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Improved enrichment of circulating tumor cells from diagnostic leukapheresis product

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Abstract

The median number of circulating tumor cells (CTCs) detected in 7.5 mL of peripheral blood by CellSearch (PB-CS) in patients with metastatic prostate cancer is in the order of 1–10, which means many samples have insufficient tumor cells for comprehensive characterization. A significant increase is obtained through diagnostic leukapheresis (DLA), however, only 2%–3% of the DLA product can be processed per CellSearch test, limiting the gain. We processed aliquots from 30 DLA products of metastatic prostate cancer patients consisting of 0.2×10^9 leukocytes using CellSearch (DLA-CS) as well as the newly introduced reduced enrichment reagent protocol (RER), which uses 10-fold less enrichment reagents than DLA-CS. The number of tumor cells and the total number of captured cells were determined using the CellTracks Analyzer. Additionally, for six DLA samples, a 1.0×10^9 leukocyte aliquot was processed (RER+), using twofold less enrichment reagents than DLA-CS. A median 2.7-fold reduction in leukocyte co-enrichment was found between DLA-CS and RER methods without any loss in tumor cell recovery (Wilcoxon Signed Ranks Test, $p = 0.953$). Using 1.0×10^9 leukocyte aliquots a fourfold increase in tumor cells was found compared to DLA-CS and a 19-fold increase compared to PB-CS was obtained. The here-introduced RER protocol results in a higher final sample purity without any loss in tumor cell recovery while using 10-fold less CellSearch capture reagent. With this improved method, 26% of the leukapheresis sample can now be processed using reagents from a single CellSearch test, enabling the obtainment of a sufficient number of CTCs for comprehensive characterization in most metastatic prostate cancer patients.

KEYWORDS
circulating tumor cells, diagnostic leukapheresis, magnetic enrichment, reagent reduction

1 | INTRODUCTION

The enumeration of circulating tumor cells (CTCs) from blood can be used for disease prognosis [1, 2], treatment outcome [3], and disease relapse prediction [4]. In most cases, immunomagnetic enrichment is employed to enrich the CTCs from hematopoietic cells. The most prominent example of enrichment methods is the FDA-cleared CellSearch system, which was designed to enrich EpCAM positive CTCs

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from 7.5 mL blood samples [1, 5]. However, in many patients with metastatic disease, the number of CTCs found in a standard 7.5 mL blood sample is too low for tumor cell characterization while in patients with non-metastatic disease, the sensitivity and specificity are insufficient to determine the presence of disseminated cancer cells [6].

Several possibilities exist to increase the number of CTCs, such as capturing both the EpCAM positive as well as the EpCAM negative fraction of CTCs. However, the capture of EpCAM-negative CTCs is hampered by a lack of markers while also the prognostic value of these additional CTCs remains unclear [7–9]. Another option is to draw blood closer to the tumor, for example, from tumor-draining veins. Although more CTCs can be recovered this way, it is an invasive procedure normally only possible during surgery [4], and not necessarily relevant or feasible in the metastatic setting. A third option is to increase the evaluated blood volume, for example, by an in-vivo capture system [10, 11] or through diagnostic leukapheresis (DLA) [12].

In a DLA procedure, continuous density separation is employed to harvest the mononuclear cells (MNCs), while most of the other blood components, such as plasma, erythrocytes, platelets, neutrophils, basophils, and eosinophils are returned to the patient. As CTCs have a similar density as MNCs [13], these are co-captured in the procedure. In a regular leukapheresis procedure, more than five liters of blood are typically processed, whereas DLA is a shortened procedure, to minimize the burden on patients, in which only two to five liters are processed. The resulting samples can then be processed using the CellSearch system. Here aliquots of only 0.2×10^9 white blood cells (WBC) are processed to ensure the sample can be analyzed [13–15]. These 0.2×10^9 aliquots only constitute 2%–3% of the collected DLA sample.

In the processing of DLA material using CellSearch, the number of nucleated cells that are non-specifically enriched and end up in the analysis cartridge limits the amount of DLA sample that can be processed per test. The processing of larger DLA aliquots frequently leads to excessively dense analysis cartridges, making identification of CTCs impossible. In this regard, the sample processing capability of the CellSearch system is underutilized, something that could be addressed by using a different imaging platform or by dividing the sample over several CellSearch cartridges. Another perspective is that the current methodology uses too much reagent per test.

