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The novel association of circulating tumour cells and circulating megakaryocytes with prostate cancer prognosis

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BACKGROUND

- Circulating tumour cell (CTC) is rare in blood.
- Previous CTC studies have mainly focused on epithelial CTCs, but not CTCs undergoing/undergone epithelial-mesenchymal transition (EMT).
- EMT has been recognised to play an important role in cancer progression and metastasis.
- Size and deformability based CTC isolation system can detect different subtypes of CTCs, including epithelial, EMTing and EMTed CTCs.

AIMS

To develop an approach for the investigation of different subtypes of circulating tumour cells (CTCs) and other cells to evaluate their potential prognostic value of prostate cancer.

MATERIALS & METHODS

- **Study population**: 81 prostate cancer patients, including 38 untreated localised and 43 progressive castration-resistant prostate cancers (CRPCs).
- **CTC isolation**: From 7.5 mL of whole blood using the Parsortix™ system from ANGLE plc (**Fig. 1**).
- **Immunofluorescence (IF)**: anti-CK, VIM, and CD45 antibodies with different fluorescence detection.
- **Fluorescence *in situ* hybridisation (FISH):** 5 rounds of FISH including 10 Probes were applied on cells after immunofluorescence.
- **Statistics:** Kendall's rank correlation was used to assess the association between CTCs and concurrent PSA level; Wilcoxon rank-sum test was applied to assess the distribution of CTCs in sub-groups divided by specific clinical features.

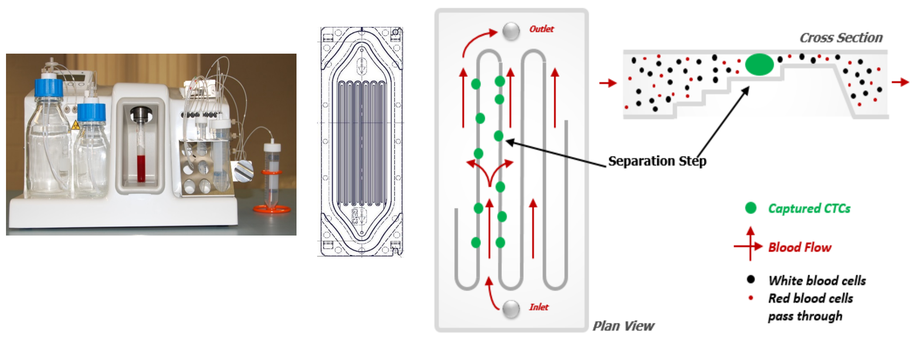

Fig. 1. Parsortix system overview and cassette design

RESULTS

1. CTC isolation and identification in prostate cancer and healthy men.

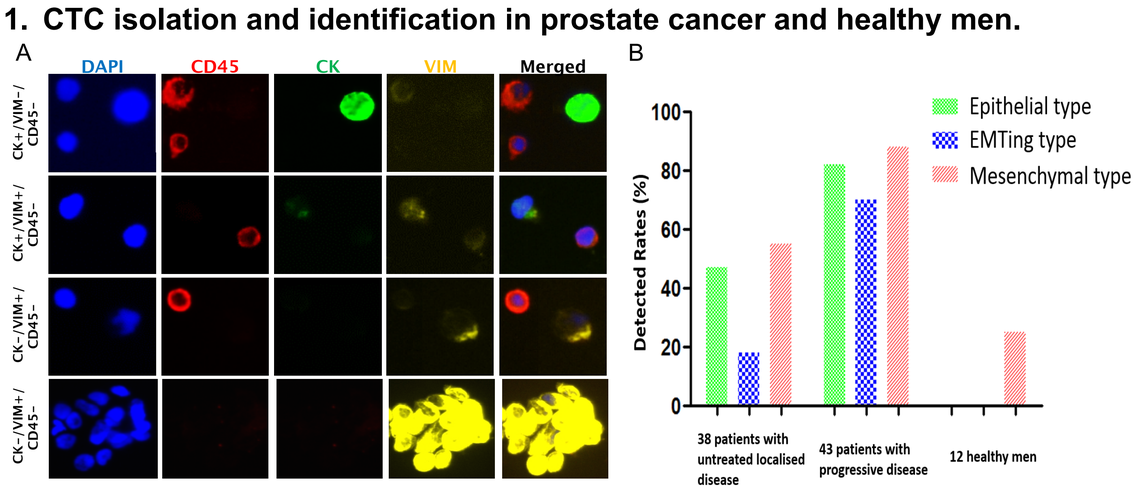

Fig. 2. Different types of detected cells with epithelial and mesenchymal properties. (**A**) Representative images of CTCs with epithelial (CK+/VIM-/CD45-), EMTing (CK+/VIM+/CD45-), and mesenchymal (CK-/VIM+/CD45-) feature and CTC cluster. **(B)** Detected rate of each subtype in untreated localised, progressive CRPC and healthy men.

