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Development and Validation of Circulating Tumor Cell Enumeration (Epic Sciences) as a Prognostic Biomarker in Men with Metastatic Castration Resistant Prostate Cancer

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Abstract

Purpose: To evaluate the prognostic significance of circulating tumor cell (CTC) number determined on the Epic Sciences platform in men with metastatic castration resistant prostate cancer (mCRPC) treated with an androgen receptor signaling inhibitor (ARSI).

Patients and Methods: A pre-treatment blood sample was collected from men with progressing mCRPC starting either abiraterone or enzalutamide as a 1st, 2nd or 3rd line systemic therapy at Memorial Sloan Kettering Cancer Center (Discovery cohort, N=171) or as a 1st or 2nd line therapy as part of the multi-center PROPHECY trial (NCT02269982) (Validation cohort, N=107). The measured CTC number was then associated with overall survival (OS) in the Discovery cohort, and progression-free survival (PFS) and OS in the Validation cohort. CTC enumeration was also performed on a concurrently obtained blood sample using the CellSearch® Circulating Tumor Cell Kit.

Results: In the MSKCC Discovery cohort, CTC count was a statistically significant prognostic factor of OS as a dichotomous (< 3 CTCs/mL versus ≥ 3 CTCs/mL; HR = 1.8, (1.3-3.0, 95% CI)) and as a continuous variable when adjusting for line of therapy, presence of visceral metastases, PSA, lactate-dehydrogenase, and alkaline-phosphatase. The findings were validated in an independent dataset from PROPHECY - (HR (95% CI) = 1.8, (1.1-3.0) for OS and 1.7 (1.1-2.9), for PFS). A strong correlation was also observed between CTC counts determined in matched samples on the CellSearch® and Epic platforms (r = 0.84).

Conclusion: The findings validate the prognostic significance of pretreatment CTC number determined on the Epic Sciences platform for predicting OS in men with progressing mCRPC starting an ARSI.

Keywords

CTC; prognosis; biomarker; prostate cancer

INTRODUCTION

Circulating tumor cells that are shed into the bloodstream from the primary tumor or metastatic sites play a key role in the development of metastases [1]. It follows both intuitively and biologically that the detection of CTCs in a patient's blood would predict for a worse outcome relative to those in whom CTCs are not detected prior to or following a therapeutic intervention. Such is the case for every tumor type studied [2-6] independent of the assay used to determine the presence or absence of CTCs in a pre-treatment blood sample, or as a quantitative measure of the number of CTCs in the blood determined both pre- and post-therapy thereby providing a non-invasive method to monitor disease status longitudinally over time [7].

Doing so is not straightforward because defining, detecting, and characterizing a CTC has its own set of challenges in that they are rare (1 in 10^5 to 1 in 10^7 nucleated cells typically) and morphologically, phenotypically and biologically diverse [1]. It is therefore essential that what is determined to be a "CTC" is both rigorously and reproducibly defined, and that the device/assay utilized to determine the number of CTCs present in a patient blood sample is analytically valid or at a minimum, achieved the level of performance to justify its use for this context. Presently there are a wide range of technologies to isolate and capture CTCs many of which are based on cell size or affinity capture[8]. Only one, the CellSearch® Circulating Tumor Cell kit and corresponding device [9] has achieved an FDA clearance as an aid to monitoring breast, colorectal, and prostate cancers. With this platform, a CTC is defined as a cell in the blood captured by an EpCAM ferrofluid that is CK^+CD45^- with an intact DAPI stained nucleus, and was shown to strongly associate with overall survival (OS) both pre- and post-therapy using a cut-off of 4 or fewer (favorable) or 5 or more CTCs/7.5 ml of blood (unfavorable) [10]. Further, a separate analysis of 5 phase III registration trials in prostate cancer further validated the prognostic significance of the conversion from unfavorable pre- to favorable counts post-therapy, and separately, a change from present (1 or more) to absent (none), CTC0) both of which serve as an indicators of favorable response to therapy that reflects patient benefit [11].

In contrast, the Epic Sciences platform is a non-selection based method in which all nucleated cells from a tube of blood are deposited on glass pathology slides (Fig. 1A) [12], stained, and imaged on a cell-by-cell basis to identify cells of interest *in silico* using computer vision. Those malignant, CTCs, and non-malignant cells, myeloid/lymphoid cells can be evaluated independently. The slides can be stored long-term at −80 °C and analyzed at a later date. A typical assay images and analyzes between 10^6-10^8 individual nucleated cells in a blood sample depending on the analytic requirements of the test being performed.

Clinically, the platform was used to analytically and clinically validate the nuclear-localized AR-V7 protein biomarker in CTCs, and show the clinical utility of the defined biomarker to inform the selection of a taxane versus an androgen receptor signaling inhibitor in the 2nd line or greater mCRPC treatment setting: level IIA evidence in the 2019 NCCN guidelines (v1.0), which lead to coverage by Center for Medicare Services and New York State approval as a Laboratory Developed Test (LDT) [13-16]. The relationship of CTC number to clinical outcomes using the platform has not been established.

The primary focus of this analysis was to clinically validate CTC number, enumerated on the Epic Sciences platform as a prognostic biomarker for overall survival (OS) and progression-free survival (PFS) in men with progressing mCRPC about to start treatment with 2nd generation ARSI, such as abiraterone or enzalutamide [17-22]. Similar to CellSearch®, a CTC was defined as any CK+, $CD45^-$ cell with an intact DAPI stained nucleus with a cluster of CTCs considered as 1 count or event (Fig. 1A & B).