As the CellSearch system was developed for the processing of 7.5 mL of whole blood, the initial enrichment takes place in a 10 mL volume. However, the volume taken up by cells in a DLA sample is much lower compared to blood, due to a large reduction of erythrocytes [15]. With this in mind, we established a reduced enrichment reagent protocol (RER) using standard CellSearch reagents and validated this by the processing of DLA samples obtained from prostate cancer patients. We compared this approach to the processing of peripheral blood with CellSearch (PB-CS), the previously reported DLA sample processing with CellSearch (DLA-CS), and an expanded version of the RER protocol (RER+) that enriches CTCs from 1.0×10^9 WBC instead of 0.2×10^9 WBC.

2 | RESULTS

2.1 | Cell volume in blood and DLA product

To assess whether increasing the concentration of WBC in the DLA product before enrichment resulted in a more concentrated sample compared to peripheral blood, we calculated the number and volume composition for both sample types based on differential blood counts from the used PB and DLA samples. Results are shown in Figure 1.

Mainly due to the large reduction in erythrocytes, the number of cells and platelets per mL during magnetic particle incubation in DLA-CS is on average only 7% (median 7%, range 4%–9%) of that in PB-CS. In the RER protocol, the DLA is concentrated, resulting in the

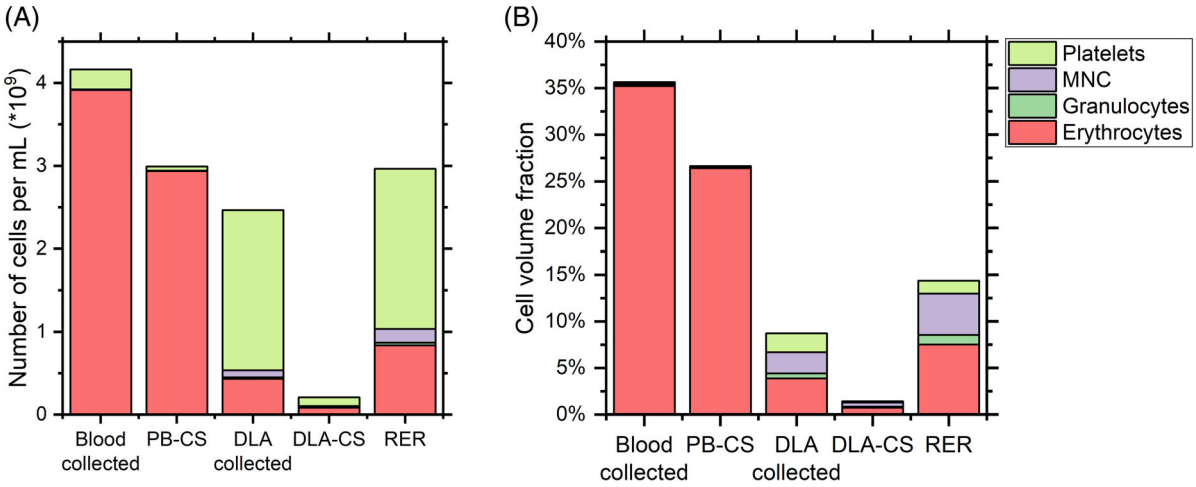

FIGURE 1 (A) Average number of cells and platelets per mL and (B) percentage of the volume consisting of cells and platelets in blood and diagnostic leukapheresis (DLA) as collected as well as during magnetic particle incubation in peripheral blood by CellSearch (PB-CS), DLA-CS, and reduced enrichment reagent protocol (RER) procedures, showing the RER processing to be more comparable to PB-CS. [Color figure can be viewed at wileyonlinelibrary.com]

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number of cells and platelets per mL to be about equal to that in PB-CS (average 98%, median 96%, range 59%–131%).

In the same way, the percentage of volume taken up by cells and platelets (volume fraction) in PB-CS was on average 26% (median 26%, range 20%–32%), and for the DLA-CS procedure on average 1.5% (median 1.4%, range 1.1%–2.2%). In the RER procedure, in RER, the reduction of the reaction volume by 10-fold increases the cell volume fraction by approximately the same factor compared to DLA-CS, resulting in a volume fraction of 16% (median 14%, range 11%–23%), which is much closer to, but still below the volume fraction in PB-CS.