2. CTCs were confirmed by repeated FISH.

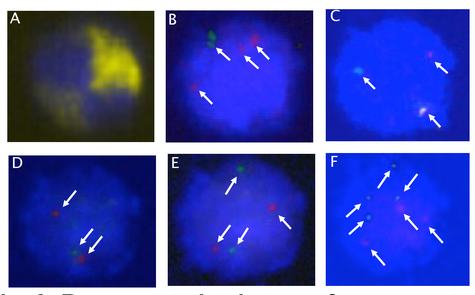

Fig. 3. Representative images from repeated FISH analysis on one CK-/VIM+/CD45- CTC (A) post-immunostaining. 1st round (**B**): *AR* (red) and 6q16 (green). 2nd round (**C**): RP11-476D17 (red) and RP11-95I21 (green). 3rd round (**D**): *C-MYC* (red) and *NKX3.1* (green). 4th round (**F**): *RB1* (red) and *PTEN* (green). 5th round (**F**): *CCND1* (red) and 16q22.1 (green).

Table 1. Percentage of detected genetic changes in sub-population of cells

	0%	<30%	30-49%	50-69%	70-99%	100%
Epithelial type, n=25	4 (16%)	4 (16%)	5 (20%)	7 (28%)	1 (4%)	4 (16%)
EMTing type, n=39	11 (28%)	6 (15%)	9 (23%)	8 (21%)	2 (5%)	3 (8%)
Mesenchymal type, n=54	8 (15%)	17 (31%)	17 (31%)	9 (17%)	2 (4%)	1 (2%)
CK-/VIM-/CD45- cells, n=77	27 (35%)	22 (29%)	18 (23%)	6 (8%)	1 (1%)	3 (4%)
CD45+ cells, n=140	60 (43%)	41 (30%)	29 (21%)	9 (6%)	1 (1%)	0 (0%)

3. Subgroups of CTCs are associated with serum PSA level, primary biopsy GS, the risk of localised tumour and metastases.

