What is the role of phenotype of cancer stem cells in diagnosis, prognosis and treatment in colorectal carcinoma?


Role of stem cells in colorectal cancer progression and prognostic and predictive characteristics of stem cells markers in colorectal cancer.

Fedyanin M1, Popova A, Polyanskaya E, Tjulandin S.

Abstract

In the last decade an increasing number of studies on tumor stem cell theory stating that there is only a small fraction of tumor cells capable of inducing tumor growth have been appeared. These cells can not only differentiate into more mature tumor cells, but also can maintain their own pool, that is the capacity for self-renewal. There are distinct subpopulations of cells within a tumor that express different combinations of stem cell markers and have different functions. The following markers are typically considered as markers of colorectal adenocarcinoma stem cells: CD133, CD144, CD24, CD166, CD44, CD29, ALDH1, LGR5, and CXCR4. However, data on the role of cancer stem cells in the process of colorectal cancer progression, their prognostic and predictive role are lacking. Researches on the phenotype, molecular and functional properties of this tumor cell subpopulation in both primary site and metastases of colorectal cancer are of great interest because they can allow developing new diagnostic and therapeutic strategies in the future.

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It is also known that the proportion of cancer stem cell like in CTCs in colon cancer cases is higher and may be used as a predictivearker related with the risk of relapse.

Ex-vivo characterization of circulating colon cancer cells distinguished in stem and differentiated subset provides useful biomarker for personalized metastatic risk assessment
Abstract

Background

Circulating tumor cells (CTCs) represent one of the most interesting target in improving diagnosis, prognosis and treatment. Herein we evaluate the possibility of using an emo-cytometric approach on the evaluation of the heterogeneous population of CTCs to improve personalized metastatic risk assessment. We benchmarked ex vivo behavior of distinct subsets of circulating colon tumor cells with correspondent clinical behavior of patients from which we isolated CTCs.

Methods

Isolation and CTC expansion were performed by a gradient protocol. In vitro characterization was determined by flow cytometry, immunofluorescence, western blotting and proteomic profiling. Cell sorter was performed with immunomagnetic beads. Confocal microscopy was used to evaluate tissue sections. Kaplan Mayer curves was cared for through Medcalc program.

Results

We collected heterogeneous CTCs, derived from the whole blood of seven patients affected by colon cancer, expressing CD133\textsuperscript{pos}CD45\textsuperscript{neg} (5 ± 1) and (2 ± 1) and CK20\textsuperscript{pos}CD45\textsuperscript{neg} of (29 ± 3) (11 ± 1) cells/ml in Dukes D and A stage respectively. Proliferation rate of 57 ± 16 %, expression for CXCR4\textsuperscript{pos} of 18 ± 7 % and detectable levels of IL-6, IL-8 and SDF-1 cytokines in conditioned culture medium characterized short-time expanded–CTCs (eCTCs). ECTCs organized in tumor sphere were CD45\textsuperscript{neg}CD133\textsuperscript{pos} while in adhesion were CXCR4\textsuperscript{pos}CK20\textsuperscript{pos}. These two subsets were separately injected in mice. The first group of xenografts developed superficial lesions within 2 weeks. In the second group, in absence of growing tumour, the survival of injected eCTCs was monitored through SDF-1 serum levels detection. The detection of human cancer cells expressing CK20, in mice tissues sections, suggested a different biological behaviour of injected eCTC-subsets:
tumorigenic for the first and disseminating for the second. The benchmarking of the experimental data with the clinical course highlights that patients with prevalence of circulating cancer stem cells (CD45^{neg}CD133^{pos}) have a lower overall survival. Conversely, patients with prevalence of circulating differentiated cells (CXCR4^{pos}CK20^{pos}) have a low disease-free survival.

**Conclusion**

On the basis of the heterogeneous composition and despite the low number of CTCs, it was possible to distinguish two subgroups of CTCs, suggesting a different clinical outcome. CTC-subsets detailing is useful to better define the metastatic–risk personalized score thus improving disease management and reducing patient care cost.

**Keywords**

Circulating colon tumor differentiated/stem cells Metastatic-risk stratification

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**Added 9.02.16**

*How is it possible that a failing chemotherapy could lead to the development of metastases and what is the contribution of CTCs as a prognostic marker to this process.*

**Does the mobilization of circulating tumour cells during cancer therapy cause metastasis?**

- Olga A. Martin,
- Robin L. Anderson,
- Kailash Narayan
- & Michael P. MacManus

**Affiliations**

**Contributions**

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Abstract

Despite progressive improvements in the management of patients with locoregionally confined, advanced-stage solid tumours, distant metastasis remains a very common — and usually fatal — mode of failure after attempted curative treatment. Surgery and radiotherapy are the primary curative modalities for these patients, often combined with each other and/or with chemotherapy. Distant metastasis occurring after treatment can arise from previously undetected micrometastases or, alternatively, from persistent locoregional disease. Another possibility is that treatment itself might sometimes cause or promote metastasis. Surgical interventions in patients with cancer, including biopsies, are commonly associated with increased concentrations of circulating tumour cells (CTCs). High CTC numbers are associated with an unfavourable prognosis in many cancers. Radiotherapy and systemic antitumour therapies might also mobilize CTCs. We review the preclinical and clinical data concerning cancer treatments, CTC mobilization and other factors that might promote metastasis. Contemporary treatment regimens represent the best available curative options for patients who might otherwise die from locally confined, advanced-stage cancers; however, if such treatments can promote metastasis, this process must be understood and addressed therapeutically to improve patient survival.

Subject terms:

- Cell migration
- Chemotherapy
- Metastasis
- Radiotherapy
- Surgical oncology

Added 8.26.16

Prognostic value of CTCs for the risk of relapse.


Hall CS1, Karhade MG1, Bowman Bauldry JB1, Valad LM1, Kuerer HM1, DeSnyder SM1, Lucci A2.

Author information

Abstract

BACKGROUND:

Circulating tumor cells (CTCs) can be identified in approximately 25% of nonmetastatic breast cancer patients, and data are emerging regarding their prognostic significance. We hypothesized that CTCs identified before resection of the primary tumor would predict worse outcomes in nonmetastatic breast cancer patients.
STUDY DESIGN:

We performed CTC enumerations on 509 patients with nonmetastatic breast cancer as part of an IRB-approved study. The CTCs (per 7.5 mL blood) were identified using the CellSearch System (Janssen). The presence of ≥1 CTC meeting morphologic criteria for malignancy was considered a positive result. Log-rank test and Cox regression analysis were applied to establish the association of CTCs with relapse-free and overall survival.

RESULTS:

Median follow-up was 48 months and mean age was 53 years. Fifty-nine percent of patients (299 of 509) had tumors larger than 2 cm, and 46% (234 of 509) had positive lymph nodes. One hundred sixty-six patients received neoadjuvant chemotherapy (NACT) before CTC assessment, and 343 patients were chemonaïve. One or more CTC was identified in 43 of 166 (26%) NACT treated patients, and in 81 of 343 (24%) chemonaïve patients. Circulating tumor cells were not associated with tumor size, grade, or lymph node status (p = NS). Detection of 1 or more CTCs predicted decreased relapse-free (log-rank p < 0.001, hazard ratio [HR] 2.72, 95% CI 1.57 to 4.72; p < 0.001) and overall survival (log-rank p = 0.02, HR 2.29, 95% CI 1.12 to 4.67; p = 0.03) at 48 months of follow-up.

CONCLUSIONS:

One or more CTCs identified before resection of the primary breast tumor predicted worse relapse-free and overall survival, irrespective of primary tumor size, grade, or lymph node positivity.

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Added 8.19.16

The value of CTCs in prostate carcinoma as tool for follow up a treatment outcome.

Decline in Circulating Tumor Cell Count and Treatment Outcome in Advanced Prostate Cancer


European Urology, June 2016

Published online: 09 June 2016

Abstract

Background
Treatment response biomarkers are urgently needed for castration-resistant prostate cancer (CRPC). Baseline and post-treatment circulating tumor cell (CTC) counts of ≥5 cells/7.5 ml are associated with poor CRPC outcome.

**Objective**

To determine the value of a ≥30% CTC decline as a treatment response indicator.

**Design, setting, and participants**

We identified patients with a baseline CTC count ≥5 cells/7.5 ml and evaluable post-treatment CTC counts in two prospective trials.

**Intervention**

Patients were treated in the COU-AA-301 (abiraterone after chemotherapy) and IMMC-38 (chemotherapy) trials.

**Outcome measures and statistical analysis**

The association between a ≥30% CTC decline after treatment and survival was evaluated using univariable and multivariable Cox regression models at three landmark time points (4, 8, and 12 wk). Model performance was evaluated by calculating the area under the receiver operating characteristic curve (AUC) and c-indices.

**Results**

Overall 486 patients (122 in IMMC-38 and 364 in COU-AA-301) had a CTC count ≥5 cells/7.5 ml at baseline, with 440, 380, and 351 patients evaluable at 4, 8, and 12 wk, respectively. A 30% CTC decline was associated with increased survival at 4 wk (hazard ratio [HR] 0.45, 95% confidence interval [CI] 0.36–0.56; \( p < 0.001 \)), 8 wk (HR 0.41, 95% CI 0.33–0.53; \( p < 0.001 \)), and 12 wk (HR 0.39, 95% CI 0.3–0.5; \( p < 0.001 \)) in univariable and multivariable analyses. Stable CTC count (<30% fall or <30% increase) was not associated with a survival benefit when compared with increased CTC count. The association between a 30% CTC decline after treatment and survival was independent of baseline CTC count. CTC declines significantly improved the AUC at all time-points. Finally, in the COU-AA-301 trial, patients with CTC ≥5 cells/7.5 ml and a 30% CTC decline had similar overall survival in both arms.

**Conclusions**

A 30% CTC decline after treatment from an initial count ≥5 cells/7.5 ml is independently associated with CRPC overall survival following abiraterone and chemotherapy, improving the performance of a multivariable model as early as 4 wk after treatment. This potential surrogate must now be prospectively evaluated.

**Patient summary**

Circulating tumor cells (CTCs) are cancer cells that can be detected in the blood of prostate cancer patients. We analyzed changes in CTCs after treatment with abiraterone and chemotherapy in two large clinical trials, and found that patients who have a decline in CTC count have a better survival outcome.

**Take Home Message**
This study establishes the association between a 30% decline in circulating tumor cells as early as 4 wk after treatment initiation and increased survival in patients with metastatic castration-resistant prostate cancer treated with abiraterone and chemotherapy in clinical trials.

**Keywords:** Castration-resistant prostate cancer, Treatment outcome, Response, Circulating tumor cells, Abiraterone, Chemotherapy.

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**The presence of CTCs in breast carcinoma may related with defect immune status of the patients.**


**Circulating Tumor Cells (CTC) Are Associated with Defects in Adaptive Immunity in Patients with Inflammatory Breast Cancer.**

Mego M¹, Gao H², Cohen EN², Anfossi S², Giordano A², Sanda T², Fouad TM³, De Giorgi U⁴, Giuliano M⁵, Woodward WA⁶, Alvarez RH⁷, Valero V⁸, Ueno NT⁸, Hortobagyi GN³, Cristofanilli M⁹, Reuben JM¹⁰.

**Author information**

**Abstract**

**BACKGROUND:**

Circulating tumor cells (CTCs) play a crucial role in tumor dissemination and are prognostic in primary and metastatic breast cancer. Peripheral blood (PB) immune cells contribute to an unfavorable microenvironment for CTC survival. This study aimed to correlate CTCs with the PB T-cell immunophenotypes and functions of patients with inflammatory breast cancer (IBC).

**METHODS:**

This study included 65 IBC patients treated at the MD Anderson Cancer Center. PB was obtained from patients prior to starting a new line of chemotherapy for CTCs enumeration by CellSearch®, and T cell phenotype and function by flow cytometry; the results were correlated with CTCs and clinical outcome.

**RESULTS:**

At least 1 CTC (≥1) or ≥5 CTCs was detected in 61.5% or 32.3% of patients, respectively. CTC count did not correlate with total lymphocytes; however, patients with ≥1 CTC or ≥5 CTCs had lower percentages (%) of CD3+ and CD4+ T cells compared with patients with no CTCs or <5 CTCs, respectively. Patients with ≥1 CTC had a lower percentage of T-cell receptor (TCR)-activated CD8+ T cells synthesizing TNF-α and IFN-γ and a higher percentage of T-regulatory lymphocytes compared to patients without CTCs. In multivariate analysis, tumor grade and % CD3+ T-cells were associated with ≥1 CTC, whereas ≥5 CTC was associated with tumor grade, stage, % CD3+ and % CD4+ T cells, and % TCR-activated CD8 T-cells synthesizing IL-17.

**CONCLUSIONS:**
IBC patients with CTCs in PB had abnormalities in adaptive immunity that could potentially impact tumor cell dissemination and initiation of the metastatic cascade.

**KEYWORDS:**
Circulating tumors cells; adaptive immunity; and inflammatory breast cancer

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PMCID:PMC4911877
DOI:10.7150/jca.13098

**Added 8.05.16**

The detection and characterization of CTCs in metastatic melanoma may provide the opportunity to personalized approach and resolve the issue of tumor diversity.


**Circulating Melanoma Cell Subpopulations: Their Heterogeneity and Differential Responses to Treatment.**

Gray ES¹, Reid AL¹, Bowyer S², Calapre L¹, Siew K², Pearce R¹, Cowell L³, Frank MH⁴, Millward M⁵, Ziman M⁶.