METHODS

Patient Selection

All studies were performed with respect to the ethical guidelines outlined in the Declaration of Helsinki.

Discovery Cohort: Blood samples were collected from patients with progressing mCRPC treated between December 2012 and September 2016 at MSKCC about to start first, second or third line of therapy. All patients provided written informed consent to an Institutional Review Board (IRB)–approved biospecimen protocol and had histologic confirmation of prostate cancer. The evaluation included a physical examination, recording the Karnofsky performance status (KPS) and laboratory studies that included a complete blood count with hemoglobin (Hgb), chemistry panel (albumin (ALB), alkaline phosphatase (ALK), lactate-dehydrogenase (LDH), PSA, and serum testosterone to confirm castrate status (<50 ng/dl). Blood draws taken more than 30 days prior to therapy initiation were excluded.

Validation Cohort: Similarly, blood samples were collected from men with progressing mCRPC in either the first or second line setting prior to starting an ARSI collected as part of the multicenter IRB approved PROPHECY trial (NCT02269982) [13, 16]. Eligibility here included 2 or more poor prognostic factors [23, 24], and all provided written informed consent. Additional details re. patient population, eligibility criteria and design have been described elsewhere [25].

Epic Sciences CTC Collection, Enumeration and Analysis

A single tube of blood (Streck™ Cell-Free DNA BCT®) was collected from each patient and after red cell lysis, all nucleated cells were deposited onto glass slides at MSKCC or shipped to Epic Sciences as whole blood and processed within 96 hours of the blood draw as previously described [12, 14] (full details are available in the Supplementary Materials). Both sites handled and processed all samples identically using established Standard Operating Procedures (SOP). Any cell that was CK+CD45^{-} with an intact nucleus was classified as a CTC, and CTC clusters, defined as at least two adjacent cells, were classified as one event in the final count. CTC counts were normalized to blood volume and expressed as the number detected per 1 mL. In the case of the PROPHECY Validation cohort, time matched blood samples were sent to a CAP/CLIA approved laboratory at MSKCC for analysis using the CellSearch® Circulating Tumor Cell kit [9]. All blood samples were collected within 30 days prior to the start of ARSI, and all enumeration results were blinded to the treating physicians and to patients. In the Validation cohort, Epic Sciences laboratory personnel were blinded to the clinical outcomes and clinical investigators were blinded to all CTC biomarker results.

Statistical Analyses

The primary endpoint of this retrospective analysis was OS, defined as the date that therapy was initiated until the date of death from any cause or of last follow-up in the MSKCC Discovery and Validation cohorts. In addition, in the Validation cohort PFS was defined as date of therapy initiation to date of radiographic progression defined by the Prostate

Cancer Working Group 2 soft tissue and bone scan criteria, clinical progression, or death, and excluded PSA progression [13, 26]. The analysis of the Discovery cohort was performed by the Epic and MSKCC statistical teams and the results used to inform the writing of a Statistical Analysis Plan for the Validation cohort (Supplementary Materials). The biomarker data were then sent to the study statistician for the Validation cohort (SH) who unblinded the data and performed the analysis. Datalocks for the Discovery and Validation cohorts were July 29th, 2020 and February 4, 2020 respectively.

In the Discovery cohort, the proportional hazards model was used to explore if CTC count (as a continuous and dichotomized variable) is prognostic of OS and the Kaplan-Meier product-limit approach to estimate the OS distribution dichotomized by CTC cut-point. To determine a poor prognosis cut-point in the Discovery cohort, the univariate hazard-ratio was plotted for each unit increase in CTC/mL value and a cut-point defined qualitatively based on the overall trend in HR and the number of patients in the high CTC group. In multivariable analysis, covariates included line of therapy, presence of visceral metastases, lactate-dehydrogenase (LDH) levels, prostate-specific antigen (PSA) levels, hemoglobin (Hgb) levels, alkaline phosphatase (ALK) levels, white-blood cell (WBC) counts, albumin levels, and CTC counts as either a continuous or dichotomized covariate. Covariates were selected based on the best subset selection method using the global χ^2 statistic and WBC, ALB, Hgb, and patient age were excluded. In the Validation cohort, the proportional hazards model was utilized to confirm the prognostic significance of CTC dichotomized at 3 or greater level and as continuous variable (modelled as log2(CTC+1), adjusting for the validated baseline risk (Halabi prognostic risk-score) as previously described [13, 24] that includes Eastern Cooperative Oncology Group performance status, site of spread, lactate dehydrogenase, opioid analgesic use, albumin, hemoglobin, prostate-specific antigen, and alkaline phosphatase.

The cut-off for poor prognosis was pre-specified in the Statistical Analysis Plan for the Validation cohort prior to unblinding and analysis (Supplementary Materials). The full details for analysis of the association between OS and PFS with CTC counts, as well as a method agreement analysis between Epic Sciences CTC counts and CellSearch® CTC counts in time matched samples in the Validation cohort are listed in the Statistical Analysis plan (Supplementary Materials).

RESULTS

Patient demographics and clinical baseline

Between March 30th, 2013 and August 8th, 2018, 218 unique samples were collected from men with progressing mCRPC prior to starting either abiraterone acetate or enzalutamide as standard of care at MSKCC in which 171 were considered evaluable (Discovery cohort, Fig. 2A). Samples were excluded if the blood draw was taken prior to 30 days of therapy initiation, or if the patient was starting a therapy beyond the 3rd line setting. Patient demographics and clinical baseline characteristics are presented in Table 1. Among the 171 patients, the median age was 68 years (range 45-87). Sixty percent were about to start first line therapy for mCRPC, 29% and 11% of the samples were taken prior to starting second- and third-line therapy respectively. Sixty patients (35%) had received a prior ARSi

and 14 (8%) a prior taxane chemotherapy. Eighty-three (48.5%) had bone only or lymph node only disease while 88 (51.5%) had multiple sites of metastases. The median follow-up time among surviving patients was 56.5 months, ranging from 5.0 months to 84.2 months and 138 had died as of July 29th, 2020.

