RNA profiling of circulating tumor cells systemically captured from diagnostic leukapheresis products in prostate cancer patients

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ARTICLE INFO

Keywords:
Circulating tumor cells
Diagnostic leukapheresis
Prostate cancer
RNA profiling

ABSTRACT

The application of circulating tumor cells (CTCs) in both clinical practice and research has been continuously limited by the rare number of targets that can be found in a tube of peripheral blood. Diagnostic leukapheresis (DLA) was used to increase the sampling volume. AdnaTest was used to process the whole leukopak, and the RNAs of captured CTCs was then profiled by NanoString nCounter platform. Spike-in experiments and leukopaks from patients with metastatic prostate cancer were used to validate this new strategy. The whole leukopak was further concentrated five times to reduce the total volume from 150 mL to 30 mL, which enabled it to be processed by 3 separate AdnaTest kits. The spike-in experiment demonstrated a reliable capture when there were more than 100 cancer cells/10 mL of concentrated leukopak. In 1 out of 5 real patient samples, CTCs were only detected in the leukopak, but not in peripheral blood. The RNA profiling of DLA CTCs indicated a more aggressive phenotype of CTCs occurred when the patient was experiencing a disease relapse, even when the serum prostate specific antigen (PSA) level was still relatively low and CTCs in peripheral blood were not detectable. We established a new protocol, integrating DLA, AdnaTest and NanoString nCounter technology, to profile RNAs from CTCs captured from a large blood screening volume. The new protocol can process the whole leukopak with sensitive CTC capture. The RNA profiling of CTCs can provide valuable information for disease monitoring.

1. Introduction

Prostate cancer (PCa) is the most common cancer type and the second leading cause of cancer death in American men. More than 268,000 new cases and 34,000 PCa-caused deaths were estimated to occur in the United States in 2022 [1]. In some Asian countries, like China, PCa has also become a prominent health issue in recent decades, with an annual 12.6% increase in incidence since 2000 [2]. Despite of the dramatic progress in novel therapeutic agents, metastatic PCa (mPCa) remains incurable [3]. The 5-year survival rate of patients with mPCa is less than 30%, while that rate for non-metastatic PCa is above 90% [3,4]. Circulating tumor cells (CTCs), defined as cancer cells that leave the tumor (primary and/or metastatic site) and enter the blood stream (directly or via the lymphatics), are considered to be the seeds for developing metastases [5]. It has been demonstrated that a higher CTC count in peripheral blood is independently associated with worse progression-free survival and overall survival in metastatic castration-resistant PCa (mCRPC) patients [6,7]. The dynamic changes of CTC quantity have also been widely adopted as a way to monitor the treatment response or disease progression [8,9]. Since CTCs may be reflective of molecular features of the tumor, they have emerged as liquid biopsy tools to predict treatment effect, thus guiding personalized precision medicine [10–12]. However, the application of CTCs in both clinical practice and research has been continuously limited by the rare number of targets that can be found in a tube of peripheral blood, which makes it even harder to detect CTCs in the early phase of metastasis, e.g., biochemical recurrence [13].

Technically, CTC detection includes three steps: sampling, CTC capture and analysis. The past decade has witnessed a robust development of

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novel CTC capture and analysis technologies with increasing sensitivity and specificity [5]. However, the source/total number of CTCs in a tube remains the same if there are no new sampling strategies, regardless of the advances in capture and/or analysis. Diagnostic leukapheresis (DLA) is a well-established and tolerated clinical procedure, in which large volumes of blood are processed, with centrifugal enrichment of mononuclear cells (MNCs) into a leukopak [14]. The remaining blood components (e.g. plasma, red blood cells, platelets, etc.) are returned to the patient. Theoretically, CTCs, if there are any in the circulation, can be co-enriched into the leukopak, due to a similar density as MNCs [14,15]. Multiple studies have demonstrated that compared to a peripheral blood, DLA can largely amplify the screening blood volume (~10 mL vs. ~5 L), resulting in a dramatic increase in the detected CTC number (~10 vs. x 10^5) [16–18]. However, due to the large volume (50–150 mL) and high density of nucleated cells (~ x 10^10/L), it is very hard to process the whole leukopak using conventional technologies, e.g. the CellSearch® system (Janssen Diagnostics). There is an unmet need to develop robust, high-throughput downstream analysis protocols coupled with the CTCs captured following DLA.

In this study, we established and validated a new strategy to process the whole leukopak using AdnaTest, a positive selection kit applying epithelial cell adhesion molecule (EpCAM) antibody-conjugated magnetic beads. The mRNA of captured CTCs was then profiled by NanoString nCounter platform which utilizes molecular barcoding and single molecule imaging to detect hundreds of genes in one single reaction.