**Author information**

**Abstract**

Metastatic melanoma is a highly heterogeneous tumor; thus, methods to analyze tumor-derived cells circulating in blood should address this diversity. Taking this into account, we analyzed, using multiparametric flow cytometry, the co-expression of the melanoma markers melanoma cell adhesion molecule and melanoma-associated chondroitin sulphate proteoglycan and the tumor-initiating markers ATP-binding cassette sub-family B member 5 (ABCB5), CD271, and receptor activator of NF-κβ (RANK) in individual circulating tumor cells (CTCs) from 40 late-stage (III-IV) and 16 early-stage (I-II) melanoma patients. CTCs were heterogeneous within and between patients, with limited co-expression between the five markers analyzed. Analysis of patient matched blood and metastatic tumors revealed that ABCB5 and RANK subpopulations are more common among CTCs than in the solid tumors, suggesting a preferential selection for these cells in circulation. Pairwise comparison of CTC subpopulations longitudinally before and 6-13 weeks after treatment initiation showed that the percentage of RANK(+) CTCs significantly increased in the patients undergoing targeted therapy (N=16, P<0.01). Moreover, the presence of ≥5 RANK(+) CTCs in the blood of patients undergoing targeted therapies was prognostic of shorter progression-free survival (hazards ratio 8.73, 95% confidence interval 1.82-41.75, P<0.01). Taken together, our results provide evidence of the heterogeneity among CTC subpopulations in melanoma and the differential response of these subpopulations to targeted therapy.

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PMCID:PMC4504811
DOI:10.1038/jid.2015.127
Relevant markers on CTCs in breast carcinoma cases with relevance with the risk of metastases.


Circulating levels of transforming growth factor-βeta (TGF-β) and chemokine (C-X-C motif) ligand-1 (CXCL1) as predictors of distant seeding of circulating tumor cells in patients with metastatic breast cancer.


Author information

Abstract

BACKGROUND:

The presence of circulating tumor cells (CTCs) in the peripheral blood is a prerequisite for the formation of distant metastases. Transforming growth factor-βeta (TGF-β) and Chemokine (C-X-C Motif) Ligand-1 (CXCL1) are cytokines involved in the colonization of distant sites by CTCs in several pre-clinical animal models. However, their role is poorly-investigated in patients with metastatic cancer. Here, we investigated whether circulating levels of TGF-β and CXCL1 are predictors of CTC seeding in preferential distant sites in patients with metastatic breast cancer.

MATERIALS AND METHODS:

CTCs were isolated from the peripheral blood of 61 patients with metastatic breast cancer by immunomagnetic separation. Plasma samples were collected from the same patients and assayed for TGF-β and CXCL1 by enzyme-linked immunoassay.

RESULTS:

Patients were grouped in CK1+/-(N<10), CK2+(N ≥ 10<50) and CK3+(N ≥ 50), according to the number (N) of cytokeratin 7/8-positive CTCs: the highest number of CK7/8-positive CTCs was detected in patients with negative Human epidermal growth factor receptor-2 (HER-2/NEU) status (p<0.0001) antigen, identified by the monoclonal antibody Ki-67 (Ki-67) ≥ 15% (p=0.003), Carcinoma antigen 15-3 (CA-15.3) ≥ 40 U/ml (p=0.004) and those with lung metastases (p=0.01). We found that elevated plasma concentrations of TGF-β and CXCL1 are predictive for the detection of CTCs. In particular, patients with CK3+ CTCs and plasma concentrations of TGF-β and CXCL1 higher than the median value had a poor prognosis in comparison to patients with CK1+/− CTCs and TGF-β and CXCL1 concentrations below the median value.

CONCLUSION:
Our study shows that elevated circulating levels of TGF-β and CXCL1 are associated with a poor prognosis, and higher detection of CTCs and propensity of these cells to seed lung metastases in patients with breast cancer.

PMID:23564790

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**Added 7.22.16**

**Value of CTCs in lung carcinoma cases.**


**Predictive and prognostic value of circulating tumor cell detection in lung cancer: a clinician's perspective.**

Tognela A1, Spring KJ2, Becker T2, Caixeiro NJ3, Bray VJ4, Yip PY5, Chua W6, Lim SH7, de Souza P8.

**Author information**

Abstract

There is increasing evidence for the use of circulating tumor cells (CTCs) as a "liquid biopsy" for early detection of lung cancer recurrence, prognosticating disease and monitoring treatment response. Further, CTC molecular analysis and interrogation of single cells hold significant potential in providing insights into tumor biology and the metastatic process. Ongoing research will likely see the translation of CTCs as a prognostic and predictive biomarker in both small cell, and non-small cell, lung cancer to routine clinical practice.

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**KEYWORDS:**

Biomarkers; Circulating tumor cells; Lung cancer; Non-small cell; Small cell

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DOI:10.1016/j.critrevonc.2014.10.001

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**Added 7.15.16**

**The role of CTCs in cases of colon carcinoma with peritoneal carcinomatosis and their indication for surgical processes.**


**Prognostic factor analysis of circulating tumor cells in peripheral blood of patients with peritoneal carcinomatosis of colon cancer origin treated with cytoreductive surgery plus an intraoperative hyperthermic intraperitoneal chemotherapy procedure (CRS + HIPEC).**
Melero JT1, Ortega FG2, Gonzalez AM1, Carmona-Saez P2, Garcia Puche JL2, Sugarbaker PH3, Delgado M4, Lorente JA5, Serrano MJ6.

**Author information**

**Abstract**

**PURPOSE:**

Complete cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) has changed the therapeutic landscape, improving overall survival in patients with peritoneal carcinomatosis with a colonic origin. The main limitation of this aggressive locoregional procedure, however, is extra-abdominal or distant spread. The objective of this study was to identify the prognostic value of circulating tumor cells (CTCs) in patients with peritoneal carcinomatosis of colonic origin undergoing CRS + HIPEC.

**PATIENTS AND METHODS:**

Fourteen patients diagnosed with peritoneal carcinomatosis from colon cancer and suitable for potentially curative treatment with CRS + HIPEC were included in this study. CTCs were isolated from the peripheral blood by immunomagnetic techniques by the use of a multi-cytokeratin-specific antibody and detected via immunocytochemical methods. The phenotypic characterization of EGFR on CTCs was analyzed by immunofluorescence.

**RESULTS:**

At baseline, 50% of the patients were positive for CTCs, with a mean value of 5.5 CTCs per 10 mL of peripheral blood. After surgery, 28.57% of the patients presented CTCs, with a mean value of 6.75 CTCs per 10 mL. A positive correlation was found between the presence of CTC-negative, epidermal growth factor receptor-positive at baseline and the patients who had symptoms of intestinal obstruction (21.4%). In addition, the presence of CTCs identified patients with distant dissemination and was also significantly correlated with progression-free survival (P = .0024).

**CONCLUSION:**

The detection and characterization of CTCs are good prognostic and predictive markers in patients with peritoneal carcinomatosis resulting from colon cancer. These analyses could be used as a new tool to identify subpopulations of patients who could benefit from CRS + HIPEC treatment.

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DOI:10.1016/j.surg.2015.09.013

Added 7.08.16

*The role circulating cancer stem cells and their possible clinical value.*

Circulating cancer stem cells: the importance to select.
Yang MH1, Imrali A1, Heeschen C1.

Author information

Abstract

It has been demonstrated that even localized tumors without clinically apparent metastasis give rise to circulating tumor cells (CTCs). A growing number of technically diverse platforms are being developed for detecting/isolating CTCs in the circulating blood. Despite the technical challenges of isolating rare CTCs from blood, recent studies have already shown the predictive value of CTCs enumeration. Thus, it is becoming increasingly accepted that CTC numbers are linked to patients' outcome and may also be used to monitor treatment response and disease relapse, respectively. Further CTCs provide a non-invasive source for tumor material, 'liquid biopsy', which is particularly important for patients, where no biopsy material can be obtained or where serial biopsies of the tumor, e.g., following treatment, are practically impossible. On the other hand the molecular and biological characterization of CTCs has still remained at a rather experimental stage. Future studies are necessary to define CTC heterogeneity to establish the crucial role of circulating cancer stem cells for driving metastasis, which represent a distinct subpopulation of CTCs that bear metastasis-initiating capabilities based on their stemness properties and invasiveness and thus are critical for the patients’ clinical outcome. As compared to non-tumorigenic/metastatic bulk CTCs, circulating cancer stem cells may not only be capable of evading from the primary tumor, but also escape from immune surveillance, survive in the circulating blood and subsequently form metastases in distant organs. Thus, circulating cancer stem cells represent a subset of exclusively tumorigenic cancer stem cells characterized by their invasive characteristics and are potential therapeutic targets for preventing disease progression. To date, only a few original reports and reviews have been published focusing on circulating cancer stem cells. This review discusses the potential importance of isolating and characterizing these circulating cancer stem cells, but also highlights current technological limitations.

KEYWORDS:
Cancer stem cells; circulating tumor cells (CTCs); drug resistance

PMID:26543330
PMCID:PMC4626824
DOI:10.3978/j.issn.1000-9604.2015.04.08

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Added 7.01.16

The CTCs role in prognosis of overall survival according their mitotic rate.

Mitosis in circulating tumor cells stratifies highly aggressive breast carcinomas

- Daniel L. AdamsEmail author,
- Diane K. Adams,
- Steingrimur Stefansson,
- Christian Haudenschild,
Enumeration of circulating tumor cells (CTCs) isolated from the peripheral blood of breast cancer patients holds promise as a clinically relevant, minimally invasive diagnostic test. However, CTC utility has been limited as a prognostic indicator of survival by the inability to stratify patients beyond general enumeration. In comparison, histological biopsy examinations remain the standard method for confirming malignancy and grading malignant cells, allowing for cancer identification and then assessing patient cohorts for prognostic and predictive value. Typically, CTC identification relies on immunofluorescent staining assessed as absent/present, which is somewhat subjective and limited in its ability to characterize these cells. In contrast, the physical features used in histological cytology comprise the gold standard method used to identify and preliminarily characterize the cancer cells. Here, we superimpose the methods, cytologically subtyping CTCs labeled with immunohistochemical fluorescence stains to improve their prognostic value in relation to survival.

Methods

In this single-blind prospective pilot study, we tracked 36 patients with late-stage breast cancer over 24 months to compare overall survival between simple CTC enumeration and subtyping mitotic CTCs. A power analysis (\(1-\beta = 0.9, \alpha = 0.05\)) determined that a pilot size of 30 patients was sufficient to stratify this patient cohort; 36 in total were enrolled.

Results

Our results confirmed that CTC number is a prognostic indicator of patient survival, with a hazard ratio 5.2, \(p = 0.005\) (95% CI 1.6–16.5). However, by simply subtyping the same population based on CTCs in cytological mitosis, the hazard ratio increased dramatically to 11.1, \(p < 0.001\) (95% CI 3.1–39.7).

Conclusions
Our data suggest that (1) mitotic CTCs are relatively common in aggressive late-stage breast cancer, (2) mitotic CTCs may significantly correlate with shortened overall survival, and (3) larger and more defined patient cohort studies are clearly called for based on this initial pilot study.

Keywords
Circulating tumor cells Mitotic index of CTCs Blood based biopsy Breast cancer cell motility

Added 6.24.16

The detection of alteration of CTCs concentration in squamous cells carcinoma may have a predictive value for possible recurrence.


Prognostic Role of Circulating Tumor Cells during Induction Chemotherapy Followed by Curative Surgery Combined with Postoperative Radiotherapy in Patients with Locally Advanced Oral and Oropharyngeal Squamous Cell Cancer.

Inhestern J1, Oertel K1, Stemmann V1, Schmalenberg H2, Dietz A3, Rotter N4, Veit J4, Görner M5, Sudhoff H6, Junghanß C7, Wittekindt C8, Pachmann K9, Guntinas-Lichius O1.

Author information

Abstract

BACKGROUND:

The prognostic role of circulating tumor cells (CTCs) after induction chemotherapy using docetaxel, cisplatin and fluorouracil (TPF) prior to surgery and adjuvant (chemo)radiation in locally advanced oral squamous cell cancer (OSCC) was evaluated.

METHODS:

In this prospective study, peripheral blood samples from 40 patients of the phase II study TISOC-1 (NCT01108042) with OSCC before, during, and after treatment were taken. CTCs were quantified using laser scanning cytometry of anti-epithelial cell adhesion molecule-stained epithelial cells. Their detection was correlated with clinical risk factors, recurrence-free (RFS) and overall survival (OS).

RESULTS:

Before starting the treatment CTCs were detected in 32 of 40 patients (80%). The median number at baseline was 3295 CTCs/ml. The median maximal number of CTCs during treatment was 5005 CTCs/ml. There was a significant increase of CTCs before postoperative radiotherapy compared to baseline before 1st cycle of IC (p = 0.011), 2nd cycle of IC (p = 0.001), 3rd cycle of IC (p = 0.004), and before surgery (p = 0.002), but not compared to end of therapy (p = 0.118). CTCs at baseline >median was also associated to risk of recurrence (p
Maximal CTCs during therapy >median was more frequently observed in tumors of the oral cavity (p=0.022) and related to higher risk of death during follow-up (p = 0.028). Patients with CTCs at baseline >median value had significant lower RFS than patients with CTCs at baseline <median value (p = 0.025). Patients with maximal CTCs values >median during the complete course of therapy had a significantly lower OS than patients with values <median (p = 0.049). Finally, the multivariate analysis revealed that OS was significantly lower in patients with maximal CTCs during treatment higher than the median value (HR=6.151; CI: 1.244-30.420).

CONCLUSIONS:

Baseline CTCs and maximal CTCs during therapy both seem to be good prognostic markers for OSCC when treated by TPF induction chemotherapy, surgery, and postoperative (chemo)radiation.

PMID:26186556  
PMCID:PMC4505900  
DOI:10.1371/journal.pone.0132901

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The increased number of CTCs in the portal vein may predict the development of a liver metastases.


A High Circulating Tumor Cell Count in Portal Vein Predicts Liver Metastasis From Periampullary or Pancreatic Cancer: A High Portal Venous CTC Count Predicts Liver Metastases.

Tien YW¹, Kuo HC, Ho BI, Chang MC, Chang YT, Cheng MF, Chen HL, Liang TY, Wang CF, Huang CY, Shew JY, Chang YC, Lee FY, Lee WH.

