Original Research

Generating human prostate cancer organoids from leukapheresis enriched circulating tumour cells

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Abstract Background: Circulating tumour cell (CTC)—derived organoids have the potential to provide a powerful tool for personalised cancer therapy but are restrained by low CTC numbers provided by blood samples. Here, we used diagnostic leukapheresis (DLA) to enrich CTCs from patients with metastatic prostate cancer (mPCa) and explored whether organoids provide a platform for \textit{ex vivo} treatment modelling.

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1. Introduction

Translational studies on advanced prostate cancer (PCa) have often been limited to static sources, such as resection of the primary tumour or biopsies [1–4]. However, cancer is an ever moving target, as dynamic evolution drives spatial and temporal heterogeneity allowing tumours to adapt and escape therapeutic interventions. We therefore require new methods that provide real-time insights into evolving cancer biology for treatment tailoring. Circulating tumour cells (CTCs) could serve as a dynamic tumour source which captures the genetic and phenotypic heterogeneity of cancer and can be obtained at multiple time points during the disease course to assess clinical progression [5]. In addition, CTCs can be obtained from peripheral blood in a relatively non-invasive manner, thus providing an easily accessible source of metastatic cells as an alternative to tumour biopsies. This is particularly beneficial for patients with primarily bone metastasis such as in metastatic PCa (mPCa). In mPCa, CTC numbers have already been shown to harbour significant prognostic and predictive value [6–10]. The possibility to obtain viable CTCs also provides the opportunity to propagate CTCs ex vivo. The development of cancer organoids has allowed us to better capture the tumour-specific characteristics than standard 2D culture methods [11]. Together, this provides the opportunity to use CTC derived organoids as a representative model of the current disease status and use for drug discovery and sensitivity-screening [12]. PCa CTCs can be cultured as organoids, although the reports thus far suggest a very low efficiency rate for success [13,14]. Previous reports suggest that one important obstacle is the high number of CTCs needed to initiate organoid propagation [13,15]. Because the median CTC count in patients with mPCa is 2–20 CTCs per 7.5 mL of blood [10], ex vivo expansion of CTCs is unlikely to be successful in most patients. Diagnostic leukapheresis (DLA) is a standardised procedure to enrich for mononuclear cells by continuous centrifugation of blood. Because CTCs have a similar density to mononuclear cells, they are enriched as well [16]. Importantly, DLA is a minimally invasive and generally safe procedure that is well tolerated by patients [17]. Within this prospective study we set out to isolate CTCs from patients with mPCa by DLA. We hypothesised that the increased number of CTCs obtained by DLA, will allow us to culture CTC derived organoids and potentially provide a platform for individualised disease modelling. Using optimised methods we validated DLA as a feasible and safe method to enrich for CTCs in patients with mPCa. CTCs could be propagated as short-term organoid cultures in 35% of the samples, from which we could obtain one stable organoid cell line. These short-term organoid cultures expressed the classical markers of PCa and maintained genomic variants previously identified in metastatic samples. Overall, our study provides an important step forward in implementing CTCs in individualised disease modelling, nevertheless identifies several challenges that require further optimisation.

2. Material and methods

Patients with mPCa were included and selected based on the presence of ≥5 per 7.5 mL of blood. The study was conducted in accordance with the Declaration of Helsinki and was approved by the medical ethical committee of the Erasmus Medical Center Rotterdam (EMC16-449), full exclusion and inclusion criteria are

Methods: We prospectively screened 102 patients with mPCa and performed DLA in 40 patients with ≥5 CTCs/7.5 mL blood. We enriched CTCs from DLA using white blood cell (WBC) depletion alone or combined with EpCAM selection. The enriched CTC samples were cultured in 3D to obtain organoids and used for downstream analyses.