2. Methods

2.1. Cell line

LNCaP-95 (LN95) cell line (obtained courtesy of Dr. Alan Meeker, Johns Hopkins Medical Institute, Baltimore, MD) was maintained in 10% charcoal-stripped FBS (CSS)-RPMI1640 medium with 1 × penicillin-streptomycin solution (ThermoFisher Scientific) at 37 °C with 5% CO₂. Cells were routinely checked for mycoplasma contamination using the e-Myco™ VALiD Mycoplasma PCR Detection Kit (iNtRON Biotechnology, Inc., South Korea). For the spike-in experiment, cells were dissociated by 1 × TrypLE Expression solution (ThermoFisher Scientific) for 3–5 min and re-suspended in culture medium before cell counting on the Countess™ Automated Cell Counter (ThermoFisher Scientific) according to the manufacturer’s instruction.

2.2. Diagnostic leukapheresis

All experiments were performed in accordance with the relevant guidelines and regulations and ethical principles of the Declaration of Helsinki. Analysis of human samples was approved by the Ethics Committee of Johns Hopkins Medicine Institutional Review Board (NA_00087,094, May 2018). Written informed consent for the use of blood products for research purposes was obtained from each blood donor. The leukopaks from healthy donors were purchased from the New York Blood Center and the DLA for patients with mPCa was performed in the American Red Cross. All procedures were well tolerated. Prior to each DLA procedure, the total blood volume (TBV) of the patient was estimated according to the formula of Nadler. No pretreatment with granulocyte colony stimulating factor was given. The procedure was performed according to the standard continuous MNC protocol. Acid citrate dextrose formula A was used as an anticoagulant at a concentration of 1:11. In each procedure, the amount of blood processed was 10,000 mL, and the final volume of each leukopak was about 150 mL. The MNCs in the leukopaks were further concentrated using Ficoll-Paque PLUS (Millipore Sigma) density gradient centrifugation at 800 × g for 20 min, followed by resuspending the cells to a final volume of 30 mL with phosphate buffered saline (PBS) containing 0.5% bovine serum albumin (BSA) (Fig. 1). For each mPCa patient, a set of 10 mL peripheral blood sample was collected at the same time using a lavender-topped ethylenediaminetetraacetic (EDTA) tube.

2.3. CTC capture and analysis

For the enrichment and molecular characterization of CTCs from the leukopak or peripheral blood, the AdnaTest ProstateCancerSelect and AdnaTest Prostate Cancer Detect kits (Qiagen) were used with modification. In brief, 100 μL antibody-coated, magnetic beads from AdnaTest

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Fig. 1. A schematic diagram of the comparison between peripheral blood-based CTC detection and DLA-based CTC detection and the work flow of processing a leukopak. CTC detection includes blood sampling, CTC capture and CTC analysis. Compared to 10 mL of peripheral blood, DLA allows the screening of up to 10 L of peripheral blood. In our optimized protocol, AdnaTest was used to capture CTCs, followed by diagnostic gene expression analyses by RT-qPCR. For each leukopak (~150 mL), the MNCs were further concentrated using Ficoll-Paque PLUS (Millipore Sigma) density gradient centrifugation at 800 × g for 20 min, followed by resuspending the cells to a final volume of 30 mL, which enabled it to be processed by 3 separate AdnaTest process.
ProstateCancerSelect kit were added into 10 mL blood sample with 40 min of incubation time at room temperature before selected cells were washed with 1 × PBS for four times. Each leukopak sample (30 mL) was tested by 3 independent assays. After washed CTCs were lysed and reversely transcribed with oligo(dt) beads by Sensiscript RT kit (Qiagen), cDNA was used to detect expression of genes including ribosomal protein L13a (RPL13A), EPCAM, prostate specific antigen (PSA), prostate specific membrane antigen (PSMA), androgen receptor-full length (AR-FL), AR splicing-variant 7 (AR-V7), and Homeobox B13 (HOXB13) by real time quantitative polymerase-chain reaction (RT-qPCR) as previously described [10]. The expression level of each gene tested in RT-qPCR was presented as the absolute copy number calculated by standard curve method as previously described [10].

2.4. nCounter low RNA input workflow for PanCancer Progression Panel

The remaining cDNAs from the AdnaTest were used for RNA profiling by the nCounter PanCancer Progression Panel (NanoString Technologies, WA). The 770 targeted genes in the panel were amplified using the nCounter Low RNA Input Kit (NanoString Technologies) which has been validated and found to be highly efficient and specific [19]. Briefly, the cDNAs of CTCs were amplified using the multiplex low-input primer pool with 7 cycles of PCR. The PCR-amplified products were then quantified by an Agilent Bioanalyzer 2100 (Agilent Technologies) and hybridized with the nCounter PanCancer Progression Panel following the standard nCounter hybridization protocol.