Author information

Abstract

Circulating tumor cells (CTCs) released from a periampullary or pancreatic cancer can be more frequently detected in the portal than the systemic circulation and potentially can be used to identify patients with liver micrometastases. Aims of this study is to determine if CTCs count in portal venous blood of patients with nonmetastatic periampullary or pancreatic adenocarcinoma can be used as a predictor for subsequent liver metastases. CTCs were quantified in portal and peripheral venous blood samples collected simultaneously during pancreaticoduodenectomy in patients with presumed periampullary or pancreatic adenocarcinoma without image-discernible metastasis. Postoperatively patients were monitored for liver metastasis by abdominal magnetic resonance imaging or computed tomography every 3 months for 1 year. Sixty patients with a pathological diagnosis of periampullary or pancreatic adenocarcinoma were included in the study. Multivariate analysis indicated that portal CTC count was a significant predictor for liver metastases within 6 months after surgery. Eleven of 13 patients with a high portal CTCs count (defined as >112 CMx Platform
estimated CTCs in 2mL blood) developed liver metastases within 6 months after surgery. In contrast, only 6 of 47 patients with a low portal CTC count developed liver metastases (P<0.0001). A value of 112 CMx Platform estimated CTCs had 64.7% sensitivity and 95.4% specificity to predict liver metastases within 6 months after surgery. We concluded that a high CTC count in portal venous blood collected during pancreaticoduodenectomy in patients with periampullary or pancreatic adenocarcinoma without metastases detected by currently available imaging tools is a significant predictor for liver metastases within 6 months after surgery.

PMID:27100430
PMCID:PMC4845834
DOI:10.1097/MD.0000000000003407

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**In head neck squamous cell carcinoma, the CTCs evaluation may have a prognostic value**

Diagnostic and Prognostic Value of Circulating Tumor Cells in Head and Neck Squamous Cell Carcinoma: a systematic review and meta-analysis

- Xiang-Lei Wu
- Qian Tu
- Gilbert Faure
- Patrice Gallet
- Chantal Kohler
- Marcelo De Carvalho Bittencourt

*Scientific Reports* 6, Article number: 20210 (2016)

doi:10.1038/srep20210

Several techniques have been developed to detect circulating tumor cells (CTC) in patients with head and neck squamous cell carcinoma (HNSCC), but their diagnostic and prognostic value are not yet fully established. A computerized retrieval of literatures was conducted without time restrictions using the electronic database in December 2014. Diagnostic accuracy variables were pooled and analyzed by the Meta-DiSc software. Engauge Digitizer and Stata software were used for pooled survival analysis. Twenty-two retrieved studies were eligible for systematic review, of which 9 conformed for the diagnostic test meta-analysis and 5 for the prognostic analysis. Subgroup analysis showed 24.6% pooled sensitivity and 100% pooled specificity of detections by using positive selection strategy, which moreover presented low heterogeneity. The presence of CTC was significantly associated with shorter disease free survival (DFS, HR 4.62, 95% CI 2.51–8.52). In conclusion, current evidence
identifies the CTC detection assay as an extremely specific, but low sensitive test in HNSCC. Also, the presence of CTC indicates a worse DFS.

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**Added 5.20.16**

*The heterogeneity of CTCs should also be in consideration in advance stage of the disease since may influence severely clinical decisions*


**Evaluation and consequences of heterogeneity in the circulating tumor cell compartment.**

Brouwer A1,2, De Laere B1, Peeters D1,3, Peeters M1,2, Salgado R1,3,4, Dirix L1,5, Van Laere S1

**Author information**

**Abstract**

A growing understanding of the molecular biology of cancer and the identification of specific aberrations driving cancer evolution have led to the development of various targeted agents. Therapeutic decisions concerning these drugs are often guided by single biopsies of the primary tumor. Yet, it is well known that tumors can exhibit significant heterogeneity and change over time as a result of selective pressure. Circulating tumor cells (CTCs) are shed from various tumor sites and are thought to represent the molecular landscape of a patient's overall tumor burden. Moreover, a minimal-invasive liquid biopsy facilitates monitoring of clonal evolution during therapy pressure and disease progression in real-time. While more information becomes available regarding heterogeneity among CTCs, comparison between these studies is needed. In this review, we focus on the genomic and transcriptional heterogeneity found in the CTC compartment, and its significance for clinical decision making.

**KEYWORDS:**

- circulating tumor cells; heterogeneity; liquid biopsy

PMID: 26980749
DOI: 10.18632/oncotarget.8015

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**Added 5.13.16**

*The contribution of CTCs in triple negative breast carcinoma is established also by a meta-analysis*


**The significant prognostic value of circulating tumor cells in triple-negative breast cancer: a meta-analysis.**
Lu YJ1, Wang P2, Wang X1, Peng J1, Zhu YW1, Shen N1.

Author information

Abstract

BACKGROUND:

The clinical validity of circulating tumor cells (CTCs) is still controversial in patients with triple-negative breast cancer (TNBC).

METHODS:

A comprehensive literature search was performed to identify relevant articles in the PubMed, Web of Science, MEDLINE, and Embase databases through September 2015. The outcomes of interest were disease progression and overall survival. The hazard ratio (HR) and 95% confidence interval (95% CI) were considered the effect indicators and were pooled in meta-analyses under a fixed- or random-effect model according to heterogeneity.

RESULTS:

Ten of the eligible studies were included for a total of 642 enrolled TNBC patients. Overall analyses revealed that the presence of CTCs predicted aggressive disease progression (HR = 2.18, 95% CI = 1.59-2.99, Pheterogeneity = 0.010, I² = 52.2%) and reduced overall survival (HR = 2.02, 95% CI = 1.59-2.57, Pheterogeneity = 0.169, I² = 26.6%). Further subgroup analyses demonstrated that CTC-positive patients also had poor disease progression and overall survival in different subsets, including cancer stage.

CONCLUSION:

Our meta-analysis provides strong evidence that detection of CTC in the peripheral blood is an independent prognosticator of poor survival outcomes for TNBC patients.

KEYWORDS:
circulating tumor cells (CTCs); prognosis; triple-negative breast cancer (TNBC)

PMID:27008698
DOI:10.18632/oncotarget.8156

Added 4.29.16

The value of CTCs is also established in Pancreatic ductal carcinoma.


Clinical Significance of Circulating Tumor Microemboli as a Prognostic Marker in Patients with Pancreatic Ductal Adenocarcinoma.
Abstract

BACKGROUND:

Characterization of circulating tumor cells (CTCs) has been used to provide prognostic, predictive, and pharmacodynamic information in many different cancers. However, the clinical significance of CTCs and circulating tumor microemboli (CTM) in patients with pancreatic ductal adenocarcinoma (PDAC) has yet to be determined.

METHODS:

In this prospective study, CTCs and CTM were enumerated in the peripheral blood of 63 patients with PDAC before treatment using anti-EpCAM (epithelial cell adhesion molecule)-conjugated supported lipid bilayer-coated microfluidic chips. Associations of CTCs and CTM with patients’ clinical factors and prognosis were determined.

RESULTS:

CTCs were abundant [mean (SD), 70.2 (107.6)] and present in 81% (51 of 63) of patients with PDAC. CTM were present in 81% (51 of 63) of patients with mean (SD) 29.7 (1101.4). CTM was an independent prognostic factor of overall survival (OS) and progression free survival (PFS). Patients were stratified into unfavorable and favorable CTM groups on the basis of CTM more or less than 30 per 2 mL blood, respectively. Patients with baseline unfavorable CTM, compared with patients with favorable CTM, had shorter PFS (2.7 vs 12.1 months; P < 0.0001) and OS (6.4 vs 19.8 months; P < 0.0001). Differences persisted if we stratified patients into early and advanced diseases. The number of CTM before treatment was an independent predictor of PFS and OS after adjustment for clinically significant factors.

CONCLUSIONS:

The number of CTM, instead of CTCs, before treatment is an independent predictor of PFS and OS in patients with PDAC.

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PMID:26861552
DOI:10.1373/clinchem.2015.248260

Added 4.22.16

The model of metastases may be hidden in CTCs enumeration and analysis.

Modelling circulating tumour cells for personalised survival prediction in metastatic breast cancer.

Ascolani G¹, Occhipinti A¹, Liò P¹.

Author information

Abstract

Ductal carcinoma is one of the most common cancers among women, and the main cause of death is the formation of metastases. The development of metastases is caused by cancer cells that migrate from the primary tumour site (the mammary duct) through the blood vessels and extravasating they initiate metastasis. Here, we propose a multi-compartment model which mimics the dynamics of tumoural cells in the mammary duct, in the circulatory system and in the bone. Through a branching process model, we describe the relation between the survival times and the four markers mainly involved in metastatic breast cancer (EPCAM, CD47, CD44 and MET). In particular, the model takes into account the gene expression profile of circulating tumour cells to predict personalised survival probability. We also include the administration of drugs as bisphosphonates, which reduce the formation of circulating tumour cells and their survival in the blood vessels, in order to analyse the dynamic changes induced by the therapy. We analyse the effects of circulating tumour cells on the progression of the disease providing a quantitative measure of the cell driver mutations needed for invading the bone tissue. Our model allows to design intervention scenarios that alter the patient-specific survival probability by modifying the populations of circulating tumour cells and it could be extended to other cancer metastasis dynamics.

PMID:25978366
PMCID:PMC4433130
DOI:10.1371/journal.pcbi.1004199

Added 4.15.16

CTCs may help us identify the primary tumor when it is unknown.


Identifying cancer origin using circulating tumor cells.

Lu SH¹,², Tsai WS³, Chang YH⁴, Chou TY⁵, Pang ST⁴, Lin PH⁴, Tsai CM⁶, Chang YC¹,².

Author information

Abstract

Circulating tumor cells (CTCs) have become an established clinical evaluation biomarker. CTC count provides a good correlation with the prognosis of cancer patients, but has only been used with known cancer patients, and has been unable to predict the origin of the CTCs. This study demonstrates the analysis of CTCs for the identification of their primary cancer source. Twelve mL blood samples were equally dispensed on 6 CMx chips, microfluidic chips coated with an anti-EpCAM-conjugated supported lipid bilayer, for CTC capture and isolation. Captured CTCs were eluted to an immunofluorescence (IF) staining panel consisting of 6 groups of
antibodies: anti-panCK, anti-CK18, anti-CK7, anti-TTF-1, anti-CK20/anti-CDX2, and anti-PSA/anti-PSMA. Cancer cell lines of lung (H1975), colorectal (DLD-1, HCT-116), and prostate (PC3, DU145, LNCaP) were selected to establish the sensitivity and specificity for distinguishing CTCs from lung, colorectal, and prostate cancer. Spiking experiments performed in 2mL of culture medium or whole blood proved the CMx platform can enumerate cancer cells of lung, colorectal, and prostate. The IF panel was tested on blood samples from lung cancer patients (n = 3), colorectal cancer patients (n = 5), prostate cancer patients (n = 5), and healthy individuals (n = 12). Peripheral blood samples found panCK(+) and CK18(+) CTCs in lung, colorectal, and prostate cancers. CTCs expressing CK7(+) or TTF-1(+), (CK20/CDX2)(+), or (PSA/PSMA)(+) corresponded to lung, colorectal, or prostate cancer, respectively. In conclusion, we have designed an immunofluorescence staining panel to identify CTCs in peripheral blood to correctly identify cancer cell origin.

**KEYWORDS:**
CMx chip; Cancer cell origin; IF panel; circulating tumor cells; immunofluorescence staining; microfluidic chip; supported lipid bilayer

PMID: 26828696
PMCID: PMC4910938
DOI: 10.1080/15384047.2016.1141839

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**How much help circulating tumor cells can become as a prognostic factor for the clinical benefit of primary surgical resection of a tumor or metastasectomies?**


**Perioperative circulating tumor cell detection: Current perspectives.**

*Kaifi JT*1,2, *Li G*1,3, *Clawson G*4, *Kimchi ET*1,2,3, *Staveley-O'Carroll KF*1,2,3.

**Author information**

**Abstract**

Primary cancer resections and in selected cases surgical metastasectomies significantly improve survival, however many patients develop recurrences. Circulating tumor cells (CTCs) function as an independent marker that could be used in the prognostication of different cancers. Sampling of blood and bone marrow compartments during cancer resections is a unique opportunity to increase individual tumor cell capture efficiency. This review will address the diagnostic and therapeutic potentials of perioperative tumor isolation and highlight the focus of future studies on characterization of single disseminated cancer cells to identify targets for molecular therapy and immune escape mechanisms.

**KEYWORDS:**
Circulating tumor cells; disseminated tumor cells; metastasectomy; surgery
Added 3.25.16

**In rectal carcinoma cases circulating tumor cells may carry information relative with responsiveness to radiotherapy.**

*BMC Cancer. 2015*

**Cytokeratin 20 positive circulating tumor cells are a marker for response after neoadjuvant chemoradiation but not for prognosis in patients with rectal cancer.**

*Sebastian Hinz, Christian Röder, Jürgen Tepel, Alexander Hendricks, Clemens Schafmayer, Thomas Becker, Holger Kalthoff*

**ABSTRACT**

**BACKGROUND:** Several studies have shown, that circulating tumor cells (CTC) have a negative prognostic value in colorectal cancer patients. Aim of this study was to evaluate the role of CTC in specifically rectal cancer patients regarding the influence on overall survival and to elucidate the impact of CTC in predicting response after chemoradiation (RCTX).

**METHODS:** In this prospective monocentric study 267 patients with rectal cancer were included. Patients with locally advanced tumors were treated with RCTX followed by surgery. The primary endpoints were: Evaluation of CTC at the time of surgery and correlation with main tumor characteristics, response to neoadjuvant RCTX and overall survival (OS). CTC were detected in the blood using CK20 RT-PCR.

**RESULTS:** Sixty-three patients were treated with neoadjuvant RCTX. In 46.8 % of the patients receiving neoadjuvant RCTX CTC were detected, which was significantly higher than in the group without RCTX (p = 0.002). Histopathologic regression after RCTX was evident in 27.8 % of the patients. In the subgroup of responders after RCTX we found CTC at a significantly lower rate than in non-responders (p = 0.03). No significant association was found between CTC detection and tumor characteristics and OS. The OS was significantly improved for responders compared to non-responders (p = 0.007).