Results: The DLA procedure resulted in a median yield of 5312 CTCs as compared with 22 CTCs in 7.5 mL of blood. Using WBC depletion, we recovered 46% of the CTCs, which reduced to 12% with subsequent EpCAM selection. From the isolated and enriched CTC samples, organoid expansion succeeded in 35%. Successful organoid cultures contained significantly higher CTC numbers at initiation. Moreover, we performed treatment modelling in one organoid cell line and identified substantial tumour heterogeneity in CTCs using single cell DNA sequencing.

Conclusions: DLA is an efficient method to enrich CTCs, although the modest success rate of culturing CTCs precludes large scale clinical application. Our data do suggest that DLA and subsequent processing provides a rich source of viable tumour cells. Therefore, DLA offers a promising alternative to biopsy procedures to obtain sufficient number of tumour cells to study sequential samples in patients with mPCa.

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described in the supplementary material and methods. DLA was performed using the standard settings, only the plasma pump rate was adjusted. CTCs from DLA were enriched by negative depletion of white blood cell (WBC) using RosetteSep™ CTC Human CD45 Depletion Cocktail (STEMCELL Technologies, Vancouver, British Columbia, Canada), with or without subsequent manual positive immunomagnetic enrichment using CellSearch EpCAM ferrofluids. The enriched DLA samples were cultured under optimised conditions to obtain PCa organoids.

3. Results

3.1. Patient characteristics and CTC screening

We screened 102 patients with mPca for eligibility and selected 45 patients who had ≥5 CTC in 7.5 mL of peripheral blood (PB) for CTC collection by DLA (Fig. 1A). Patients with metastatic castration-resistant PCa (mCRPC) screened for study participation tended to have a higher CTC burden than those patients with metastatic hormone-sensitive PCa (mHSPC) (median of
We were able to effectively process a median of 25.5 mL WBCs. Using the autologous RBCs from 40 mL of PB, we achieved an optimal WBC:RBC ratio for depletion of 1825 CTC/7.5 mL vs 1 resp. P < 0.001, supplementary figure 1A). Moreover, 50% of the patients with mCRPC had 5 or more CTCs, whereas 39% of the patients with mHSPC were included. The overall median CTC count of the included patient population was 22 CTCs per 7.5 mL PB prior to DLA, with 10 CTCs/7.5 mL and 26 CTCs/7.5 mL for patients with mHSPC and mCRPC, respectively (Fig. 1B). The CTC burden was monitored in 24 patients and was found to remain stable in between screening, before the DLA and after completion of the DLA procedure (P = 0.37, supplementary figure 1B).

3.2. Diagnostic leukapheresis

After initial screening, a total of 40 patients successfully underwent DLA, as four patients withdrew and the DLA procedure was terminated in one patient because of an adverse event (grade III vasovagal reaction directly after start of the procedure). No other grade ≥III adverse events were observed. The baseline characteristics of the patient population and DLA procedure are described in Table 1. Four patients were included twice at separate time points during their treatment course, resulting in serial samples for subjects 9, 11, 24 and 22 (annotated as e.g. 9-I/II). We examined the impact of DLA density settings on CTC enrichment in four patients, by performing the DLA procedure in two fractions of both 5L blood at 2% and 5% haematocrit, respectively. As there was a trend towards higher CTC yield when DLA was performed at 5% haematocrit (Supplementary figure 1C, P = 0.125), we continued the DLA procedure using high DLA density settings and processed 5L of total blood volume, which limited the procedure time to approximately 2 h. The median CTC concentration of the DLA product was 64/mL compared to 2.5/mL in PB samples pre-DLA (P < 0.001), with an estimated median yield of 5312 CTCs in 96 mL of DLA product (Fig. 1C). Using these DLA settings, we were able to retrieve a median of 36% of the estimated CTCs available given the processed blood volume and the CTC count in PB (Supplementary figure 1D).