2.5. Data analysis

Data generated by the nCounter PanCancer Progression Panel were processed by nSolver Analysis Software version 4.0 (NanoString Technologies) and Microsoft Excel (Microsoft, WA). The data were first normalized as previously reported [19]. The normalized data were then analyzed by the Advanced Analysis Module in the nSolver Analysis Software version 4.0 to reveal the differentially expressed genes with a preset threshold of statistical significance. To control for multiple testing, an adjusted p-value (i.e., false discovery rate (FDR) q-value) threshold of 0.01 was used for statistical significance. The unsupervised hierarchical clustering analysis was performed as previously described [20]. Differentially expressed genes were represented by different color spectrum from the lowest (blue color) to the highest (yellow color) expressions on the heatmap of clustering analyses.

2.6. Gene set enrichment analysis (GSEA)

GSEA was applied to determine the potential functional pathways associated with the differentially expressed genes between different CTC samples. The software was acquired from the Broad Institute Gene Set Enrichment Analysis website (http://software.broadinstitute.org/gsea/index.jsp) [21]. Thirty-seven pre-defined gene sets were used as the reference sets, which were downloaded from the NanoString website (http://www.nanostring.com). The log2 transformed and median normalized data were first ranked according to the signal-to-noise ratio. Then, the GSEA algorithm generated an enrichment score, which estimated whether certain gene sets were enriched in a sample or just randomly distributed. A gene set with nominal p-value (NOM p) < 0.01 and FDR q-value < 0.25 was considered as significantly enriched [22].

3. Results

3.1. Spike-in experiments to validate the efficiency of CTC detection in leukopaks

In order to investigate whether the AdnaTest kit is applicable for a sample with ultra-high density of nucleated cells, like DLA products, leukopaks from healthy donors were used for spike-in experiments. Since DLA may introduce other background cell types to the sample, we first aimed to validate whether all 6 markers (EpCAM, AR-FL, PSA, PSMA, HOXB13, AR-V7) which were previously used for CTC detection in peripheral blood could still be utilized. AdnaTest was applied to 7 leukopaks from healthy donors without any spike-in. According to the positive thresholds that were previously reported, EpCAM and AR-FL were found to be positive even in non-cancerous leukopaks. On the other hand, PSA, PSMA, HOXB13 and AR-V7 were all negative, and thus were selected for further CTC diagnosis in DLA products (Supplemental Table 1).

LN95 cells (from 50 to 1000) were spiked into healthy leukopaks to mimic DLA products from cancer patients with different numbers of CTCs. The same number of LN95 cells were spiked in parallel into PBS as a control sample to demonstrate how the background cells in a leukopak may affect AdnaTest capture (Fig. 2a). All four markers were undetectable in “0 cell” groups. The Log_R0 (copy numbers) were 1.13 ± 0.47, 2.07 ± 0.12, 2.80 ± 0.10, 3.24 ± 0.03 for PSA; 2.21 ± 0.60, 2.99 ± 0.09, 3.69 ± 0.23, 4.29 ± 0.05 for PSMA; 1.55 ± 0.69, 2.37 ± 0.06, 2.79 ± 0.22, 3.38 ± 0.03 for HOXB13; 0.43 ± 0.46, 1.31 ± 0.21, 2.10 ± 0.30, 2.82 ± 0.02 for AR-V7, when 50, 100, 500 and 1000 LN95 cells were spiked into the leukopak, respectively (Fig. 2b–e). When comparing the copy numbers between the PBS group and the leukopak group under the same spiking-cell number, no significant differences were observed, except for the situation when only 50 LN95 cells were used. In this setting, the PBS groups demonstrated significantly higher copy numbers than the leukopak groups in all 4 gene markers (P < 0.05).

3.2. CTC detection in leukopaks from metastatic prostate cancer patients

Three patients with mPCa were recruited. One of the three patients underwent three DLA at different time points, so in total five leukopaks were collected. The serum PSA levels at the time of DLA were listed in Table 1, which may be related to disease burden. According to the DLA protocol, 10,000 mL of peripheral blood was screened during each procedure. The MNC concentrations were 3.9 × 10^6/L, which was about one order of magnitude higher than those of peripheral blood. In one out of five experiments, CTCs were not found in the peripheral blood sample, but were detected in the leukopak. In three experiments, CTCs were detected in both peripheral blood and the leukopak. Due to the nature of the AdnaTest procedure, the exact CTC number cannot be counted or compared, however, the copy numbers of diagnostic markers were constantly higher in leukopaks than in paired peripheral blood. Representative comparison of copy numbers was shown in Supplemental Table 2.