In the PROPHECY Validation cohort, 118 patients were enrolled from May 2015 until January 2017 of whom EPIC data was available from 107 patients. The median age was 73 years; of these men, 71% were first line mCRPC, and 29% were second line mCRPC after progression on abiraterone or enzalutamide. The median PSA, LDH and alkaline phosphatase were 22.1 ng/ml, 110 and 200 U/L, respectively and demographics have been previously published [13]. The majority of patients had multiple sites of metastases and 22% had bone only disease. The median follow-up time among surviving patients was 31 months (range 3.4-42.3) and 83 patients had died.

CTC detection rate and survival analysis in the Discovery cohort

At least one CTC, defined as any CK+, $CD45^{-}$ cell with an intact nucleus, was detected in 91.8%, (157 of 171) of patients in whom ≥ 3 CTC/mL, ≥ 5 CTC/mL, and ≥ 10 CTC/mL were detected in 28.7%, 21.6%, and 14.0% of patients respectively. A histogram of pre-treatment CTC count by patient sample in the Discovery cohort is presented in Fig. 2B and was numerically higher in patients with multiple sites of metastases (Supplementary Fig. 1).

Qualitatively, the survival times decreased significantly after 3 or more CTCs/mL were detected as shown in a plot of OS times versus CTC/mL along with an estimate (solid line) of median survival per unit increase in CTC/mL value Fig. 2C. This was also visualized in a plot of the univariate HR versus CTC/mL dichotomization cut-off point (Supplementary Fig. 2) in which a plateau in the HR was observed after approximately the 3/mL cut-off point. Kaplan-Meier analysis is presented in Fig. 2D in which patients were dichotomized at < 3 CTCs/mL (CTC-low), and those with ≥ 3 (CTC-high) and shorter median survival times were observed in each bin with increasing CTC counts (33 versus 13 months respectively). A demographic comparison between the CTC ≥ 3 and < 3 is presented in Supplementary Table 1.

The proportional hazards model was utilized to test for CTC number adjusting for line of therapy, presence of visceral metastases, and known blood based prognostic factors including lactate-dehydrogenase (LDH) and PSA. Application of the model validated the prognostic significance of the CTC ≥ 3 CTCs/mL threshold with overall survival (HR (95% CI) = 2.0 (1.3 – 3.0); $P = 0.001$) (Table 2) and this threshold was chosen for external validation based on the prognostic significance and the prevalence of patients above this threshold. Patients in the CTC ≥ 3 group also had higher PSA and LDH levels, and a higher proportion had multiple sites of metastatic spread relative to those with lymph node or bone only (41% versus 61%), explained in part by the presence of a higher burden of disease (Supplementary Table 1). Here again, CTC counts were also strongly adversely prognostic on a continuous scale when other baseline prognostic factors were considered, further validating the relationship of higher CTCs to an inferior survival outcome. (Table 2).

Validation of CTC count as a prognostic biomarker in the PROPHECY cohort

A blinded and independent analysis was performed to validate the above associations with OS and to assess the prognostic significance of CTC in predicting PFS in the PROPHECY Validation cohort [13] (Fig. 3A). Here, CTCs were detected in 83.2%, (89 of 107) of baseline pre-treatment samples of which 36% (39 of 107) had ≥ 3 CTCs/mL (histogram in Fig. 3B and boxplot of CTC counts by site of spread is shown in Supplementary Fig. 3). In univariate analysis, the median OS was 12.1 mo (95% CI=10.4-20.4) for CTC ≥ 3/mL versus 25.0 mo (95% CI=19.2-30.4) for CTC < 3/mL, respectively. The univariate HR for death was 2.5 (95% CI 1.6-3.9). The median PFS times on abiraterone or enzalutamide were 3.7 (95% CI=2.9-6.0) and 7.5 months (95% CI=5.5-9.5) in patients with CTC ≥3 and < 3 respectively. The univariate HR for PFS was 2.2 (95% CI 1.4-3.3) (Table 3, Fig. 3C & D). In multivariable analysis, adjusting for clinical prognostic factors (prognostic risk-score [27]), CTC counts dichotomized at the ≥ 3 cutpoint were again statistically significantly associated with poor OS (HR = 1.8 (95% CI=1.1-3.0); $P = 0.03$) and poor PFS (HR = 1.7 (95% CI 1.1-2.9); $P = 0.03$) (Table 3). CTC count as a continuous variable was also significantly associated with OS (HR = 1.3 (95% CI 1.1-1.6), $P = 0.002$) and PFS (HR = 1.3 (95% CI 1.1-1.5), $P = 0.01$, Table 3).

Thirty-six patients (33.6%) had received prior abiraterone or enzalutamide, and the HR for survival CTC counts were similar as both a continuous (HR = 2.3 (95% CI 1.4-3.6) and dichotomized variable (HR = 1.4 (95% CI 1.2-1.6) when adjusting for this factor, as well as when the risk-score was included in this model, [HR = 1.7 (95% CI 1.0-2.8) for ≥3 and < 3 CTC/mL and HR = 1.7 (95% CI 1.0-2.8) as a $\log_2$+1 transform] (Supplementary Table 3).