The number of cells and platelets per mL in the RER procedure is similar to that in PB-CS, while the cell volume concentration is below that of PB-CS. As the concentration of capture reagents in the assay is the same for all three procedures, the capture reagent per cell is approximately the same for PB-CS and RER, and ~12-fold higher in DLA-CS. The capture reagent per cell volume is compared to PB-CS ~twofold higher in the RER protocol, and ~10-fold higher in DLA-CS. This decrease in capture reagent per cell is expect to lead to a decrease in WBC co-enrichment, while sufficient particles for specific binding are expect to be present in the RER procedure to facilitate efficient enrichment of the CTCs.

2.2 | CTC recovery

Aliquots of 30 DLA samples were processed using DLA-CS and RER protocols, together with peripheral blood samples using the PB-CS protocol. In Figure 2A the number of recovered CTCs after DLA-CS and RER processing are compared, in Table S1 the number of CTCs detected with each method for all patients can be found. Linear regression was performed on log-transformed data to be robust against outliers. This resulted in a regression of $\log_{10}(CTC_{RER}) = -0.036 + 0.977 \log_{10}(CTC_{DLA\text{-}CS})$ with an R^2 of 0.95. Using the non-parametric Wilcoxon Signed Ranks Test the paired samples did not show a significant difference ($p = 0.957$), indicating the RER procedure has similar CTC recovery as the DLA-CS procedure. To make a first step toward processing the entire DLA sample, we evaluated the use of RER adjusted for a fivefold larger sample size (RER+) using six out of the 30 patient samples. The number of CTCs found in all samples with the different enrichment methods is shown in Figure 2B.

The DLA-CS of an 0.2×10^9 WBC aliquot leads to a median 5.6-fold (mean 5.6, range 1.4–14.6-fold) increase in CTCs compared to the 7.5 mL whole blood PB-CS (Wilcoxon Signed Ranks test, $p < 0.001$). RER leads to a similar increase (median 4.4, mean 5.5, range 2–18.6-fold, $p < 0.001$). In the six samples for which also the RER+ protocol was performed, the fivefold increase in sample input led to a 3.6-fold median increase in CTCs compared to DLA-CS, or a total median increase in CTC of 19.3-fold (mean 23.2, range 4.3–50.1-fold) compared to PB-CS. The lower increase seen in the number of CTC compared to the increase in input could be a result of the larger tube used, resulting in a lower magnetic force, or simply be due to the small sample size.

2.3 | WBC carry-over

We expected the lower amount of reagents to result in a lower non-specific binding of magnetic particles, and therefore a lower non-specific cell capture. To examine this, we evaluated the total number of cells present in the enriched samples, as shown in Figure 3. Although we expected the total number of cells to be sample dependent, we find as shown in Figure 3A that the total number of cells in the enriched sample when using DLA-CS is not predictive of the total number of total cells found using RER, as there is no correlation ($R^2 = 0.003$) in the number of cells between the two methods, with a linear regression of $\log_{10}(cells_{RER}) = 4.336 + 0.056 \log_{10}(cells_{DLA\text{-}CS})$. We also observed no correlation between the number of CTCs and total cell number when comparing DLA-CS and RER, see Figure S2.