3.3. Establishment and validation of CTC RNA profiling using NanoString

Besides determining a patient to be CTC-positive or not, downstream analyses on captured CTCs to reveal their molecular features may be more informative for diagnosis and prognosis. Here we established a protocol using the nCounter PanCancer Progression Panel coupled with the matched Low RNA Input Kit to detect the expression levels of 770 mRNAs for the CTCs captured from leukopaks using AdnaTest. To validate the feasibility, 100, 500 and 1000 LN95 cells were spiked into three leukopaks from healthy donors, respectively. Then, four samples were generated from each leukopak (Fig. 3a). Sample 1 was an aliquot of the non-cancerous leukopak before spiking, which should contain only white blood cells and no cancer cells, thus, the pre-spiking white blood cells. Sample 2 contained the same number of LN95 cells in PBS. Then the sample underwent the AdnaTest capture process. Sample 3 was an aliquot of the leukopak after magnetic beads pulling out cancer cells, thus, the post-capture white blood cells. Sample 4 were cells pulled out by magnetic beads, which were confirmed to be a combination of cancer cells and some white blood pulled out by non-specific binding (Supplemental Fig. 1). All 12 samples were analyzed together and this experiment was repeated three times.

When comparing the gene profiling between Sample 2 (pure LN95)
In this study, we validated the feasibility of increasing sampling volume and sample amount as an opportunity to assess whether the dynamic tracking of RNA expression patterns between post-capture white blood cells and captured CTCs (Fig. 4b). The three tests of the same leukopak were clustered together, respectively. Volcano plots demonstrated significances versus means of differential fold changes for the comparisons of gene expressions between every two CTC samples (Fig. 5a–c). Using a statistical cutoff of FDR < 0.01, significant differences were found in 96 genes between the second DLA and the first one, in 152 genes between the third and the first sample, and in 55 genes between the second and the third sample. Functional pathways associated with differentially expressed genes were analyzed by GSEA. When comparing to the first CTC sample, GSEA demonstrated 3 gene sets were significantly enriched in the second and the third CTC samples, including ECM Structure (NES = 1.57, FDR = 0.107, NOM p < 0.001), Cell Motility (NES = 1.54, FDR = 0.079, NOM p < 0.001) and Sprouting Angiogenesis (NES = 1.43, FDR = 0.184, NOM p < 0.001) (Fig. 5d–f). When comparing the second and the third CTC samples, none of the gene sets were significantly enriched in either one.

4. Discussion

CTC as a modality of liquid biopsy has been widely studied in a variety of cancer types. Low yield and the lack of appropriate downstream analyses for clinical use have limited application and development [23]. In this study, we validated the feasibility of increasing sampling volume and sample amount as an opportunity to assess whether the dynamic tracking of RNA expression patterns between post-capture white blood cells and captured CTCs (Fig. 4b). The three tests of the same leukopak were clustered together, respectively. Volcano plots demonstrated significances versus means of differential fold changes for the comparisons of gene expressions between every two CTC samples (Fig. 5a–c). Using a statistical cutoff of FDR < 0.01, significant differences were found in 96 genes between the second DLA and the first one, in 152 genes between the third and the first sample, and in 55 genes between the second and the third sample. Functional pathways associated with differentially expressed genes were analyzed by GSEA. When comparing to the first CTC sample, GSEA demonstrated 3 gene sets were significantly enriched in the second and the third CTC samples, including ECM Structure (NES = 1.57, FDR = 0.107, NOM p < 0.001), Cell Motility (NES = 1.54, FDR = 0.079, NOM p < 0.001) and Sprouting Angiogenesis (NES = 1.43, FDR = 0.184, NOM p < 0.001) (Fig. 5d–f). When comparing the second and the third CTC samples, none of the gene sets were significantly enriched in either one.
by doing DLA, and processing the whole leukopak by using the AdnaTest procedure. This increased CTC yield then enabled bulk RNA profiling using the NanoString nCounter technology.

CTC detection includes blood sampling, CTC capture and CTC analysis [5]. However, even the best capture technology cannot “create” more CTCs in a tube of blood, therefore, the sampling step determines the total number of CTCs that can be captured in an assay. Recently, several medical wire-based in vivo capture technologies have attempted to increase sampling by placing the CTC capture device in the vein [24,25]. Another way to increase the blood sampling volume without introducing new devices is apheresis [14,15]. Apheresis is a well-established, safe technique that has a long history and is widely available. The common uses include, but are not limited to, self-MNC preservation, component blood transfusion, etc. [26]. Several studies have validated and confirmed that DLA followed by conventional CTC capture technologies can significantly increase the CTC yield. Tamminga and colleagues used CellSearch® to detect CTCs in DLA products from patients with non-small cell lung cancer, and demonstrated that the number of CTCs in 7.5 mL DLA product were a median of 9.2 times (interquartile range = 5.6–24.0) higher than that in 7.5 mL blood [16]. Fischer and colleagues found that