3.3. CTC enrichment from DLA material

We compared two methods to enrich and isolate CTCs from the DLA product: depletion of WBCs using the RosetteSep™ method, with or without subsequent positive selection using EpCAM directed antibodies. For the RosetteSep™ method to function, WBCs are cross-linked with RBCs leading to erythrocyte rosetting of WBC allowing for gradient separation. Because the DLA product contains a high WBC and relative low RBC concentration[21], we needed to add RBCs to achieve an optimal WBC:RBC ratio for depletion of WBCs. Using the autologous RBCs from 40 mL of PB, we were able to effectively process a median of 25.5 mL out of the 96 mL DLA obtained. We compared the impact of WBC depletion alone and subsequent EpCAM enrichment using twelve paired samples (Supplementary Fig. 2). WBC depletion alone was found to reduce the WBC concentration from 93.6*10^6/mL to 0.12*10^6/mL, resulting in a WBC depletion factor of 3.1 log_{10}-fold. With subsequent EpCAM enrichment of CTCs, we further reduced the WBC concentration to 4.1 log_{10}-fold. However, this was at expense of a substantial CTC loss, as the median CTC recovery reduced from 54% with WBC depletion alone to 11.5% with additional EpCAM selection (P < 0.001). We therefore chose to use WBC depletion alone for the

| N = 37 | Patient characteristics |
|--------|------------------------|
| Age, years (median, range) | 70 (49–83) |
| WHO status at registration | | |
| 0 | 12 |
| 1 | 23 |
| 2 | 2 |
| Hormone status at time of inclusion | | |
| HSPC | 12 |
| CRPC | 25 |
| Gleason score at diagnosis | | |
| ≤6 | 2 |
| 7 | 8 |
| 8 | 9 |
| 9–10 | 13 |
| Missing | 5 |
| M-stage at diagnosis | | |
| M0 | 5 |
| M1 | 21 |
| Mx | 9 |
| Missing | 2 |
| Type of prior therapy | | |
| Local therapy | 16 |
| (i.e. radical prostatectomy or RT on prostate) | |
| ADT (i.e. chemical or surgical) | 25 |
| Chemotherapy | 17 |
| Hormonal therapy (other than ADT) | 16 |
| Radioisotope therapy | 8 |
| Other | 5 |

| Diagnostic leukapheresis characteristics |
|----------------------------------------|
| Total duration of DLA (minutes; median, range) | 104 (25–925) |
| Total processed blood volume (mL; median, range) | 5112 (1153–10001) |
| Volume of collected DLA product (mL; median, range) | 96 (18–178) |
| Missing | 3 |

HSPC, hormone-sensitive prostate cancer; CRPC, castration-resistant prostate cancer.

*a 4 subjects underwent DLA twice.*
majority of the remaining samples and only applied additional EpCAM selection if WBC depletion insufficiently enriched the sample. In the overall population, we reduced the WBC concentration by 3.21 log_{10}-fold and recovered 46% from the CTCs with WBC depletion alone (Fig. 2).

3.4. CTC derived organoids

After 18 DLA samples we performed an interim analysis to assess the success rate of organoid cultures from isolated and enriched CTC samples. In nine out of eighteen samples we obtained organoid samples of which seven could be maintained for at least six weeks and thus we proceeded to the second stage of our prospective study. In total, we established CTC derived organoids in 14 out of 40 DLA samples (35% success rate, Supplementary figure 3). At this point we terminated recruitment as the primary endpoint of 50% success rate was no longer a feasible goal. Both the absolute CTC yield after enrichment and the tumour cell percentage in culture was found to be higher in samples that resulted in organoid propagation ex vivo (P < 0.001 and P < 0.01 resp, Fig. 3A and B). Moreover, only one out of nine mHSPC samples, with the highest CTC yield within the population (subject 38; 64,155), could be propagated as organoids. The majority of the organoid cultures could be maintained for six to eight weeks until proliferation stalled, thus providing limited number of organoids for downstream applications (Fig. 3C). Two organoid cultures (EMC-PCa-25 and EMC-PCa-41) could be expanded and maintained for over six months, the latter yielding a stable cell line. Validation of the PCa origin of the organoids was shown by quantitative real-time PCR (qRT-PCR) analysis of prostate (cancer)-specific transcripts (Table 2, Supplementary figure 4). The vast majority of the isolated samples were positive for AR and/or KLK3 (PSA) while expression of AR-V7 was identified in only one sample. Three of 14 organoid cultures (subject 16, 41, and 93) expressed the TMPRSS2-ERG fusion transcript. We performed additional validation of PCa origin using patient-specific somatic SNVs previously identified in metastatic biopsies (Table 2, Supplementary table 2 and 3) [4]. We validated PCa origin in the matched organoid cultures from subject 9-I/II, 79 and 24-I/II by detection of the SNVs in TP53 and PTEN resp. Subject 79 was a patient with neuroendocrine PCa (NEPC), of whom the CTC-derived organoids maintained the NEPC features as they lacked AR and KLK3 expression.