**CONCLUSIONS:** Responders after neoadjuvant RCTX had a lower incidence of CTC compared to non-responders, which might be a result of effective systemic and local treatment prior to surgery.
Interestingly, detection of CTC did not correlate with tumor stage and OS, which is in contrast to previous reports of patients with colon cancer.

**Added 3.18.16**

CTCs can also be utilized in purpose to identify the organ of the primary origin of cancer.

*CancerBiolTher. 2016 Jan*

**Identifying Cancer Origin Using Circulating Tumor Cells.**

*Lu SH, Tsai WS, Chang YH, Chou TY, Pang ST, Lin PH, Tsai CM, Chang YC.*

**ABSTRACT**

Circulating tumor cells (CTCs) have become an established clinical evaluation biomarker. CTC count provides a good correlation with the prognosis of cancer patients, but has only been used with known cancer patients, and has been unable to predict the origin of the CTCs. This study demonstrates the analysis of CTCs for the identification of their primary cancer source. 12 mL blood samples were equally dispensed on six CMx chips, microfluidic chips coated with an anti-EpCAM-conjugated supported lipid bilayer, for CTC capture and isolation. Captured CTCs were eluted to an immunofluorescence (IF) staining panel consisting of six groups of antibodies: anti-panCK, anti-CK18, anti-CK7, anti-TTF-1, anti-CK20/anti-CDX2, and anti-PSA/anti-PSMA. Cancer cell lines of lung (H1975), colorectal (DLD-1, HCT-116), and prostate (PC3, DU145, LNCaP) were selected to establish the sensitivity and specificity for distinguishing CTCs from lung, colorectal, and prostate cancer. Spiking experiments performed in 2 mL of culture medium or whole blood proved the CMx platform can enumerate cancer cells of lung, colorectal, and prostate. The IF panel was tested on blood samples from lung cancer patients (n = 3), colorectal cancer patients (n = 5), prostate cancer patients (n = 5), and healthy individuals (n = 12). Peripheral blood samples found panCK+ and CK18+ CTCs in lung, colorectal, and prostate cancers. CTCs expressing CK7+ or TTF-1+, (CK20/CDX2)+, or (PSA/PSMA)+ corresponded to lung, colorectal, or prostate cancer, respectively. In conclusion, we have designed an immunofluorescence staining panel to identify CTCs in peripheral blood to correctly identify cancer cell origin.
How CTCs may predict the success or failure in colorectal carcinoma when it is treated with monoclonal antibodies against EGFr.


Circulating tumor cells as a longitudinal biomarker in patients with advanced chemorefractory, RAS-BRAF wild-type colorectal cancer receiving cetuximab or panitumumab.

Musella V1, Pietrantonio F2, Di Buduo E1, Iacovelli R2, Martinetti A2, Sottotetti E2, Bossi I2, Maggi C2, Di Bartolomeo M2, de Braud F2, Daidone MG1, Cappelletti V1.

Abstract
A still relevant number of patients with RAS-BRAF wild-type colorectal cancer (CRC) do not respond to treatment with antiepidermal growth factor receptor (EGFR) monoclonal antibodies cetuximab and panitumumab, suggesting that additional biomarkers to guide patient selection are urgently needed. Circulating tumor cells (CTCs) may represent such a biomarker. In this prospective study, 38 patients with advanced RAS-BRAF-wild-type CRC received third-line therapy with cetuximab-irinotecan or panitumumab. Peripheral blood samples for CTC status determination were collected at baseline, during treatment at early (2-4 weeks) and at later (8-10 weeks) times. CTC enrichment was done with the AdnaTest ColonCancerSelect kit, whereas CTC detection was done with the AdnaTest ColonCancerDetect kit. CTC status positivity was defined according to the kit manufacturer's thresholds. Fifty percent of patients were defined as CTC positive at baseline and the overall RECIST response rate was 26%. CTC baseline status was not associated with treatment response, whereas early CTC status and CTC status changes during treatment were significantly associated with tumor response. Kaplan-Meier analysis showed a significantly shorter progression-free survival (median, 2.0 versus 4.0 months, p = 0.004) and overall survival (4.7 versus 11.4, p = 0.039) in patients with early CTC + status compared with CTC - ones. In multivariable analysis including classical prognostic factors, the CTC status changes profile during treatment was an independent predictor of both progression-free survival (p < 0.001) and overall-survival (p = 0.001). CTC status assessed early during treatment with anti-EGFR monoclonal antibodies may predict treatment failure in advance compared to imaging-based tools.

What is the utility of CTCs as a predictive parameter in pancreatic carcinoma cases?

Circulating Tumor Cell Phenotype Predicts Recurrence and Survival in Pancreatic Adenocarcinoma.


ABSTRACT

OBJECTIVE: We assessed circulating tumor cells (CTCs) with epithelial and mesenchymal phenotypes as a potential prognostic biomarker for patients with pancreatic adenocarcinoma (PDAC).

BACKGROUND: PDAC is the fourth leading cause of cancer death in the United States. There is an urgent need to develop biomarkers that predict patient prognosis and allow for better treatment stratification.

METHODS: Peripheral and portal blood samples were obtained from 50 patients with PDAC before surgical resection and filtered using the Isolation by Size of Epithelial Tumor cells method. CTCs were identified by immunofluorescence using commercially available antibodies to cytokeratin, vimentin, and CD45.

RESULTS: Thirty-nine patients (78%) had epithelial CTCs that expressed cytokeratin but not CD45. Twenty-six (67%) of the 39 patients had CTCs which also expressed vimentin, a mesenchymal marker. No patients had cytokeratin-negative and vimentin-positive CTCs. The presence of cytokeratin-positive CTCs (P< 0.01), but not mesenchymal-like CTCs (P= 0.39), was associated with poorer survival. The presence of cytokeratin-positive CTCs remained a significant independent predictor of survival by multivariable analysis after accounting for other prognostic factors (P< 0.01). The detection of CTCs expressing both vimentin and cytokeratin was predictive of recurrence (P= 0.01). Among patients with cancer recurrence, those with vimentin-positive and cytokeratin-expressing CTCs had decreased median time to recurrence compared with patients without CTCs (P= 0.02).

CONCLUSIONS: CTCs are an exciting potential strategy for understanding the biology of metastases, and provide prognostic utility for PDAC patients. CTCs exist as heterogeneous populations, and assessment should include phenotypic identification tailored to characterize cells based on epithelial and mesenchymal markers.

Added 2.26.16

Why we shouldn’t rely in positive selection method of CTCs based on one marker only.

BMC Cancer. 2012 May

Circulating tumour cells escape from EpCAM-based detection due to epithelial-to-mesenchymal transition.
ABSTRACT

BACKGROUND:
Circulating tumour cells (CTCs) have shown prognostic relevance in metastatic breast, prostate, colon and pancreatic cancer. For further development of CTCs as a biomarker, we compared the performance of different protocols for CTC detection in murine breast cancer xenograft models (MDA-MB-231, MDA-MB-468 and KPL-4). Blood samples were taken from tumour bearing animals (20 to 200 mm²) and analysed for CTCs using 1. an epithelial marker based enrichment method (AdnaTest), 2. an antibody independent technique, targeting human gene transcripts (qualitative PCR), and 3. an antibody-independent approach, targeting human DNA-sequences (quantitative PCR). Further, gene expression changes associated with epithelial-to-mesenchymal transition (EMT) were determined with an EMT-specific PCR assay.

METHODS: We used the commercially available Adna Test, RT-PCR on human housekeeping genes and a PCR on AluJ sequences to detect CTCs in xenografts models. Phenotypic changes in CTCs were tested with the commercially available "Human Epithelial to Mesenchymal Transition RT-Profiler PCR Array".

RESULTS: Although the AdnaTest detects as few as 1 tumour cell in 1 ml of mouse blood spiking experiments, no CTCs were detectable with this approach in vivo despite visible metastasis formation. The presence of CTCs could, however, be demonstrated by PCR targeting human transcripts or DNA-sequences - without epithelial pre-enrichment. The failure of CTC detection by the AdnaTest resulted from downregulation of EpCAM, whereas mesenchymal markers like Twist and EGFR were upregulated on CTCs. Such a change in the expression profile during metastatic spread of tumour cells has already been reported and was linked to a biological program termed epithelial-mesenchymal transition (EMT).

CONCLUSIONS: The use of EpCAM-based enrichment techniques leads to the failure to detect CTC populations that have undergone EMT. Our findings may explain clinical results where low CTC numbers have been reported even in patients with late metastatic cancers. These results are a starting point for the identification of new markers for detection or capture of CTCs, including the mesenchymal-like subpopulations.

Date: 26 February 2016

Added 2.11.16

Cancer stem cells and tumor-associated macrophages: a roadmap for multitargeting strategies

C Raggi1,4, H S Mousa1,4, M Correnti1, A Sica2,3 and P Invernizzi1

1. Liver Unit and Center for Autoimmune Liver Diseases, Humanitas Clinical and Research Center, Milan, Italy
2. Laboratory of Molecular Immunology, Humanitas Clinical and Research Center, Milan, Italy
Abstract
The idea that tumor initiation and progression are driven by a subset of cells endowed with stem-like properties was first described by Rudolf Virchow in 1855. ‘Cancer stem cells’, as they were termed more than a century later, represent a subset of tumor cells that are able to generate all tumorigenic and nontumorigenic cell types within the malignancy. Although their existence was hypothesized >150 years ago, it was only recently that stem-like cells started to be isolated from different neoplastic malignancies. Interestingly, Virchow, in suggesting a correlation between cancer and the inflammatory microenvironment, also paved the way for the ‘Seed and Soil’ theory proposed by Paget a few years later. Despite the time that has passed since these two important concepts were suggested, the relationships between Virchow’s ‘stem-like cells’ and Paget’s ‘soil’ are far from being fully understood. One emerging topic is the importance of a stem-like niche in modulating the biological properties of stem-like cancer cells and thus in affecting the response of the tumor to drugs. This review aims to summarize the recent molecular data concerning the multilayered relationship between cancer stem cells and tumor-associated macrophages that form a key component of the tumor microenvironment. We also discuss the therapeutic implications of targeting this synergistic interplay.

Abbreviations:
c-Myc, avian myelocytomatosis virus oncogene cellular homolog; CCL-2, chemokine C-C motif ligand 2; CSC, cancer stem cell; CSF-1, colony-stimulating factor 1; EMT, epithelial-to-mesenchymal transition; M-CSF, macrophage colony-stimulating factor; NF-κB, nuclear factor-κB; SOX2, sex-determining region-Y-related high-mobility group box 2; TAM, tumor-associated macrophage; Wnt, wingless-type MMTV integration site family member

Added 2.5.16

There are markers in CTCs in urothelial carcinoma that have clinical relevance with the response to radiation therapy.

Cancer BiolTher. 2014 Jun 15

Application of a telomerase-based circulating tumor cell (CTC) assay in bladder cancer patients receiving postoperative radiation therapy: a case study.

Ju M, Kao GD, Steinmetz D, Chandrasekaran S, Keefe SM, Guzzo TJ, Christodouleas JP, Hahn SM, Dorsey JF.

ABSTRACT

BACKGROUND: Muscle invasive bladder carcinoma is an often lethal disease that requires aggressive treatment. Improved assays would contribute to better risk prediction and clinical management of this disease. A telomerase-based assay to detect circulating tumor cells (CTCs) may usefully fulfill this role.
METHODS: Two patients (C1 and C2) were enrolled onto an IRB-approved bladder biomarker study before initiating post-operative radiation therapy (RT) for muscle invasive bladder carcinoma. Blood samples were taken at predefined intervals: before, during, and after RT and then retrospectively correlated with imaging studies and disease course.

RESULTS: C1 began RT for positive resection margins on surgical pathology, at which time CTCs were undetectable and pelvic imaging demonstrated no evidence of disease. However, following the completion of treatment, the patient's CTC count was found to have increased to 202 CTCs/mL, and MRI demonstrated new abdominal and pelvic masses consistent with progressive disease. C1 ultimately died of disease with distant and local failure. Conversely, C2 was found to have 632 CTCs/mL before the initiation of RT for positive surgical margins, although imaging demonstrated no visible masses. At the conclusion of RT, repeat imaging showed changes that were indeterminate for either tumor recurrence or post-radiation effects. However, the patient's CTC count had dropped to 184 CTCs/mL. Furthermore, a second follow-up assay performed 6 months later revealed no detectable CTCs and repeat imaging showed complete resolution of worrisome imaging changes, thus excluding tumor progression.

CONCLUSION: To our knowledge this is the first report of a telomerase-based assay to identify CTCs in bladder cancer patients. Further studies are required to fully determine the ultimate clinical utility of this assay. However, the two patient vignettes described here illustrate how serial CTC assays may track the disease course and inform the management of bladder cancer patients undergoing adjuvant RT and potentially chemotherapy.  THIS IS NOT THE FIRST REPORT BECAUSE WE HAVE NUMEROUS BLADDER CA. PATIENTS OVER 12 YRS

How important the cancer stem cells in breast carcinoma are? What is the clinical importance of them?


Breast cancer stem cells: current advances and clinical implications.


ABSTRACT

There is substantial evidence that many cancers, including breast cancer, are driven by a population of cells that display stem cell properties. These cells, termed cancer stem cells (CSCs) or tumor initiating cells, not only drive tumor initiation and growth but also mediate tumor metastasis and therapeutic resistance. In this chapter, we summarize current advances in CSC research with a major focus on breast CSCs (BCSCs). We review the prevailing methods to isolate and characterize BCSCs and recent evidence documenting their cellular origins and phenotypic plasticity that enables them to transition between mesenchymal and epithelial-like states. We describe in vitro and clinical evidence that these cells mediate metastasis and treatment resistance in breast cancer, the development of novel strategies to isolate circulating tumor cells (CTCs) that contain CSCs and the use of patient-derived xenograft (PDX) models in preclinical breast cancer research. Lastly, we highlight several signaling pathways that regulate BCSC self-renewal and describe clinical implications of targeting these cells for breast cancer treatment. The development of strategies to effectively target BCSCs has the potential to significantly improve the outcomes for patients with breast cancer.
Are there markers that predict the area of metastases in colorectal carcinoma? Learn more... THE TEST THAT R.G.C.C. OFFERS FOR THIS IS: METASTAT®

Gastroenterology. 2013 Sep

Isolation and phenotypic characterization of colorectal cancer stem cells with organ-specific metastatic potential.
Gao W, Chen L, Ma Z, Du Z, Zhao Z, Hu Z, Li Q.