![Fig. 3](image-url)

Fig. 3. (a) A schematic diagram of the spike-in experiment: one-hundred, five-hundred and one-thousand LN95 cells were spiked into three leukopaks from healthy donors, respectively. Then four different samples were generated from each leukopak as described in the manuscript (HD-healthy donor, WBC-white blood cells). (b) Correlation between the genes detected in Sample 2 (the same number of pure LN95 cells as spiked into the leukopak) and Sample 4 (cells that pulled out by AdnaTest). (c) Correlation between the genes detected in Sample 1 (pre-spiking white blood cells) and Sample 3 (post-spiking white blood cells). (d) Heatmap demonstrating the differentially expressed genes among all four groups. Up-regulated genes are in yellow, down-regulated in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

![Fig. 4](image-url)

Fig. 4. (a) A schematic diagram of the patient sample collection: one patient with metastatic prostate cancer underwent 3 times of DLA at different time points as described in the manuscript. (b) Heatmap demonstrating the differentially expressed genes among all CTCs and CTC-depleted leukopaks (white blood cells) at three different time points. Each leukopak was processed by three AdnaTest assays as described before. Up-regulated genes are in yellow, down-regulated in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
CTCs can be detected in more than 90% of non-metastatic breast cancer patients using DLA products analyzed by CellSearch®, and the number of enumerated CTCs per mL of DLA product was about one order of magnitude higher than that in 1 mL of peripheral blood [14].

It is challenging, however, to process the whole leukopak, because it has both ultra-high MNC density (~10^10/L) and large physical volume (~150 mL) at the same time. Studies utilizing CellSearch® can only process a small aliquot of leukopak (1–2 mL) after diluting the sample, which attenuated the effect of increased blood screening volume. Tamminga and colleagues tried to optimize the process using a size-based CTC capture technology and found 10–20 mL of DLA product could be processed [27]. However, it was still only 10–20% of the total volume. Wu and colleagues applied two different types of magnetic beads to capture CTCs from highly concentrated blood samples and saw promising results, but it was only validated in 1 mL of mimicking DLA products [28]. Here we further concentrated the whole leukopak five times to reduce the total volume to 30 mL which enabled it to be processed by 3 separate AdnaTest applications. We thus evaluated whether the AdnaTest can still reliably capture CTCs in such ultra-concentrated blood samples. When there were more than 100 LN95 cells/10 mL concentrated leukopak, the copy numbers correlated well with the increasing spiking cell number, and there was no significant difference between PBS background and leukopak background, by using our optimized protocol. The further RNA profiling also indicated that though the “captured CTC” samples were contaminated by some white blood cells, the “post-capture” leukopak didn’t show any evidence of missed cancer cells. In 1 out of 5 real patient samples, CTCs were only detected in the leukopak, but not in peripheral blood.

Profiling the molecular features of CTCs is the key to understand metastatic diseases, as well as to facilitate precision medicine [5]. Due to the low yield, it is very challenging to do the bulk sequencing for CTCs captured from a conventional peripheral blood sample. Single cell sequencing is a way to analyze these rare cells, however, the low CTC yield may not represent the molecular features of the whole tumor [29].

DLA can move this towards a real biopsy by increasing the number of CTCs that can be sequenced [30,31]. Lambros and colleagues performed single cell sequencing on CTCs captured from DLA products in patients suffering from mPCa [31]. They found that the intra-patient heterogeneity and clonal evolution could be better demonstrated by CTCs in a leukopak than in peripheral blood. Despite of the advances in single cell sequencing, robust high-throughput downstream analysis for DLA CTCs in clinical settings is still an unmet need. In this study, we established a new protocol for CTC RNA profiling using NanoString nCounter technology, which utilizes molecular barcoding and single molecule imaging to detect hundreds of genes in a single reaction [32]. Each color-coded barcode is attached to a target-specific probe corresponding to a certain transcript which can be individually counted. We applied this technology to a series of real patient samples. The RNA profiling of DLA CTCs indicated a more aggressive phenotype of CTCs occurred when the patient was experiencing a disease relapse, even at that time, the serum PSA level was still relatively low and CTCs in peripheral blood were not detectable.

This study has several limitations. First, the patient cohort was small. The value of this new protocol to monitor disease progression, or guiding precision medicine, need to be further validated in a larger patient cohort. Second, due to the nature of AdnaTest, the CTCs cannot be visualized by immunofluorescence, thus the exact numbers of CTCs cannot be determined. Last but not least, as a future direction, it will be promising to use this workflow to capture and analyze CTCs from patients at the early phase of metastasis, like patients with biochemical recurrence of PCa, in whom the CTC burden may be very low.