3.5. Characterisation of CTC-derived organoid cell line

From the organoid culture samples, we were able to generate one stable organoid cell line, which enabled us to perform in-depth genomic and phenotypical characterisation. WGS of EMC-PCa-41 revealed a triploid genome with an estimated tumour cell purity of 99%, an overall tumour mutational burden of 2.13 somatic mutations per mega base pairs and no predominant mutational signature (Fig. 3A). We identified multiple CNAs including a focal amplification on chromosome Xq, encompassing the AR locus and a focal deep deletion on

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**Fig. 2. Efficiency of CTC enrichment and isolation techniques from diagnostic leukapheresis.** (A-B) Two CTC enrichment methods were compared (depicted on the X-axis) [1]: white blood cell (WBC) depletion (n = 40) and (2) WBC depletion followed by EpCAM enrichment (n = 15). The boxplot depicts the median, upper and lower quartiles, whiskers indicate 1.5 times the interquartile range (IQR). Individual data points are shown. **A)** WBC depletion factor after CTC enrichment. To calculate the WBC depletion factor, the number of WBCs before enrichment was divided by the number of WBCs after enrichment. WBC concentration was measured by a hematology analyser. Y-axis is a logarithmic scale. **B)** Relative CTC recovery (%) after CTC enrichment. To calculate CTC recovery, the estimate CTC count after the enrichment was divided by the estimate CTC count before the enrichment. Estimate CTC counts were extrapolated from 1 mL samples.
10q causing loss of PTEN. Furthermore, EMC-PCa-41 was characterised by multiple inter- and intra-chromosomal rearrangements, including the interstitial deletion leading to the TMPRSS2-ERG fusion as was identified by qRT-PCR (Table 2, Supplementary figure 4). Moreover, ERG expression in the organoids was validated by immunohistochemistry (Fig. 3B). Overall, EMC-PCa-41 harbours genomic features which are frequently identified in mCRPC tumours, such as alterations encompassing the AR, PTEN and ERG gene [4]. Next, we determined the sensitivity of EMC-PCa-41 to commonly used treatments for mCRPC, enzalutamide and taxane chemotherapeutics (Fig. 4C and D). Both androgen depleted culture conditions (minus R1881) and enzalutamide treatment could only partially inhibit cell proliferation of EMC-PCa-41, suggesting resistance. Interestingly, subject 41 started with enzalutamide after the DLA procedure and switched treatment after only two months due to rising PSA levels (Supplementary figure 5).

3.6. CTC and organoid heterogeneity by single cell copy number alterations

To investigate the heterogeneity within CTCs and early-stage organoid cultures, we performed low-pass WGS.
on multiple single cells obtained from the two long-term mCRPC organoid samples with matched CTCs and WBCs that served as normal controls (subject 25 and 41). The genomic profiles revealed several single cells without any distinct CNA that clustered together with WBC controls. Additional validation excluded these single cells, as they lacked tumour-specific SNVs and were likely non-malignant (supplementary figure 6). We then performed a t-SNE analysis to identify clusters of tumour cells bearing similar copy number profiles to assess heterogeneity and extract consensus profiles. The CTCs and organoid cells isolated from subject 25 separated into five distinct clusters (Fig. 5A). The organoid cells isolated from early cultures clustered separately (cluster 1) from the CTCs and displayed unique focal amplifications on chromosome 11p, 14 and 20. Cluster 3, encompassing 8 of 18 CTCs, harboured a triploid genome and closely resembled the focal amplifications previously identified in the matched organoid cell line EMC-PCa-41.