ABSTRACT

BACKGROUND & AIMS: Migrating cancer stem cells (MCSCs) are believed to form metastases. We sought to identify markers of MCSCs from human colorectal cancers (CRCs) and determine their roles in organ-specific metastasis.

METHODS: To identify colorectal MCSCs that contribute to organ-specific metastasis, we developed a model of liver or lung metastasis using primary tumor cells from patients with CRC who had liver and lung metastases. Distinct organ-specific metastatic cells were isolated by 6 cycles of selecting for cells that formed liver and lung tumors after subcutaneous injection into mice. Microarray analysis was used to identify markers of the organ-specific MCSCs. We then measured levels of these markers in CRC cell lines and 128 CRC samples. We characterized the functional roles of these markers in organ-specific metastasis.

RESULTS: We identified CD110 and CDCP1 as cell surface markers of MCSCs from human colorectal tumors that metastasized to liver and lung. We observed a distinct pattern of CD110 and CDCP1 in a panel of primary colorectal tumor samples and their matched liver or pulmonary metastases, indicating that these proteins might serve as biomarkers of organ-specific metastasis. Functional studies showed that thrombopoietin attracts CD110(+) CSCs and increases their self-renewal to promote formation of liver metastases. CDCP1 promoted adhesion of CRC cells to the lung endothelium.

CONCLUSIONS: We isolated MCSCs from primary human CRCs and found that the CD110(+) and CDCP1(+) subpopulations mediate organ-specific metastasis. These findings might be used to aid in selection of patients for postoperative adjuvant therapy.

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Realities and myths about the stage of a cancer and the time of spreading CTCs and disseminated tumor cells.

Cancer Growth Metastasis. 2015 Nov

Cancer Metastases: Early Dissemination and Late Recurrences.
Friberg S, Nyström A.

ABSTRACT

Background: Metastatic cells from a primary tumor can occur before the primary cancer is detected.
cells can also remain in the patient for many years after removal of the primary tumor without proliferating. These dormant malignant cells can awaken and cause recurrent disease decades after the primary treatment. The purpose of this article is to review the clinical evidence for early dissemination and late recurrences in human malignant tumors. We used the following definitions: dormancy of cells may be defined as a nonproliferating state or an arrest in the cell cycle that results in a prolonged G0 phase. If one accepts the term "late metastases" to indicate a period exceeding 10 years from the removal of the primary tumor, then the two malignancies in which this occurs most frequently are cutaneous malignant melanoma (CMM) and renal cell carcinoma (RCC).

**Methods:** PubMed, Web of Science, and Scopus were searched with the keywords "metastases," "early dissemination," "late recurrences," "inadvertently transmitted cancer," "tumor growth rate," "dormancy," "circulating tumor cells," and "transplantation of cancer."

**Results:** Several case reports of early dissemination and late recurrences of various types of malignancies were found. Analyses of the growth rates of several malignant tumors in the original host indicated that the majority of cancers had metastasized years before they were detected. CMM, RCC, and malignant glioblastoma were the three most common malignancies resulting from an organ transplantation. CMM and RCC were also the two most common malignancies that showed dormancy. In several cases of transplanted CMM and RCC, the donor did not have any known malignancy or had had the malignancy removed so long ago that the donor was regarded as cured.

**Conclusion:** (1) Metastases can frequently exist prior to the detection of the primary tumor. (2) Metastatic cells may reside in organs in the original host that are not usually the site of detectable secondary tumors, for example, the kidneys and heart. (3) Metastatic cells remain dormant for decades after the primary tumor has been removed. (4) Dormancy might be reversible and lead to late recurrences.

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**Added 12.11.15**

**The role of c-MET is promising as a marker for recurrences prediction.**


**c-MET immunostaining in colorectal carcinoma is associated with local disease recurrence.**

*Al-Maghrabi J, Eman E, Gomaa W, Saggaf M, Buhmeida A, Al-Qahtani M, Al-Ahwal M.*

**ABSTRACT**

**BACKGROUND:**
Increased mesenchymal-epithelial transition factor gene (c-MET) expression in several human malignancies is related to increased tumour progression. The aim of the present study is to explore the relationship between immunohistochemical expression of c-MET in colorectal carcinoma (CRC) and the clinicopathological characteristics and follow up data, to compare the expression of c-MET in primary CRC and its metastasis in lymph nodes and to test its validity as independent prognostic factor.

**METHODS:**
Hundred and thirty-five archival CRC and nodal metastases samples were collected from King Abdulaziz University Hospital, Saudi Arabia. Tissue microarrays were constructed and immunohistochemistry was done to detected c-MET protein expression. Appropriate statistical analysis was performed.
RESULTS:
High c-MET immunostaining was significantly associated with tumour size larger than 5 cm (p

CONCLUSION:
c-MET is a new promising target that may help in understanding the pathogenesis of CRC, and to be used as independent prognostic biomarker to predict local disease recurrence in CRC. Further molecular in vitro and in vivo studies are required to pursue c-MET as potential molecular marker of metastases and test the possibility of its incorporation as a new targeted therapeutic target.

Additional 12.04.15

Be familiar with relationship between Heat shock proteins and their relevance or radioresistance and radiosensitivity.

Oncotarget. 2015 Oct 27
doi: 10.18632/oncotarget.6248

Radioresistant human lung adenocarcinoma cells that survived multiple fractions of ionizing radiation are sensitive to HSP90 inhibition.

ABSTRACT:
Despite the common usage of radiotherapy for the treatment of NSCLC, outcomes for these cancers when treated with ionizing radiation (IR) are still unsatisfactory. A better understanding of the mechanisms underlying resistance to IR is needed to design approaches to eliminate the radioresistant cells and prevent tumor recurrence and metastases. Using multiple fractions of IR we generated radioresistant cells from T2821 and T2851 human lung adenocarcinoma cells. The radioresistant phenotypes present in T2821/R and T2851/R cells include multiple changes in DNA repair genes and proteins expression, upregulation of EMT markers, alterations of cell cycle distribution, upregulation of PI3K/AKT signaling and elevated production of growth factors, cytokines, important for lung cancer progression, such as IL-6, PDGFB and SDF-1 (CXCL12). In addition to being radioresistant these cells were also found to be resistant to cisplatin. HSP90 is a molecular chaperone involved in stabilization and function of multiple client proteins implicated in NSCLC cell survival and radioresistance. We examined the effect of ganetespib, a novel HSP90 inhibitor, on T2821/R and T2851/R cell survival, migration and radioresistance. Our data indicates that ganetespib has cytotoxic activity against parental T2821 and T2851 cells and radioresistant T2821/R and T2851/R lung tumor cells. Ganetespib does not affect proliferation of normal human lung fibroblasts. Combining IR with ganetespib completely abrogates clonogenic survival of radioresistant cells. Our data show that HSP90 inhibition can potentiate the effect of radiotherapy and eliminate radioresistant and cisplatin-resistant residual cells, thus it may aid in reducing NSCLC tumor recurrence after fractionated radiotherapy.

Additional 11.30.15

Urothelial carcinoma can also been assessed and monitored by CTCs.


Detection of circulating tumor cells in metastatic and clinically localized urothelial carcinoma.
ABSTRACT

OBJECTIVE:
To examine the incidence and prognostic value of circulating tumor cells (CTCs) in urothelial cancer (UC). The detection of CTCs is prognostic in several cancer types.

METHODS:
A total of 44 subjects with UC were assessed for CTCs using CellSearch Technology and 7.5 mL of peripheral blood, sorted by magnetic separation (epithelial cell adhesion molecule positive) and immunofluorescent staining (positive for cytokeratin 8, 18, or 19, negative for CD45, positive for 4',6-diamidino-2-phenylindole) to identify the CTCs.

RESULTS:
Five (17%) of 30 subjects with clinically localized and 7 (50%) of 14 subjects with metastatic UC had ≥1 detectable CTC (range 1-177). Six subjects had ≥5 CTCs. Fluorescence in situ hybridization analysis was performed in 20 samples from 18 unique subjects using the UroVysion probe set. Copy number gains consistent with neoplasm were observed in those with measurable CTCs but not in any of the CTC-negative samples tested. With a median follow-up of 337 days, all 7 patients with metastasis and detectable CTCs had died compared with 3 (43%) of the 7 with metastasis but without detectable CTCs.

CONCLUSION:
CTCs are commonly observed in metastatic UC. CTCs were observed in 50% of the patients with metastatic UC tested. Fluorescence in situ hybridization analysis confirmed the aneusomic chromosomal content in the CTCs. These findings suggest that measurable CTCs might be prognostic for shortened survival in patients with metastatic UC, although the optimal threshold for a "positive" finding is unknown. CTCs were also detected in a subset of patients with clinically localized disease, identifying a potential high-risk, preoperative group for future study.

Added 11.20.15

In the absence of reliable biomarker in sarcomas CTCs become an important 'player' in the field.


Universal marker and detection tool for human sarcoma circulating tumor cells.


ABSTRACT:
To date, no specific marker exists for the detection of circulating tumor cells (CTC) from different types of sarcomas, though tools are available for detection of CTCs in peripheral blood of patients with cancer for epithelial cancers. Here, we report cell-surface vimentin (CSV) as an exclusive marker on sarcoma CTC regardless of the tissue origin of the sarcoma as detected by a novel monoclonal antibody. Utilizing CSV as a probe, we isolated and enumerated sarcoma CTCs with high sensitivity and specificity from the blood of patients bearing different types of sarcoma, validating their phenotype by single cell genomic amplification, mutation detection, and FISH. Our results establish the first universal and specific CTC marker described for enumerating CTCs from different types of sarcoma, thereby providing a key prognosis tool to monitor cancer metastasis and relapse.
Learn about the role of CTCs in ovarian carcinoma.

Tumour Biol. 2015 Oct 26

**Circulating tumor cells as trigger to hematogenous spreads and potential biomarkers to predict the prognosis in ovarian cancer.**

Gasparri ML, Savone D, Besharat RA, Farooqi AA, Bellati F, Ruscito I, Panici PB, Papadia A

**ABSTRACT:**

Despite several improvements in the surgical field and in the systemic treatment, ovarian cancer (OC) is still characterized by high recurrence rates and consequently poor survival. In OC, there is still a great lack of knowledge with regard to cancer behavior and mechanisms of recurrence, progression, and drug resistance. The OC metastatization process mostly occurs via intracoelomatic spread. Recent evidences show that tumor cells generate a favorable microenvironment consisting in T regulatory cells, T infiltrating lymphocytes, and cytokines which are able to establish an "immuno-tolerance milieu" in which a tumor cell can become a resistant clone. When the disease responds to treatment, immunoediting processes and cancer progression have been stopped. A similar inhibition of the immunosuppressive microenvironment has been observed after optimal cytoreductive surgery as well. In this scenario, the early identification of circulating tumor cells could represent a precocious signal of loss of the immune balance that precedes cancer immunoediting and relapse. Supporting this hypothesis, circulating tumor cells have been demonstrated to be a prognostic factor in several solid tumors such as colorectal, pancreatic, gastric, breast, and genitourinary cancer. In OC, the role of circulating tumor cells is still to be defined. However, as opposed to healthy women, circulating tumor cells have been demonstrated in peripheral blood of OC patients, opening a new research field in OC diagnosis, treatment monitoring, and follow-up.

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The value of CTCs have been also established for sarcomas.


**Significance of Circulating Tumor Cells in Soft Tissue Sarcoma.**

Chiara Nicolazzo and Angela Gradilone

**ABSTRACT:**

Circulating tumor cells can be detected from the peripheral blood of cancer patients. Their prognostic value has been established in the last 10 years for metastatic colorectal, breast, and prostate cancer. On the contrary their presence in patients affected by sarcomas has been poorly investigated. The discovery of EpCAM mRNA expression in different sarcoma cell lines and in a small
cohort of metastatic sarcoma patients supports further investigations on these rare tumors to deepen the importance of CTC isolation. Although it is not clear whether EpCAM expression might be originally present on tumor sarcoma cells or acquired during the mesenchymal-epithelial transition, the discovery of EpCAM on circulating sarcoma cells opens a new scenario in CTC detection in patients affected by a rare mesenchymal tumor.

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**Added 10.30.15**

**CD44 as a biomarker and the presence of cancer stem cells may become a very important prognostic factor for breast carcinoma.**


_Published online 2015 Oct 4._ doi: 10.1155/2015/158682  

**Prognostic Value of Cancer Stem Cells Markers in Triple-Negative Breast Cancer.**  
Francesca Collina, Maurizio Di Bonito, Valeria Li Bergolis, Michelino De Laurentis, Carlo Vitagliano, Margherita Cerrone, Francesco Nuzzo, Monica Cantile, Gerardo Botti

**ABSTRACT:**  
Triple-negative breast cancer (TNBC) has a significant clinical relevance of being associated with a shorter median time to relapse and death and does not respond to endocrine therapy or other available targeted agents. Increased aggressiveness of this tumor, as well as resistance to standard drug therapies, may be associated with the presence of stem cell populations within the tumor. Several stemness markers have been described for the various histological subtypes of breast cancer, such as CD44, CD24, CD133, ALDH1, and ABCG2. The role of these markers in breast cancer is not clear yet and above all there are conflicting opinions about their real prognostic value. To investigate the role of CSCs markers in TNBC cancerogenesis and tumor progression, we selected 160 TNBCs samples on which we detected protein expression of CD44, CD24, CD133, ALDH1, and ABCG2 by immunohistochemistry. Our results highlighted a real prognostic role only for CD44 in TNBCs. All other CSCs markers do not appear to be related to the survival of TNBC patients. In conclusion, despite the fact that the presence of the cancer stem cells in the tumor provides important information on its potential aggressiveness, today their detection by immunohistochemistry is not sufficient to confirm their role in carcinogenesis, because specific markers probably are not yet identified.