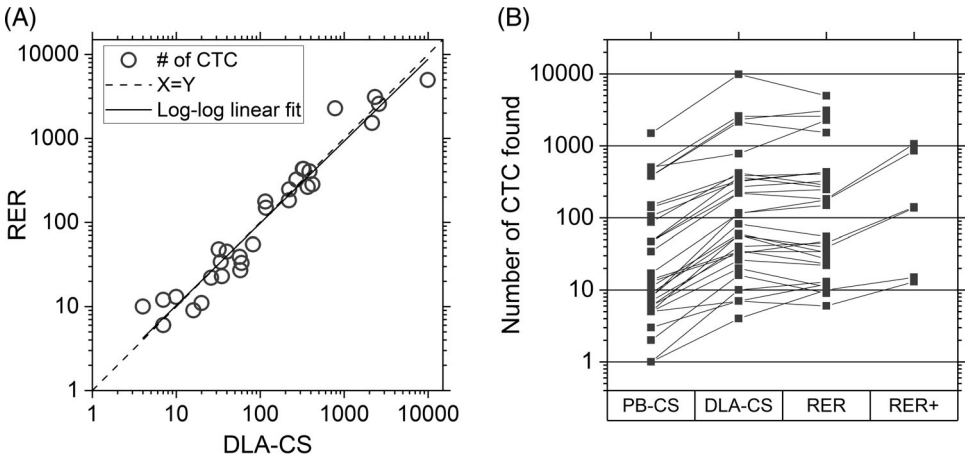

FIGURE 2 (A) Comparison of circulating tumor cell (CTC) recovery between diagnostic leukapheresis sample processing with CellSearch (DLA-CS) and reduced enrichment reagent protocol (RER) procedures using 0.2×10^9 WBC from 30 DLA samples of metastatic prostate cancer patients and (B) number of CTCs detected in 30 peripheral blood and regular DLA samples using CellSearch, RER, and RER+ (6 samples) procedures, showing an increase in CTCs when moving from PB-CS to DLA-CS or RER and a further increase in CTCs when moving to RER+.

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The total number of cells (Figure 3B) showed a median 26.1-fold (mean 51.6, range 0.03–288.4-fold) increase when moving from PB-CS to DLA-CS. Comparing DLA-CS to RER a median reduction in total cells of 2.7-fold was observed (mean 6.6, range 0.2–62.7-fold, Wilcoxon Signed Ranks test, $p < 0.001$). To place this decrease into perspective, a gallery showing image examples from cartridges containing different numbers of cells after enrichment is shown in Figure S1. Here also the presence of disrupted cells likely due to necrosis or shear stress as well as the clumps of cells often seen after the magnetic enrichment of DLA material can be seen.

Comparing RER to RER+ for the six samples where both were performed, a median increase of 5.2-fold was observed (mean 8.6, range 0.75–30.3-fold), which is in line with the fivefold larger number of cells processed as well as the fivefold larger reagent volume used. Most of the RER+ samples contain too many cells to be imaged when placed into a single CellSearch cartridge.

2.4 | Purity and relative recovery

As the number of CTCs remained similar while the total number of cells in the final sample decreased, the resulting purity of RER samples was improved compared to DLA-CS samples. In Figure 4A the sample purity of all matched blood and DLA samples processed with CellSearch, RER, and RER+ are shown. It can be seen that although the number of CTCs increased, the purity of the resulting samples when processing DLA using CellSearch was reduced compared to blood (Wilcoxon Signed Ranks test, $p < 0.001$). However, when using RER a

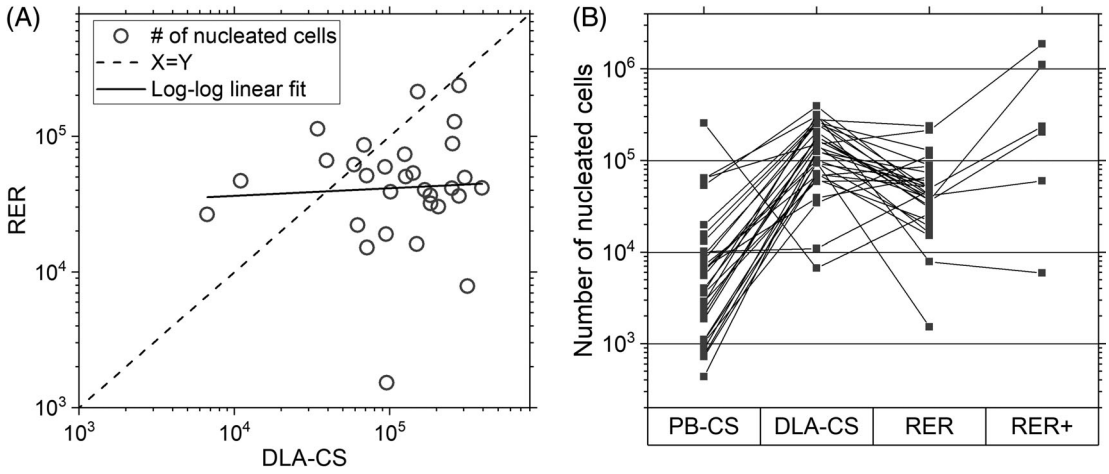

FIGURE 3 (A) Comparison of the total number of cells after processing of diagnostic leukapheresis (DLA) samples with DLA-CS and reduced enrichment reagent protocol (RER) protocols for 30 leukapheresis samples of prostate cancer patients and (B) the total number of cells after processing whole blood and DLA samples using CellSearch, RER, and RER+ (6 samples) protocols, showing a decrease in co-enriched WBC in the RER method compared to DLA-CS.