4. Discussion

This study confirms that DLA is a safe and efficient method to harvest large amounts of CTCs from patients with mPCa. Furthermore, we have optimised WBC depletion methods to efficiently recover CTCs and remove WBCs from DLA samples. From the isolated and enriched CTC fractions, we were able to establish organoid cultures in 35% of the samples which were mostly of short-term nature, although one sample led to a stable organoid line. Our study shows that DLA is a promising method to obtain viable tumour cells from patients with mPCa for subsequent downstream analyses such as single cell sequencing. Unfortunately, the modest success rate to expand organoid cultures precluded us from using CTC-derived organoid cultures as a platform to select personalised treatment options for now.

The use of living cells, directly obtained from patients as ‘real-life’ drug screening models, is an appealing prospect in our quest to improve personalised cancer treatment. Indeed, phenotyping living tumour cells has the advantage to directly measure the response to treatment compared with ‘phenotyping after fixation’–based stratification [18]. Previous reports on CTC cultures indicated that one of the main factor for success is the number of cells to initiate expansion [13,15]. Indeed, we found that samples that were successfully propagated as organoids contained significantly higher number and percentage of CTCs after enrichment and isolation. Although DLA allows us to obtain vast numbers of CTCs, processing of the DLA material provides new challenges due to the excess of WBCs present in the sample. The currently available methods for WBC depletion or CTC enrichment require an excess of magnetic beads or RBCs to achieve the appropriate ratio to capture all cells by antibodies. This limited the DLA volume that can be (cost)effectively processed. In our study, we were limited to one-fourth of the DLA sample from 5L blood and thus the number of CTCs obtained for culture. Further optimisation of DLA sample processing could therefore tremendously impact the amount of viable tumour cells acquired and benefit downstream applications.

In our study, we obtained DLA samples from 40% of the screened patients and were able to generate organoids in 35% of the samples including two long-term cultures. This is a substantial improvement compared to previous reports that used CTCs for ex vivo organoid cultures [19]. The article of Gao et al. [13] described the formation of one (long-term) organoid culture of 17 patient samples (success rate of 6%). Noteworthy, patients were preselected based on >100 CTCs per 10 mL of PB, although the number of patients screened was not reported. Lambros et al. [14] described the formation of one neuroendocrine organoid culture of 14 patient

Table 2

| Subject ID | Expression prostate (cancer) transcripts | Somatic variant (variant allele frequency) |
|------------|----------------------------------------|------------------------------------------|
| 9-I        | AR and AR-V9                           | TP53 c.407A>T (99.7%)                    |
| 9-II       | NT                                     | TP53 c.407A>T (83.9%)                    |
|            |                                        | (99.9%)                                  |
| 16         | AR, KLK3 and TMPRSS2-ERG               | NT                                       |
| 21         | AR and KLK3                            | PIK3CA c.3140A>G (1.9%)                  |
| 24-I       | PSA                                    |                                          |
| 24-II      | AR and KLK3                            | PIK3CA c.3140A>G (45%)                   |
| 25         | AR, KLK3 and AR-V9                     | PLCG2 c.655G>A (27.5%)                  |
| 38         | AR and KLK3                            | NT                                       |
| 41         | AR, KLK3 and TMPRSS2-ERG               | NT                                       |
| 79         |                                        | TP53 c.733G>A (97%)                      |
| 91         | AR and KLK3                            | NT                                       |
| 93         | AR, KLK3 and TMPRSS2-ERG               | NT                                       |
| 94         | AR and KLK3                            | NT                                       |
| 97         | AR and KLK3                            | NT                                       |
samples (success rate of 7%) which was used for genetic profiling. Whether these organoids could be maintained as long- or short-term cultures is unclear. Unfortunately, most of the organoids obtained in our study could be maintained as short-term cultures with limited proliferative capacity, implicating that the current culture techniques do not provide an optimal environment for sustained viability. We were able to maintain two samples for \( \frac{C21}{C20} \) passages (subject 25 and 41), eventually leading to one stable organoid line (EMC-PCa-41). Obtaining preclinical models of PCa has been notoriously difficult, presumably due to the low