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**Added 10.26.15**

**Learn more about the contribution and importance of CTCs in lung carcinomas.**

*Lung Cancer, Volume 76, Issue 1: April 2012, Pages 19–25*  

*Circulating tumour cells, their role in metastasis and their clinical utility in lung cancer.*  
John D. O'Flaherty, Steven Gray, Derek Richard, Dean Fennell, John J. O’Leary, Fiona H. Blackhall, Kenneth J. O’Byrne

**ABSTRACT:**
Circulating tumour cells (CTCs) have attracted much recent interest in cancer research as a potential biomarker and as a mean of studying the process of metastasis. It has long been understood that metastasis is a hallmark of malignancy, and conceptual theories on the basis of metastasis from the nineteenth century foretold the existence of a tumour “seed” which is capable of establishing discrete tumours in the “soil” of distant organs. This prescient “seed and soil” hypothesis accurately predicted the existence of CTCs; microscopic tumour fragments in the blood, at least some of which are capable of forming metastases. However, it is only in recent years that reliable, reproducible methods of CTC detection and analysis have been developed. To date, the majority of studies have employed the CellSearch™ system (Veridex LLC), which is an immunomagnetic purification method. Other promising techniques include microfluidic filters, isolation of tumour cells by size using microporous polycarbonate filters and flow cytometry-based approaches. While many challenges still exist, the detection of CTCs in blood is becoming increasingly feasible, giving rise to some tantalizing questions about the use of CTCs as a potential biomarker. CTC enumeration has been used to guide prognosis in patients with metastatic disease, and to act as a surrogate marker for disease response during therapy. Other possible uses for CTC detection include prognostication in early stage patients, identifying patients requiring adjuvant therapy, or in surveillance, for the detection of relapsing disease. Another exciting possible use for CTC detection assays is the molecular and genetic characterization of CTCs to act as a “liquid biopsy” representative of the primary tumour. Indeed it has already been demonstrated that it is possible to detect HER2, KRAS and EGFR mutation status in breast, colon and lung cancer CTCs respectively. In the course of this review, we shall discuss the biology of CTCs and their role in metastagenesis, the most commonly used techniques for their detection and the evidence to date of their clinical utility, with particular reference to lung cancer.

How appreciated is the value of CTCs in inflammatory breast carcinoma?

Circulating Tumor Cells and Recurrence After Primary Systemic Therapy in Stage III Inflammatory Breast Cancer.


Abstract

Inflammatory breast cancer (IBC) is rare and aggressive, with poor survival. While circulating tumor cells (CTCs) predict outcome in non-IBC patients, little data exists regarding their prognostic significance in IBC. This prospective study analyzed blood samples for CTCs from 63 stage III IBC patients to determine if CTCs present after primary systemic chemotherapy predicted relapse. CTC identification was not associated with tumor characteristics, lymph node positivity, or complete pathologic response to systemic therapy. At mean follow-up of 38 months, multivariable analysis demonstrated that detection of one or more CTCs predicted shortened relapse-free (log-rank P = 0.005, hazard ratio [HR] = 4.22, 95% confidence interval [CI] = 1.67 to 10.67, Cox P = 0.002) but not overall survival (log-rank P = 0.54, HR = 1.53, 95% CI = 0.41 to 5.79, Cox P = 0.53). All statistical tests were two-sided. In this study, CTCs after primary chemotherapy identified IBC patients at high risk for relapse.

How well informed you are about the contribution of CTCs and CSCs in resistance mechanism in therapy approach and in the risk of relapse?


EMT, CTCs and CSCs in tumor relapse and drug-resistance.
Mitra A, Mishra L, Li S.

A B S T R A C T

Tumor relapse and metastasis are the primary causes of poor survival rates in patients with advanced cancer despite successful resection or chemotherapeutic treatment. A primary cause of relapse and metastasis is the persistence of cancer stem cells (CSCs), which are highly resistant to chemotherapy. Although highly efficacious drugs suppressing several subpopulations of CSCs in various tissue-specific cancers are available, recurrence is still common in patients. To find more suitable therapy for relapse, the mechanisms underlying metastasis and drug-resistance associated with relapse-initiating CSCs need to be identified. Recent studies in circulating tumor cells (CTCs) of some cancer patients manifest phenotypes of both CSCs and epithelial-mesenchymal transition (EMT). These patients are unresponsive to standard chemotherapies and have low progression free survival, suggesting that EMT-positive CTCs are related to co-occur with or transform into relapse-initiating CSCs. Furthermore, EMT programming in cancer cells enables in the remodeling of extracellular matrix to break the dormancy of relapse-initiating CSCs. In this review, we extensively discuss the association of the EMT program with CTCs and CSCs to characterize a subpopulation of patients prone to relapses. Identifying the mechanisms by which EMT-transformed CTCs and CSCs initiate relapse could facilitate the development of new or enhanced personalized therapeutic regimens.

What is the rational of CTCs usage in clinical practice in hormone refractory cases of prostate cancer?


Published online 2015 Jun 9. doi: 10.1186/s12885-015-1478-4

Categorical versus continuous circulating tumor cell enumeration as early surrogate marker for therapy response and prognosis during docetaxel therapy in metastatic prostate cancer patients.
Mark Thalgott, Brigitte Rack, Matthias Eiber, Michael Souvatzoglou, Matthias M. Heck, Caroline Kronester, Ulrich Andergassen, Victoria Kehl, Bernd J. Krause, Jurgen E. Gschwend, Margitta Retz, and Roman Nawroth

A B S T R A C T

Background:
Circulating tumor cell (CTCs) counts might serve as early surrogate marker for treatment efficacy in metastatic castration-resistant prostate cancer (mCRPC) patients. We prospectively assessed categorical and continuous CTC-counts for their utility in early prediction of radiographic response, progression-free (PFS) and overall survival (OS) in mCRPC patients treated with docetaxel.

Methods:
CTC-counts were assessed in 122 serial samples, as continuous or categorical (
Results:
Categorical CTC-count status predicted PD at q4 already after one cycle (q1) and after 4 cycles (q4) of chemotherapy with an odds ratio (OR) of 14.9 (p=0.02) and 18.0 (p=0.01). Continuous CTC-values predicted PD only at q4 (OR 1.04, p=0.048). Regarding PFS, categorical CTC-counts at q1 were independent prognostic markers with a hazard ratio (HR) of 3.85 (95% CI 1.1-13.8, p=0.04) whereas early continuous CTC-values at q1 failed significance (HR 1.02, 95% CI 0.99-1.05, p=0.14). For OS early categorical and continuous CTC-counts were independent prognostic markers at q1 with a HR of 3.0 (95% CI 1.6-15.7, p=0.007) and 1.02 (95% CI 1.0-1.04, p=0.04).

Conclusion:
Categorical CTC-count status is an early independent predictor for TR, PFS and OS only 3 weeks following treatment initiation with docetaxel whereas continuous CTC-counts were an inconsistent surrogate marker in mCRPC patients. For clinical practice, categorical CTC-counts may provide complementary information towards individualized treatment strategies with early prediction of treatment efficacy and optimized sequential treatment.

Do you know that CTCs analysis may contribute and improve the staging of breast cancer patients?

Circulating tumor cells in breast cancer beyond the genotype of primary tumor for tailored therapy.
Ren C, Han C, Fu D, Wang D, Chen H, Chen Y, Shen M.

Abstract

Although TNM staging based on tumor, node lymph status and metastasis status is the most widely used method in the clinic to classify breast cancer (BC) and assess prognosis, it offers limited information for different BC subgroups. Circulating tumor cells (CTCs) are regarded as minimal residual disease and are proven to have a strong relationship with BC. Detection of ≥5 CTCs per 7.5 mL in peripheral blood predicts poor prognosis in metastatic BC irrespective of other clinical parameters, whereas, in early-stage BC, detection of CK19+ CTCs are also associated with poor prognosis. Increasing data and clinical trials show that CTCs can improve prognostic accuracy and help tailor treatment for patients with BC. However, heterogeneous CTCs in the process of an epithelial-mesenchymal transition (EMT) in BC make it a challenge to detect these rare cells. Moreover, the genotypic and phenotypic features of CTCs are different from primary BC tumors. Molecular analysis of CTCs in BC may benefit patients by identifying those amenable to tailored therapy. We propose that CTCs should be used alongside the TNM staging system and the genotype of primary tumor to guide tailored BC diagnosis and treatment.

2015 UICC.

Are you aware that CTCs level may predicts the response to adjuvant radiotherapy in prostate cancer?
The significance of circulating tumor cells in prostate cancer patients undergoing adjuvant or salvage radiation therapy

L E Lowes, M Lock, G Rodrigues, D D'Souza, G Bauman, B Ahmad, V Venkatesan, A L Allan and T Sexton

ABSTRACT

BACKGROUND:
Following radical prostatectomy, success of adjuvant and salvage radiation therapy (RT) is dependent on the absence of micrometastatic disease. However, reliable prognostic/predictive factors for determining this are lacking. Therefore, novel biomarkers are needed to assist with clinical decision-making in this setting. Enumeration of circulating tumor cells (CTCs) using the regulatory-approved CellSearch System (CSS) is prognostic in metastatic prostate cancer. We hypothesize that CTCs may also be prognostic in the post-prostatectomy setting.

METHODS:
Patient blood samples (n=55) were processed on the CSS to enumerate CTCs at 0, 6, 12 and 24 months after completion of RT. CTC values were correlated with predictive/prognostic factors and progression-free survival.

RESULTS:
CTC status (presence/absence) correlated significantly with positive margins (increased likelihood of CTCneg disease; P=0.032), and trended toward significance with the presence of seminal vesicle invasion (CTCpos; P=0.113) and extracapsular extension (CTCneg; P=0.116). Although there was a trend toward a decreased time to biochemical failure (BCF) in baseline CTC-positive patients (n=9), this trend was not significant (hazard ratio (HR)=0.3505; P=0.166). However, CTC-positive status at any point (n=16) predicted for time to BCF (HR=0.2868; P=0.0437).

CONCLUSIONS:
One caveat of this study is the small sample size utilized (n=55) and the low number of patients with CTC-positive disease (n=16). However, our results suggest that CTCs may be indicative of disseminated disease and assessment of CTCs during RT may be helpful in clinical decision-making to determine, which patients may benefit from RT versus those who may benefit more from systemic treatments.

Added 8.21.15

What could be the contribution of CTCs in the refractory or recurrence non-small cell lung carcinoma cases?


Prognostic value of circulating tumor cells’ reduction in patients with extensive small-cell lung cancer.

Nicola Normanno, Antonio Rossi¹, Alessandro Morabito¹, Simona Signoriello¹, Simona Bevilacqua¹, Massimo Di Maio¹, Raffaele Costanzo, Antonella De Luca, Agnese Montanino, Cesare Gridelli, Gaetano Rocco,
ABSTRACT

Objectives: Circulating tumor cells (CTCs) have been hypothesized to be a prognostic factor in small-cell lung cancer (SCLC), and different cutoffs have been proposed to identify patients at high risk. We assessed the prognostic value of CTCs in patients with extensive SCLC.

Materials and methods: CTCs were assessed with the Cell search system in 60 extensive SCLC patients. CTC count at baseline or after one cycle of chemotherapy (cycle-1) or as change after chemotherapy were analyzed separately. Primary outcome was overall survival. The accuracy of prognostic role was assessed by Harrell's c-index. Optimal cutoffs were determined by Harrell's c-index. Improvement was estimated by calculating the difference of c-indexes of models including clinical variables with or without CTCs.

Results: CTCs were identified in 90% (54/60) of patients at baseline, in which CTC count ranged from 0 to 24,281. CTC count was strongly associated with the number of organs involved. The prognostic accuracy was only marginally increased by the addition to clinical information of optimal cycle-1. Conversely, a reduction of CTC count higher than 89% following chemotherapy significantly improved prognostic accuracy (bootstrap p-value = 0.009) and was associated with a lower risk of death (HR 0.24, 95% CI 0.09–0.61). When previously proposed cutoffs were applied to our cohort, they showed only marginal improvement of the prognostic accuracy.

Are you aware that CTCs are playing major role of hepatic or pulmonary metastases in colorectal carcinoma? Find out why in the relevant research article below.

Localization of Circulating Tumor Cells in Patients With Hepatic Metastases
Long R.Jiao, Christos Apostolopoulos, Jimmy Jacob, Richard Szydlo, Natalia Johnson, Nicole Tsim, Nagy A. Habib, R.Charles Coombes, and Justin Stebbing

ABSTRACT

Purpose: There are few data on the impact of immediate and differing surgical interventions on circulating tumor cells (CTCs), nor their compartmentalization in different anatomic vascular sites.

Patients and Methods: CTCs from consecutive patients with colorectal liver metastases were quantified before and immediately after open surgery, laparoscopic resection, open radiofrequency ablation (RFA), or percutaneous RFA. For individuals undergoing open surgery, either hepatic resections or open RFA, CTCs were examined in both systemic and porta circulation by measuring CTCs in samples derived from the peripheral vein, an artery, the hepatic portal vein, and the hepatic vein.

Results:
A total of 29 consecutive patients with colorectal liver metastases with a median age of 55 years (range, 30 to 88 years) were included. CTCs were localized to the hepatic portosystemic macrocirculation with significantly greater numbers than in the systemic vasculature. Surgical procedures led to a statistically significant fall in CTCs at multiple sites measured. Conversely, RFA, either open or percutaneous, was associated with a significant increase in CTCs.

Conclusion:
Surgical resection of metastases, but not RFA, immediately decreases CTC levels. In patients with colorectal liver metastases, CTCs appear localized to the hepatic (and pulmonary) macrocirculations. This may explain why metastases in sites other than the liver and lungs are infrequently observed in cancer.

