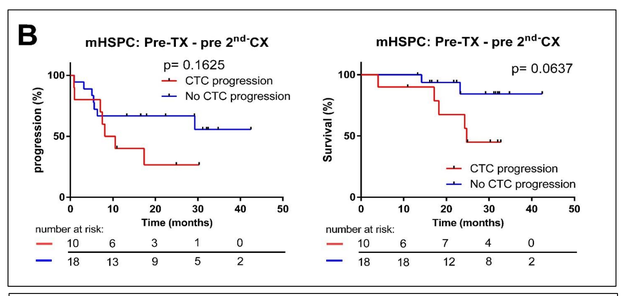


Figure 3: mHSPC patients with ≥ 6 CTCs pre-TX had shorter time to progression (3 vs 17 months, HR: 2.1, p= 0.1944) and a significantly shorter median OS (18.3 vs undefined months, HR: 7.451, p= 0.0124 and compared to patients with <6 CTCs per 7.5mL of blood.

4. Longitudinal CTC sampling to predict/monitor treatment response





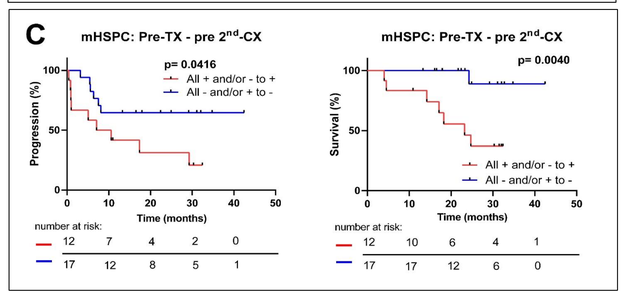


Figure 4. CTC dynamics during DOC treatment. A) The presence of ≥6 CTCs at any time point and an increase from <6 CTCs pre-TX to ≥6 CTCs at any time point was associated with poor PFS and OS. **B-C)** CTC progression and a switch from CTC negative to CTC positive between pre-TX and pre 2nd-CX was significantly associated with shorter time to progression and death)

5. M-CTC number and M-CTC progression can identify a subgroup of patients who have shorter PFS and/or OS even after a PR to DOC

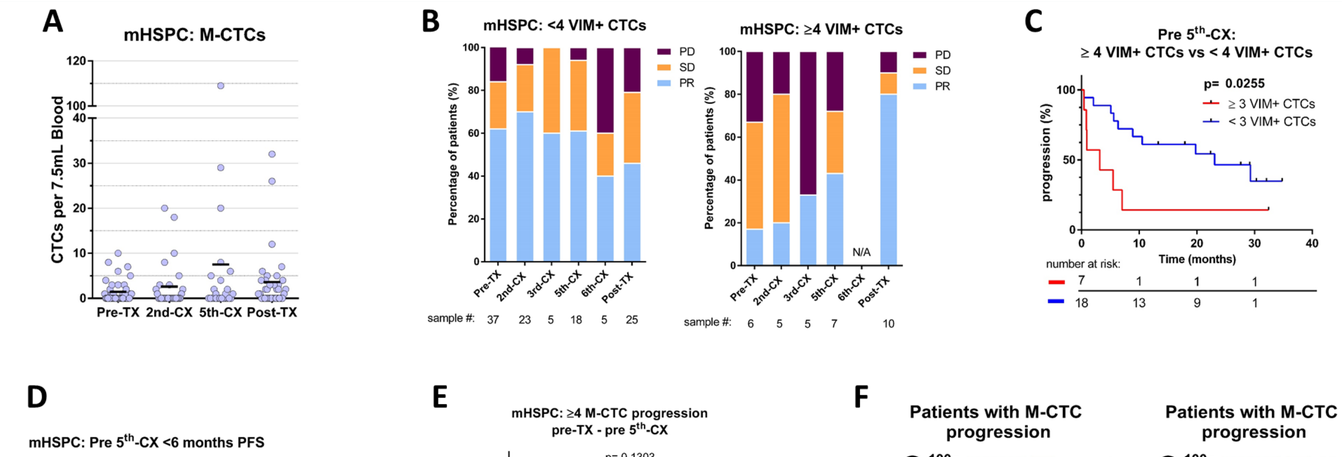
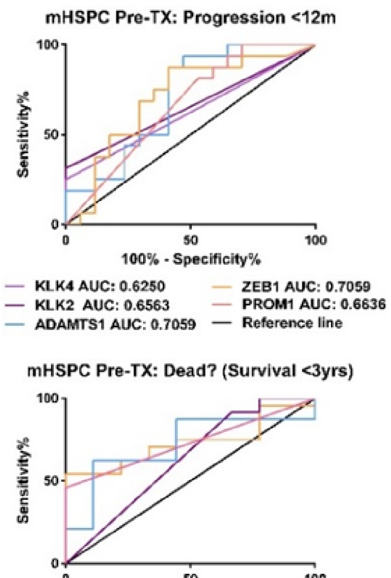


Figure 5. A) Highest mean numbers of M-CTCs were detected pre 5th-CX and post-TX **B)** We found a paradoxical relationship between increasing M-CTCs and inital PR to DOC - 80% patients who had ≥4 M-CTCs post-TX had PR. **C-D)** Survival and ROC analysis revealed high numbers of M-CTCs pre 5th CX were related to poor PFS. **E)** Patients with M-CTC progression from pre-TX to pre 5th-CX had shorter time to progression (3 months vs 23 months **F)** The majority of patient who had M-CTC progression experienced progression or death <6 months post-TX and 60% these patients were those with inital PR.