Fig. 4. Genetic and phenotypic characterisation of the CTC-derived stable organoid cell line, EMC-PCa-41. (A) CIRCOS plot representing the characterisation of EMC-PCa-41 as obtained by whole genome sequencing. The first outer track depicts the genomic ideogram. The second track displays the copy number profile with amplifications marked in light-green, deep amplification in dark-green, deletions in blue and deep deletions in dark-blue. Deep deletion refers to high-level losses resulting in (near) homozygous losses while deep amplification refers to high-level amplifications resulting in many additional copies as defined by GISTIC2. The third track depicts the lower-allele frequency (LAF) values of individual copy number segments (LAF values ≤0.33 in pink and LAF values >0.33 in black). The fourth track displays the number of mutations per 5 megabase pairs (Mb), with regions of mutational frequency above 20 Mb marked in blue. The fifth track highlight regions marked by regional hypermutation (kataegis). Inner circle displays structural variants, with deletions in black, translocations in dark blue, insertions in yellow, inversions in light-blue and tandem duplication in red. (B) Representative overview and detailed images of immunohistochemical staining on EMC-PCa-41 organoids of AR, PSA, cytokeratin 8/18 (CK8/18) and ERG, and counterstained with haematoxylin. Top row depicts negative control in which the primary antibody was omitted. Scale bar represents 10 μm in size in the detailed images and 100 μm in the overviews. (C) Drug sensitivity of the organoid cell line EMC-PCa-41 towards the anti-androgen treatment enzalutamide and taxane chemotherapeutics, as compared to the established PCa organoid lines. Data shown are the average of 3 individual cell viability experiments with three technical replicates, scale bars represent SEM. Hormone sensitivity of EMC-PCa-41 was determined by cell viability in androgen depleted conditions (minus R1881) and compared with the AR-positive cell organoid cell line MSK-PCa-2. Chemosensitivity was compared with the AR-negative cell line MSK-PCa-1.
proliferative capacity of PCa, as well as overgrowth of benign epithelial and stromal cells. Therefore, establishing PCa cell lines from liquid biopsy samples provides several advantages, including lack of normal epithelial cells and the possibility to obtain metastatic samples from patients with bone only disease in a minimally invasive manner. The CTC-derived organoid cell line EMC-PCa-41 was found to harbour enzalutamide resistance, similarly to the patient. Noteworthy, the single cell sequencing of the CTCs and the established organoid line did suggest a selective pressure that resulted in \textit{ex vivo} expansion of one clone. Thus EMC-PCa-41 probably does not reflect the genetic tumour heterogeneity of the patient. Nevertheless, EMC-PCa-41 provides a unique novel model that harbours genomic alterations similar to a large subset of patients with mCRPC including the \textit{TMPRSS2-ERG} gene fusion, which is underrepresented in the currently available models of PCa [4,20].

Treatment modalities for mPCa have profoundly changed and expanded during the last decade. Understanding how PCa cells adapt to the selective pressure of treatment is becoming increasingly important to further improve outcome. Therefore, we need patient-derived materials that reflect the current status of the patient’s cancer, including the spatial and temporal tumour heterogeneity. Here we show that DLA enable in-depth studies into intratumour heterogeneity in mPCa by performing single cell whole genome DNA sequencing on CTCs. The single cell analysis distinguished clusters of cells with unique copy number alterations which is in line with a previous study [14]. Within our study we were also able to obtain matched samples in four patients, which shows that longitudinal CTC sampling by DLA is feasible, providing a platform to study clonal evolution and adaptation to treatment. Moreover, DLA based CTC enrichment provides substantial amount of easily accessible biomaterial that enable in-depth characterisation in a large patient population. In addition, enriched CTCs could be used to assess treatment sensitivity using short-term assays as an alternative to stable \textit{ex vivo} cultures. Overall, our study provides an important step forward in implementing CTCs in individualised disease modelling, nevertheless identifies several challenges that require further optimisation to enable the development of a personalised drug screening platform.