5. Conclusion

In conclusion, we established a new protocol, integrating DLA, AdnaTest and NanoString nCounter technology, to profile RNAs from CTCs captured from a large blood screening volume. The new protocol can process the whole leukopak (~150 mL) with sensitive CTC capture.
RNA profiling of CTCs may provide valuable information for disease monitoring. Further work is needed to validate this new protocol in a larger patient cohort with low CTC burden.

Ethics approval and consent to participate

All experiments were performed in accordance with the relevant guidelines and regulations and ethical principles of the Declaration of Helsinki. Analysis of human samples was approved by the Ethics Committee of Johns Hopkins Medicine Institutional Review Board (NA_00087_094, May 2018). Written informed consent for the use of blood products for research purposes was obtained from each blood donor.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Funding

LD and WX were supported by National Natural Science Foundation of China (8207847, 82103485) and Innovative Research Team of High-Funding for Cue Biopharma, Inc., a founder with equity interest in Keystone University and licensed to Qiagen, and A

Authors contributions

Conceptualization: LD, JL, SRA, WX and KJP; Data curation: LD, XD; Methodology: LD, XD, CL, ZZ, CYH and MDK; Project administration: SW

Acknowledgements

We thank NanoString Technologies for the free reagents and technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mtbio.2022.100474.

References

[1] R.L. Siegel, K.D. Miller, H.E. Fuchs, A. Jemal, Cancer statistics, 2022, CA A Cancer J. Clin. 72 (1) (2022) 1–33, https://doi.org/10.3322/caac.21706.

[2] W. Chen, R. Zheng, P.D. Baade, S. Zhe, H. Zeng, F. Bray, A. Jemal, X.Q. Yu, J. He, Cancer statistics in China, 2015, CA A Cancer J. Clin. 66 (2) (2016 Mar) 115–132, https://doi.org/10.3322/caac.21338.

[3] L. Dong, R.C. Zieren, W. Xue, T.M. de Reijke, K.J. Pienta, Metastatic prostate cancer remains incurable, why? Asian journal of urology 6 (1) (2019 Jan) 26–41, https://doi.org/10.1016/j.ajur.2018.11.005.

[4] J.N. Wu, K.M. Fish, C.P. Evans, R.W. de'Verre White, M.A. Dall'Era, No improvement noted in overall or cause-specific survival for men presenting with metastatic prostate over a 20-year period, Cancer 120 (6) (2014 Mar 15) 818–823, https://doi.org/10.1002/cncr.28485.

[5] C.J. Kim, L. Dong, S. Amend, Y.K. Cho, K. Pienta, The role of liquid biopsies in prostate cancer management, Lab Chip 21 (17) (2021 Sep 7) 5326–5328, https://doi.org/10.1039/d1lc00485a.

[6] G. Heller, K. Fizzini, R. McCormack, A. Molina, D. MacLean, L.J. Webb, F. Saad, J.S. de Bono, H.I. Scher, The added value of circulating tumor cell enumeration to standard markers in assessing prognosis in a metastatic castration-resistant prostate cancer population, Clin. Cancer Res. 23 (8) (2017 Apr 15) 1967–1973, https://doi.org/10.1158/1078-0432.ccr-16-1224.

[7] J.S. De Bono, H.I. Scher, R.B. Montgomery, C. Parker, M.C. Miller, H. Tissing, G.V. Doyle, L.W. Terstappen, K.J. Pienta, D. Raghavan, Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer, Clin. Cancer Res. 14 (19) (2008 Oct 1) 6302–6309, https://doi.org/10.1158/1078-0432.ccr-08-1028.

[8] D. Lorente, D. Olmos, J. Mateo, D. Dolling, D. Bianchi, G. Seed, P. Florb, M. Crespo, L. Figueiredo, S. Miranda, H.I. Scher, Circulating tumor cell accumulation as a biomarker of disease progression in metastatic castration-resistant prostate cancer patients with low baseline CTC counts, Ann. Oncol. 29 (7) (2018 Jul 1) 1554–1566, https://doi.org/10.1093/annonc/mdy172.

[9] G. Heller, R. McCormack, T. Kheoa, A. Molina, M.R. Smith, R. Dreicer, F. Saad, R. de Wit, D.T. Aftab, M. Hirmand, A. Limon, Circulating tumor cell number as a response measure of prolonged survival for metastatic castration-resistant prostate cancer: a comparison with prostate-specific antigen across five randomized phase III clinical trials, J. Clin. Oncol. 36 (6) (2018 Feb) 572, https://doi.org/10.1200/jco.2017.75.2998.