Last, one-hundred and six patients were evaluable for a post therapy PSA50 response, and 53 patients were evaluable for soft tissue response. Of the patients in the ≥ 3 CTC/mL group, 4/38 (11%) had confirmed PSA declines, and 2/20 (10%) had RECIST response, while in the < 3 CTC/mL group 21/68 (31%) had confirmed PSA declines while 4/33 (12%) had RECIST response.

Comparison of CTC counts between Epic Sciences and the CellSearch® platforms

Time matched samples from a single blood draw taken at baseline in the PROPHECY cohort (n=102) were analyzed after overnight shipping within 48 hours for CellSearch® CTC and Epic counts in independent blinded laboratories and a method agreement analysis was performed as described in the Statistical Analysis Plan (Supplementary Materials). CTCs were detected in 75% of samples on the CellSearch® platform and in 85% of samples on the Epic Platform with the counts on the two platforms strongly correlated (r = 0.84; Supplementary Fig. 4). As a dichotomized variable (Epic ≥ 3/mL and CellSearch® ≥ 5/7.5 mL), 73% concordance was observed and of the 37 samples in the Epic ≥ 3/mL group, 81% (30) had unfavorable, 5 or more cells/7.5 of blood, CellSearch® counts. Survival analyses for CellSearch® CTC counts were also performed (Supplementary Fig. 5 & Supplementary Table 4), and the HR for CellSearch® CTC counts as a dichotomized variable (≥ 5 versus 4 or fewer) was comparable in the same multivariable model for OS (HR = 1.7 (1.0-2.9), p = 0.03) and PFS (HR = 1.5 (0.9-2.3); p = 0.11). HR adjusting for prior abiraterone or enzalutamide were also comparable and are presented in Supplementary Table 5:

CTC nuclear localized AR-V7 in the context of total counts on the Epic Sciences platform

In an exploratory analysis, we examined the association of the Epic nuclear AR-V7 protein detection in CTCs with OS after adjustment for Epic CTC enumeration in both cohorts. In the MSKCC Discovery and PROPHECY Validation cohorts, 9.9% (17 of 171) and 10.4% (11 of 107) were positive by the nuclear localized CTC AR-V7 Epic Sciences assay at baseline and of the positive cases, 71% (12 of 17) and 73% (8 of 11) also had ≥ 3 CTC/mL respectively. In multivariable analysis of OS of the Discovery cohort, CTC AR-V7 remained associated with OS (HR (95% C.I.) = 2.21 (1.24, 3.93)) along with Epic CTC enumeration (HR (95% C.I.) = 1.83 (1.20, 2.79)). In the Validation cohort, nuclear localized AR-V7 also remained associated with OS in multivariable modeling (HR 2.30 95% CI 1.16-4.55) and a similar HR for Epic CTC enumeration was observed (HR 1.63 95% CI 0.96-2.78) (Supplementary Table 6). These data indicate that while CTC enumeration is strongly prognostic of OS, CTC AR-V7 nuclear detection remained prognostic for poor AR therapy outcomes even after adjusting for CTC burden.

DISCUSSION

The presence of circulating tumor cells (CTCs) in blood reflects the ability of cancer cell to detach from the primary or metastatic focus to develop new sites of spread that results a worsening prognosis. Here we show, that the number of CTCs, defined as any nucleated cell that CK+, $CD45^{-}$, enumerated with Epic Sciences platform as a continuous and dichotomized variable is independently prognostic for survival in univariate and multivariable modeling in men with progressing mCRPC about to start a 2^{nd} generation ARSI such as abiraterone or enzalutamide. The findings were validated using an independent cohort of men with high risk mCRPC treated similarly where an additional finding was the comparable association with progression free survival. Separately, CTC counts measured on the Epic Sciences platform were shown to correlate strongly to CTC counts obtained using the CellSearch® Circulating Tumor Cell kit, an FDA cleared predicate device/assay that applies similar criteria to define a CTC.

Much of the success in drug discovery in advanced prostate cancer can be attributed the availability of biomarkers reported using analytically and clinically valid devices and assays for the context of use being studied. Pre-treatment contexts to inform treatment selection include an understanding of a patient's prognosis and predicting and selecting a treatment that is most likely to provide benefit and avoiding those which will not. Validated pre-treatment nomograms are available to determine patient risk, while changes in disease manifestations present at the start of therapy relative to post-treatment to determine efficacy include the measured level of PSA and those assessed by imaging [24, 27-29]. Each has limitations and additional genomic biomarkers, AR splice variant detection such as AR-V7, and measures of disease burden such as CTC enumeration or ctDNA quantification may provide improved discrimination of outcomes as well as better monitoring biomarkers [17, 30-33]. Importantly, our data show that CTC nuclear AR-V7 protein detection was strongly associated with worse survival in this mCRPC AR therapy context after adjusting for CTC enumeration although a larger cohort will be needed to assess the additive value of both biomarkers.

CTC counts measured using the FDA cleared CellSearch® Circulating Tumor Cell kit is a validated pre- and post-treatment biomarker of prognosis and response in breast, colorectal, and prostate cancers [2-7]. In this case, a CTC was defined as an intact nucleated cell captured from blood by and EpCAM ferrofluid that stained positive for markers of epithelial origin (cytokeratins), and negative for CD45, a marker of leukocyte lineage [9]. Further study in mCRPC patients showed the “added value” of the CTC test result to understand the prognosis of patients specifically predicted to have a favorable outcome using standard measures, [34], and that changes in count at 12 week combination with LDH, shown to meet the Prentice criteria as a surrogate for overall survival in a phase 3 registration trial in the post-chemotherapy mCRPC setting [35]. Also shown, was that both a post-treatment CTC conversion from unfavorable to favorable (≥ 5 to < 5 cells/7.5 ml of blood), the FDA cleared outcome measure, and a newly developed outcome measure, CTC0, representing change from any (1 or more) pretreatment to none post-treatment were shown to have higher concordance with survival than PSA [11]. CTCs also serve as a source of tumor material for the biologic characterization of an individual patients disease for a predictive biomarker to guide treatment choice.