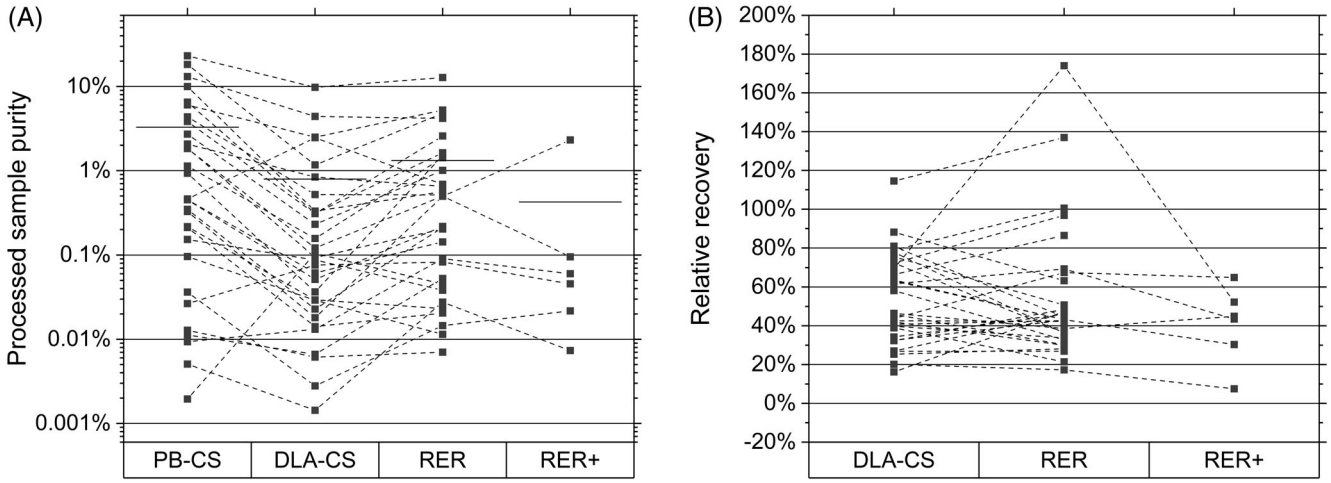

FIGURE 4 (A) Processed sample purity and (B) relative circulating tumor cell (CTC) recovery compared to blood. Results represent final samples as found after enrichment of 7.5 mL of blood, 0.2×10^9 or 1.0×10^9 WBC from diagnostic leukapheresis (DLA) and processed using CellSearch, reduced enrichment reagent protocol (RER), or RER+, showing increases purity for RER compared to DLA-CS.

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median 2.2-fold improvement (mean 5.8, range 0.3–41.2-fold, Wilcoxon Signed Ranks test, $p = 0.004$) in sample purity compared to DLA-CS was found.

In Figure 4B it can be seen that both the DLA-CS (mean 53%, median 46%) and RER (mean 54%, median 43%) resulted in similar recovery compared to PB-CS, which indicates a loss of CTCs in the DLA procedure. The increase to 1.0×10^9 WBC in RER+ seems to show a small reduction in relative recovery (mean 41%, median 44%), which might also be a result of the small sample size.

3 | DISCUSSION

The standard CellSearch test was developed to detect CTCs in 7.5 mL of peripheral blood. In the majority of metastatic cancer patients, the number of CTCs is insufficient for tumor characterization, hence larger blood volumes are needed [6]. DLA typically collects CTCs from 1 to 5 liters of blood [15], allowing also the detection of CTCs in a larger portion of non-metastatic cancer patients [12]. The concentration of CTCs per tube of blood does not significantly decrease after the DLA procedure suggesting a fast replenishment. This indicates that processing multiple passages of the complete blood volume can lead to a further increase in harvested CTCs [16]. Adaptation of the CellSearch peripheral blood test to DLA resulted in a DLA-CS protocol that uses aliquots of 0.2×10^9 leukocytes [12–15] meaning that in our sample set, the DLA product of on average 112 mL blood could be processed per CellSearch test. Although one could perform >30 DLA-CS tests to process the complete DLA product, it would be cost and time prohibitive. The major limitation to processing larger volumes was the large number of leukocytes remaining after enrichment [14]. Together with the presence of clumped and broken cells, this high background impedes efficient identification and subsequent interrogation of CTCs [12, 17].