[10] E.S. Antonarakis, C. Lu, H. Wang, B. Luber, M. Nakazawa, J.C. Roeser, Y. Chen, T.A. Mohammad, Y. Chen, H.L. Fedor, T.L. Lotan, AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer, N. Engl. J. Med. 371 (11) (2014 Sep 11) 1028–1038, https://doi.org/10.1056/nejma1315915.

[11] A.J. Armstrong, S. Halabi, J. Luo, D.M. Nanus, P. Giannakakou, R.Z. Szumewitz, D.C. Danila, P. Healy, M. Anand, C.J. Rothwell, J. Rasmussen, Prospective multicenter validation of androgen receptor splice variant 7 and hormone therapy resistance in high-risk castration-resistant prostate cancer: the PROPHECY study, J. Clin. Oncol. 37 (13) (2019 May 1) 1120, https://doi.org/10.1002/jco.20180371.

[12] H.I. Scher, R.P. Graf, N.A. Schreiber, B. McLaughlin, D. Lu, J. Louw, D.C. Danila, L. Dugan, A. Johnson, G. Heller, M. Fleisher, Nuclear-specific AR-V7 protein localization is necessary to guide treatment selection in metastatic castration-resistant prostate cancer, Eur. Urol. 71 (6) (2017 Jun 1) 874–882, https://doi.org/10.1016/j.eururo.2016.11.024.

[13] S. Grisanti, A. Antonelli, C. Almici, C. Foroni, M. Sodano, L. Triggiani, D. Greco, C. Palumbo, M. Martini, S.M. Magrini, Analysis of circulating tumor cells in prostate cancer patients at PSA recurrence and review of the literature, Anticancer Res. 36 (6) (2016 Jun 1) 2975–2981, https://ar.iaiournals.org/contents/6/6/2975long.

[14] J.C. Fischer, D. Niederacher, S.A. Topp, E. Henisch, S. Schumacher, N. Schmitz, L.Z. Fohrdiehl, C. Vay, I. Hoffmann, N.S. Kasprowicz, P.G. Hepp, Diagnostic leukapheresis enables reliable detection of circulating tumor cells of nonmetastatic prostate cancer patients, Proc. Natl. Acad. Sci. USA 110 (41) (2013 Oct 8) 16580–16585, https://doi.org/10.1073/pnas.1313594110.

[15] T.N. Fehm, F. Meier-Stiegen, C. Driemel, B. Jager, F. Reinhardt, J. Naskou, A. Franken, H. Neubauer, R.P. Neves, G. van Dalum, E. Ruckhaberle, Diagnostic leukapheresis for CTC analysis in breast cancer patients: CTC frequency, clinical experiences and recommendations for standardized reporting, Cytometry A 93 (12) (2018 Dec) 1213–1219, https://doi.org/10.1002/cya.23669.

[16] M. Tamminga, K.C. Andreu, H. van den Bos, T.J. Hilsemmer, A. Mertink, D.C. Spierings, P. Lansdorp, W. Timens, E. Schuuring, L.W. Terstappen, H.J. Groen, Leukapheresis increases circulating tumour cell yield in non-small cell lung cancer, counts related to tumour response and survival, Br. J. Cancer 126 (3) (2022 Feb) 409–418, https://doi.org/10.1038/s41416-021-01634-0.

[17] K.C. Andreu, A. Mertink, L.L. Zonne, L.W. Terstappen, N.H. Stockeclin, R.P. Neves, C. Driemel, R. Lampignano, L. Yang, H. Neubauer, T. Fehm, Toward a real liquid biopsy in metastatic breast and prostate cancer: diagnostic Leukapheresis increases CTC yields in a European prospective multicenter study (CTCTrap), Int. J. Cancer 142 (10) (2018 Nov 15) 2584–2591, https://doi.org/10.1002/cncr.31752.

[18] A. Franken, C. Driemel, B. Behrens, F. Meier-Stiegen, V. Endris, A. Stenzinger, D. Niederacher, J.C. Fischer, N.H. Stockeclin, E. Ruckhaberle, T. Fehm, Label-free
enrichment and molecular characterization of viable circulating tumor cells from diagnostic leukapheresis products, Clin. Chem. 65 (4) (2019 Apr 1) 549–558, https://doi.org/10.1373/clinchem.2018.296814.

[19] L. Dong, C.Y. Huang, E.J. Johnson, L. Yang, R.C. Zieren, K. Horie, C.J. Kim, S. Warren, S.R. Amend, W. Xue, K.J. Pienta, High-throughput simultaneous mRNA profiling using nCounter technology demonstrates that extracellular vesicles contain different mRNA transcripts than their parental prostate cancer cells, Anal. Chem. 93 (8) (2021 Feb 17) 3717–3725, https://doi.org/10.1021/acs.analchem.0c03185.