Significant here as well is that concordance of the predicted overall survival of patients with mCRPC determined with CTC counts obtained with the non-selection based Epic Sciences Platform and the FDA cleared predicate CellSearch® platform when a similar definition of a CTC was used. In this context, both the CellSearch® and Epic Sciences platforms define a CTC as any circulating cell of epithelial lineage, cytokeratin positive, without leukocyte lineage, CD45 negative. At the same time, it should be noted that this definition of a CTC identifies and enumerates only a subset of the intact malignant cells that be present in blood while excluding those undergoing an epithelial to mesenchymal (EMT) transition, or lineage plasticity that results in a transition to a neuroendocrine/stem cell like phenotype that grow independent of AR signaling [36, 37]. While the results between the two platforms for the CTC definition used here were similar, the Epic platform has the additional advantages of bio-banking un-stained sample at −80 °C allowing the immunofluorescence or genomic analysis to be completed at a later date (years), and the ability to isolate plasma for cell-free, proteomic, or metabolomic analysis from the same sample.

Recognized as well is that non-malignant cells with epithelial lineage can also disseminate into the blood through other mechanisms that may affect prognosis in addition to those derived from the cancer itself. They include those from cardiovascular related events or viral infection [38-40] considered in the same context as applied here [41]. On the Epic Sciences platform, CK+, $CD45^{-}$ cells have been observed in a small fraction of healthy donor blood samples, albeit at a lower frequency than from mCRPC patient blood samples [42], and the true tissue origin of each CTC detected without deeper characterization is unknown, such as through methylation analysis or transcriptomics. In prior sequencing analysis of CTCs detected in mCRPC patient blood samples on the platform, the majority of $CK+CD45^{-}$ cells were found to have some level of cancer related genomic copy-number alterations (CNAs), while other cells were found to be absent of CNAs [43] perhaps owing to the fact that a fraction of these cells are not of tumor origin, or owing to low coverage and the technical challenges of sequencing each individual cell.

In conclusion, we observed that CTC counts, defined as any CK+CD45^{-} cell detected on the Epic Sciences platform is a statistically significant and independently validated prognostic factor for OS in men with progressing mCRPC about to start either abiraterone or enzalutamide. Future studies of CTC counts while on therapy relative to baseline are needed to determine the significance of changes in counts to patient outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

Conflict of Interest Disclosures:

Howard I. Scher

Leadership - Asterias Biotherapeutics

Stock and Other Ownership Interests - Asterias Biotherapeutics

Honoraria - Research to Practice

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Andrew J. Armstrong

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Patents, Royalties, Other Intellectual Property - Co-inventor of a biomarker technology that has been licensed to Qiagen

Travel, Accommodations, Expenses - Dendreon; Medivation; Sanofi

Jun Luo

Consulting or Advisory Role - Janssen Oncology; Sun Pharma; Tolero Pharmaceuticals

Research Funding - Astellas Pharma (Inst); Calibr (Inst); cardiff Oncology (Inst); Constellation Pharmaceuticals (Inst); Gilead Sciences (Inst); Mirati Therapeutics (Inst); Orion Pharma GmbH (Inst); Sanofi (Inst)

Patents, Royalties, Other Intellectual Property - Jun Luo is a co-inventor of a technology assigned to Johns Hopkins University who licensed to Tokai Pharmaceuticals. (Inst); Jun Luo is a co-inventor of a technology licensed to A&G Pharmaceuticals (Inst); Jun Luo is a co-inventor of a technology licensed to Qiagen (Inst)

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Research Funding - Abbvie (Inst); Amgen (Inst); Astellas Pharma (Inst); AstraZeneca (Inst); AVEO (Inst); Bayer (Inst); Boehringer Ingelheim (Inst); Bristol-Myers Squibb (Inst); Clovis Oncology (Inst); Dendreon (Inst); Endocyte (Inst); Exelixis (Inst); Genentech (Inst); Immunomedics (Inst); Inovio Pharmaceuticals (Inst); Janssen (Inst); Karyopharm Therapeutics (Inst); Lilly (Inst); Medivation (Inst); Merck (Inst); Millennium (Inst); Newlink

Genetics (Inst); Novartis (Inst); Progenics (Inst); Rexahn Pharmaceuticals (Inst); Sanofi (Inst); Stem CentRx (Inst)

Travel, Accommodations, Expenses - Amgen; Immunomedics; Sanofi

(OPTIONAL) Uncompensated Relationships - ATLAB Pharma; Phosplatin Therapeutics; Telix Pharmaceuticals

Qian Yang

No Relationships to Disclose

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Consulting or Advisory Role - Astellas Pharma; AstraZeneca; Bayer; Bristol-Myers Squibb; Constellation Pharmaceuticals; Exelixis; Genentech; Innocrin Pharma; Janssen; Merck Sharp & Dohme; Michael J. Hennessy Associates; Myovant Sciences; Pfizer; Sanofi; Vizuri

Speakers' Bureau - Bayer; Exelixis; Sanofi

Research Funding - Acerta Pharma (Inst); Astellas Pharma (Inst); Bayer (Inst); Bristol-Myers Squibb (Inst); Calithera Biosciences (Inst); Dendreon (Inst); Exelixis (Inst); Innocrin Pharma (Inst); Janssen Oncology (Inst); Novartis (Inst); Pfizer (Inst); Sanofi/Aventis (Inst)