\textbf{Author contributions}

L.M. contributed to writing - original draft preparation, methodology, investigation, formal analysis, and visualisation. L.v.D. contributed to writing - original draft preparation, methodology, investigation, formal analysis, project administration, and visualisation. J.K. contributed to writing - original draft preparation, methodology, investigation, and formal analysis. A.d.J. contributed to writing - reviewing and editing, investigation, project administration, formal analysis, and visualisation. T.W. contributed to project administration. S.E.-S. contributed to methodology and investigation. C.B. contributed to investigation. A.S. contributed to investigation and visualisation. R.N. contributed to writing - reviewing and editing, methodology, and investigation. N.S. contributed to writing - reviewing and editing, and supervision. J.v.R. contributed to writing - reviewing and editing, data curation, software, and visualisation. H.v.d.W. contributed to writing - reviewing and editing, data curation, software, and visualisation. P.t.B. contributed to supervision and resources. Y.S. contributed to resources. P.H. contributed to resources. R.d.W. contributed to writing -

Fig. 5. Clustering of individual circulating tumour cells and organoid cells based on low-pass whole genome sequencing data. t-SNE plot (k-nearest neighbour algorithm; Louvain method) of tumour cells, clustering by absolute copy number values (0.01 Mb). Copy number values were obtained by low-pass whole genome sequencing of single tumour cells after whole genome amplification, white blood cells were taken along as negative controls (not displayed in t-SNE).
reviewing and editing, methodology, and resources. S.S. contributed to writing - reviewing and editing. J.M. contributed to writing - reviewing and editing, supervision, Funding acquisition. W.v.W. contributed to writing - reviewing and editing, supervision, Funding acquisition. M.L. contributed to conceptualisation, methodology, writing - original draft preparation, supervision, Funding acquisition.

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Role of the funding agency

KWF (Dutch Cancer Society) had no role in the design of the study; the collection, analysis or interpretation of the data; preparing and submitting the manuscript for publication. The content of this manuscript is solely the responsibility of the authors. KWF was informed about the progress and results of the presented study.

Data availability

The CTC-derived organoid cell line EMC-PCa-41 will be made available to academic institutions under the Erasmus MC Biological Uniform Material Transfer Agreement. The WGS data from metastatic biopsies were part of the CPCT-02 study, which has been made available by the Hartwig Medical Foundation. Both WGS and clinical data are freely available for academic use from the Hartwig. Medical Foundation through standardised procedures and request forms can be found at https://www.hartwigmedicalfoundation.nl. The WGS data from the organoid cell line as analysed in this manuscript will be made available at publication.

Conflict of interest statement

Ronald de Wit has an advisory role and/or received speaker fees from; Sanofi, Merck, Lilly, Roche, Bayer, Janssen, Clovis and research funding (Institutional); Sanofi, Bayer.

Stefan Sleijfer is the chair of Center for Personalised Cancer Treatment (CPCT), chair of Dutch Science Agenda Personalized Medicine and SkylineDx.

Harmen van de Werken has stock and/or other ownership interests in Cergentis and has received honoraria from Bayer.

Martijn Lolkema has an advisory role and/or received speaker fees from; Incyte, Amgen, Janssen Cilag B.V., Bayer, Servier, Roche, Pfizer Sanofi Aventis Netherlands BV, Astellas and has received (Institutional) research funding from Sanofi, JnJ, Merck and Astellas.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2021.03.023.

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