[20] M.B. Eisen, P.T. Spellman, P.O. Brown, D. Botstein, Cluster analysis and display of genome-wide expression patterns, Proc. Natl. Acad. Sci. USA 95 (25) (1998 Dec 8) 14863–14868, https://doi.org/10.1073/pnas.95.25.14863.

[21] A. Subramanian, P. Tamayo, V.K. Mootha, S. Mukherjee, B.L. Ebert, M.A. Gillette, A. Paulovich, S.L. Pomeroy, T.R. Golub, E.S. Lander, J.P. Mesirov, Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles, Proc. Natl. Acad. Sci. USA 102 (43) (2005 Oct 25) 15545–15550, https://doi.org/10.1073/pnas.0506580102.

[22] M. Li, H. Li, Q. Chen, W. Wu, X. Chen, L. Ran, G. Si, X. Tan, A novel and robust long noncoding RNA panel to predict the prognosis of pancreatic cancer, DNA Cell Biol. 39 (7) (2020 Jul 1) 1282–1289, https://doi.org/10.1089/dna.2019.5241.

[23] B. Rupp, H. Ball, F. Wuchu, D. Nagrath, S. Nagrath, Circulating tumor cells in precision medicine: challenges and opportunities, Trends Pharmacol. Sci. 43 (5) (2022 May) 378–391, https://doi.org/10.1016/j.tips.2022.02.005.

[24] G. Theil, J. Bialek, C. Weiβ, F. Lindner, P. Fornara, Strategies for isolating and propagating circulating tumor cells in men with metastatic prostate cancer, Diagnostics 12 (2) (2022 Feb 15) 497, https://doi.org/10.3390/diagnostics12020497.

[25] O. Vermesh, A. Aalipour, T.J. Ge, Y. Saenz, Y. Guo, I.S. Alam, S.M. Park, C.N. Adelson, Y. Minnake, J. Vilches-Moure, E. Godoy, An intravascular magnetic wire for the high-throughput retrieval of circulating tumour cells in vivo, Nature biomedical engineering 2 (9) (2018 Sep) 696–705, https://doi.org/10.1038/s41551-018-0257-3.

[26] D. Zhang, Y. Zhu, Y. Jin, N.M. Kaweme, Y. Dong, Leukapheresis and hyperleukocytosis, Past and future, Int. J. Gen. Med. 14 (2021 Jul) 3457–3467, https://doi.org/10.2147/ijgm.s217877.

[27] M. Tamminga, K.C. Andree, T.J. Hillemann, M. Jayat, E. Schuuring, H. van den Bos, D.C. Spierings, P.M. Lansdorp, W. Timena, L.W. Terzappen, H.J. Groen, Detection of circulating tumor cells in the diagnostic leukapheresis product of non-small-cell lung cancer patients comparing cellsearch® and ISET, Cancers 12 (4) (2020 Apr) 896, https://doi.org/10.3390/cancers12040896.

[28] J. Wu, R. Baha, R. Guglielmi, B. Behrens, G. Van Dulum, G. Flüggen, A. Koch, S. Patel, W.T. Knoefel, N.H. Stoecklein, R.P. Neves, Magnetic-based enrichment of rare cells from high concentrated blood samples, Cancers 12 (4) (2020 Apr) 933, https://doi.org/10.3390/cancers12040933.

[29] M. Peralta, N. Osman, J.G. Goetz, Circulating tumor cells: towards mechanical phenotyping of metastasis, iScience (2022 Feb 22), 103969, https://doi.org/10.1016/j.isci.2022.103969.

[30] F. Reinhardt, A. Franken, F. Meier-Stiegen, C. Driemel, N.H. Stoecklein, J.C. Fischer, D. Niederacher, E. Rückgabeberle, T. Fehm, H. Neubauer, Diagnostic leukapheresis enables reliable transcriptomic profiling of single circulating tumor cells to characterize inter-cellular heterogeneity in terms of endocrine resistance, Cancers 11 (7) (2019 Jul) 903, https://doi.org/10.3390/cancers11070903.

[31] M.B. Lambros, G. Seed, S. Sumanasuriya, V. Gil, M. Crespo, M. Fontes, R. Chandler, N. Mehra, G. Fowler, B. Ebbs, P. Flohr, Single-cell analyses of prostate cancer liquid biopsies acquired by apheresis, Clin. Cancer Res. 24 (22) (2018 Nov 15) 5625–5644, https://doi.org/10.1158/1078-0432.ccr-18-0862.

[32] H.F. Tsang, V.W. Xue, S.P. Koh, Y.M. Chiu, L.P. Ng, S.C. Wong, NanoString, a novel digital color-coded barcode technology: current and future applications in molecular diagnostics, Expert Rev. Mol. Diagn. 17 (1) (2017 Jan 2) 95–103, https://doi.org/10.1080/14737559.2017.1266533.