Travel, Accommodations, Expenses - Bayer; Exelixis; Janssen Oncology; Merck; Pfizer; Sanofi; UroToday

Russell Zelig Szmulewitz

Honoraria - Astellas Pharma

Consulting or Advisory Role - Abbvie; Amgen; Astellas Pharma; AstraZeneca; Exelixis; Janssen Oncology; Merck; Pfizer; Sanofi

Research Funding - Abbvie; Astellas Pharma; Incyte; Janssen Oncology; Macrogenics

Patents, Royalties, Other Intellectual Property - Patent licensed by University of Chicago of which I am co-inventor to Corcept Therapeutics for combination AR/GR inhibition in prostate cancer

Travel, Accommodations, Expenses - Corcept Therapeutics

Daniel Costin Danila

Honoraria - Angle; Astellas Pharma; AxImmune; Bayer; Clovis Oncology; Janssen Oncology; Pfizer; Pfizer; ScreenCell

Consulting or Advisory Role - Angle; Astellas Pharma; AxImmune; Bayer; Clovis Oncology; Janssen Scientific Affairs; Pfizer; Pfizer; Sanador

Research Funding - Genentech; Janssen Research & Development (Inst); Prostate Cancer Foundation

Patents, Royalties, Other Intellectual Property - GENE EXPRESSION PROFILE ASSOCIATED WITH PROSTATE CANCER

Travel, Accommodations, Expenses - American Austrian Open Medical Institute; Astellas Pharma; Cambridge Healthtech Institute; Genzyme; Janssen Biotech; Janssen Scientific Affairs; Pfizer; Prostate Cancer Foundation; ScreenCell; Stop Cancer

Rick Wenstrup

Employment - Epic Sciences; Epic Sciences

Leadership - Epic Sciences

Stock and Other Ownership Interests - Epic Sciences; Epic Sciences

Consulting or Advisory Role - Blueprint Genetics; Resolys Bio

Patents, Royalties, Other Intellectual Property - patent royalties, AssurexHealth

Travel, Accommodations, Expenses - Epic Systems

Mithat Gonen

No Relationships to Disclose

Susan Halabi

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Consulting or Advisory Role - Eisai; Ferring

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HIGHLIGHTS

- In this study, a CTC is any CK+, $CD45^{-}$ cell and clusters are counted as one event.
- The findings validate CTC count on the Epic platform as a prognostic biomarker.
- Comparable associations with OS and PFS were observed using the CellSearch® device.

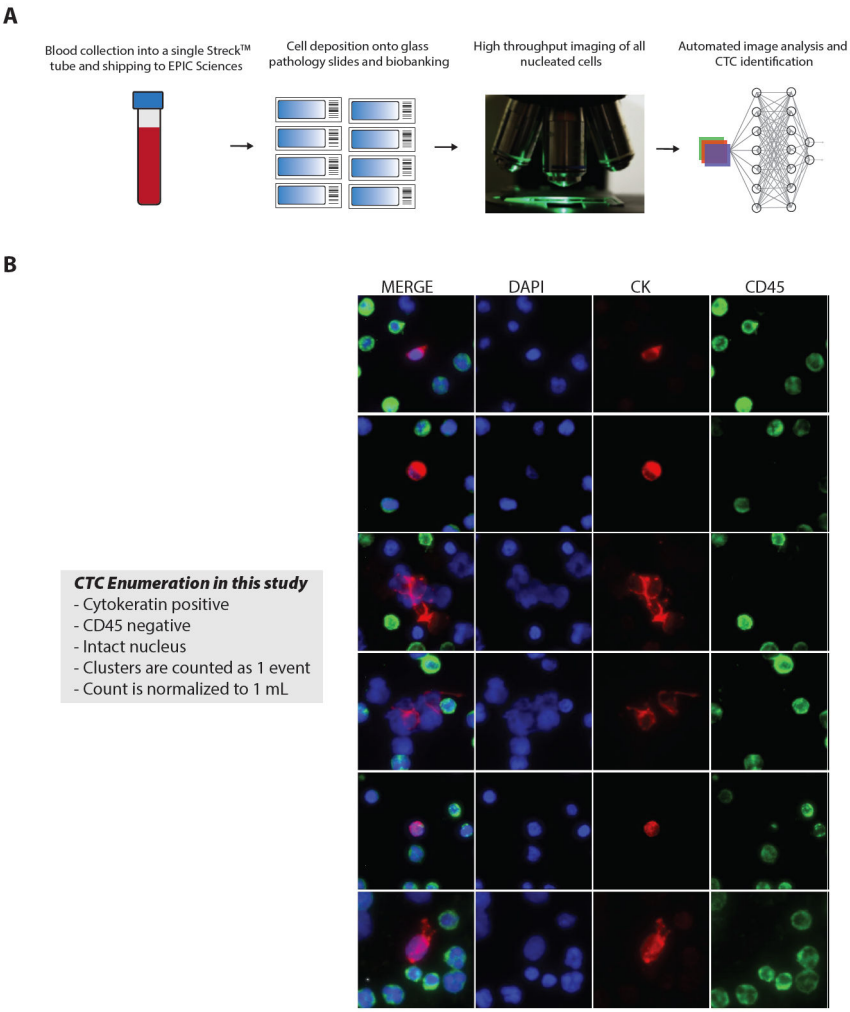

Figure 1: The Epic Sciences platform for CTC detection and enumeration.