J Clin Oncol 27:6160-6165. © 2009 by American Society of Clinical Oncology

**Do you know that CTCs are correlated stronger with the clinical reality?**

ANTICANCER RESEARCH 32:2881(2012)

**Comparative Chemosensitivity of Circulating Human Prostate Cancer and Primary Cancer Cells**

RHIANA MENEN1,2,3, MING ZHAO1, LEI ZHANG1, MOHAMED K. HASSANEIN1, VLADIMER BOBEK4,5, KATARINA KOLOSTOVA4, MICHAELBOUVET2 and ROBERT M. HOFFMAN1,2.
1AntiCancer Inc.. San Diego, CA, USA.;
2Department of Surgery, University of California, San Diego, CA, USA.;
3Department of Surgery, University of California, San Francisco-Eastbay Oakland, CA, USA.;
4Department of Tumor Biology; Third Faculty of Medicine, Charles University, Prague, Czech Republic;
5Department of Surgery. Third Faculty of Medicine, Charles University and Faculty Hospital Kralovske Vinohrady, Prague, Czech Republic

Abstract:
The chemosensitivity of circulating PC-3 human prostate cancer cells, isolated from nude mice orthotopically implanted with PC-3, was compared to that of the parental PC-3 cells. PC-3 and circulating PC-3, both labeled with green fluorescent protein (GFP), were seeded in 96-well plates. The MTT assay was then performed at 24, 48 and 72 hours, comparing control cultures to cultures treated with cisplatin at 1, 2.5, 5 and 10 Ì¼m/ml, and docetaxel at 10, 20, 25 and 50 Ì¼m/ml at each time point. The circulating tumor cells (CTCs) exhibited a significantly increased sensitivity to both cisplatin and docetaxel when compared to PC-3 parental cells, with docetaxel having the greater efficacy. The future goal, based on the studies, is the culture of CTCs from cancer patients' peripheral blood for chemosensitivity testing, for improved individualized therapy.

**Molecular characterization of circulating tumor cells in patients with ovarian cancer improves their prognostic significance -- a study of the OVCAD consortium.**

ABSTRACT

OBJECTIVE:
The study aims at identifying novel markers for circulating tumor cells (CTCs) in patients with epithelial ovarian cancer (EOC), and at evaluating their impact on outcome.

METHODS:
Microarray analysis comparing matched EOC tissues and peripheral blood leucocytes (N=35) was performed to identify novel CTC markers. Gene expression of these novel markers and of EpCAM was analyzed using RT-qPCR in blood samples taken from healthy females (N=39) and from EOC patients (N=216) before primary treatment and six months after adjuvant chemotherapy. All samples were enriched by density gradient centrifugation. CTC positivity was defined by over-expression of at least one gene as compared to the healthy control group.

RESULTS:
CTC were detected in 24.5% of the baseline and 20.4% of the follow-up samples, of which two thirds were identified by overexpression of the cyclophilin C gene (PPIC), and just a few by EpCAM overexpression. The presence of CTCs at baseline correlated with the presence of ascites, sub-optimal debulking, and elevated CA-125 and HE-4 levels, whereas CTC during follow-up occurred more often in older and platinum resistant patients. PPIC positive CTCs during follow-up were significantly more often detected in the platinum resistant than in the platinum sensitive patient group, and indicated poor outcome independent from classical prognostic parameters.

CONCLUSIONS:
Molecular characterization of CTC is superior to a mere CTC enumeration or even be the rationale for CTC diagnostics at all. Ultimately CTC diagnostics may lead to more personalized treatment of EOC, especially in the recurrent situation.

The paradigm of personalized therapy in oncology.

ABSTRACT

INTRODUCTION:
Currently, anticancer therapy is mainly based on histology and on giving the same treatment to presumed homogeneous patients. The switch from histology-driven therapy to molecular clinical oncology is correlated with a better understanding of the 'molecular taxonomy' of each tumor that can provide us with targets for specific drugs. Cancer therapy is moving irreversibly towards personalized therapy that benefits selected patients. Once the potential therapeutic targets are identified, the availability of predictive biomarkers is the key element and their prospective evaluation should be a parallel component of the clinical evaluation of a new
drug.

**AREAS COVERED:**
The state of the art in clinical results of personalized therapy. The authors discuss the finding that, in patients with advanced disease, a limited number of targeted agents improve overall survival, whilst the majority only have an effect on response rate and/or time to tumor progression, with efficacy limited in time due to acquired resistance.

**EXPERT OPINION:**
The mechanisms leading to resistance are related to tumor cell heterogeneity and in part explained by the cancer stem cell model and genetic instability. The steps toward the optimization of tailored therapy need validated predictive biomarkers, pharmacogenetics analysis and a close collaboration between bench and bedside.

Authors: Gasparini G, Longo R.

Uploader: Ioannis Papasotiriou

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**Added 1.14.13**


**Circulating Tumor Cells: Personalized Medicine in Interventional Oncology?**

**ABSTRACT**

Innovative technologic advancements have expanded the ability of interventional radiologists to capture and visualize directly tumor cells that have intravasated into the circulation. The detection of these circulating tumor cells (CTCs) is revolutionizing the understanding of the pathogenesis of metastasis and is paving the way for exquisitely sensitive techniques to detect malignancy, monitor recurrence, and prognosticate outcomes. In this review, the prevailing theories on the pathobiology of metastasis and the tools that have been developed to investigate CTCs are summarized. The tremendous impact CTCs are likely to have in oncology is discussed, with particular emphasis on their relevance to interventional oncology.

Authors: Sheth RA, Hesketh R, Deipolyi AR, Oklu R.

Uploader: Ioannis Papasotiriou

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**Added 1.14.13**

**Front Oncol.** 2012; 2: 69. Published online 2012 Jul 2. doi: 10.3389/fonc.2012.00069 PMCID: PMC3387782

**Circulating tumor cells: the substrate of personalized medicine?**

**ABSTRACT**

Circulating tumor cells (CTCs) are believed to be responsible for the development of metastatic disease. Over the last several years there has been a great interest in understanding the biology of CTCs to understand metastasis, as well as for the development of companion diagnostics to predict patient response to anti-cancer targeted therapies. Understanding CTC biology requires innovative technologies for the isolation of these rare
cells. Here we review several methods for the detection, capture, and analysis of CTCs and also provide insight on improvements for CTC capture amenable to cellular therapy applications.

Authors: Greene BT, Hughes AD, King MR

Uploader: Ioannis Papasotiriou

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**Single-cell analysis of circulating tumor cells identifies cumulative expression patterns of EMT-related genes in metastatic prostate cancer.**

**ABSTRACT**

**BACKGROUND:**
Prostate tumors shed circulating tumor cells (CTCs) into the blood stream. Increased evidence shows that CTCs are often present in metastatic prostate cancer and can be alternative sources for disease profiling and prognostication. **Here we postulate that CTCs expressing genes related to epithelial-mesenchymal transition (EMT) are strong predictors of metastatic prostate cancer.**

**METHODS:**
A microfiltration system was used to trap CTCs from peripheral blood based on size selection of large epithelial-like cells without CD45 leukocyte marker. These cells individually retrieved with a micromanipulator device were assessed for cell membrane physical properties using atomic force microscopy. Additionally, 38 CTCs from eight prostate cancer patients were used to determine expression profiles of 84 EMT-related and reference genes using a microfluidics-based PCR system.

**RESULTS:**
Increased cell elasticity and membrane smoothness were found in CTCs compared to noncancerous cells, highlighting their potential invasiveness and mobility in the peripheral circulation. Despite heterogeneous expression patterns of individual CTCs, genes that promote mesenchymal transitioning into a more malignant state, including IGF1, IGF2, EGFR, FOXP3, and TGFβ3, were commonly observed in these cells. An additional subset of EMT-related genes (e.g., PTPRN2, ALDH1, ESR2, and WNT5A) were expressed in CTCs of castration-resistant cancer, but less frequently in castration-sensitive cancer.

**CONCLUSIONS:**
The study suggests that an incremental expression of EMT-related genes in CTCs is associated with metastatic castration-resistant cancer. Although CTCs represent a group of highly heterogeneous cells, their unique EMT-related gene signatures provide a new opportunity for personalized treatments with targeted inhibitors in advanced prostate cancer patients.

Authors: Chen CL, Mahalingam D, Osmulski P, Jadhav RR, Wang CM, Leach RJ, Chang TC, Weitman SD, Kumar AP, Sun L, Gaczynska ME, Thompson IM, Huang TH.

Uploader: Ioannis Papasotiriou
Prognostic significance of CTCs and CSCs of tumor drainage vein blood in Dukes stage B and C colorectal cancer patients.

ABSTRACT

The clinical significance of circulating tumor cells (CTCs) including cancer stem cells (CSCs) (CTC/CSC) in the tumor drainage vein blood of patients with colorectal cancer (CRC) is unclear. In this study, we investigated the prognostic value of CTC/CSC that express carcinoembryonic antigen (CEA) cytokeratin 19 (CK19), CK20 and/or CD133 (CEA/CK/CD133) mRNA in the tumor drainage blood of CRC patients with Dukes' stage B and C. We examined tumor drainage blood from 197 patients with Dukes' stage B and C CRC. CTCs that expressed CEA, CK19, CK20 and CD133 mRNA were detected using the quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) assay. Each mRNA level was normalized with GAPDH mRNA levels. In the relationship between the expression of CEA/CK/CD133 in the tumor drainage blood and clinicopathological factors, a significant correlation was observed between CEA/CK/CD133 expression and Dukes' stage (p<0.041). In CRC patients with Dukes' stage B and C, disease-free (DFS) and overall survival (OS) of patients with CEA/CK/CD133 positive in the tumor drainage blood were significantly worse than that of marker gene negative patients. In contrast, in patients with Dukes' stage A, no significant differences were shown between these groups. By Cox progression analysis, it was shown that CEA/CK/CD133 mRNA in tumor drainage blood was an independent prognostic factor for DFS and OS in patients with Dukes' stage B and C. These results suggest that detecting CEA/CK/CD133 mRNA in tumor drainage blood by the real-time RT-PCR method would have a prognostic value in CRC patients with Dukes' stage B and C.

Authors: Shimada R, Iinuma H, Akahane T, Horiuchi A, Watanabe T.

Uploader: Ioannis Papasotiriou

Added 12.21.2010

Relationship of circulating tumor cells to the effectiveness of cytotoxic chemotherapy in patients with metastatic non-small-cell lung cancer.

ABSTRACT

The aim of this study was to investigate the relationship of the number of circulating tumor cells (CTCs) with the effectiveness of cytotoxic chemotherapy in patients with metastatic non-small-cell lung cancer (NSCLC). We prospectively evaluated CTCs in the peripheral blood of patients with previously untreated metastatic NSCLC. From May 2008 through August 2010, 33 patients (23 men and 10 women; median age, 64 years; range, 46-74 years) were enrolled. All patients received combination chemotherapy with gemcitabine and carboplatin. The CTCs were captured from samples of peripheral blood with a semiautomated system using an antibody against epithelial cell adhesion molecule. Blood samples with one or more CTC per 7.5 ml were defined as positive. Of total 33 patients, 12 (36.4%) had positive CTCs and 5 (15.2%) had five or more CTCs before chemotherapy. There were no differences in response rates to cytotoxic chemotherapy between CTC-
positive patients and CTC-negative patients. On the other hand, the rate of progressive disease in cytotoxic chemotherapy was significantly higher in CTC-positive patients (66.7%) than in CTC-negative patients (23.8%, p = 0.02). In conclusion, the number of CTCs could be a useful predictive factor for the effectiveness of cytotoxic chemotherapy in patients with metastatic NSCLC.

Authors: Hirose T, Murata Y, Oki Y, Sugiyama T, Kusumoto S, Ishida H, Shirai T, Nakashima M, Yamaoka T, Okuda K, Ohnishi T, Ohmori T.

Uploader: Ioannis Papasotiriou

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**Barriers to the use of personalized medicine in breast cancer.**

**ABSTRACT**

**PURPOSE:**
Personalized medicine—the use of genomics and molecular diagnostics to direct care decisions—may improve outcomes by more accurately individualizing treatment to patients. Using qualitative research, we explored care delivery barriers to the use of personalized medicine for patients with breast cancer using examples of BRCA and gene expression profile testing.

**METHODS:**
We conducted 51 interviews with multidisciplinary stakeholders in breast cancer care: clinicians (n = 25) from three academic and nine nonacademic organizations, executives (n = 20) from four major private insurers, and patient advocates (n = 6).

**RESULTS:**
Barriers were common to the BRCA and gene expression profile tests and were classified under two categories: poor coordination of tests relative to treatment decisions and reimbursement-related disincentives. Perception of specific barriers varied across groups. Difficulty coordinating diagnostics relative to decisions was the most frequent concern by clinicians (60%), but only 35% of payers and 17% of advocates noted this barrier. For 60% of payers, drug- and procedure-based reimbursement was a significant barrier, but only 40% of clinicians and none of the advocates expressed the same concern. The opinion that patient out-of-pocket expenses are a barrier varied significantly between advocates and clinicians (83% v 20%, P < .007), and advocates and payers (83% v 15%, P < .004). Barriers were reported to result in postponement or avoidance of tests, delayed treatment decisions, and proceeding with decisions before test results.

**CONCLUSION:**
Poorly coordinated diagnostic testing and the current oncology reimbursement model are barriers to the use of genomic and molecular diagnostic tests in cancer care.

Authors: Weldon CB, Trosman JR, Gradishar WJ, Benson AB 3rd, Schink JC.

Uploader: Ioannis Papasotiriou
Moving toward personalized medicine in castration-resistant prostate cancer.

ABSTRACT

Recent advances in research technologies have allowed improved molecular characterization of castration-resistant prostate cancer (CRPC). These efforts hold promise for development of therapies that target alterations unique to an individual patient's prostate cancer. Targets include androgens and the androgen receptor pathway, pathways associated with hormone-resistant disease, and the immune system. In aggregate, this will allow physicians to choose treatments based on a particular tumor profile. As these approaches are developed, CRPC treatment is becoming an example of truly personalized medicine.

Authors: Van Allen EM, Pomerantz M.

Uploader: Ioannis Papasotiriou

Cancer Stem Cells and Chemosensitivity.