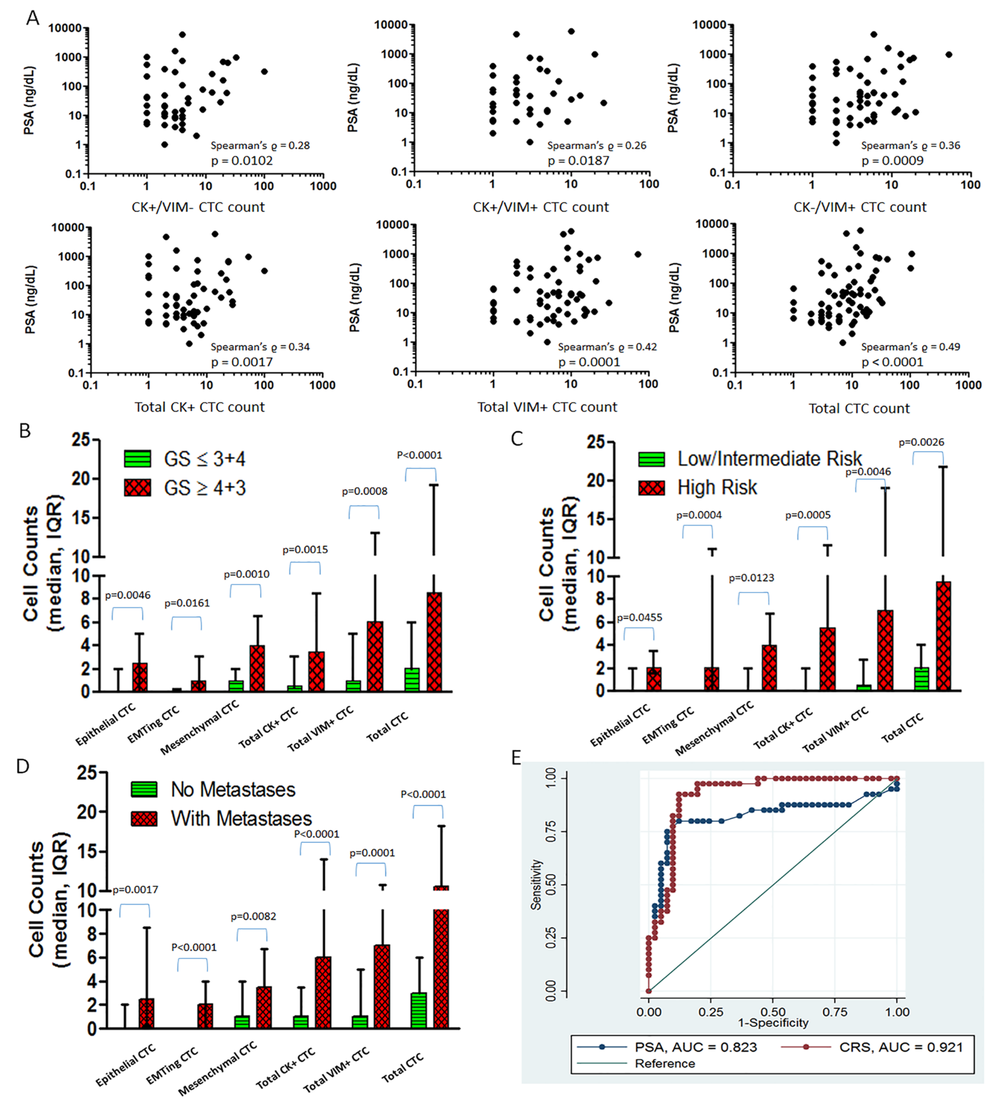

Fig. 4. The correlation of CTC count with clinical features. (A) Association of PSA with epithelial CTCs, EMTing CTCs, mesenchymal CTCs, total CK+ CTCs, total VIM+ CTCs and total CTCs. The numbers of all types of CTCs were higher in patients with higher GS of primary tumour (B), patients with untreated high-risk localised disease (C), and patients with metastases (D). (E) ROC analysis of the efficiencies of serum PSA level (blue, AUC = 0.823) and combined risk score (CRS) with CTCs (red, AUC = 0.921) in discriminating metastatic prostate cancer patients from those without metastasis.

4. Circulating megakaryocytes identification and association with better survival.

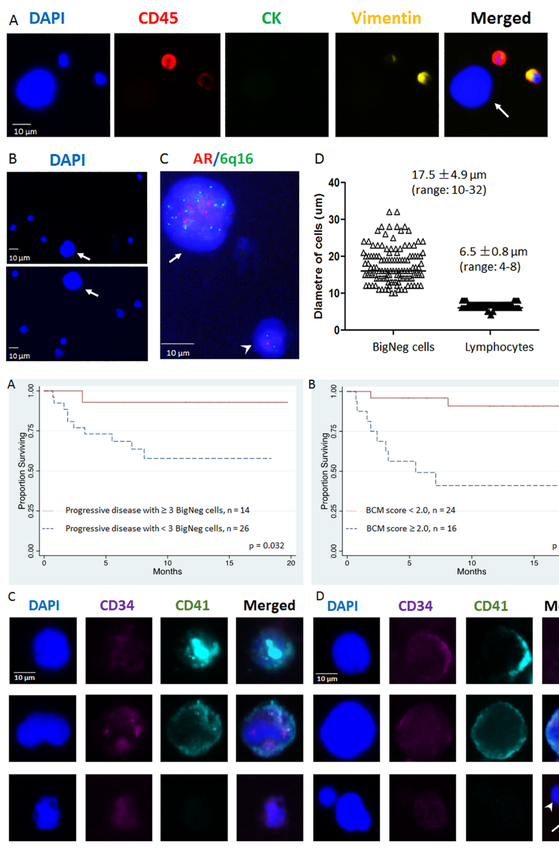

Fig. 5. Representative IF/FISH images and nuclear size of BigNeg cells. (**A**) One BigNeg cell (arrowed) with a big and bright Nucleus and negative for CD45, CK and VIM and two adjacent CD45+/VIM+ lymphocytes. (**B**) BigNeg cells (arrowed) were identified under low resolution image. (**C**) FISH analysis by probes of *AR* (red) and 6q16 (green) showed polyploidy of the BigNeg cell (arrowed) and an adjacent diploid lymphocyte (arrow-head). (**D**) Comparison of nucleus diameter between BigNeg cells and lymphocytes.

Fig. 6. BigNeg cell count was associated with survival and their megakaryocyte nature was confirmed by IF. Kaplan-Meier curve for overall survival showed progressive prostate cancer patients with less than three BigNeg cells had significantly shorter survival rates (p=0.032) (**A**); with BCM (Barts CTC and megakaryocyte score) ≥ 2.0 had even poorer survival (p=0.0003) (**B**). (**C**) PMA-treated K562 cells have CD34 expression with or without CD41 expression. (**D**) megakaryocytes were positive for CD34 with or without CD41 staining while the lymphocyte was negative for both CD34 and CD41.

CONCLUSIONS

- The size-based system, Parsortix™, can efficiently harvest both CK+ CTCs and CK-/Vimentin+/CD45- cells.
- Genetic alterations were detected in a large proportion of VIM+/CD45- circulating cells, indicating that Parsortix™ captures CTCs under EMT.
- EMTing CTC counting correlated the best with metastases. Combination of EMTing CTCs and PSA greatly increase the accuracy of metastasis prediction.
- Combination of circulating megakaryocytes and mesenchymal CTCs has great power to predict CRPC patient survival.

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