A) Schematic of blood collection, shipping, bio-banking, and CTC analysis and detection.

B) Example CTC images. A CTC is defined in this study as any CK+CD45- cell detected in circulation with an intact nucleus. A cluster of CTCs is counted as one event.

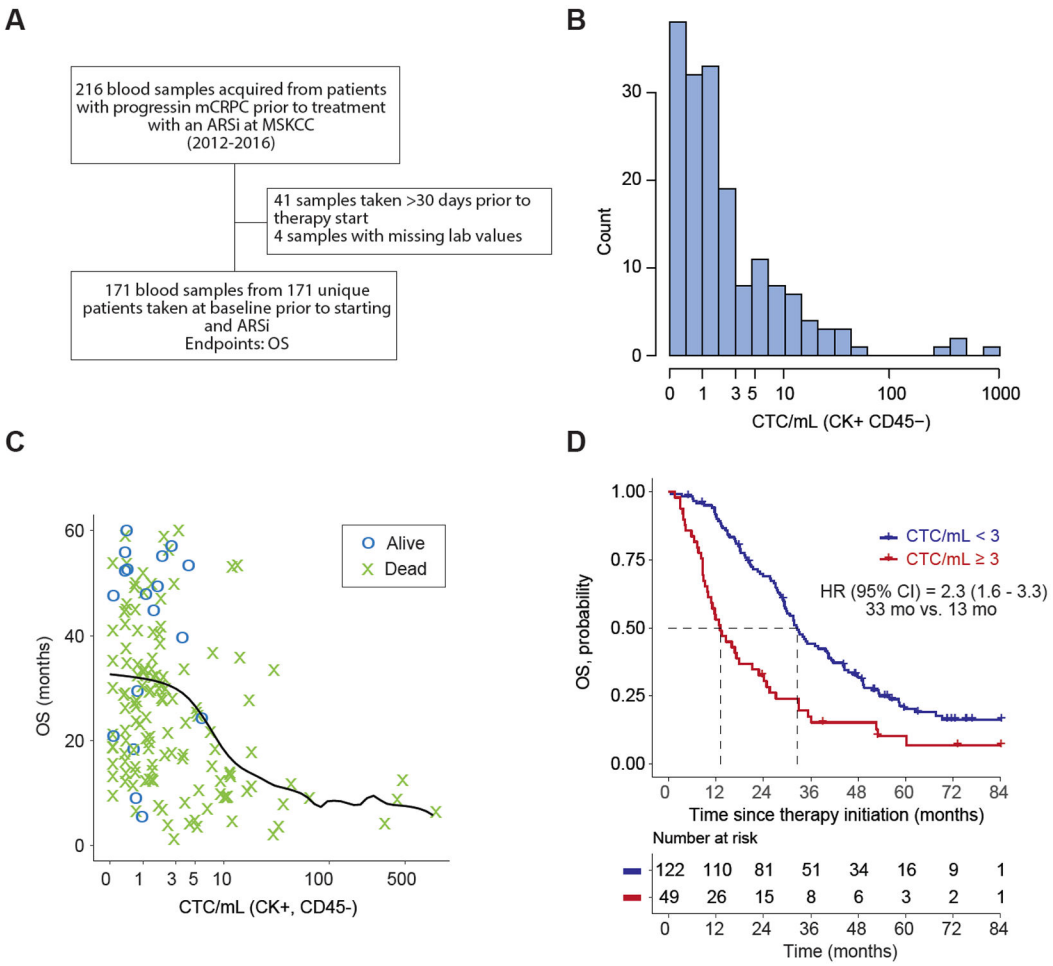

Figure 2: CTC detection frequency and prognostic associations with OS in the MSKCC Discovery cohort.

A) Patient selection. **B)** Histogram of CTC/mL values in the cohort. **C)** Plot of survival times versus CTC/mL. An estimate of the median survival using a gaussian kernel density estimate (KDE) shown. **D)** Kaplan-Meier estimate dichotomized at the 3 CTC/mL cutoff.

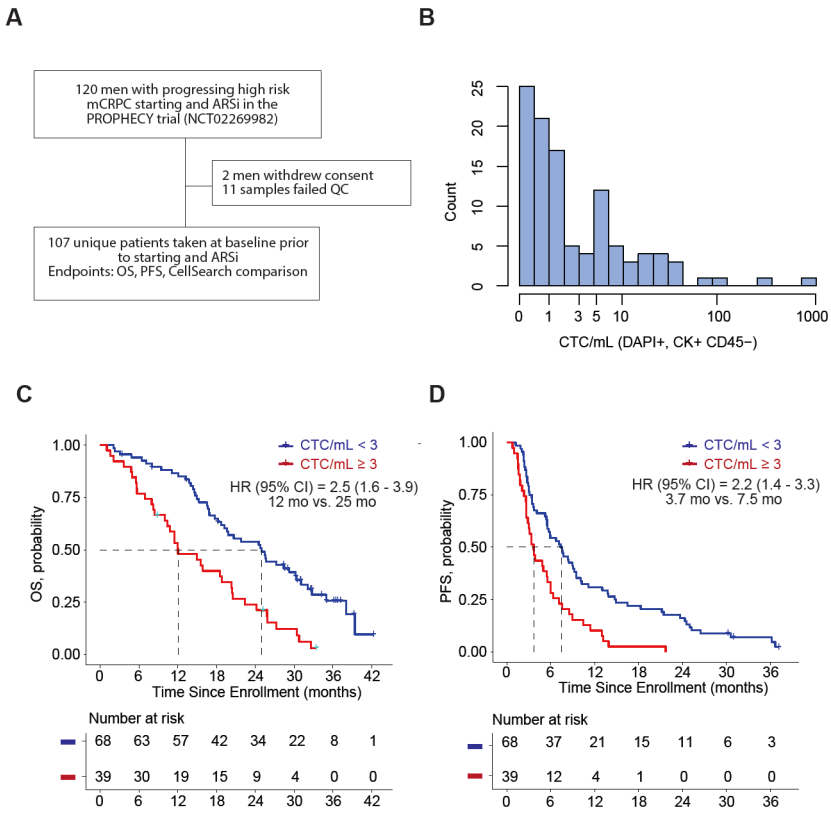

Figure 3: CTC detection frequency and prognostic associations with OS in the PROPHECY Validation cohort.