ABSTRACT

Cancer lethality is mainly due to the onset of distant metastases and refractoriness to chemotherapy. Thus, the development of molecular targeted agents able to restore or increase chemosensitivity will provide valuable therapeutic options for cancer patients. Growing evidence indicates that a cellular subpopulation with stem cell-like features, commonly referred to as cancer stem cells (CSCs), is critical for tumor generation and maintenance. Recent advances in stem cells biology are revealing that this cellular fraction shares many properties with normal adult stem cells and represents the prominent tumorigenic population able to propagate the parental tumor in animal models. CSCs seem to be protected against widely used chemotherapeutic agents by means of different mechanisms such as marked proficiency in DNA damage repair, high expression of ATP-binding cassette drug transporters and activation of PI3K/AKT and Wnt pathways. Moreover, microenvironmental stimuli such as those involved in epithelial-mesenchymal transition and hypoxia indirectly contribute to chemoresistance by inducing in cancer cells a stem-like phenotype. Understanding how CSCs overcome chemotherapy-induced death stimuli, and integrating such knowledge into clinical research methodology, has become a priority in the process of identifying innovative therapeutic strategies aimed at improving the outcome of cancer patients.

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Circulating tumor cells in breast cancer: A tool whose time has come of age.

ABSTRACT

Currently, CTCs are being integrated into clinical trial design as a surrogate for phenotypic and genotypic markers in correlation with development of molecularly targeted therapies. As CTCs play a crucial role in tumor dissemination, translational research is implicating CTCs in several biological processes, including epithelial to mesenchymal transition. In this mini-review, we review CTCs in metastatic breast cancer, and discuss their clinical utility for assessing prognosis and monitoring response to therapy. We will also introduce their utility in pharmacodynamic monitoring for rational selection of molecularly targeted therapies and briefly address how they can help elucidate the biology of cancer metastasis.

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Circulating and disseminated tumor cells in the management of advanced prostate cancer.

ABSTRACT

Management of prostate cancer is recognized as one of the most important medical problems. Latest findings concerning the role of circulating (CTC) and disseminated tumor cells (DTC) have provided new insights into the biology of metastasis with important implications for the clinical management of prostate cancer patients. Most of the established methods of circulating/disseminated tumor cell enrichment use density-gradient centrifugation and immunomagnetic procedures. Reverse transcriptase polymerase chain reaction is another used detection technique. Novel methods, the CTC-chip and the epithelial immunospot assay already showed promising results. For localized and metastatic prostate cancer, significant correlations between spreading tumor cells and well-established indicators of disease activity have been demonstrated. Careful randomized prospective trials will be required to justify the routine use of CTCs/DTCs for therapy decision making.

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Detection of circulating tumor cells and tumor stem cells in patients with breast cancer by using flow cytometry: a valuable tool for diagnosis and prognosis evaluation.

ABSTRACT

Circulating tumor stem cells (CTSC), a subpopulation of circulating tumor cells (CTC), may lead to recurrent diseases. The aim of this study was to detect CTC (CD45(-)EpCAM(+)) and CTSC (CD45(-)EpCAM(+))CD44(+)CD24(-)) of breast cancer (BC) patients, as well as to explore their clinical relevance. CTC and CTSC in peripheral blood (PB) of 45 female BC patients were detected by using flow cytometry (FCM).
SKBR-3 cells were mixed with MNC of four healthy volunteers at different ratios in order to evaluate the sensitivity of FCM. Real-time quantitative polymerase chain reaction (QRT-PCR) was conducted and compared with FCM. The expression of EPCAM between CTC < 50 and ctc ≥ 50 groups (19.98 ± 23.93 versus 29.46 ± 29.27 × 10⁻⁵), and the expression of cd44 between ctsc negative and positive groups (0.85 ± 0.91 versus 0.81 ± 0.75) were statistically the same. FCM had higher specificity than qrt-pcr. Statistical differences were obtained between ctc /< 50 and ctc ≥ 50 groups among different TNM stages, histology stages, estrogen receptor (ER) status and progesterone receptor (PR) status (p /< 0.05). Statistical differences between ctsc negative and positive groups within different TNM stages and regional lymph node metastasis (RLNM) status (p /< 0.05) were also obtained. Moreover, the percentage of ctc on CD45 negative cells (CD45(-)c) among different clinical pathology was statistically different, p="0.000." Additionally, the percentage of ctsc on CD45(-)c with TNM stage was rising (0: 0.00 ± 0.00‰, i: 0.03 ± 0.05‰, ii: 0.06 ± 0.14‰, iii: 0.10 ± 0.09‰, iv: 0.29 ± 0.35‰, p="0.034")." Statistical difference in the percentage of ctsc on CD45(-)c among different RLNM status (p="0.001") was also obtained. FCM to detect ctc and ctsc may be used to diagnose disease at early stage, to guide clinical therapy or to predict prognosis.


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Review Article Enabling personalized cancer medicine through analysis of gene-expression patterns

ABSTRACT

Therapies for patients with cancer have changed gradually over the past decade, moving away from the administration of broadly acting cytotoxic drugs towards the use of more-specific therapies that are targeted to each tumour. To facilitate this shift, tests need to be developed to identify those individuals who require therapy and those who are most likely to benefit from certain therapies. In particular, tests that predict the clinical outcome for patients on the basis of the genes expressed by their tumours are likely to increasingly affect patient management, heralding a new era of personalized medicine.

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Personalized medicine: hope or hype?

Personalized Medicine:

Medicine has always been personalized. For years, physicians have incorporated environmental, behavioural, and genetic factors that affect disease and drug response into patient management decisions. However, until recently, the 'genetic' data took the form of family history and self-reported race/ethnicity. As genome
sequencing declines in cost, the availability of specific genomic information will no longer be limiting. Rather, our ability to parse these data and our decision whether to use it will become primary. As our understanding of genetic association with drug responses and diseases continues to improve, clinically useful genetic tests may emerge to improve upon our previous methods of assessing genetic risks. Indeed, genetic tests for monogenic disorders have already proven useful. Such changes may usher in a new era of personalized medicine. In this review, we will discuss the utility and limitations of personal genomic data in three domains: pharmacogenomics, assessment of genetic predispositions for common diseases, and identification of rare disease-causing genetic variants.

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**Personalized medicine in oncology: present status, trends and reality-few thoughts**

The last few years the scientific and medical community has realized that treating cancer on average has minimal effect on the outcome of cancer treatment strategy and benefit. Recent discoveries in cancer pharmacogenomics lead us to start change our understanding about how cancer progresses and that each patient has its own profile and feature of its cancer, therefore, we have to base our treatment strategy to this profile. Recently there is an effort to introduce genetic test derived from the tumor sample where the discovery of genetic abnormalities may help us make the right decisions to cancer therapy for each individual. But in all these efforts we base the main analytic process to the level of genetic material (DNA) focusing on the discovery mutations, variations and abnormalities that may influence the therapy decisions. The point that actually makes us feel confident that we may be able to achieve better outcomes in cancer may also mislead us to the wrong decisions. To be specific we need to start put critical questions and try to provide answers to these.

1. For example we know that genetic material (DNA) holds only the information of the phenotype spectrum and we may never be sure that this information (irrelevant if they are normal or aberrant) will be expressed as protein and phenotype on cancer cells. So the question is: does all this information in a genetic level actually be relevant with the phenotype of the disease? How close all the information is to epigenetic (gene expression analysis) and proteomic (protein production) level?

2. We know from clinical reality that the primary tumor varies from the lymph node cancer and the metastases which may behave differently than the primary tumor because of the variation due to the genetic instability. So the next question is does the analysis from the tissue sample derived from the primary tumor will reflect also the same features of the distant cancer colonies and metastases?

3. Scientists have well proved and established the genetic heterogeneity and pleomorphy on a tumor which is consisted from normal cells as well as from several subset of cancer cells with different features and behavior. From all these subclones only one or very few clones will have the ability to metastasize and develop a distant metastases. These cells are known as tumor initiating cells (TIC) or cancer stem cell like (CSCs) which may be able to invade migrate and metastasize to a distant organ very early. So from the analysis of a primary tumor where the TIC may not be present any more, this information is relevant with the behavior of the disease in a macroscopic level as the body of a patient.
4. Scientists have recently developed awareness that cancer cells may change not only the gene sequence but also the gene expression rate and their profile (epigenetic level) which provides them a plasticity of phenotype in time. How relevant a genetic level analysis will be to this plasticity feature, since genetic mutation and variant happen much less frequent than the gene expression profile in a tumor? What does this mean to a patient treatment? Where is the role of the micro-environment to this part? From only these few preliminary questions a healthcare practitioner, a scientist and patients may realize that we may have done a progress by changing our mentality by introducing personalized medicine approach but we barely scratch the surface. We need to have a more robust and extensive analytic approach to each individual based on cancer kinetics and progression. If we do not manage to realize all these fast we may conclude to a big failure of personalized medicine approach and loose valuable time on cancer treatment which at the end cost even more patient lives.

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From pharmacogenetics to personalized medicine: a vital need for educating health professionals and the community.

ABSTRACT

The field of pharmacogenetics will soon celebrate its 50th anniversary. Although science has delivered an impressive amount of information in these 50 years, pharmacogenetics has suffered from lack of integration into clinical practice. There are several reasons for this, including the unmet need for education at medical schools and the lack of awareness about the impact of genetic medicine on healthcare in the community. Recently, the FDA announced that it considers pharmacogenomics one of three major opportunities on the critical path to new medical products. This notion by the FDA is filling the regulatory void that existed between drug developers and drug users. However, in order to bring pharmacogenetic testing to the prescription pad successfully, healthcare professionals and policy makers, as well as patients, need to have the necessary background knowledge for making educated treatment decisions. To effectively move pharmacogenetics into everyday medicine, it is therefore imperative for scientists and teachers in the field to take on the challenge of disseminating pharmacogenetic insights to a broader audience.

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Pharmacogenomics. scientific Journal

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Added 11.1.12
Future of Personalized Medicine in Oncology: A Systems Biology Approach

ABSTRACT The development of cost-effective technologies able to comprehensively assess DNA, RNA, protein, and metabolites in patient tumors has fueled efforts to tailor medical care. Indeed validated molecular tests assessing tumor tissue or patient germline DNA already drive therapeutic decision making. However, many theoretical and regulatory challenges must still be overcome before fully realizing the promise of personalized molecular medicine. The masses of data generated by high-throughput technologies are challenging to manage, visualize, and convert to the knowledge required to improve patient outcomes. Systems biology integrates engineering, physics, and mathematical approaches with biologic and medical insights in an iterative process to visualize the interconnected events within a cell that determine how inputs from the environment and the network rewiring that occurs due to the genomic aberrations acquired by patient tumors determines cellular behavior and patient outcomes. A cross-disciplinary systems biology effort will be necessary to convert the information contained in multidimensional data sets into useful biomarkers that can classify patient tumors by prognosis and response to therapeutic modalities and to identify the drivers of tumor behavior that are optimal targets for therapy. An understanding of the effects of targeted therapeutics on signaling networks and homeostatic regulatory loops will be necessary to prevent inadvertent effects as well as to develop rational combinatorial therapies. Systems biology approaches identifying molecular drivers and biomarkers will lead to the implementation of smaller, shorter, cheaper, and individualized clinical trials that will increase the success rate and hasten the implementation of effective therapies into the clinical armamentarium.

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The origin of Cancer Stem Cells

Cancer stem cells (CSCs), also known as tumor-initiating cells (TICs), are cancer cells that possess capability of proliferation, differentiation, and self-renewal. It is widely believed that CSCs play critical role in the initiation, metastasis, and relapse of cancers, but the origin of CSCs remains unclear. Up to date, several hypotheses have been described, and cell fusion and horizontal gene transfer, which may occur during development and tissue repair process, are considered as important origins of CSCs. In addition, critical gene mutations in stem cells, progenitor cells or even differentiated cells may also contribute to the formation of CSCs, and cell microenvironment is critical to CSC self-renewal and differentiation. The ongoing efforts to identify the CSCs origins may shed more light on understanding of cancer initiation and progression, as well as the development of novel cancer therapies.

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Uploader: Panagiotis Apostolou

BREAST CANCER, LOCALLY ADVANCED AND METASTATIC
CANCER STEM CELL-LIKE CELLS: A THERAPEUTIC MODEL IN BREAST CANCER PATIENTS, WHERE ANY OTHER RECOMMENDED THERAPY HAS FAILED


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Introduction
Nowadays, the difficulty to treat metastatic breast cancers is high. Many clinical therapeutic lines have failed and there is no suggested therapeutic approach (in literature) concerning this type of tumors. The last decades, circulating tumor cells (CTCs) are the state - of - the -art in cancer therapy. In the present study, CTCs were isolated and identified. CSCs (cancer stem cells) were isolated from the above population of CTCs and their gene pattern was compared with those from the primary tumor as well as with those from the metastatic tumor. This study attempts to find a correlation between the CTCs and the metastatic regions in comparison with the primary tumor as well as to find out if all the CSCs have the same hallmarks in the selected breast cancer stem cell populations.

Materials and methods
In order the protocol to be performed, CTCs from patient's blood samples were isolated and then cultured in appropriate conditions. CSCs were identified and isolated from the population of CTCs. mRNA was extracted and was used for Microarray hybridization assays. The same procedure was repeated for primary tumor as well for metastatic tumor samples. The expression pattern of primary tumor cells was compared with those in the metastatic tumor. Finally, the therapeutic approach which was based on the above findings, was designed.

Results
The results showed that the gene expression pattern of metastatic sites is similar to that of metastatic sites and less to that of the primary site. Then, the patients followed a therapeutic approach based on the data of the clinical results which were evaluated within a six- month period showing that the patients showed an objective response rate.

Conclusion
The results showed that the entity that determines the clinical outcome in breast cancer, is the subpopulation of CTCs, the circulating CSCs. Moreover, there are various types of CSCs in one patient, and not only one, as they have different growth mechanisms in primary and secondary tumors.

Disclosure
All authors have declared no conflicts of interest.

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