Here, we introduced the novel RER protocol, which is comparable to or better than the DLA-CS protocol while using only 10% of the enrichment reagents. The RER+ protocol uses 50% of the enrichment reagents to process a five times larger sample, thereby overcoming the cost and time restrictions of the DLA-CS procedure.

The main difference between the DLA-CS and RER protocol lies in the reduced reagent volume, but there are other differences as well. CellSearch is semi-automated and RER is a manual procedure, causing a higher operator dependence and likely more variability. The magnetic incubation in DLA-CS is eight times 3 min, and in RER three times 10 min magnetic incubation is used. In both the DLA-CS and RER protocols, staining is done using the same reagents at the same concentrations. However, RER uses a smaller staining volume and staining is performed at 37°C. In the DLA-CS protocol, the system removes a portion of the unbound magnetic particles before the sample is transferred to the cartridge. The reason for this is that these hinder the imaging and identification of CTCs. In the RER protocol, this step is not necessary because there are much fewer ferrofluid particles present.

The lower number of total cells in the enriched samples when using the RER protocol is likely due to the lower amount of ferrofluid used. Surprisingly, there is no correlation seen in the total number of cells captured in DLA-CS and RER. A possible reason for this could be a threshold effect: By using a reduced reagent volume, in samples where a high number of healthy cells is able to bind magnetic particles non-specifically, the reduced number of magnetic particles causes many of these cells to bind insufficient particles to be retained during separation. The specific antibody–antigen binding of the particles would in these cases outcompete the non-specific interaction, resulting in a retained binding efficiency for CTCs. If this is the case, an even lower reagent volume may further reduce non-specific capture without a loss of capture efficiency. As a too-high sample concentration or too-low magnetic particle concentration will inevitably lead to a reduction in CTC recovery, further optimization is needed.

To access the potential of DLA, the entire sample will need to be processed. The RER protocol demonstrates the possibility of enriching CTCs from aliquots of 0.2×10^9 WBC using only 10% of the CellSearch enrichment reagents while obtaining a comparable number of CTCs. At the same time, the amount of non-specifically enriched cells is significantly lowered, resulting in a better final sample purity. A proof of principle using six patient samples showed that this method also allows a five times larger DLA sample to be processed using only half of the standard CellSearch reagents, with only a slight reduction in recovery compared to a 0.2×10^9 WBC sample. As we have used less than 50% of the regular amount of reagents for the staining, with this approach a total sample of 2.0×10^9 WBC could potentially be processed using a single CellSearch test, representing 26% of the leukapheresis sample or the leukapheresis product of 1.1 liter of blood. To analyze such a sample, a single CellSearch cartridge will not suffice. In most cases, either multiple cartridges will be needed, or sample imaging needs to be migrated to another system in which the cells can be distributed over a larger surface, such as an entire glass slide. For applications in which (initial) identification of all CTCs is not needed, such as CTC culture or generation of patient-derived xenografts, this is not an issue [15]. In these cases, only a portion of the resulting sample could be stained and imaged to estimate the total number of CTCs.

Using the here presented RER and RER+ protocols it has become possible to cost and time efficiently obtain a sufficient number of CTCs for comprehensive characterization in most metastatic prostate cancer patients. This opens up the possibility of processing a much larger DLA sample in a cost-effective manner.

4 | CONCLUSIONS

The newly presented RER protocol can be used for the processing of standard leukapheresis aliquots using 10-fold less CellSearch capture reagents, without any loss in CTC recovery, but with a 2.7-fold decrease in leukocyte co-enrichment, thereby realizing a higher sample purity. The RER+ method allows for enrichment of CTCs out of

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the leukapheresis product obtained from more than 1 L of blood, using the reagents from a single CellSearch test. This new way of processing DLA samples for CTC enrichment allows for the obtainment of sufficient CTCs for comprehensive characterization in most metastatic prostate cancer patients and is thereby the next step in realizing the full potential of DLA.

5 | MATERIALS AND METHODS

5.1 | Patient samples

DLA samples were obtained from 28 metastatic hormone sensitive prostate cancer patients before initiation of treatment and with >2 CTCs in a 7.5 mL sample of blood. In two cases, patients underwent a second leukapheresis procedure after becoming hormone-resistant resulting in a total of 30 samples. Leukapheresis was performed per the optimized procedure described by Mout et al. [18] on a Spectra Optia (Terumo, Lakewood, United States). Samples were collected in accordance with the Declaration of Helsinki as part of a study approved by the medical ethical committee of the Erasmus Medical Center (PICTURES study [MEC20-0422]).