6. CTC mRNA detection to predict and montior DOC response in PCa

- Using the Fluidgm BioMark HD, patient CTC mRNA was subject to mutliplex RT-qPCR analysis of a custom 30 predictive/prognostic gene panel.
- Data used: microarray analysis of DOC-R PCa cell lines and published literature
- Final candidate 30 gene panel consisted of genes that were: PCa specific and/or commonly upreguated in DOC resistance and had low/no expression in leukocytes



	<12m		Dead?
KLK2	0.43	ADAMTS1	0.36
KLK4	0.38	SNAI1	0.34
ADAMTS1	0.36	KLK2	0.32
ZEB1	0.36	AR	0.29
FOLH1	0.28	KLK3	0.28
MCAM	0.26	MCAM	0.22
SNAI1	0.26	KLK4	0.21
SERPINE2	0.25	SDC2	0.21
IGFBP3	0.23	POU5F1	0.19
POU5F1	0.22	IGFBP3	0.16
SOX2	0.16	FOLH1	0.14
PODXL	0.14	ZEB1	0.13
KLK3	0.14	SERPINE2	0.13
SDC2	0.12	ACKR3	0.07
HSPB8	0.11	HSPB8	0.06
AR	0.10	NANOG	0.00
PLSCR4	0.06	SYDE1	-0.03
PRKD1	0.06	PRKD1	-0.03
SYDE1	0.02	SOX2	-0.11
ACKR3	-0.01	TWIST2	-0.14
FSTL1	-0.03	PODXL	-0.16
COL5A1	-0.04	FBLN1	-0.18
FBLN1	-0.06	FSTL1	-0.19
TWIST2	-0.09	AXL	-0.22
NANOG	-0.10	COL5A1	-0.23
LTBP1	-0.13	LTBP1	-0.26
AXL	-0.15	PLSCR4	-0.29
PROM1	-0.34	PROM1	-0.42
SPRY4		SPRY4	

Figure 6. Pre-TX, high/ expression of KLK2, KLK4, ADAMTS1 and ZEB1 was signficantly correlated with and preditive of progression <12 months post-TX.
High/expression of ADAMTS1, SNAI1 and KLK2 was also associated with death <3 years post-TX.
High expression of PROM1/CD133 was associated with good prognosis

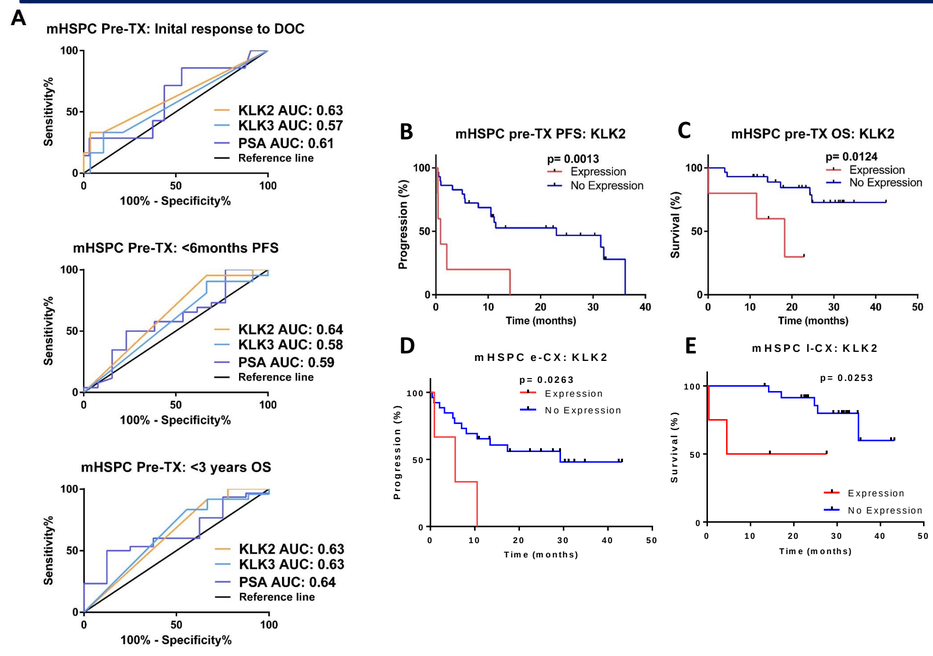


Figure 7. A) Pre-TX, expression of KLK2 was a better preidictive/ prognositic biomaker of inital PD post-TX and progression <6 months post-TX compared to KLK3 epression and serum PSA levels, and performed as well as KLK3 and PSA to predict OS. **B-C)** KLK2 expression pre-TX was significantly predictive of shorter time to progression (1 vs 23 months) and death (18 vs undefined months). **D-E)** KLK2 expression during early (e-CX) and late (l-CX) DOC treatment was associated with shorter time to progression (6 vs 29 months) and death (16 vs undefined months)

Conclusions

- **Pre-TX total CTC and E-CTC numbers were elevated in patient who were non-responsive to DOC, and the detection of ≥ 6 CTCs pre-TX was significantly predictive of poor PFS and OS and these biomarkers may be used in the clinical an early indicators of resistance**
- **CTC dynamics during treatment such as the detection of ≥ 6 CTCs during early or late treatment, CTC progression and changing CTC score status can be used to monitor treatment response and used as early predictors of the development of resistance**
- **KLK2 expression is a promising biomarker of resistance to DOC and may have clinical utility if used as an alternative to or in conjunction with serum PSA**