A) Patient selection. **B)** Histogram of CTC/mL values in the cohort. **C)** Kaplan-Meier estimate OS dichotomized at the 3 CTC/mL cut-off point, and PFS (**D**).

Table 1:

Patient Demographics

	MSKCC Discovery Set	PROPHECY Validation set
Unique Patients, no. (%)	171	107
Unique Blood Samples, no. (%)	171	107
Median Age in years (range)	68 (45,87)	73 (44,92)
Death events, no. (%)	138 (80.7%)	83 (77.6%)
Median Follow Up of Survivors in months (range)	56.5 (5.0, 84.2)	31 (3.4, 42.3)
Therapy Line - no. (%)		
pre-1st	103 (60.2%)	76 (71%)
pre-2nd	49 (28.7%)	31 (29%)
pre-3rd	19 (11.1%)	0 (0%)
Sites of Metastases – no. (%)		
Lymph Node Only	24 (14.0%	3 (2.8%)
Bone Only	59 (34.5%)	23 (21.5%)
Lung Only	1 (0.6%)	0 (0%)
Multiple Sites	88 (51.5%)	79 (73.8%)
Prior Taxane Chemotherapy – no. (%)	14 (8.2%)	20 (18.7%)
Prior ARSi – no. (%)	60 (35.1%)	40 (37.4%)
Baseline lab values - median (range)		
PSA ng/mL	18.1 (0.0900, 2010)	22.1 (0.1, 4194.9)
ALB g/L	4.2 (3.3, 4.9)	4.0 (2.7, 4.9)
ALK U/L	96 (42, 2170)	110 (91, 150)
HGB g/dL	12.6 (8.2, 15.7)	12.8 (8.7, 15.9)
LDH U/L	208 (124, 2120)	200 (100, 618)
WBC x 10^9/L	5.9 (2.6, 12.1)	6.4 (3.7, 22.3)
CellSearch® CTC count/7.5mL	n/a	4 (0, 12,972)

Abbreviations: PSA - prostate specific antigen, ALB - albumin, ALK - alkaline phosphatase, HGB - hemoglobin, LDH - lactate dehydrogenase, WBC - white blood cell

Table 2:

Proportional hazards models of overall survival (OS) with Epic Sciences CTC count represented continuously and dichotomized at 3 CTC/mL in the Discovery cohort

	Model with Dichotomized CTC Counts (≥ 3/mL vs <3)		Model with Continuous CTC Counts*	
	HR (95% CI)	**P**	**HR (95% CI)**	**P**
Overall Survival				
Univariate Analysis				
CTC	2.3 (1.6, 3.3)		1.3 (1.2, 1.5)	
Multivariable Analysis				
Presence of visceral metastases	1.7 (1.1, 3.1)	0.02	1.8 (1.1, 3.1)	0.02
More than one line of therapy (Yes vs. No)	2.5 (1.8, 3.6)	< 0.001	2.6 (1.8, 3.8)	<0.001
ALK*	1.3 (1.0, 1.6)	0.05	1.2 (1.0, 1.5)	0.10
LDH*	1.8 (1.3, 2.4)	0.001	1.7 (1.1, 2.4)	0.008
PSA*	1.1 (1.0, 1.3)	0.03	1.1 (1.0, 1.3)	0.03
CTC	2.0 (1.3, 3.0)	0.001	1.2 (1.1, 1.4)	0.001

*log2(x+1) transformed; CTC – Circulating Tumor Cell; ALK – alkaline-phosphatase; LDH – lactate-dehydrogenase; PSA – prostate specific antigen

Table 3:

Proportional hazards models of overall survival (OS) and progression free-survival (PFS) with Epic Sciences CTC count represented continuously and dichotomized at 3 CTC/mL in the Validation cohort.

	Model with Dichotomized CTC Counts (≥ 3/mL vs. <3ml)		Model with Continuous CTC Counts*	
	HR (95% CI)	**P**	**HR (95% CI)**	**P**
Overall Survival				
Univariate Analysis				
CTC	2.5 (1.6-3.9)		1.4 (1.2-1.6)	
Multivariable Analysis				
CTC	1.8 (1.1-3.0)	0.03	1.3 (1.1-1.6)	0.002
prognostic risk-score [22] (continuous)	1.01 (1.00-1.02)	0.01	1.00 (0.99-1.01)	0.48
Progression Free Survival				
Univariate Analysis				
CTC	2.2 (1.4-3.3)		1.3 (1.2-1.5)	
Multivariable Analysis				
CTC	1.7 (1.1-2.9)	0.03	1.3 (1.1-1.5)	0.01
prognostic risk-score [22] (continuous)	1.01 (1.00-1.01)	0.07	1.00 (0.99-1.01)	0.67

CTC – Circulating Tumor Cell; ALK – alkaline-phosphatase; LDH – lactate-dehydrogenase; PSA – prostate specific antigen;

*log2(x+1) transformed