5.2 | Relative recovery, cell concentration, and cell volume calculation

To compare the CTC recovery from DLA to that found in PB-CS (CTC_{PB}), the expected number of CTCs (CTC_{exp}) was calculated using Equation (1)

$$CTC_{exp} = \frac{MNC_{DLA}}{MNC_{PB}} \times CTC_{PB} \quad (1)$$

where MNC_{DLA} and MNC_{PB} are the total number of MNCs in the processed DLA aliquot and PB-CS sample respectively.

During a DLA procedure, the MNC population is targeted for extraction. The sample however contains impurities; some erythrocytes, granulocytes, and platelets are co-captured during the procedure. To compare the cell density in RER to that of PB-CS and DLA-CS, we calculated the total number of cells per mL as well as what percentage of the volume is taken up by cells (volume fraction) at the time of magnetic particle incubation.

For this calculation we have represented the different cell types as having a volume of: erythrocytes 90 pL, lymphocytes 187 pL, monocytes 413 pL, neutrophils 299 pL, eosinophils 344 pL, basophils 344 pL, and platelets 10.5 pL [19–22]. In our calculation we accounted for the following volume changes: Before PB-CS and DLA-CS the sample is diluted to 14 mL and centrifuged. The AutoPrep then aspirates the diluted plasma and some of the platelets, leaving about 4 mL. This sample is diluted again with 6 mL of CellSearch dilution buffer before the magnetic particles are added [23]. In RER processing, the DLA samples are concentrated by the removal of plasma to reach a concentration of 0.2×10^9 WBC/mL.

Differential blood counts of DLA and whole blood samples were taken on the same day and determined on a DxH 500 hematology analyzer (Beckman Coulter, Utrecht, The Netherlands). The number of blood cells as well as their total volume per mL during magnetic particle incubation was then calculated for the blood and DLA product during PB-CS, DLA-CS, and RER processing.

5.3 | CellSearch processing

Patient blood samples were stored in CellSave vacutainers (Menarini-Silicon Biosystems, Bologna, Italy) and processed using the CellSearch system according to the manufacturer's instructions using the CTC-kit (Menarini-Silicon Biosystems). DLA samples were stored in CellSave vacutainers in 10 mL aliquots and shipped overnight to the University of Twente. For DLA samples, an aliquot containing 0.2×10^9 WBC was placed in a 15 mL conical tube (Menarini-Silicon Biosystems) and processed on the CellSearch Autoprep according to the manufacturer's instructions whenever possible using the CTC-kit (https://documents.cellsearchctc.com/). All samples were processed within 48 h after collection.

5.4 | Reduced enrichment reagent procedures

5.4.1 | Enrichment

For the RER procedure, an aliquot containing 0.2×10^9 WBC was placed into a 12 × 75 mm 4.5 mL centrifuge tube (Greiner bio, Alphen aan de Rijn, The Netherlands) and centrifuged at 400 RCF for 5 min. Subsequently, the supernatant was aspirated until 1 mL remained. The sample was then incubated with 15 µL CellSearch ferrofluid for 10 min in a magnet (iMag, Becton Dickinson, San Jose, CA, United States) after which 15 µL CellSearch capture enhancement reagent was added. The sample was mixed and incubated twice more for 10 min in the magnet, mixing again after each incubation. Next, the sample was supplemented with 2 mL "Cell buffer" (phosphate buffered saline (Merck, Darmstadt, Germany) supplemented with bovine serum albumin (Merck), EDTA (Merck), casein (Merck) and mouse serum (Invitrogen, Carlsbad, United States)) and placed in the magnet for 20 min after which the unbound fraction was aspirated using a glass Pasteur pipet and syringe pump set to 1 mL/min. The bound fraction was resuspended in 1 mL of Cell buffer before performing a second separation of 10 min in the magnet.

For the RER+ procedure, samples containing 1.0×10^9 WBC were first incubated with 20 µg/mL DNase I (Roche, Basel, Switzerland) together with 20 µM $MgSO_4$ to prevent aggregation. The enrichment was performed analogously to the RER procedure. However, as the RER+ protocol uses a five times larger sample input, all volumes up to the separation step in Figure 5 were multiplied by

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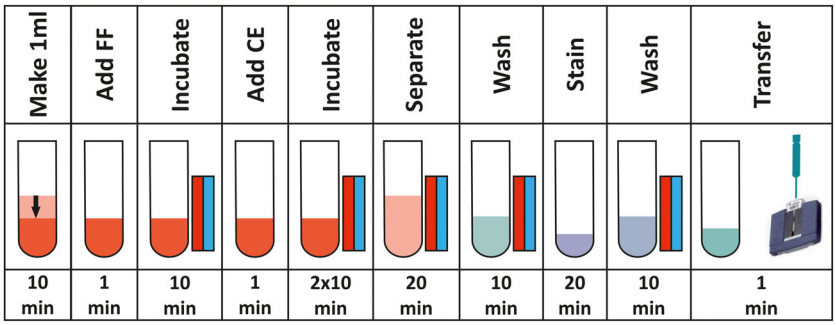

FIGURE 5 Schematic representation of the reduced enrichment reagent protocol (RER) procedure to process diagnostic leukapheresis (DLA) aliquots. The bottom row indicates the time for each step. The red/blue rectangle represents the BD iMag. CE, capture enhancement reagent; FF, ferrofluid. [Color figure can be viewed at wileyonlinelibrary.com]

5, and larger consumables were used where needed. Specifically, aspirating supernatant to 5 mL in a 12 mL round bottom tube (Greiner bio), and using 75 µL CellSearch ferrofluid and 75 µL CellSearch capture enhancement. RER+ samples were transferred to 12 × 75 mm centrifuge tubes after the initial separation and processed further using the standard RER protocol. Staining of RER and RER+ samples was performed identically.

5.4.2 | Staining

After aspiration of the unbound fraction the sample was resuspended in 300 µL staining solution, consisting of 50 µL permeabilization reagent, 50 µL nuclear stain, and 50 µL staining reagent, supplemented with 150 µL Cell buffer. All staining reagents were taken from a CellSearch CTC kit. The sample was incubated at 37°C for 20 min, after which 700 µL Cell buffer was added and the sample was placed in the magnet for 10 min. The unbound fraction was aspirated and the bound fraction was resuspended in 150 µL CellSearch fixation reagent supplemented with 175 µL phosphate-buffered saline. The sample was then manually transferred to a CellSearch sample cartridge using a 230 mm glass Pasteur pipet pre-coated with bovine serum albumin, and placed into a CellSearch Magnest.

A schematic representation of the 0.2×10^9 WBC RER procedure steps and times is shown in Figure 5.

5.5 | Enumeration of CTCs and total cell number

PB-CS, DLA-CS, RER, and RER+ samples were scanned using the CellTracks Analyzer II. The number of CTCs was determined using the standard CellTracks Analyzer II software, where two reviewers reached a consensus score. To minimize inter-reviewer variability, the number of CTCs in each pair of samples was scored by the same reviewers. The total number of cells in each cartridge was determined by processing the CellTracks Analyzer archives using StarDist segmentation [17] followed by a deep learning approach [24].

5.6 | Statistics

To make the linear regression robust against the large spread in the number of CTCs as well as the total number of cells, both counts were log-transformed before regression. Because CTC counts are not normally distributed, we used the non-parametric Wilcoxon Signed Ranks Test to compare the number of CTCs as well as the total number of cells enriched using each method. All tests were performed using Origin 2019b (OriginLab Corporation, Northampton, MA, United States).

AUTHOR CONTRIBUTIONS

Michiel Stevens: Conceptualization; methodology; investigation; writing – original draft; writing – review and editing; visualization. **Anouk Mentink:** Investigation; writing – review and editing. **Afroditi Nanou:** Writing – review and editing; investigation. **Frank Coumans AW:** Software; writing – review and editing. **Khrystany Isebia T:** Resources; writing – review and editing; investigation. **Jaco Kraan:** Resources; writing – review and editing; investigation. **Paul Hamberg:** Resources; writing – review and editing. **John Martens WM:** Supervision; writing – review and editing; funding acquisition. **Leon WMM Terstappen:** Funding acquisition; methodology; writing – review and editing; supervision.

DATA AVAILABILITY STATEMENT

All original data is available upon reasonable request.

INFORMED CONSENT STATEMENT

Written informed consent was obtained before any study procedures were performed.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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